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Merocyclophanes A and B, Antiproliferative Cyclophanes from the Cultured Terrestrial Cyanobacterium *Nostoc* sp

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Abstract

The cell extract of a cultured terrestrial *Nostoc* sp. (UIC 10062), obtained from a sample collected at Grand Mere State Park in Michigan, displayed antiproliferative activity against the HT-29 human colon cancer cell line. Bioactivity-guided fractionation of the cell extract, combined with LC-MS analysis, led to the isolation of two cyclophanes, named merocyclophanes A and B (**1** and **2**). Their structures were determined by various spectroscopic techniques including HRESIMS, and 1D and 2D NMR analyses. The stereoconfiguration was assigned on the basis of X-ray crystallographic and CD analyses. The structures of merocyclophanes A and B (**1** and **2**) established a hitherto unknown [7.7]paracyclophane skeleton in nature, as characterized by α -branched methyls at C-1/14. Merocyclophanes A and B (**1** and **2**) displayed antiproliferative activity against the HT-29 human colon cancer cell line with IC₅₀ values of 3.3 and 1.7 μ M, respectively.

Keywords

Nostoc sp.; Cyanobacterium; Antiproliferative activity; HT-29 human colon cancer cells; [7.7]paracyclophanes

1. Introduction

Cyanobacteria (blue-green algae) have been shown to be prolific producers of bioactive secondary metabolites (Tan, 2007; Wagoner et al., 2004; Harada, 2004). Several terrestrial cyanobacterial species belonging to the order *Nostocales* have been reported to produce

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:

naturally occurring paracyclophanes. Of these, nostocyclyne A, an acetylenic [14]paracyclophane, was isolated from the natural bloom of *Nostoc* sp. and displayed antimicrobial activity against Gram-positive bacteria *S. aureus* and *B. subtilis* (Ploutno et al., 2000). Additionally, cylindrocyclophanes and nostocyclophanes, the two classes of cyanobacterial [7.7]paracyclophanes isolated from the cultured *Nostoc* sp. and *Cylindrospermum* sp., have exhibited a broad spectrum of biological activities, including antibacterial, antifungal and cytotoxic activities (Moore et al., 1990; Chen et al., 1991; Moore et al., 1992; Bui et al., 2007; Chlipala et al., 2010). As regards their formation, the polyketide biosynthetic pathway to these natural cyclophanes was determined by an isotope precursor administration experiment, and involves dimerization of two acetate-derived nonaketides and subsequent modification by chlorination, oxidation and/or methylation, resulting in diverse chemical structures (Bobzin et al., 1993).

Recently, several cyclindrocyclophanes were reported from a terrestrial *Nostoc* sp. (UIC 10022A) obtained from the material collected in the city of Chicago. These compounds displayed inhibitory activity against the 20S proteasome and were found to be cytotoxic against cancer cell lines, including HT-29, NCI-H460, SF268 and MCF7 cells (Chlipala et al., 2010). Herein, structure elucidation and biological activity of two additional cyclophanes, named merocyclophanes A and B (**1** and **2**), that were isolated from a second *Nostoc* sp. (UIC 10062) are reported. This strain was obtained from a sample collected at Grand Mere State Park in Michigan, and the merocyclophanes were named in recognition of the collection site. Their structures were determined using various spectroscopic methods including HRESIMS, and 1D and 2D NMR analyses. The stereoconfiguration was assigned by a combination of X-ray crystallographic and CD analyses, and the structures of merocyclophanes A and B (**1** and **2**) established a new [7.7]paracyclophane carbon skeleton, as characterized by the presence of α -branched methyls at C-1/14.

2. Results and Discussion

Nostoc sp. (UIC 10062) was isolated from a sample collected at Grand Mere State Park in Michigan, and cultured in Z media (Falch et al., 1995). The freeze-dried cells were extracted with a mixture of CH_2Cl_2 and MeOH (1:1, v/v) and dried *in vacuo*, with the resulting extract (156.6 mg) subjected to bioassay-guided fractionation. This cell extract showed antiproliferative activity against the HT-29 human colon cancer cell line (IC_{50} 13.1 $\mu\text{g/mL}$), and was fractionated using Diaion HP-20 resin and an increasing amount of iPrOH in H_2O . Fractions eluting at 70 (5.0 mg) and 80 (6.5 mg) % iPrOH were found to be active, with IC_{50} values of 0.9 and 1.2 $\mu\text{g/ml}$, respectively. LC-MS and UV analyses of the active fractions also indicated the presence of two cyclophanes with molecular weights of 552 and 566. Reversed-phase HPLC of these fractions yielded merocyclophanes A (**1**, 2.4 mg, 0.11%) and B (**2**, 0.9 mg, 0.04%) as minor compounds (Fig. 1).

Merocyclophane A (**1**) was obtained as white amorphous powder, and the molecular formula was determined as $\text{C}_{36}\text{H}_{56}\text{O}_4$ by HRESIMS analysis (m/z 551.4170 [M-H] $^+$). The total numbers of proton and carbon signals, determined by analysis of ^1H and 2D NMR spectra, were only half of those required by the molecular formula, thus indicating the presence of C_2 axis of symmetry in **1**. The structure of **1** was elucidated by analysis of 2D NMR spectra including COSY, HSQC and HMBC (Fig. 2). The appearance of only two aromatic singlets (H-10/23, δ_{H} 6.04 and H-12/25, δ_{H} 6.00) indicated the presence of two tetrasubstituted aromatic rings. The carbon chemical shifts of C-9/22 (δ_{C} 158.5) and C-13/26 (δ_{C} 156.9), together with HMBC correlations from H-7/20 (δ_{H} 3.10) to C-9/22 (δ_{C} 158.5) and C-13/26 (δ_{C} 156.9), identified these partial structures as two 2,5-dialkylresorcinol moieties. Sequential COSY correlations from H1/14 to H7/20 combined with HMBC correlations from H-10/23 (δ_{H} 6.04) and H-12/25 (δ_{H} 6.00) to C-1/14 (δ_{C} 41.8) and from H-7/20 (δ_{H}

3.10) to C-9/22 (δ_C 158.5) and C-13/26 (δ_C 156.9) completed the [7.7]paracyclophane core. Additional sequential COSY correlations from the H-7/20 to the triplet methyls H₃-30/34 via three methylenes (H₂-27/31, H₂-28/32 and H₂-29/33) further expanded the carbon chains. Structure determination was completed by an HMBC correlation from the doublet methyl (H₃-35/36, δ_H 1.15) to the aromatic carbon (C-11/24, δ_C 146.6) combined with a COSY correlation between H-1/14 (δ_H 2.30) and H-35/36 (δ_H 1.15), placing methyl groups at C-1/14.

Structure **1** established the presence of four stereogenic centers, whose stereoconfiguration was determined by a combination of X-ray crystallographic and CD spectral analyses. Merocyclophane A (**1**) was crystallized from CH₃CN. Single-crystal X-ray analysis established the relative configuration of **1** as shown in Fig. 3. The absolute configurations at C-1/14 and C-7/20 were established by comparison of the CD spectrum of **1** with those reported for the nostocyclophanes (Chen et al., 1991). Cotton effects observed between 220 ~ 230 nm and between 270 ~ 280 nm arise from $\pi - \pi^*$ transitions of a benzene chromophore. According to the benzene sector rule, the chlorine-bearing stereogenic carbons in the nostocyclophanes, three carbons away from the benzene chromophore, should not affect Cotton effects in these regions (Smith, 1998). The CD spectrum of **1** exhibited negative Cotton effects at 227 ($\Delta\epsilon$, -4.35) and 277 ($\Delta\epsilon$, -2.73), similar to those observed for the nostocyclophanes, suggesting the same absolute configuration. Therefore, the absolute configurations of the four stereogenic carbons C-1/14 and C-7/20 in **1** were assigned as “*R*” and “*S*”, respectively.

Merocyclophane B (**2**) was obtained as purple amorphous powder, and the HRESIMS data (m/z 565.3954 [M-H][•]) established the molecular formula as C₃₆H₅₄O₅. The ¹H NMR spectrum of **2** closely resembled **1**, except for one of the two aromatic rings. This resulted in an unsymmetric structure. The presence of a hydroxyquinone in **2** was suggested by analysis of the UV spectrum. A chromophore corresponding to the quinone absorption was observed at 521 nm, which shifted to 413 nm in the presence of acid (0.04 v/v % TFA in MeOH). This was consistent with the observed color change from deep purple to yellow upon addition of TFA. The appearance of the down-fielded carbon chemical shift of C-23 (δ_C 132.6 compared to δ_C 104.5 in **1**) further supported the presence of the hydroxyquinone moiety in **2**. However, significant line broadening of NMR signals was observed in the hydroxyquinone moiety, causing a number of expected correlations to be absent in the HMBC spectrum, even at the elevated temperature (343 K). Thus, the carbon chemical shifts of C-21, C-22, C-25 and C-26 could not be detected. The presence of the unsymmetrical hydroxyquinone moiety in **2** raised the possibility of two conformations arising from hindered rotation around the C1–C24 and C20–C21 bonds, resulting in the formation of two diastereotopic atropisomers. A torsion energy scan carried out using MM3 force-field calculation showed high rotational energy barriers between the two possible atropisomers (see Supplementary data). However, a slow conversion of **1** into **2** was observed during prolonged storage, indicating **2** to be an oxidation artifact of **1**. This non-enzymatic oxidation suggests that both atropisomers are present in a nearly equal amount, which is supported by the identical CD spectrum observed between **1** and **2**. The stereoconfiguration of **2** (C-1/14, C-7/20) was determined by comparison of the CD spectrum with that of **1**. Negative Cotton effects observed at 227 ($\Delta\epsilon$, -2.22) and 274 ($\Delta\epsilon$, -2.03) nm indicated the same absolute configuration of **2** as determined for **1**.

The structures of merocyclophanes A and B (**1** and **2**) differ from the previously described cylindrocyclophanes and nostocyclophanes by the presence of α -branched methyls at C-1/14 as shown in Fig. 4, whereas cylindrocyclophanes have branched methyls at C-2/15 (β -branching). Isotope precursor administration experiments performed by Bobzin et al. indicated the acetate origin of these β -branched methyl groups (Bobzin et al., 1993).

However, the C-methylation in the merocyclophanes occurred at α -position, suggesting that these methyl groups are likely derived from SAM (*S*-adenosyl methionine) (Jones et al, 2010).

Merocyclophanes A and B (**1** and **2**) were tested for their antiproliferative activity against the HT-29 human colon cancer cell line. Both **1** and **2** displayed antiproliferative activity with IC₅₀ values of 3.3 μ M and 1.7 μ M, respectively. Merocyclophanes A and B (**1** and **2**) possessed similar levels of antiproliferative activity as those reported for cylindrocyclophanes A–F against various cell lines (0.5 – 5 μ g/mL). A SAR study on cylindrocyclophane A suggested that the 2,5-dialkylresorcinol moiety was the pharmacophore needed for the antiproliferative activity of cylindrocyclophanes, and that the activity was significantly enhanced by the presence of [7.7]paracyclophane core structure (Yamakoshi et al, 2009). The structure of merocyclophane A (**1**) differs from that of cylindrocyclophane A by the position of methyl groups and the lack of alkyl hydroxy groups; however, both compounds showed a similar level of activity. This indicates that replacement of the hydroxyl groups at C-1/14 or C-2/15 by methyl groups does not significantly change the antiproliferative activity of natural [7.7]paracyclophanes.

Taxonomic identification of the strain UIC 10062 was conducted on the basis of microscopic examination and phylogenetic analysis using a 16S rRNA gene sequence. Microscopic observation of the cultured UIC 10062 suggested this strain to be a *Nostoc* sp. (see Supplementary data) (Wehr et al., 2003; Castenholz et al., 2001). For phylogenetic analysis, a nearly 1.3 kb sequence of the partial PCR-amplified 16S rRNA gene of UIC 10062 was aligned with the 16S rRNA gene sequences of *Bergey's* reference strains and other related species retrieved from GenBank. Phylogenetic trees were constructed using three different methods (neighbor-joining, minimum evolution and maximum parsimony) and showed nearly identical topology, particularly in the clade that includes UIC 10062, with very similar bootstrap values (the neighbor-joining tree shown in Fig. 5). The resulting phylogenetic tree indicated that UIC 10062 formed a monophyletic clade with *Nostoc* sp. GSV224, *Nostoc* sp. ATCC53789, *Nostoc* sp. PCC9305 and *Nostoc punctiforme* PCC73102 (the *Bergey's* reference strain for *Nostoc* cluster 1), and thus UIC 10062 was designated within this cluster. The comparison of 16S rRNA gene sequences among three [7.7]paracyclophane-producing cyanobacteria (*Nostoc* sp. UIC 10062, *Nostoc* sp. UIC 10022A and *Cylindrospermum licheniforme* UTEX 2014) showed that variation in 16S rRNA gene sequences did not correlate with chemical variability. The two genetically quite distant strains of *Nostoc* sp. UIC 10022A and *C. licheniforme* UTEX 2014 (5% sequence divergence) have been identified to produce cylindrocyclophanes. However, the 16S rRNA gene sequence of *C. licheniforme* UTEX 2014 showed a higher sequence homology to *Nostoc* sp. UIC 10062 (96%) than to UIC 10022A (95%). Merocyclophanes produced by *Nostoc* sp. UIC 10062 have α -branched methyls at C-1/14, which are biosynthetically distinct from the β -branched methyls found in the cylindrocyclophanes. Also, among these three strains, only *Nostoc* sp. UIC 10022A was known to produce chlorinated cyclophanes. Considering the quite distant phylogenetic relationships of 16S rRNA genes among cyclophane-producing species, it can be hypothesized that there has been a potential horizontal gene transfer between species, and these genes have evolved to have different modification patterns due to different environmental conditions.

3. Conclusion

Chemical investigation of *Nostoc* sp. (UIC 10062), guided by antiproliferative assay against the HT-29 human colon cancer cell line, led to isolation of two new [7.7]cyclophanes, named merocyclophanes A (**1**) and B (**2**). Their structure determinations established a new carbon skeleton, as characterized by the presence of α -branched methyls at C1/14. Both

merocyclophanes A and B displayed antiproliferative activity against the HT-29 human colon cancer cell line. The strain UIC 10062 was taxonomically classified to a *Nostoc* sp. based on analysis of the microscopic morphology and 16S rRNA gene sequence.

4. Experimental

4.1. General Experimental Procedures

Optical rotations were measured using a Perkin-Elmer 241 polarimeter. UV spectra were measured on a Shimadzu UV spectrometer UV2401 and scanned from 190 to 360 nm. CD spectra were recorded on a JASCO J-710 CD spectrometer. IR spectra were acquired using a Jasco FTIR-410 Fourier transform infrared spectrometer. 1D and 2D NMR spectra were obtained on a Bruker Avance DRX 600 MHz NMR spectrometer with a 5 mm CPTXI Z-gradient. ^1H and ^{13}C NMR chemical shifts were referenced to the solvent signals ($\text{MeOH-}d_4$ and $\text{DMSO-}d_6$). The HMBC spectrum was recorded with the average $^3J_{\text{CH}}$ of 8 Hz and the HSQC spectrum was measured with the average $^1J_{\text{CH}}$ of 140 Hz. Low- and high-resolution ESI mass spectra were obtained using a Shimadzu IT-TOF LC mass spectrometer.

4.2. Biological Material

Nostoc sp. (UIC 10062) was isolated from a sample collected at the north pond of Grand Mere State Park in Michigan in 2007 (N 42°00.679', W 86°32.417'). The unialgal strain (UIC 10062) was produced through micropipette isolation techniques (Chlipala et al., 2009). The strain was cultured in a 22 L glass flask containing Z media (18 L) with sterile aeration (Falch et al., 1995). Cultures were illuminated with fluorescent lamps at 1.03 klx with an 18/6 h light/dark cycle. The temperature of the culture room was maintained at 22 °C. After 6 weeks, the biomass of cyanobacteria was harvested by centrifugation and then freeze-dried.

4.3. Strain Identification

Morphological studies were performed using a cultivated cyanobacterium UIC 10062 (See biological material) with microscopic observation for morphological characterization conducted using a Zeiss Axiostar Plus light microscope equipped with a Canon PowerShot A620 camera. The following parameters were selected to characterize its morphology: thallus morphology, shape of trichome, morphology of terminal cells, shape and arrangement of vegetative cells, presence and arrangement of heterocystes and akinetes (see Supplementary data). Taxonomic identification of the cyanobacterial specimen was made in accordance with the modern taxonomic system (Komárek et al., 2003).

4.4. DNA Extraction, PCR Amplification and Sequencing

Cell mass (258 mg), obtained from a static culture of *Nostoc* sp. (UIC 10062), was centrifuged at $14,000 \times g$ for 5 min, and pretreated with lysozyme and proteinase K as follows prior to using the Wizard Genomic DNA purification kit. The cell pellet was re-suspended in lysis buffer (2.5 mL, 10 mM Tris, 0.1M EDTA, 0.5% w/v SDS, 20 $\mu\text{g}/\text{mL}$ pancreatic RNase, pH 8.0) containing lysozyme (1 mg/mL) and incubated at 37°C for 1 hr. To this mixture, proteinase K was added to a final concentration of 100 $\mu\text{g}/\text{mL}$, and incubated at 50°C for 1 h. After incubation, the cell material was recovered by centrifugation at $14,000 \times g$ for 3 min. DNA was extracted from this pretreated cell material using the Wizard Genomic DNA purification kit (Promega), where the protocol was slightly modified to include mechanical disruption after the addition of the nuclei lysis solution (step 6). A portion of the 16S rRNA gene was PCR-amplified using the cyanobacteria-specific primers 106F and 1509R (Martínez-Murcia et al., 1995). For a total volume of 50 μL , the reaction mixture contained DNA (5 μL , approximately 90 ng), Phusion HF Buffer (10 μL , 5x), dNTP mix (1 μL , 10 mM), each primer (1 μL , 10 μM), Phusion high-fidelity DNA

polymerase (0.5 μL), and H_2O (31.5 μL). The reaction was performed in a Bio-Rad C1000 thermal cycler as following reaction program: initial denaturation for 30 s at 98°C, 35 amplification cycles of 10 s at 98°C, 30 s at 53°C and 30s at 72°C, and a final extension for 10 min at 72°C. PCR products were purified using a MinElute PCR purification kit (Qiagen) and sequenced using the cyanobacteria-specific primers 106F and 1509R as well as the internal primers, 359F and 781R (Nübel et al., 1997). The resulting 16S rRNA gene sequence was deposited in the NCBI GenBank under the accession number JN714978.

4.5. Phylogenetic Analysis

Phylogenetic and molecular evolutionary analyses were conducted using MEGA 5.0 (Tamura et al., 2011). The resulting sequence chromatograms were visually inspected, and the total sequence of 1,290 nucleotides was aligned with 40 cyanobacterial species retrieved from GenBank as well as *Gloeobacter violaceus* PCC7421 as an outgroup. Cyanobacterial reference strains were firstly selected from *Bergey's Manual* (Castenholz, 2001), and only sequences of at least 1 kb were retrieved from GenBank. Multiple sequence alignment was performed with ClustalW in MEGA 5.0 with standard gap opening and extension penalties. The evolutionary history was inferred using the neighbor-joining (NJ), minimum evolution (ME) and maximum parsimony (MP) methods. One thousand replicates were used to evaluate the robustness of branches in the inferred trees generated. Subgroups with greater than 75% consistency in the tree are labeled at the respective nodes.

4.6. Extraction and Isolation

Freeze-dried biomass (2.2 g) from 18 L of culture was harvested and extracted with CH_2Cl_2 -MeOH (250 mL, 1:1) three times and concentrated *in vacuo* to yield an extract (0.2 g), which was fractionated using Diaion HP-20 resin with iPrOH- H_2O (50 mL) to generate 8 sub-fractions (0:10, 2:8, 4:6, 6:4, 7:3, 8:2, 9:1 and 10:0 v/v). Fractions eluting with iPrOH- H_2O 7:3 v/v, (5.0 mg) and 8:2 v/v, (6.5 mg) were found to be active in the antiproliferative assay against HT-29 cells with IC_{50} values of 0.9 and 1.2 $\mu\text{g}/\text{mL}$, respectively. LC-MS analysis of these fractions indicated the presence of new compounds, and were subjected to reversed-phase HPLC (Varian C_8 semi-preparative column, 10 mm \times 250 mm, 3 mL/min) eluting with gradient of MeOH- H_2O (7:3 to 9:1 v/v for 45 min). Merocyclophanes A and B (**1** and **2**) were eluted at 37.9 min (**1**, 2.4 mg, 0.11%) and 31.6 min (**2**, 0.9 mg, 0.04%), respectively.

4.6.1. Merocyclophane A (1)—White amorphous powder; $[\alpha]_{\text{D}}^{25} - 29$ (*c* 0.024, MeOH); UV (MeOH) λ_{max} (log ϵ) 207 (4.55), 221 (4.02), 272 (3.24) nm; CD (MeOH) λ_{max} ($\Delta\epsilon$) 213 (−6.40), 227 (−4.35), 277 (−2.73); IR (neat) ν_{max} 2954, 2920, 2851, 1650, 1541, 1507 cm^{-1} ; for ^1H and ^{13}C NMR spectroscopic data, see Table 1; HRESIMS m/z 551.4170 $[\text{M}-\text{H}]^{\bullet}$ (calcd for $\text{C}_{36}\text{H}_{55}\text{O}_4$, 551.4100)

4.6.2. Merocyclophane B (2)—Purple amorphous powder; $[\alpha]_{\text{D}}^{25} - 19$ (*c* 0.070, MeOH); UV (MeOH) λ_{max} (log ϵ) 207 (4.25), 221 (3.98), 273 (3.57), 324 (2.76), 521 (2.59) nm; CD (MeOH) λ_{max} ($\Delta\epsilon$) 211 (−3.66), 227 (−2.22), 274 (−2.03) nm; IR (neat) ν_{max} 2955, 2924, 2855, 1664, 1635, 1595, 1527 cm^{-1} ; for ^1H and ^{13}C NMR spectroscopic data, see Table 2; HRESIMS m/z 565.3954 $[\text{M}-\text{H}]^{\bullet}$ (calcd for $\text{C}_{36}\text{H}_{54}\text{O}_5$, 565.3893)

4.7. X-ray Crystallographic Analysis of Merocyclophane A (1)

Crystals for X-ray analysis were grown from CH_3CN . A small single crystal, roughly 30 \times 30 \times 30 μm , was selected for data collection at sector-22-BM, and encased in Paratone-N oil and cooled to 100K to minimize crystal degradation and X-ray radiation damage. The MAR 225 CCD detector was set at a distance of 85 mm from the crystal using a wavelength of

0.80 Å to collect data near a resolution of 0.86 Å. 36 images were collected, each with a rotation sweep of 10°. The images were indexed, and the intensities were integrated and scaled with XDS (Kabsch, 1993). The WinGX package was used for structure solution and refinement. The space group was identified as C2 with one molecule in the asymmetric unit (Farrugia, 1999). SHELX was used for structure solution and refinement on F2. Six CH₃CN molecules were found in the asymmetric unit, with two of them sitting on 2-fold axes. Four CH₃CN molecules are each involved in hydrogen bonding with the four aromatic hydroxyl groups; the remaining two CH₃CN molecules reside in channels formed at the interface of the larger macrocycles as they pack together in the unit cell. Crystal data: C₃₆H₅₆O₄·5CH₃CN, MW = 758.1, monoclinic, space group C2 (5); *a* = 14.735 (2) Å, *b* = 10.840 (5) Å, *c* = 29.842 (3) Å, β = 95.226 (4)°, *V* = 4747 (2) Å³; *Z* = 4, *D*_c = 0.057 mg/m³; μ = 0.057 mm⁻¹; *F*000 = 1656. Reflections collected / unique = 13719 / 5724 (*R*_{int} = 0.0815); final *R*1 = 0.0882, *wR*2 = 0.2285 for reflections with *I* > 2σ (*I*); *R*1 = 0.1311, *wR*2 = 0.2572 for all unique data. Crystallographic data (ID No. 818958) have been deposited with the Cambridge crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (deposit@ccdc.cam.ac.uk).

4.8. HT-29 Antiproliferative Assay

The antiproliferative activity of **1** and **2** against the HT-29 cancer cell line was evaluated according to a previously established protocol (Seo et al., 2001).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

- Bobzin SC, Moore RE. Biosynthetic origin of [7.7]paracyclophanes from cyanobacteria. *Tetrahedron*. 1993; 49:7615–7626.
- Bui HTN, Jansen R, Pham HTL, Mundt S. Carbamidocyclophanes A-E, chlorinated paracyclophanes with cytotoxic and antibiotic activity from the Vietnamese cyanobacterium *Nostoc* sp. *J. Nat. Prod.* 2007; 70:499–503. [PubMed: 17311455]
- Castenholz, RW.; Phlyum, BX. Cyanobacteria. In: Boone, DR.; Castenholz, RW., editors. *Bergey's Manual of Systematic Bacteriology*. Vol. Vol. 1. New York: Spinger; 2001. p. 473-599.
- Chen JL, Moore RE, Patterson GML. Structures of nostocyclophanes A-D. *J. Org. Chem.* 1991; 56:4360–4364.
- Chlipala G, Sturdy M, Kronic A, Lantvit DD, Shen Q, Porter K, Swanson SM, Orjala J. Cyclindrocyclophanes with proteasome inhibitory activity from the cyanobacterium *Nostoc* sp. *J. Nat. Prod.* 2010; 73:1529–1537. [PubMed: 20825206]
- Falch BS, König GM, Wright AD, Sticher O, Angerhofer CK, Pezzuto JM, Bachmann H. Biological-activities of cyanobacteria – Evaluation of extracts and pure compounds. *Planta Med.* 1995; 61:321–328. [PubMed: 7480178]
- Farrugia LJ. 'WinGX' suite for small-molecule single-crystal crystallography. *J. Appl. Cryst.* 1999; 32:837–838.

- Harada KI. Production of secondary metabolites by freshwater cyanobacteria. *Chem. Pharm. Bull.* 2004; 52:889–899. [PubMed: 15304980]
- Jones AC, Monroe EA, Eisman EB, Gerwick L, Sherman DH, Gerwick WH. The unique mechanistic transformations involved in the biosynthesis of modular natural products from marine cyanobacteria. *Nat. Prod. Rep.* 2010; 27:1048–1065. [PubMed: 20442916]
- Kabsch W. Automatic processing of rotation diffraction data from crystals of initially unknown symmetry and cell constants. *J. Appl. Cryst.* 1993; 26:795–800.
- Komárek, J.; Komárková, J.; Kling, H. Filamentous Cyanobacteria. In: Wehr, JD.; Sheath, RG., editors. *Freshwater Algae of North America*. San Diego: Academic Press; 2003. p. 117-196.
- Martínez-Murcia AJ, Acinas SG, Rodríguez-Valera F. Evaluation of prokaryotic diversity by restriction digestion of 16S rDNA directly amplified from hypersaline environments. *FEMS Microbiol. Ecol.* 1995; 17:247–256.
- Moore BS, Chen JL, Patterson GML, Moore RE. [7.7]Paracyclophanes from blue-green algae. *J. Am. Chem. Soc.* 1990; 112:4061–4063.
- Moore BS, Chen JL, Patterson GML, Moore RE. Structures of cylindrocyclophanes A-F. *Tetrahedron.* 1992; 48:3001–3006.
- Nübel U, GarciaPichel F, Muyzer G. PCR primers to amplify 16S rRNA genes from cyanobacteria. *Appl. Environ. Microbiol.* 1997; 63:3327–3332. [PubMed: 9251225]
- Ploutno A, Carmeli S. Nostocyclone A, a novel antimicrobial cyclophane from the cyanobacterium *Nostoc* sp. *J. Nat. Prod.* 2000; 63:1524–1526. [PubMed: 11087597]
- Rajaniemi P, Hrouzek P, Kastovska K, Willame R, Rantala A, Hoffmann L, Komarek J, Sivonen K. Phylogenetic and morphological evaluation of the genera *Anabaena*, *Aphanizomenon*, *Trichormus* and *Nostoc* (Nostocales, Cyanobacteria). *J. Syst. Evol. Microbiol.* 2005; 55:11–26.
- Seo E-K, Kim N-C, Mi Q, Chai H, Wall ME, Wani MC, Navarro HA, Burgess JP, Graham JG, Cabieses F, Tan GT, Farnsworth NR, Pezzuto JM, Kinghorn AD. Macharistol, a new cytotoxic cinnamylphenol from the stems of *Machaerium aristulatum*. *J. Nat. Prod.* 2001; 64:1483–1485. [PubMed: 11720542]
- Smith HE. Chiroptical properties of the benzene chromophore. A method for the determination of the absolute configurations of benzene compounds by application of the benzene sector and benzene chirality rules. *Chem. Rev.* 1998; 98:1709–1740. [PubMed: 11848945]
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 2011; 28:2731–2739. [PubMed: 21546353]
- Tan LT. Bioactive natural products from marine cyanobacteria for drug discovery. *Phytochemistry.* 2007; 68:954–979. [PubMed: 17336349]
- Wagoner RMV, Drummond AK, Wright JLC. Biogenetic diversity of cyanobacteria metabolites. *Adv. Appl. Microbiol.* 2007; 61:89–217. [PubMed: 17448789]
- Wehr, JD.; Sheath, RG. *Freshwater algae of North America*. San Diego: Academic Press; 2003. p. 117-196.
- Yamakoshi H, Ikarashi F, Minami M, Shibuya M, Sugahara T, Kanoh N, Ohori H, Shibata H, Iwabuchi Y. Syntheses of naturally occurring cytotoxic [7.7]paracyclophanes, (–)-cylindrocyclophane A and its enantiomer, and implications for biological activity. *Org. Biomol. Chem.* 2009; 7:3772–3781. [PubMed: 19707682]

Highlights

- Two [7.7] paracyclophanes named merocyclophanes A and B were obtained.
- Both displayed antiproliferative activity against the HT-29 cancer cell line.
- *Nostoc* sp. (UIC 10062) was obtained from Grand Mere State Park in Michigan,
- *Nostoc* sp. was classified based on microscopic and 16S rRNA gene sequence analysis

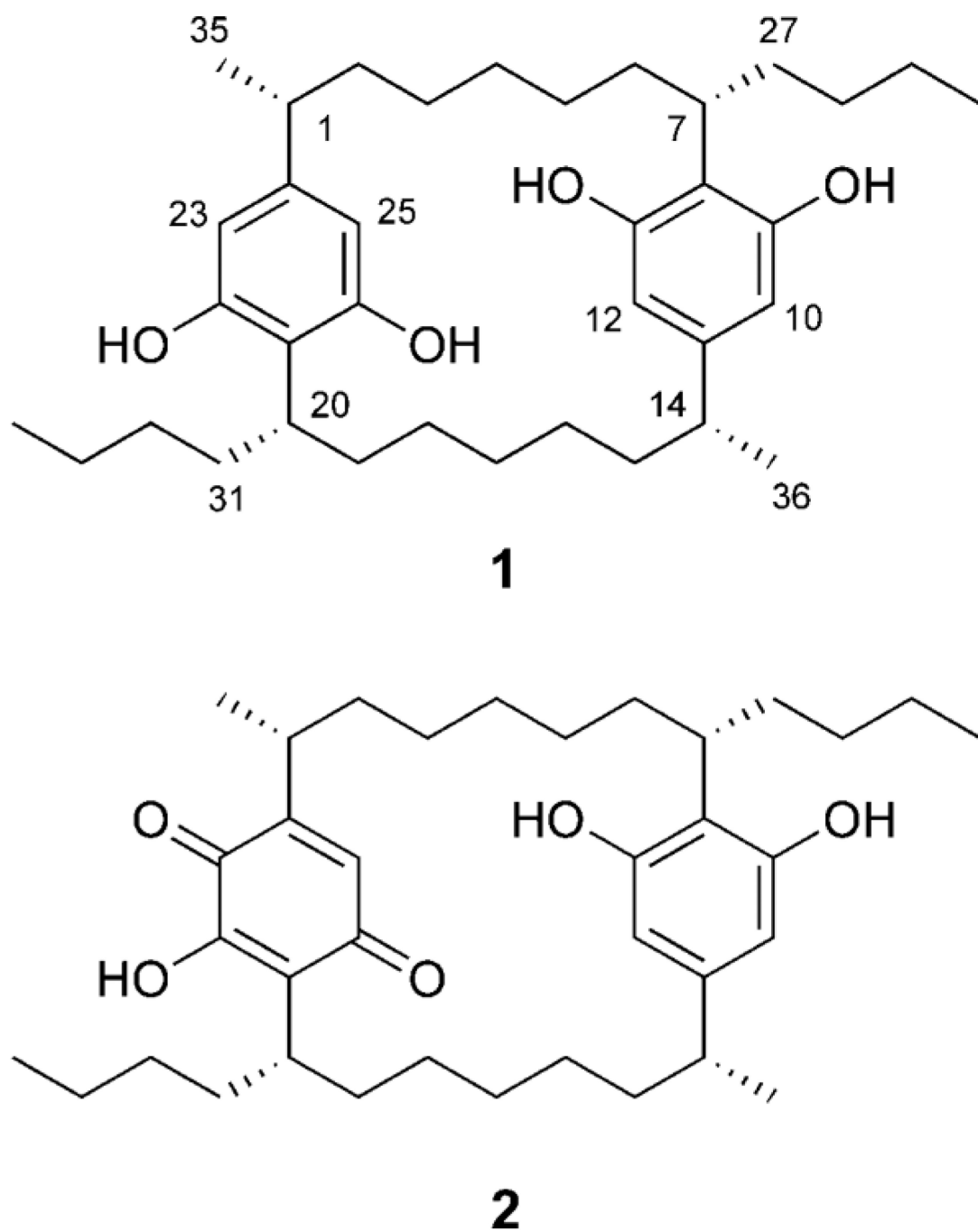


Fig. 1.
Structures of merocyclophanes A (1) and B (2).

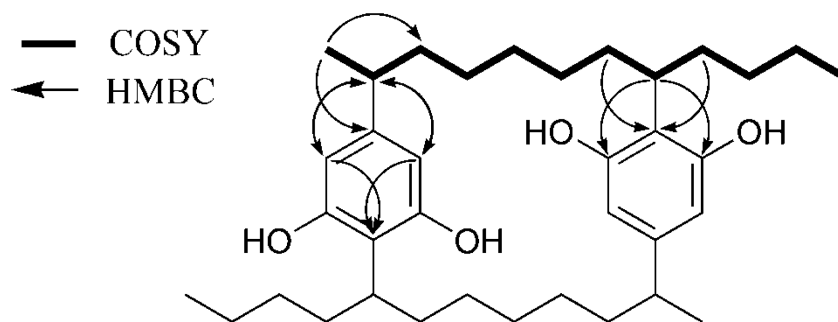


Fig. 2.
Key 2D correlations used for structure determination of **1**

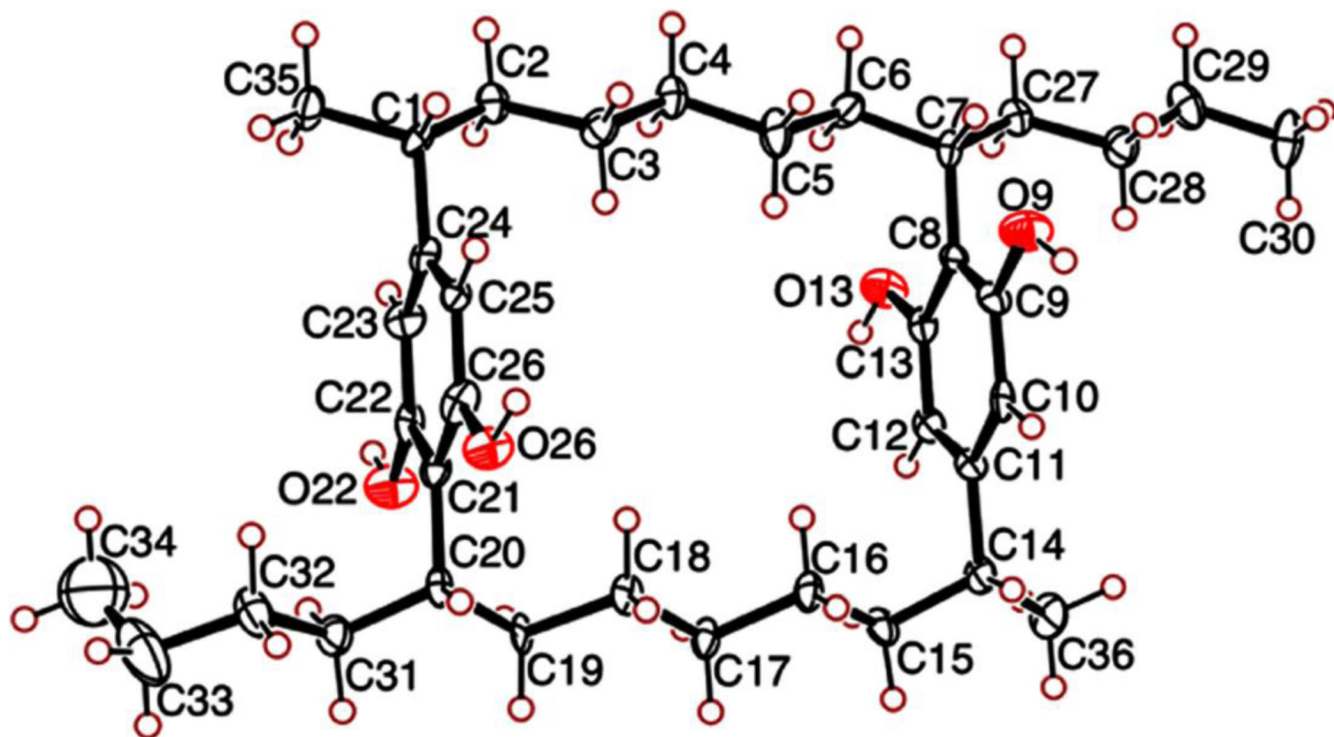


Fig. 3.
ORTEP drawing of merocyclophane A (1)

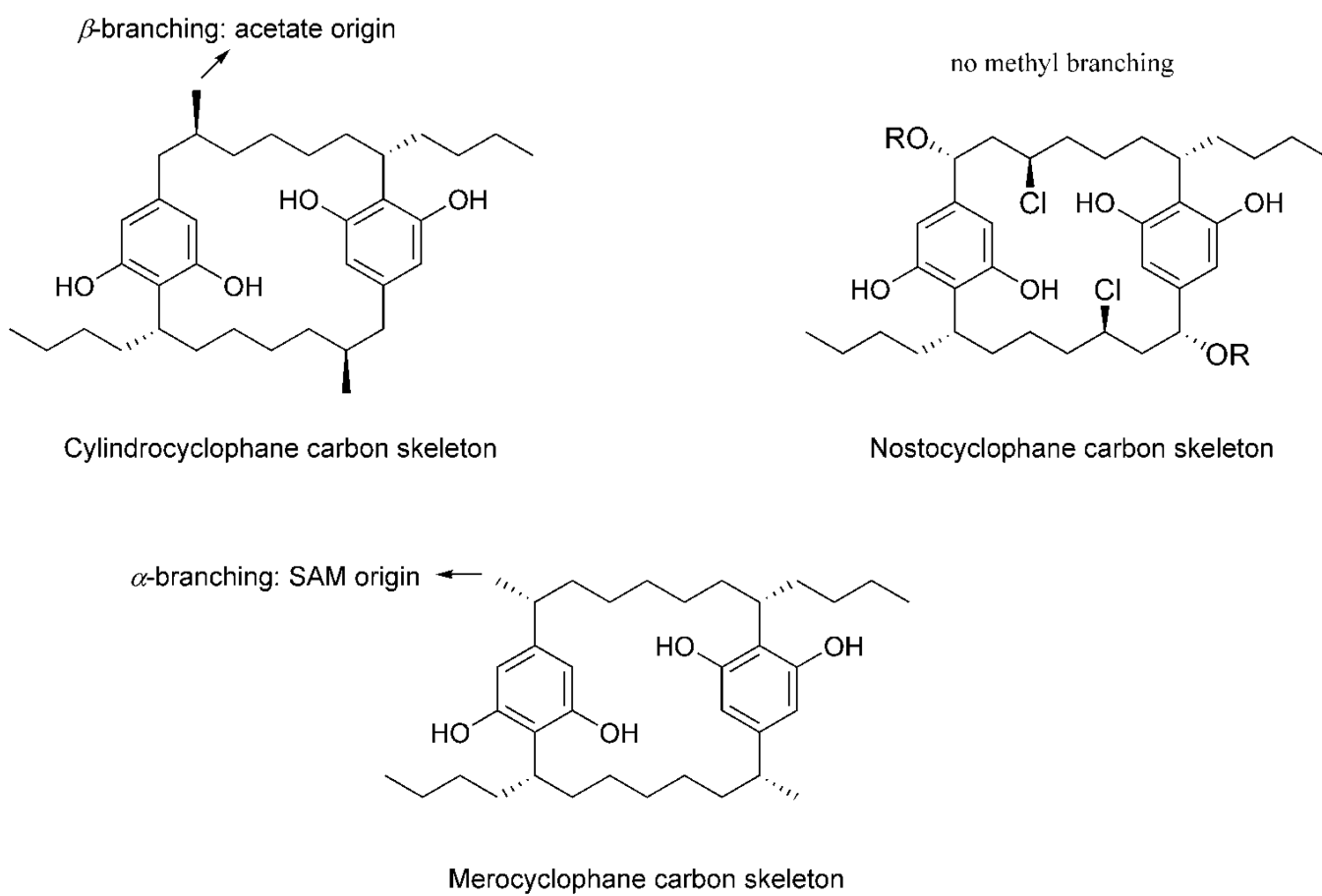


Fig. 4.
Comparison of carbon skeletons between the cylindrocyclophanes, the nostocyclophanes and merocyclophane A

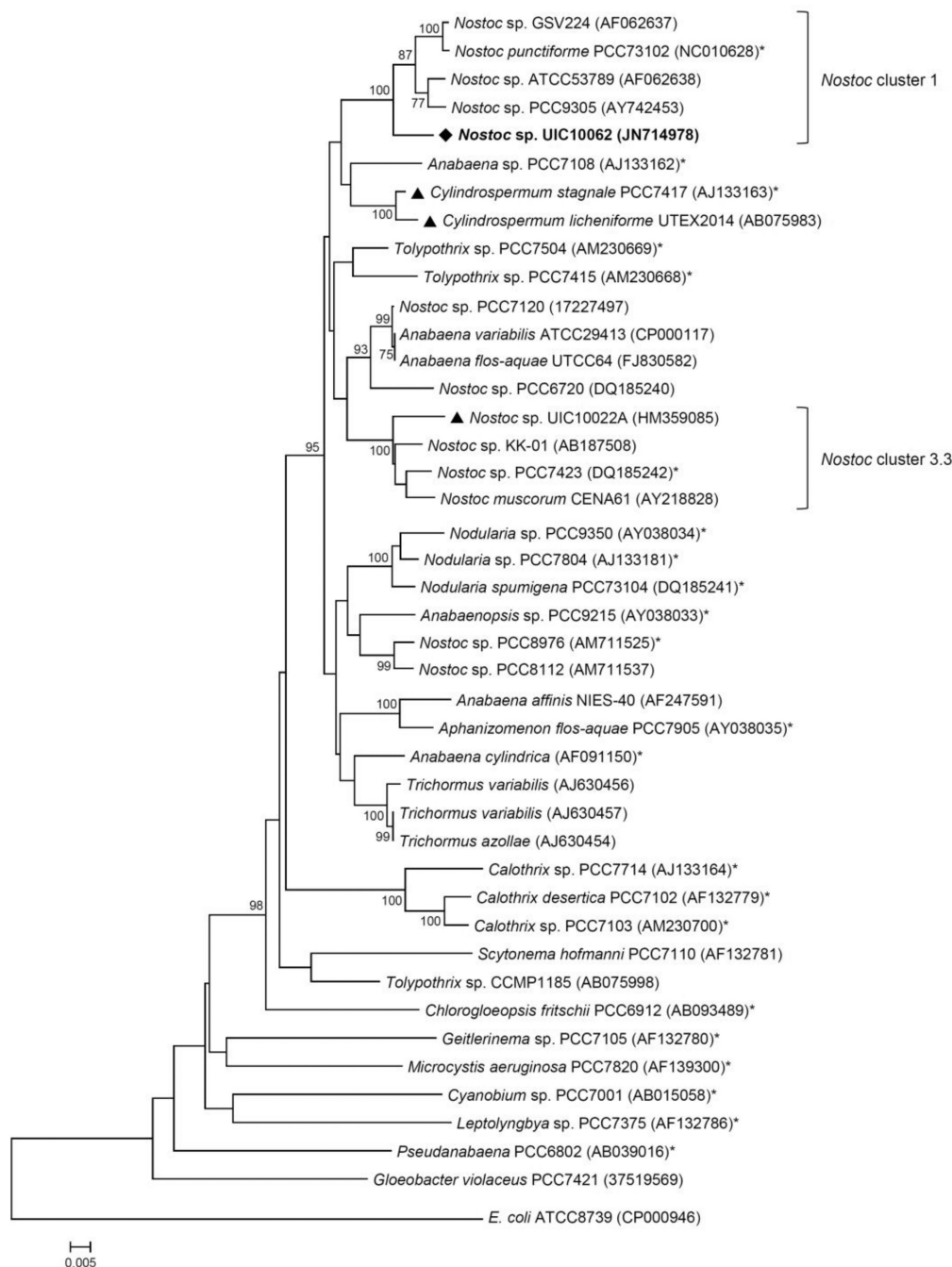


Fig. 5. Phylogenetic relationships of 16S rRNA genes from cyanobacteria. Evolutionary distances were determined using the neighbor-joining method with 1000 replicate bootstrap resampling to construct the phylogenetic tree. Strains were obtained from NCBI with the accession number given in parentheses. Strains marked with a (*) were obtained as Bergey's reference strains. Cyanobacterial strains previously reported to produce cylindrocyclophanes are denoted by a triangle (▲). Only bootstrap values greater than or equal to 75% are displayed.

Table 1

NMR spectroscopic data of merocyclophane A (1) in MeOH-*d*₄ (600 MHz)

Merocyclophane A (1)					
NO.	δ_C^d	δ_H	mult. (J in Hz)	COSY	HMBC
1 / 14	41.8	2.30	dqd (10.8, 6.9, 3.8)	2/15, 35/36	35/36, 10/23, 12/25, 11/24
2 / 15	40.5	1.32 1.45	m m	1/14, 3/16	1/14, 3/16, 11/24, 35/36
3 / 16	30.7	0.65 0.93	m m	2/15, 4/17	1/14, 2/15, 5/18
4 / 17	32.4	0.88 1.27	m m	3/16, 5/18	
5 / 18	30.6	0.67 0.98	m m	4/17, 6/19	
6 / 19	35.1	1.31	m	5/18, 7/20	7/20
7 / 20	36.7	1.97 3.10	qd (12.4, 4.0) m	6/19, 27/31	5/18, 6/19, 8/21, 9/22, 13/26, 27/31, 28/32
8 / 21	116.1				
9 / 22	158.5				
10 / 23	104.5	6.04	s		14/1, 8/21, 12/25, 9/22
11 / 24	146.6				
12 / 25	109.1	6.00	s		14/1, 8/21, 10/23, 13/26
13 / 26	156.9				
27 / 31	34.7	1.49 1.92	m m	7/20, 28/32	28/32, 29/33, 7/20
28 / 32	31.6	1.11 1.19	m m	27/31, 29/33	
29 / 33	23.8	1.25 1.29	m m	28/32, 30/34	
30 / 34	14.4	0.83	t (7.1)	29 / 33	28/32, 29/33
35 / 36	23.5	1.15	d (6.9)	1 / 7	1/14, 2/15, 11/24

^a assigned from the HSQC and HMBC spectra

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Table 2NMR spectroscopic data of merocyclophane B (**2**) in DMSO-*d*₆ (600 MHz)

No.	Merocyclophane B (2)		
	δ_C^a	δ_H	mult. (J in Hz)
1	28.7	2.86	m
2	37.6	1.22	m
		1.44	m
3	29.0	0.62	m
		0.94	m
4	30.3	0.84	m
		1.24	m
5	28.9	0.66	m
		0.94	m
6	33.0	1.27	m
		1.89	m
7	34.3	3.06	m
8	113.8		
9	155.4		
10	102.5	5.97	s
11	144.3		
12	107.3	5.95	s
13	156.7		
14	39.6	2.26	
		1.21	m
15	38.6	1.40	m
		0.62	m
16	29.0	0.94	m
		0.84	m
17	30.3	1.24	m
		0.69	m
18	28.9	0.95	m
		1.32	m
19	33.1	1.63	m
		2.80	br m
20	35.5	2.80	br m
21	nd ^b		
22	nd ^b		
23	132.6	6.30	br s
24	149.1		
25	nd ^b		
26	nd ^b		
27	32.8	1.45	m

No.	Merocyclophane B (2)		
	δ_C^a	δ_H	mult. (<i>J</i> in Hz)
28	29.7	1.80	m
		1.05	m
		1.12	m
29	21.9	1.19	m
30	13.8	0.79	t (7.3)
31	32.9	1.40	m
		1.70	m
32	29.7	1.05	m
		1.12	m
33	21.9	1.19	m
34	13.8	0.79	t (7.3)
35	20.0	1.01	br d
36	22.4	1.08	d (6.9)
9-OH		8.53	br s
13-OH		8.54	br s
26-OH		10.24	br s

^a assigned from the HSQC and HMBC spectra,

nd^b no signals observed at 25°C presumably due to signal broadening