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Ribocyclophanes A – E, Glycosylated Cyclophanes with Antiproliferative Activity from Two Cultured Terrestrial Cyanobacteria

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Abstract

The cell extracts of two cultured freshwater *Nostoc* spp., UIC 10279 and UIC 10366, both from the suburbs of Chicago, showed antiproliferative activity against MDA-MB-231 and MDA-MB-435 cancer cell lines. Bioassay-guided fractionation led to the isolation of five glycosylated cylindrocyclophanes, named ribocyclophanes A – E (**1** – **5**) and cylindrocyclophane D (**6**). The structure determination was carried out by HRESIMS, and 1D and 2D NMR analyses, and confirmed by a single crystal X-ray crystallography. The structures of ribocyclophanes A – E (**1** – **5**) contain a β -D-ribofuranose glycone in the rare 1C_4 conformation. Among isolated compounds, ribocyclophane D (**4**) showed antiproliferative activity against MDA-MB-435 and MDA-MB-231 cancer cells with an IC₅₀ value of less than 1 μ M.

SYNOPSIS TOC

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Dedicated to Susan Band Horwitz, of Albert Einstein College of Medicine, Bronx, NY, for her pioneering work on bioactive natural products.

Supporting Information Available. Taxonomic identification and phylogenetic tree of UIC 10279 and UIC 10366 strains; ¹H NMR, COSY, HSQC, HMBC spectra of **1** – **5**; DEPT-Q spectrum of **1** and **4**, ECD spectra of **1** – **5**, and antiproliferative data for **1** – **6** against MDA-MB-435 and MDA-MB-231 cancer cell lines. The Supporting Information is available free of charge on the ACS Publications website at DOI:

Polyketides (PK), one of the largest families of natural products, are not commonly found in cyanobacteria compared to other major natural products families, such as non-ribosomal peptides (NRP) and PK-NRP hybrids.^{1–3} However, [7.7]paracyclophanes represent a family of cyanobacterial polyketides that has been found in heterocyst-forming cyanobacteria of the order Nostocales.^{4–11} The 22-membered [7.7]paracyclophane core structure, possessing a C_2 axis of symmetry, has been shown to be produced by dimerization of two halogenated alkyl-resorcinol units made by a Type I / Type III polyketide synthase hybrid.^{12–14} The alkyl-resorcinol units, termed cylindrofridins, were isolated by Preisitsch *et al.* and proposed as biosynthetic intermediates.¹⁵ The identification of the biosynthetic intermediates by Nakamura *et al.* confirmed the biosynthetic origin of these alkyl-resorcinol units as well as their dimerization.^{13,14,16} The core [7.7]paracyclophane structure is further modified by tailoring steps such as methylation, acetylation, carbamate formation, glycosylation and/or halogenation, to generate diverse structural variants.¹⁷ Cyanobacterial cyclophanes have been shown to possess a broad range of biological activities, including antibacterial, antifungal and cytotoxic activities.^{6–11}

Several *Nostoc* spp. from the University of Illinois at Chicago (UIC) cyanobacterial library have been identified to produce [7.7]paracyclophanes. Chlorinated cylindrocyclophanes were obtained from *Nostoc* sp. (UIC 10022A).⁷ These chlorinated cylindrocyclophanes exhibited 20S proteasome-inhibitory activity and cytotoxicity against several cancer cell lines. Carbamidocyclophanes F and G were reported from UIC 10274, containing terminal chlorines as well as an uncommon carbamate moiety.¹⁰ In addition, the isolation of merocyclophanes A and B from *Nostoc* sp. (UIC 10062) from Grand Mere State Park in Michigan was reported, as well as merocyclophanes C and D from the extract of UIC 10110, a *Nostoc* sp. from Colorado. Merocyclophanes A–D featured a new [7.7]paracyclophane carbon skeleton characterized by the presence of branched methyls at α positions (C-1/14).^{8,11} The extract of a cultured *Nostoc* sp. (UIC 10279), obtained from a sample collected in the southwest suburbs of Chicago, displayed antiproliferative activity against the human melanoma cancer cell line MDA-MB-435. HPLC-based activity profiling followed by LCMS and ¹H NMR-based dereplication suggested the presence of three glycosylated cyclophanes as active metabolites. Concurrently, a second *Nostoc* sp. (UIC 10366), obtained from a collection in the northwestern suburbs of Chicago, also displayed antiproliferative activity against MDA-MB-435 cancer cells. LCMS and ¹H NMR-based dereplication also suggested the presence of glycosylated cyclophanes similar to UIC 10279, including one unique to UIC 10366. During isolation, a fifth minor compound was identified from UIC

10366. Herein, we report the isolation, structure determination and antiproliferative activities of five glycosylated cyanobacterial [7.7]paracyclophanes, named ribocyclophanes A – E (**1** – **5**).

Results and Discussion

Strain UIC 10279 was obtained from a sample collected southwest of Chicago near the suburb of Joliet, Illinois, while strain UIC 10366 was obtained from a sample collected near Elgin, Illinois, a suburb northwest of Chicago. The strains were both designated as *Nostoc* sp. based on morphological and 16S rRNA gene sequence analysis (Supporting Information, S1 and S2). Each strain was cultured in Z media,¹⁸ and the freeze-dried cells were extracted with a mixture of CH₂Cl₂ and MeOH (1:1, v/v). The resulting extracts displayed antiproliferative activity against the MDA-MB-435 cancer cell line. Both extracts were fractionated using Diaion HP-20 resin with an increasing amount of iPrOH in H₂O. Fractions eluting at 50% and 60% iPrOH showed antiproliferative activity for both strains. To facilitate the identification of potentially active compounds, the active fractions were subjected to our HPLC-based activity profiling that combines HPLC-based fractionation in a 96 well plate with the cell-based activity assay. Three active compounds were identified in each strain. Dereplication of the active fractions of UIC 10279 by LC-MS and ¹H NMR spectroscopy indicated the presence of glycosylated cyclophanes with molecular weights of 848, 716 and 700 Da. Dereplication of the active peaks of UIC 10366 by LC-MS and ¹H NMR spectroscopy indicated the presence of the previously reported cylindrocyclophane D as well as two potentially new glycosylated cyclophanes with molecular weights of 848 Da and 758 Da.⁴ Active fractions for each strain were subjected to reversed-phase HPLC to yield six pure compounds between the two extracts. During isolation from the extract of UIC 10366, a low abundance compound, ribocyclophane E (**5**), was also obtained. Ribocyclophanes A (**1**, 0.5%), B (**2**, 0.1%) and C (**3**, 0.3%) were isolated from UIC 10279, and ribocyclophanes A (**1**, 0.5%), D (**4**, 0.5%), E (**5**, <0.1%) as well as the known compound cylindrocyclophane D (**6**, 0.2%) were isolated from UIC 10366.

Ribocyclophane A (**1**), a white amorphous powder obtained from UIC 10279, displayed a deprotonated molecule at *m/z* 847.4895 ([M-H][−]) in HRESIMS analysis, corresponding to a molecular formula of C₄₆H₇₁O₁₄. The ¹H NMR spectrum of **1** showed signals characteristic of cylindrocyclophanes with two singlet aromatic protons (H-10/23, δ_H 6.19; H-12/25, δ_H 6.09), benzylic doublet and multiplet methines (H-1/14, δ_H 3.65; H-7/20, δ_H 3.16), shielded diastereotopic methylenes (H₂-3/16 and H₂-5/18, δ_H 0.5–1.0) and a doublet methyl (H₃-35/36, δ_H 1.05). Only half the number of signals were observed in the ¹H and ¹³C NMR spectra, as indicated by the molecular formula, suggesting the presence of an element of symmetry in **1**. The ¹H NMR spectrum of **1** showed an additional set of proton signals adjacent to oxygen (δ_H 3.0–4.0) and an anomeric proton (δ_H 4.85) compared to those observed for the cylindrocyclophanes, suggesting the presence of a glycoside moiety in **1**. The structure and position of a sugar moiety were determined by 2D NMR analysis. Sequential COSY correlations from the anomeric proton H-1'/6' (δ_H 4.85) to the diastereotopic methylene protons H₂-5'/10' (δ_H 3.19 and 3.30) via three hydroxylated methine protons, H-2'/7' (δ_H 3.73), H-3'/8' (δ_H 3.70) and H-4'/9' (δ_H 3.59), together with an HMBC correlation from H₂-5'/10' to C-1'/6', indicated the sugar moiety to be a

pentopyranose (Figure 1). The HMBC correlation observed from the anomeric protons H-1'/6' to C-1/14 established the linkage between the pentopyranose and the cylindrocyclophane aglycone.

To determine the relative configuration of the sugar moiety, $^3J_{\text{HH}}$ analysis and an NOE experiment were initially attempted. The small $^3J_{\text{HH}}$ value (1.8 Hz), observed between H-1'/6' and H-2'/7' indicated that these two protons are in an either ax-eq or eq-eq relationship. The $^3J_{\text{HH}}$ values between H-2'/7', H-3'/8' and H-4'/9' could not be measured directly from the ^1H NMR spectrum due to the broad signals observed; however, the broad line shapes of NMR signals observed for all of the signals indicated small $^3J_{\text{HH}}$ values between these protons. In addition, no NOE correlation was observed between H-1'/6' and H-3'/8', and between H-2'/7' and H-4'/9', complicating the determination of the relative configuration for the sugar moiety. To unambiguously determine the $^3J_{\text{HH}}$ values of the glycone moiety, spin simulation was performed using Spinworks. The $^3J_{\text{HH}}$ values measured using spin simulation were 3.0 (H-2'/7' and H-3'/8'), 3.0 (H-3'/8' and H-4'/9'), 1.2 (H-4'/9' and H-5'a/10'a) and 2.4 Hz (H-4'/9' and H-5'b/10'b), respectively. These small J values clearly demonstrated that all the protons are in ax-eq or eq-eq relationships, thus suggesting β -D-ribose (1C₄), β -L-ribose (4C₁), β -D-xylose (1C₄) or β -L-xylose (4C₁) as a possible glycone moiety. All of these possible sugar moieties adopt thermodynamically unfavorable conformations where three or all four oxygen atoms are in axial positions. To unambiguously disclose the relative configuration of the sugar moiety as well as its spatial conformation, single crystal X-ray crystallographic analysis was carried out using a crystal obtained from a mixture of EtOH and H₂O (1:1) by slow evaporation. The crystal structure (Figure 2) of **1** revealed that the relative configuration of the glycone moiety is a β -ribose where oxygen atoms at C-1', C-2' and C-4' were axially positioned. The absolute configuration of **1** was determined by measuring the optical rotation of the glycone moiety and ECD analysis of the aglycone moiety, each liberated by acid hydrolysis (1N HCl). The negative rotation ($[\alpha]_{\text{D}} - 9$), observed for the glycone moiety, indicated the absolute configuration to be a β -D-ribose with 1C₄ conformation (Figure 2). Negative Cotton effects observed at 230 nm and 280 nm for the aglycone moiety are identical to those observed for cylindrocyclophane A, indicating the same absolute configuration.⁶ The potential energy of a 1C₄ conformation is approximately four times higher than that of 4C₁ conformation for β -D-ribose.¹⁹ The adoption of a thermodynamically unfavorable conformation for the glycone moiety could be rationalized by a favored spatial arrangement to avoid a steric interaction between the aglycone and glycone moieties in the overall structure (Figure 3). The β -D-ribose glycone with the preferred 4C₁ conformation occupies a much larger spatial volume than that with a 1C₄ conformation, creating a steric effect with the aromatic ring and branched methyl of the cylindrocyclophane aglycone. This steric effect forces the sugar moiety into a 1C₄ conformation, and the adoption of a 1C₄ conformation increases the overall stability of **1** by reducing the spatial volume of the glycone moiety. The selection of the unfavorable 1C₄ conformation is also supported by the anomeric effect, in which heteroatom substituents preferentially assume an axial orientation when adjacent to a heteroatom within a ring. The glycone moiety of **1** obtained from UIC 10366 was found to have the same negative specific rotation and identical ^1H NMR signals as those observed for **1** from UIC 10279, suggesting

that the glycone produced by UIC 10366 is also a β -D-ribofuranose. The crystal structure of **1** allowed the assignment of the stereocenters of the cylindrocyclophane aglycone as 1*R*, 2*S*, 7*R*, 14*R*, 15*S*, 20*R* which are identical to those reported for cylindrocyclophane A.

Ribocyclophane B (**2**) was obtained as a white amorphous powder from UIC 10279, and HRESIMS analysis (m/z 715.4477 [M-H][−]) suggested the molecular formula of **2** as C₄₁H₆₄O₁₀. The ¹H NMR spectrum of **2** showed the overall signal distribution pattern similar to that observed for **1**. The appearance of four singlet aromatic (δ_H 6.07, 6.09, 6.20 and 6.24) and two doublet methyl (δ_H 1.05 and 1.06) signals indicated the structure of **2** to be non-symmetrical. The integration of each signal of the glycone moiety corresponded to that of one aromatic proton signal of the aglycone moiety, suggesting the presence of only one sugar moiety in **2**. The newly appeared signal of H-14 (δ_H 3.74) compared to **1**, together with the mass difference of 132 between **2** and **1**, demonstrated that one of the glycoside moieties in **1** was replaced by a hydroxy group in **2**. The ECD spectrum of **2**, showed positive Cotton effects at 221 and 275 nm, identical to those observed for **1**, suggesting the same absolute configuration for **2**.

Ribocyclophane C (**3**) was obtained as a white amorphous powder from UIC 10279. HRESIMS analysis (m/z 699.4533 [M-H][−]) suggested the molecular formula of **3** as C₄₁H₆₄O₉. The ¹H NMR spectrum of **3** closely resembled that of **2**, and four aromatic and two doublet methyl signals were observed, thus indicating the structure of **2** to be also non-symmetrical due to the presence of one glycone moiety. The notable difference was the replacement of an oxygenated methine proton H-14 in **2** by diastereotopic methylene protons (δ_H 1.81 and 2.61) in **3**. This, together with the 16 mass difference between **2** and **3**, which corresponded to one oxygen atom, suggested the absence of the hydroxy group at C-14. This was confirmed by NMR analysis (Table 2), demonstrating the structure of **3** to be the deoxygenated analogue of **2**. The positive Cotton effects observed at 222 and 274 nm indicated the same absolute configuration of **3** as determined for **2**.

Ribocyclophane D (**4**) was obtained as a white amorphous powder from UIC 10366. HRESIMS analysis (m/z 757.4489 [M-H][−]) suggested the molecular formula of **4** as C₄₃H₆₆O₁₁. The ¹H NMR spectrum of **4** was similar to that of **2** and **3**, suggesting the compound was non-symmetrical due to differing moieties on the cylindrocyclophane scaffold. The difference in molecular formula between **2** and **4** suggested the presence of an acetate group. The presence of a singlet at δ_H 2.0 in the ¹H NMR spectrum and signals at δ_C 21.3 and δ_C 172.6 in the ¹³C NMR spectrum confirmed the presence of an acetate moiety. HMBC correlations from H-14 to C-37 confirmed the placement of the acetate moiety α to the resorcinol core. The positive Cotton effects observed at 222 and 273 nm indicated the same absolute configuration as all previously described [7.7]paracyclophanes.

Ribocyclophane E (**5**) was obtained as a minor component from UIC 10366 during the isolation of **4**. HRESIMS analysis showed an isotopic pattern consistent with the presence of one chlorine atom and suggested a molecular formula of C₄₃H₆₇ClO₁₁ (m/z 793.9098 [M-H][−] and 795.9101 [M-H][−] in a 3:1 ratio). Comparing the ¹H NMR spectrum to the spectra for **1** – **4** indicated **5** to be structurally different. The ¹H NMR spectrum of **5** was more similar to the ¹H NMR spectra of the cylindrofridins published by Preisitsch et al.¹⁵ NMR analysis

allowed the assignment of two aliphatic chains as well as a ribose. The signal at δ_{H} 3.83 indicated a proton adjacent to a halogen, suggesting the presence of the chlorine atom on one of the aliphatic chains. HSQC and ^{13}C spectra confirmed the presence of the chlorine, with the signal at δ_{H} 3.83 correlating to C-7 at δ_{C} 65.1. COSY correlations from H-7 to H-6 and H-27 confirmed the placement of the chlorine along the aliphatic chain. The presence and positions of two resorcinol-like groups were deduced by HMBC correlations from H-23/25 to C-1 and likewise from H-10/12 to C-14, and H-20 to C-22/26 and H-31/19 to C-21. No correlations were observed from H-7 to C-9/13 or H-6/27 to C-8, as would be present in other [7.7]paracyclophane HMBC spectra. This is consistent with cylindrofridin-like structures that are linear. HMBC correlation from H-1 to C-1' confirmed the position of the ribose and a correlation from H-14 to C-37, along with the strong singlet signal at δ_{H} 2.07, suggested the presence of an acetate moiety. The Cotton effects observed at 222 and 274 nm indicated the same absolute configuration as the previously described cylindrofridins and [7.7]paracyclophanes.¹⁵ We were unable to establish the configuration of the chiral center at C-7.

Ribocyclophanes A – D (**1** – **4**) are glycosylated analogues of the cylindrocyclophanes. Ribocyclophane E (**5**) is a glycosylated analog of the cylindrofridins, and a likely biosynthetic intermediate of **4**. A notable structural feature of **1** – **4** is the presence of a β -D-ribofuranose glycone moiety linked to the benzoyl carbon of the cylindrocyclophane aglycone. This glycone moiety exclusively adopted the thermodynamically unfavorable $^1\text{C}_4$ conformation in which three oxygen atoms are in an axial position and one oxygen atom is in an equatorial position. The crystal structure of **1** showed that no hydrogen bonding is involved between the glycone and aglycone moieties to favor the shift of conformational equilibrium toward $^1\text{C}_4$ conformation. This suggested that an avoidance of steric crowding between the β -D-ribofuranose glycone and the cylindrocyclophane aglycone is likely to be the major driver of the adoption of the $^1\text{C}_4$ conformation.

Ribocyclophanes A – E (**1** – **5**) and cylindrocyclophane D (**6**) were tested for their antiproliferative activity against MDA-MB-231 and MDA-MB-435 cancer cell lines. Ribocyclophane D (**4**) showed the strongest activity among six compounds with IC_{50} values of less than 1 μM in both cell lines (IC_{50} 0.8 μM and 0.6 μM , respectively), whereas moderate activity was observed for ribocyclophane C (**3**) (IC_{50} 1.2 μM and 1.3 μM , respectively). Ribocyclophane E (**5**) showed no antiproliferative activity against either cell line at 25 μM , corroborating the findings of Preisitsch et al. that an unclosed [7.7]paracyclophane core structure does not retain antiproliferative activity.¹⁵ Ribocyclophanes A, B, and cylindrocyclophane D all had modest activity against the two cell lines as shown in Supporting Information S18. Interestingly, the large glycone moieties do not seem to change the antiproliferative activity, as ribocyclophane D has similar activity levels as smaller [7.7]paracyclophanes such as the merocyclophanes.^{8,11}

Experimental Section

General Experimental Procedures

Optical rotations were measured on a Perkin-Elmer 241 polarimeter. ECD spectra were recorded on a Jasco J-710 ECD spectrometer. UV and IR spectra were recorded on a

Shimadzu UV spectrometer UV2401 and a Thermo Nicolet 6700 FT-IR spectrometer, respectively. 1D and 2D NMR spectra including ^1H NMR, COSY, TOCSY, HSQC, HMBC and T-ROESY spectra were obtained on a Bruker Avance 600 MHz NMR spectrometer with a 5 mm CPTXI Z-gradient probe, whereas a Bruker Avance II 900 MHz NMR spectrometer with a 5 mm ATM CPTCI Z-gradient probe was used to acquire the DEPT-Q spectrum. ^1H and ^{13}C NMR chemical shifts were referenced to the DMSO- d_6 solvent signals (δ_{H} 2.49 and δ_{C} 39.7, respectively) or MeOH- d_4 solvent signals (δ_{H} 3.31 and δ_{C} 49.15, respectively). A mixing time of 60 ms was set for the TOCSY experiment and 200 ms for the T-ROESY experiment. 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 145 Hz. HRESIMS and LC-MS data were obtained on a Shimadzu IT-TOF LC-MS spectrometer.

Biological Material

Nostoc sp. (UIC 10279) was isolated from a sample collected in the southwest suburbs of Chicago near the town of Joliet, Illinois. The unialgal strain (UIC 10279) was produced through micropipette isolation techniques.²⁰ Taxonomic identification of the strain UIC 10279 was carried out using a partial 16S rRNA gene sequence (GenBank Accession No. JX962720, See SI, S2). *Nostoc* sp. (UIC 10366) was isolated from a sample collected in the northwest suburbs of Chicago near the town of Elgin, Illinois, through micropipette isolation.²⁰ Taxonomic identification of the strain UIC 10366 was carried out using a partial 16S rRNA gene sequence (GenBank Accession No. MF622942, see SI, S2). Both strains were cultured in four 2.8 L Fernbach flasks each containing inorganic Z media (4×2 L) with sterile aeration.¹⁸ 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 7 weeks, the biomasses were harvested by centrifugation and then freeze-dried.

Extraction and Isolation

The freeze-dried cells were each extracted with CH_2Cl_2 -MeOH (1:1 v/v) three times and concentrated in vacuo to yield organic extracts (0.5 g of UIC 10279 and 0.65 g of UIC 10366). The resulting extracts were fractionated using Diaion HP-20 resin with an increasing amount of iPrOH in H_2O . The fractions eluting at 50% and 60% iPrOH were found to be active in the antiproliferative assay against MDA-MB-435 cells (95% and 97% at 25 $\mu\text{g/mL}$, respectively). HPLC-based activity profiling of the active fractions, which associates the HPLC chromatogram with biological activity, identified three active peaks for each strain. Dereplication of these peaks by LC-MS and ^1H NMR indicated these peaks to be new cyclophanes with the molecular weights of 848, 758, 716 and 700 Da. The fractions containing active peaks were combined and subjected to reversed-phase HPLC (Varian C_8 column, 10 mm $\times$ 250 mm, 3 mL/min) eluting with gradient using aqueous MeOH from 60 to 80 % over 40 min which led to the isolation of ribocyclophanes A (**1**, 15 mg, 0.5 %), B (**2**, 3 mg, 0.1 %), and C (**3**, 10 mg, 0.3 %) from UIC 10279; and ribocyclophanes D (**4**, 15 mg, 0.5%), E (**5**, 1 mg, >0.1%), and cylindrocyclophane D (**6**, 6 mg, 0.2%) from UIC 10366.

Ribocyclophane A (**1**): white amorphous powder; $[\alpha]_{\text{D}}^{25}$ - 6 (c 0.12, MeOH); UV (MeOH) λ_{max} (log ϵ) 208 (4.41), 223 (3.88), 276 (3.13) nm; ECD (MeOH 1.0 mM) λ_{max} (ϵ) 208 (2.87), 224 (1.27), 282 (1.04) nm; IR (neat) ν_{max} 3365, 2930, 2857, 1628, 1432, 1381, 1321

cm^{-1} ; ^1H and ^{13}C NMR, COSY, and HMBC data, Table 1; HRESIMS m/z 847.4894 $[\text{M-H}]^-$ (calcd for $\text{C}_{46}\text{H}_{71}\text{O}_{14}$, 847.4844). Spectroscopic data of **1** isolated from UIC 10366 was identical to that from UIC 10279.

Ribocyclophane B (2): white amorphous powder; $[\alpha]_{\text{D}}^{25} - 6$ (c 0.07, MeOH); UV (MeOH) λ_{max} (log ϵ) 207 (4.10), 221 (3.65), 275 (2.90) nm; ECD (MeOH 0.7 mM) λ_{max} (ϵ) 208 (1.62), 222 (0.99), 284 (0.86) nm; IR (neat) ν_{max} 3378, 2930, 2858, 1631, 1469, 1432, 1372 cm^{-1} ; ^1H and ^{13}C NMR data, Table 2; HRESIMS m/z 715.4471 $[\text{M-H}]^-$ (calcd for $\text{C}_{41}\text{H}_{63}\text{O}_{10}$, 715.4421)

Ribocyclophane C (3): white amorphous powder; $[\alpha]_{\text{D}}^{25} + 6$ (c 0.16, MeOH); UV (MeOH) λ_{max} (log ϵ) 209 (4.54), 223 (4.03), 275 (3.23) nm; ECD (MeOH 0.7 mM) λ_{max} (ϵ) 211 (2.59), 224 (1.44), 282 (2.00) nm; IR (neat) ν_{max} 3382, 2926, 2856, 1691, 1620, 1592, 1431, 1369 cm^{-1} ; ^1H and ^{13}C NMR data, Table 2; HRESIMS m/z 699.4533 $[\text{M-H}]^-$ (calcd for $\text{C}_{41}\text{H}_{63}\text{O}_9$, 699.4472)

Ribocyclophane D (4): white amorphous powder; $[\alpha]_{\text{D}}^{25} - 3$ (c 0.14, MeOH); UV (MeOH) λ_{max} (log ϵ) 208 (4.38), 220 (3.85), 280 (3.05) nm; ECD (MeOH 1.0 mM) λ_{max} (ϵ) 211 (2.82), 228 (1.21), 284 (0.9); IR (neat) ν_{max} 3380, 2931, 2862, 1625, 1433, 1372, 1256 cm^{-1} ; ^1H and ^{13}C NMR data, Table 3; HRESIMS m/z 757.4548 $[\text{M-H}]^-$ (calcd for $\text{C}_{43}\text{H}_{65}\text{O}_{11}$, 757.4526)

Ribocyclophane E (5): white amorphous powder; $[\alpha]_{\text{D}}^{25} + 7$ (c 0.14, MeOH); UV (MeOH) λ_{max} (log ϵ) 211 (3.82), 223 (3.61), 278 (2.98) nm; ECD (MeOH 0.7 mM) λ_{max} (ϵ) 208 (2.12), 226 (1.12), 277 (−0.8); IR (neat) ν_{max} 3405, 2930, 2858, 1567, 1426, 1387, 1257, 830 cm^{-1} ; ^1H and ^{13}C NMR data, Table 3; HRESIMS m/z 793.4310 $[\text{M-H}]^-$ (calcd for $\text{C}_{43}\text{H}_{66}\text{ClO}_{11}$, 793.4293)

Single Crystal X-ray Crystallographic Analysis of **1**

A small single crystal, roughly $10 \times 10 \times 30 \mu\text{m}$, was encased in Paratone-N oil and cooled to 100K in a nylon loop. Data was collected at the Advanced Photon Source, LS-CAT, sector 21, on a MAR 300mm CCD detector. 240 images of width 1° were collected at a crystal-to-detector distance of 91 mm, and wavelength $0.77 \text{ \AA}$, to a resolution of $0.8 \text{ \AA}$. The images were indexed, and the intensities were integrated and scaled with XDS.²¹ The unit cell is monoclinic, space group $P2_1$, with two molecules in the asymmetric unit, and eight disordered MeOH molecules. The structure was solved by SHELXS, and refined with SHELXL.²² Crystal data: $2\text{C}_{46}\text{H}_{72}\text{O}_{14} \cdot 8\text{C}_2\text{H}_6\text{O}$, monoclinic, space group $P2_1$ (No. 4), $a = 10.752$ (2) $\text{\AA}$, $b = 34.601$ (4) $\text{\AA}$, $c = 16.762$ (2) $\text{\AA}$, $\beta = 90.480$ (8) $^\circ$, $V = 6235.7$ (16) $\text{\AA}^3$, $Z = 4$, $D_c = 1.10$, $\mu = 0.081$, $F(000) = 2256$. Reflections collected/unique = 55588/23169 ($R_{\text{int}} = 0.0514$), final $R_1 = 0.0996$, $wR_2 = 0.2478$ for reflections with $I > 2\sigma I$; $R_1 = 0.1063$, $wR_2 = 0.2552$ for all reflections; goodness-of-fit = 1.019 for all unique data. Bijvoet analysis: Flack $\times = -0.4$ (6), Parsons $z = -0.24$ (15), Probability $P_2(\text{true}) = 1.000$. Crystallographic data (ID entry CCDC 898167) have been deposited in the Cambridge Crystallographic Data Centre.

Acid Hydrolysis of **1**

Approximately 5 mg of **1** was hydrolyzed using 1N HCl (1 mL) at 100 °C for 1 h. The resulting hydrolysate was air-dried, and extracted with H₂O and EtOAc to yield the H₂O (1.3 mg) and EtOAc (3.9 mg) fractions. The negative specific rotation observed for the H₂O fraction suggested the sugar moiety to be D-ribopyranose: $[\alpha]^{25}_D - 9$ (c 0.13, H₂O). Reversed-phase HPLC of the EtOAc fraction (C₁₈, 60 – 80% aqueous MeOH) yielded the pure aglycone moiety (0.5 mg; HRESIMS m/z 583.4113 [M-H]⁻). The ECD spectrum of the aglycone moiety displayed negative Cotton effects at 230 nm and 280 nm, indicating the same absolute configuration as that reported for cylindrocyclophane A.⁶

Antiproliferative Assay

The human melanoma cell line MDA-MB-435 and human breast cancer cell line MDA-MB-231 were purchased from the American Type Culture Collection. The cells were propagated at 37 °C in 5% CO₂ in RPMI 1640 medium supplemented with fetal bovine serum (10%), penicillin (100 units/mL) and streptomycin (100 µg/mL). Cells in log phase growth were harvested by trypsinization. A total of 5,000 cells were seeded per well of a 96-well plate and incubated overnight at 37 °C in 5% CO₂. Samples dissolved in DMSO were then sequentially diluted and added to the appropriate wells (total volume 100 µL). Each compound was tested at the following concentrations (µg/mL): 25, 5.0, 1.0, 0.2 and 0.04. The cells were incubated in the presence of test substance for ninety-six hours at 37 °C and evaluated for viability with a commercial absorbance assay (CellTiter 96 AQueous One Solution Cell Proliferation Assay, Promega Corp). Activity was expressed as the percentage of viable cells present relative to the negative (solvent) control. The positive control was vinblastine tested at 1 ng/mL, which had 49% viable cells after treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Key 2D NMR correlations used for the determination of the structure of ribocyclophane A (1).

Figure 2.
ORTEP drawing of ribocyclophane A (**1**).

Figure 3.

The β -D-ribofuranose glycone adopts the less favorable 1C_4 conformation to avoid steric interactions with the cylindrocyclophane aglycone moiety.

2 $R_1 = \text{Ribose}, R_2 = \text{OH}$

3 $R_1 = \text{Ribose}, R_2 = \text{H}$

4 $R_1 = \text{Ribose}, R_2 = \text{OAc}$

6 $R_1 = \text{OAc}, R_2 = \text{OAc}$

Table 1NMR Spectroscopic Data of Ribocyclophane A (1) in MeOH-*d*₄

No.	Ribocyclophane A (1)			
	δ_C , ^a type	δ_H , ^b mult. (J in Hz)	COSY ^b	HMBC ^b
1/14	90.1, CH	3.65, d (12.2)	2/15	2/15, 3/16, 10/23, 11/24, 12/25, 35/36, 1'/6'
2/15	41.8, CH	1.65, m	1/14, 3/16, 35/36	NA ^c
3/16	34.9, CH ₂	0.66, m	2/15, 4/17	NA ^d
		0.75, m		
4/17	30.0, CH ₂	0.82, m	3/16, 5/18	NA ^c
		1.44, m		
5/18	30.9, CH ₂	0.71, m	4/17, 6/19	NA ^d
		0.96, m		
6/19	35.5, CH ₂	1.32, m	5/18, 7/20	4/17, 5/18, 7/20, 27/31
		2.03, ddd (4.2)		
7/20	37.0, CH	3.16, m	6/19, 27/31	5/18, 6/19, 8/21, 9/22, 13/26, 27/31, 28/32
8/21	117.9, C			
9/22	158.9, C			
10/23	105.5, CH	6.19, s		1/14, 12/25, 8/21, 9/22
11/24	142.1, C			
12/25	109.3, CH	6.09, s		1/14, 10/23, 8/21, 13/26
13/26	157.1, C			
27/31	34.8, CH ₂	1.47, m	7/20, 28/32	6/19, 7/20, 8/21, 28/32, 29/33
		1.95, m		
28/32	31.9, CH ₂	1.04, m	27/31, 29/33	NA ^d
		1.17, m		
29/33	24.0, CH ₂	1.18, m	28/32, 30/34	NA ^d
		1.28, m		
30/34	14.8, CH ₃	0.79, t (7.2)	29/33	28/32, 29/33
35/36	16.7, CH ₃	1.05, d (6.0)	2/15	1/14, 2/15, 3/16
1'/6'	104.6, CH	4.85, d (1.8)	2'/7'	1/14, 5'/10', 2'/7, 3'/8'
2'/7'	72.8, CH	3.73, d (1.8, 3.0)	1'/6', 3'/8'	NA ^c
3'/8'	66.9, CH	3.70, t (3.0)	2'/7', 4'/9'	NA ^c
4'/9'	71.6, CH	3.59, ddd (2.4, 3.0)	3'/8', 5'/10'	NA ^c
5'/10'	65.6, CH ₂	3.19, dd (12.6, 2.4)	4'/9'	1'/6', 3'/8', 4'/9'
		3.30, dd (12.6)		

Table 2NMR Spectroscopic Data of Ribocyclophanes B (2) and C (3) in MeOH-*d*₄

No.	Ribocyclophane B (2)		Ribocyclophane C (3)	
	$\delta_{\text{C}}, ^a, ^b$ type	$\delta_{\text{H}} ^b$, mult. (<i>J</i> in Hz)	$\delta_{\text{C}}, ^a, ^b$ type	$\delta_{\text{H}} ^b$, mult. (<i>J</i> in Hz)
1	88.5, CH	3.65, d (10.2)	88.4, CH	3.66, d (10.2)
2	40.1, CH	1.66, m	40.1, CH	1.66, m
3	33.4, CH ₂	0.65, m	33.3, CH ₂	0.66, m
		0.76, m		0.79, m
4	28.6, CH ₂	0.83, m	28.6, CH ₂	0.81, m
		1.42, m		1.43, m
5	29.0, CH ₂	0.72, m	29.3, CH ₂	0.74, m
		0.95, m		0.97, m
6	33.9, CH ₂	1.32, m	34.0, CH ₂	1.32, m
		2.04, m		2.00, m
7	35.4, CH	3.16, m	35.4, CH	3.11, m
8	116.1, C		114.7, C	
9	157.7, C		156.9, C	
10	103.5, CH	6.24, s	106.5, CH	6.02, s
11	142.2, C		139.4, C	
12	107.6, CH	6.07, s	108.5, CH	5.97, s
13	156.0, C		155.7, C	
14	80.2, CH	3.74, d (9.6)	44.3, CH ₂	1.81, t (12.6)
				2.61, dd 13.2, 3.6)
15	40.7, CH	1.56, m	35.4, CH	1.57, m
16	33.4, CH ₂	0.65, m	35.3, CH ₂	0.62, m
		0.76, m		1.01, m
17	28.6, CH ₂	0.83, m	28.8, CH ₂	0.81, m
		1.42, m		1.34, m
18	29.0, CH ₂	0.72, m	29.3, CH ₂	0.74, m
		0.95, m		0.97, m
19	33.9, CH ₂	1.32, m	34.0, CH ₂	1.32, m
		2.04, m		2.00, m
20	35.4, CH	3.16, m	35.4, CH	3.16, m
21	116.1, C		116.4, C	
22	157.7, C		157.2, C	
23	103.7, CH	6.20, s	103.9, CH	6.20, s
24	140.4, C		140.3, C	
25	107.8, CH	6.09, s	107.6, CH	6.09, s
26	155.0, C		155.7, C	
27	33.3, CH ₂	1.47, m	33.3, CH ₂	1.48, m
		1.95, m		1.94, m

No.	Ribocyclophane B (2)		Ribocyclophane C (3)	
	$\delta_{\text{C}},^{a,b}$ type	δ_{H}^b , mult. (<i>J</i> in Hz)	$\delta_{\text{C}},^{a,b}$ type	δ_{H}^b , mult. (<i>J</i> in Hz)
28	30.2, CH ₂	1.04, m 1.17, m	30.3, CH ₂	1.06, m 1.17, m
29	22.4, CH ₂	1.19, m 1.29, m	22.3, CH ₂	1.20, m 1.29, m
30	13.1, CH ₃	0.79, t (7.2)	13.1, CH ₃	0.82, t (7.2)
31	33.3, CH ₂	1.47, m 1.95, m	33.3, CH ₂	1.48, m 1.94, m
32	30.2, CH ₂	1.04, m 1.17, m	30.3, CH ₂	1.06, m 1.17, m
33	22.4, CH ₂	1.19, m 1.29, m	22.3, CH ₂	1.20, m 1.29, m
34	13.1, CH ₃	0.79, t (7.2)	13.3, CH ₃	0.79, t (7.2)
35	15.4, CH ₃	1.05, d (7.2)	15.4, CH ₃	1.05, d (6.6)
36	15.4, CH ₃	1.06, d (6.6)	19.3, CH ₃	0.95, d (6.6)
1'	103.0, CH	4.85, d (2.4)	103.0, CH	4.85, d (2.4)
2'	71.1, CH	3.73, brs	71.1, CH	3.73, brs
3'	65.2, CH	3.70, t (3.0)	65.3, CH	3.70, t (3.0)
4'	70.1, CH	3.60, brs	70.0, CH	3.59, brs
5'	63.9, CH ₂	3.20, dd (12.6, 2.4) 3.31, overlapped	64.0, CH ₂	3.20, dd (12.6, 2.4) 3.31, overlapped

^a assigned from the HSQC and HMBC spectra,

^b recorded at 600 MHz

Table 3NMR Spectroscopic Data of Ribocyclophanes D (4) and E (5) in MeOH-*d*₄

No.	Ribocyclophane D (4)		Ribocyclophane E (5)	
	δ_{C}^a , type	δ_{H}^c , mult. (<i>J</i> in Hz)	δ_{C}^b , type	δ_{H}^c , mult. (<i>J</i> in Hz)
1	90.1, CH	3.65, d (10.0)	87.6, CH	4.0, m
2	41.7, CH	1.64, m	40.4, CH	1.77, m
3	34.9, CH ₂	0.65, m	33.9, CH ₂	0.97, m
		0.76, m		1.26, m
4	30.0, CH ₂	0.84, m	22.7, CH ₂	1.32, m
		1.43, m		
5	30.8, CH ₂	0.7, m	27.6, CH ₂	1.33, m
		0.94, m		1.44, m
6	35.5, CH ₂	1.33, m	39.4, CH ₂	1.54, m
		2.03, m		1.62, m
7	37.0, CH	3.16, m	65.1, CH	3.83, m
8	118.7, C		102.7, CH	6.15, s
9	159.0, C		159.9, C	
10	109.6, CH	6.13, s	106.0, CH	6.17, s
11	139.3, C		144.1, C	
12	105.3, CH	6.18, s	106.0, CH	6.17, s
13	157.1, C		159.9, C	
14	83.6, CH	5.0, d (10.4)	80.8, CH	5.4, m
15	40.2, CH	1.77, m	39.9, CH	1.79, m
16	34.6, CH ₂	0.71, m	30.6, CH ₂	1.29, m
		0.78, m		
17	29.8, CH ₂	0.84, m	29.8, CH ₂	1.05, m
		1.43, m		1.34, m
18	30.7, CH ₂	0.7, m	29.6, CH ₂	1.09, m
		0.94, m		
19	35.4, CH ₂	1.33, m	34.5, CH ₂	1.47, m
		2.03, m		1.92, m
20	37.0, CH	3.16, m	36.4, CH	3.19, m
21	117.8, C		118.2, C	
22	159.0, C		158.3, C	
23	105.5, CH	6.2, s	107.3, CH	6.22, br
24	142.1, C		141.9, C	
25	109.2, CH	6.08, s	107.3, CH	6.22, br
26	157.1, C		158.3, C	
27	35.0, CH ₂	1.49, m	39.4, CH ₂	1.6, m
		1.91, m		1.68, m
28	31.8, CH ₂	1.05, m	27.6, CH ₂	1.29, m

No.	Ribocyclophane D (4)		Ribocyclophane E (5)	
	$\delta_{\text{C}},^a$ type	δ_{H}^c , mult. (<i>J</i> in Hz)	$\delta_{\text{C}},^b$ type	δ_{H}^c , mult. (<i>J</i> in Hz)
29	24.0, CH ₂	1.17, m	23.4, CH ₂	1.34, m
		1.19, m		1.27, m
		1.29, m		1.33, m
30	14.8, CH ₃	0.79, t (7.3)	14.3, CH ₃	0.92, t (7.3)
31	34.8, CH ₂	1.49, m	34.5, CH ₂	1.5, m
		1.95, m		1.94, m
32	31.9, CH ₂	1.05, m	31.7, CH ₂	1.07, m
		1.17, m		1.17, m
33	24.1, CH ₂	1.22, m	23.8, CH ₂	1.22, m
		1.29, m		1.30, m
34	14.7, CH ₃	0.82, t (7.3)	15.1, CH ₃	0.82, t (7.2)
35	17.0, CH ₃	1.05, d (6.4)	16.5, CH ₃	0.99, d (6.5)
36	16.7, CH ₃	0.96, d (6.4)	14.8, CH ₃	0.85, d (6.5)
37	172.6, C		173.1, C	
38	21.3, CH ₃	2.0, s	21.2, CH ₃	2.07, s
1'	104.5, CH	4.85, d (2.0)	103.8, CH	4.89, br
2'	72.8, CH	3.72, brs	72.6, CH	3.76, br
3'	67, CH	3.7, t (3.2)	66.9, CH	3.78, br
4'	71.6, CH	3.6, brs	71.3, CH	3.67, br
5'	65.7, CH ₂	3.2, dd (12.3, 2.5)	65.6, CH ₂	3.32, overlapped
		3.32, overlapped		3.47, overlapped

^a assigned from the DEPT-Q spectrum acquired at 226 MHz,

^b assigned from HSCQ and HMBC spectra,

^c recorded at 900 MHz