Cephalocyclidin A, a Novel Pentacyclic Alkaloid from *Cephalotaxus harringtonia* var. *nana*

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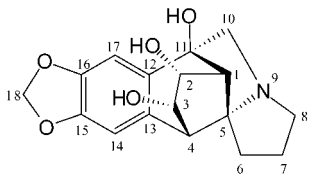
A novel alkaloid with an unprecedented fused-pentacyclic skeleton and six-consecutive asymmetric centers, cephalocyclidin A (**1**), has been isolated from the fruits of *Cephalotaxus harringtonia* var. *nana*, and the structure was elucidated on the basis of spectroscopic data. The relative and absolute stereochemistry of **1** was determined by a combination of NOESY correlations, X-ray crystallographic data, and the exciton chirality method.

Introduction

Cephalotaxus alkaloids are a family of cytotoxic heterocyclic natural products elaborated by trees of the genus *Cephalotaxus* (Cephalotaxaceae), some of which showed potent antileukemic activity.[1] Although a number of *Cephalotaxus* alkaloids with various side chains have been isolated so far, there are very few reports on backbone skeletons other than that of cephalotaxine (**2**).[1] Recently, we have isolated six new cytotoxic alkaloids, cephalezomines A−F, from the leaves of *Cephalotaxus harringtonia* var. *nana*.[2] Our continuing search for structurally unique and biogenetically interesting *Cephalotaxus* alkaloids resulted in the isolation of cephalocyclidin A (**1**), a novel alkaloid with an unprecedented fused-pentacyclic ring system from the fruits of *C. harringtonia* var. *nana*. This paper describes the isolation and structural elucidation of **1**.



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Results and Discussion

Isolation of Cephalocyclidin A (1). The fruits of *C. harringtonia* var. *nana* were extracted with MeOH, and the extract was partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, which were adjusted to pH 10 with saturated Na_2CO_3, were extracted with $CHCl_3$. $CHCl_3$-soluble materials were subjected to a silica gel column ($CHCl_3$/MeOH, 1:0 → 0:1), in which a fraction eluted with $CHCl_3$/MeOH (1:1) was purified by C_{18} HPLC [30% CH_3CN/0.03 M $(NH_4)_2CO_3$] to afford cephalocyclidin A (**1**, 6 mg, 0.0006% yield) as a colorless solid together with known related alkaloids, cephalotaxine (**2**)[3] and drupacine.[4]

Structure of Cephalocyclidin A (1). Cephalocyclidin A (**1**) showed the pseudomolecular ion peak at *m*/*z* 318 $(M + H)^+$ in the FABMS spectrum, and the molecular formula, $C_{17}H_{19}NO_5$, was established by HRFABMS [*m*/*z* 318.1316, $(M + H)^+$, Δ −2.6 mmu]. IR absorptions implied the presence of hydroxyl (3450 cm^{-1}) functionality. Analysis of ^1H and ^{13}C NMR data (Table 1) and the HMQC spectrum provided evidence that **1** possessed 17 carbon signals due to four sp^2 and two sp^3 quaternary carbons, two sp^2 and four sp^3 methines, and five sp^3 methylenes. Among them, one sp^3 quaternary carbon (δ_C 77.89) and two sp^3 methylenes (δ_C 55.31, δ_H 2.66 and 3.12; δ_C 69.94, δ_H 2.92 and 3.16) were ascribed to those bearing a nitrogen, while one sp^3 and two sp^2 quaternary carbons (δ_c 82.47, 146.51, and 147.39, respectively), two sp^3 methines (δ_c 68.73 and 73.67), and one sp^3 methylene (δ_c 101.04) were ascribed to those bearing an oxygen atom.

Partial structure **a** (C-1−C-4) with two hydroxy groups at C-2 and C-3, partial structure **b** (C-6−C-8), and a 1,2,4,5-tetrasubstituted benzenoid ring with a methylene dioxide unit were deduced from analyses of 2D NMR ($^1H-^1H$ COSY, HOHAHA,[5] HMQC, and HMBC[6]) data of **1** in $CDCl_3/CD_3OD$ (4:1) (Figure 1). HMBC correlations for H-1, H-6, and H-7 of C-5 (δ_C 77.89), H-6 of C-4 (δ_C 56.78), H-1 of C-6 (δ_C 35.06), and H-6 of C-1 (δ_C 56.68) gave rise to the connectivity of partial structures **a** and **b** through a spiro carbon (C-5). In addition, HMBC correlations for H-8 to C-10 (δ_C 69.94) and H-10 to C-8

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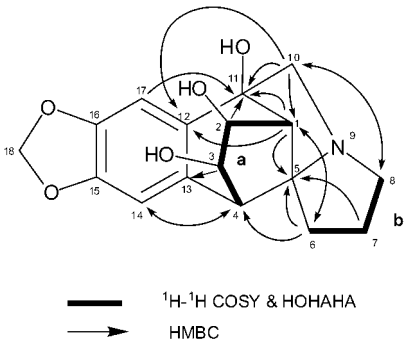

Figure 1. Selected 2D NMR correlations for cephalocyclidin A (**1**).

Table 1. ^{1}H and ^{13}C NMR Data of Cephalocyclidin A (1) in $CDCl_3/CD_3OD$ (4:1) at 310 K

	δ_H	δ_C	HMBC (^{1}H)
1	2.71 (1H, d, 7.5)	56.68	2, 4, 6, 10b, 11-OH
2	4.27 (1H, t, 7.5)	68.73	4
3	4.09 (1H, dd, 5.0, 7.5)	73.67	1, 11-OH
4	2.80 (1H, d, 5.0)	56.78	1, 2, 6, 14
5		77.89	1, 6, 7, 10b
6a	1.76 (1H, m)	35.06	1, 7, 8
6b	1.86 (1H, m)		
7a	1.95 (1H, m)	25.93	6
7b	2.04 (1H, m)		
8a	2.66 (1H, m)	55.31	6, 10a
8b	3.12 (1H, m)		
10a	2.92 (1H, d, 8.6)	69.94	8a
10b	3.16 (1H, d, 8.6)		
11		82.47	1, 2, 10, 17, 11-OH
12		136.96	1, 4, 10, 14
13		126.89	3, 4, 17
14	6.52 (1H, s)	110.18	4
15		146.51	17, 18
16		147.39	14, 18
17	6.98 (1H, s)	103.58	
18a	5.86 (1H, s)	101.04	
18b	5.88 (1H, s)		

(δ_C 55.31) suggested that both C-8 and C-10 were connected to a nitrogen atom (N-9). HMBC correlations for H-1, H-2, and H-10 to C-11 (δ_C 82.47) indicated the presence of a quaternary carbon (C-11) with a hydroxy group between C-1 and C-10. Connectivities between C-11 and C-12 and between C-4 and C-13 were elucidated by HMBC cross-peaks for H-14 (δ_H 6.52) to C-4 (δ_C 56.78) and H-17 (δ_H 6.98) to C-11 (δ_C 82.47), H-10 to C-12 (δ_C 136.96), and H-3 to C-13 (δ_C 126.89). Thus, the structure of cephalocyclidin A was elucidated to be **1** possessing a fused-pentacyclic ring system consisting of a 1-azaspiro-[4.4]nonane (C-1−C-8 and N-9), a pyrrolidine (C-1, C-5, N-9, and C-10−C-11), and a benzocycloheptane (C-1−C-4 and C-11−C-17) rings.

Stereochemistry of Cephalocyclidin A (1). The relative stereochemistry of H-1, H-4; the three hydroxyl groups at C-2, C-3, and C-11; and the N-9 at C-5 in cephalocyclidin A (**1**) were deduced from NOESY correlations as shown in computer-generated 3D drawing (Figure 2).[7]

X-ray analysis (Figure 3) of the crystal of cephalocyclidin A (**1**) hydrochloride[8] obtained from benzene−MeOH confirmed the proposed structure with a unique fused-pentacyclic ring system. The relative stereochemistry of C-1, C-2, C-3, C-4, C-5, and C-11 was coincident with that deduced from NOESY correlations, as described above.

The absolute stereochemistry of cephalocyclidin A (**1**) was elucidated by applying the exciton chirality method[9] after introduction of the *p*-methoxycinnamoyl chromophore into the hydroxyl groups at C-2 and C-3. The sign of the first Cotton effect [λ_{max} 325 nm ($\Delta\epsilon$ −6.6)] was negative, while that of the second one [λ_{max} 283 nm (5.8)] was positive (Figure 4), indicating that the chirality between the two *p*-methoxycinnamoyloxy groups at C-2 and C-3 of the derivative (**3**) was as shown in Figure 5 (left-handed screw). Thus, the absolute configurations at C-2 and C-3 were assigned as *R* and *S*, respectively,[10] indicating 1*R*, 2*R*, 3*S*, 4*S*, 5*R*, and 11*S* configurations. These results were compatible with the absolute stereochemistry of the hydrochloride of **1** deduced through the Flack parameter,[11] $\chi = 0.01(5)$, in the X-ray analysis (Figure 3).

Plausible Biogenesis of Cephalocyclidin A (1). A plausible biogenetic pathway for cephalocyclidin A (**1**) is proposed as shown in Scheme 1. The biogenetic origin of **1** seems to be a cephalotaxine-type alkaloid such as the 2-*O*-demethyl form of cephalotaxine (**2**),[12] in which an additional ring might be constructed by C-1−C-11 bond formation by aldol-type reaction after oxidation at C-11, followed by reduction of a ketone at C-2 to generate cephalocyclidin A (**1**).

Cytotoxicity of Cephalocyclidin A (1). Cephalocyclidin A (**1**) exhibited cytotoxicity against murine lymphoma L1210 cells (IC_{50} 0.85 μg/mL) and human epidermoid carcinoma KB cells (IC_{50} 0.80 μg/mL) in vitro.

Experimental Section

General Procedures. ^{1}H and ^{13}C NMR spectra were recorded on a 600 MHz spectrometer equipped with an X32 computer and a Eurotherm temperature control unit. 1D NMR spectra were measured at 310 K or 300 K with 16K data points, which were multiplied by a Gaussian filter and zero-filled to 32K data points before Fourier transformation. 2D NMR spectra were measured at 310 K, and NOESY and HOHAHA spectra in the phase sensitive mode were recorded using the TPPI method. HOHAHA spectra were recorded by spin-lock field preceded and followed by 2.5 ms trim pulses. NOESY spectra were measured with mixing times of 800 ms. Typically 256 FIDs of 2K data points and 32 scans each were employed. Chemical shifts were measured using residual CD_3-OD (δ_H 3.31 and δ_C 49.00) as an internal standard. Standard pulse sequences were employed for 2D NMR experiments. HMBC spectra were recorded using a 50 ms delay time for long-range C−H coupling with *Z*-axis PFG. FABMS was measured by using glycerol as a matrix. CD spectra were measured in millidegrees and normalized into $\Delta\epsilon_{max}$ [L mol^{-1} cm^{-1}]/L[nm] units.

(7) Conformational search and molecular mechanics calculations were conducted by the Macromodel program: Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440−467.

(8) Cephalocyclidin A (**1**) turned out its hydrochloride during extract and purification using chloroform. Accordingly, the hydrochloride of **1** was used for structure elucidation described in this paper.

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(10) The dihedral angle of HO−C-2−C-3−OH in the crystal structure of **1** was 22(1)°. ^{1}H−^{1}H vicinal coupling constants ($J_{1/2}$ = 7.3 Hz, $J_{2/3}$ = 7.6 Hz, and $J_{3/4}$ = 4.7 Hz) in the five-membered ring (C1−C5) of **3** in CD_3OD at 300 K were almost the same as those ($J_{2/3}$ = 7.5 Hz, $J_{3/4}$ = 7.8 Hz, and $J_{4/5}$ = 4.8 Hz) of cephalocyclidin A (**1**) under the same conditions, indicating that the five-membered ring (C-1−C-5) of compounds **1** and **3** took similar conformations to each other.

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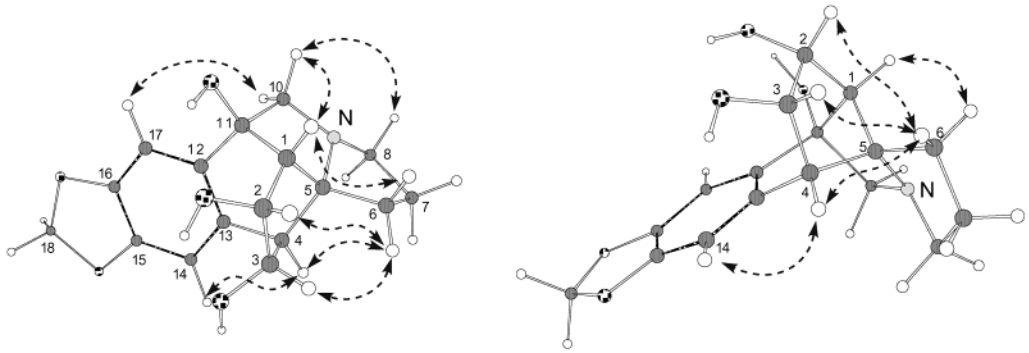


Figure 2. Key NOESY correlations (arrows) and relative stereochemistry for cephalocyclidin A (**1**); the right-hand stereostructure is represented by rotating 90° along the *x* axis of the left one.

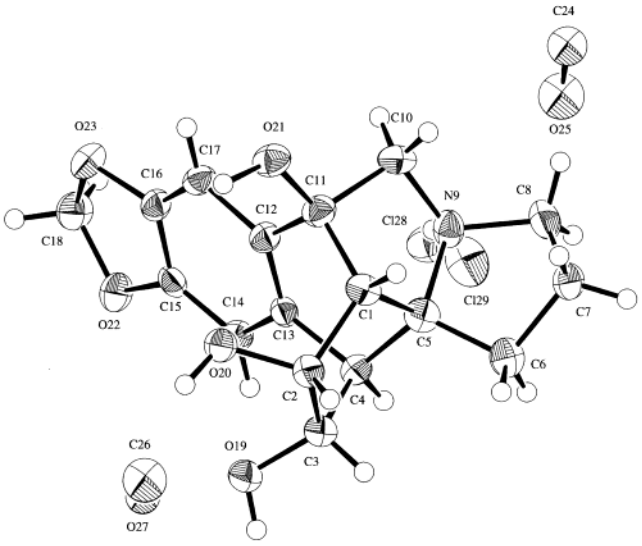


Figure 3. Molecular structure of cephalocyclidin A (**1**) hydrochloride obtained by X-ray analysis (ORTEP drawing; ellipsoids are drawn at the 35% probability level). Two MeOH molecules are contained in the crystal, and a chlorine atom is disordered with an occupancy ratio of 0.3:0.7.

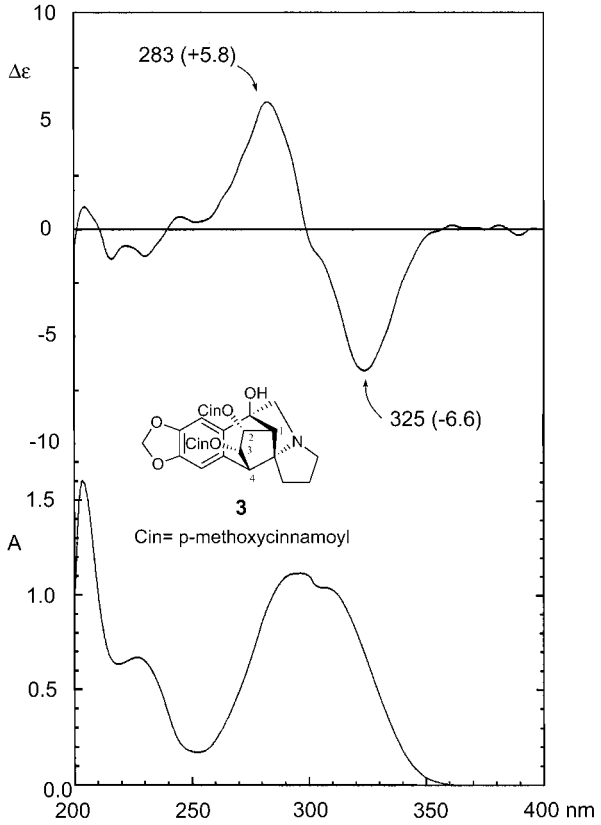


Figure 4. CD and UV spectra of 2,3-*O*-bis-*p*-methoxycinnamate (**3**) of cephalocyclidin A (**1**).

Material. The fruits of *C. harringtonia* var. *nana* (Cephalotaxaceae) were collected in Sapporo (Hokkaido, Japan) in 1999. The botanical identification of *C. harringtonia* var. *nana* was made by Mr. N. Yoshida, Graduate School of Pharmaceutical Sciences, Hokkaido University. Voucher specimens have been deposited in the herbarium of Hokkaido University.

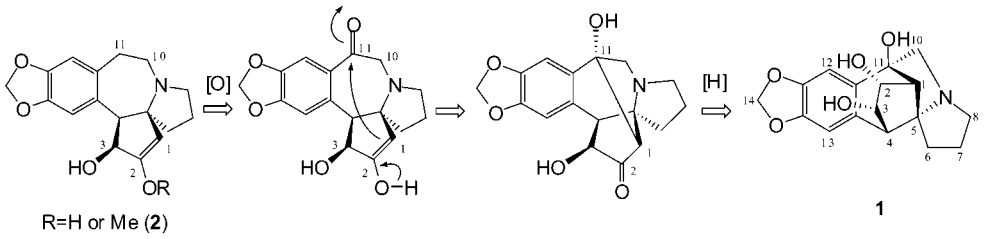
Isolation. The fruits of *C. harringtonia* var. *nana* (955 g) were crushed and extracted with MeOH (1 L) three times. The MeOH extract (78 g) was treated with 3% tartaric acid (pH 2) and then partitioned with EtOAc. The aqueous layer was treated with saturated, aqueous Na_2CO_3 to pH 10 and extracted with $CHCl_3$ to give a crude alkaloidal fraction (0.9 g). This fraction was subjected to silica gel column chromatography ($CHCl_3$/MeOH, 1:0 → 0:1), in which a fraction eluted with $CHCl_3$/MeOH (1:1) was purified by C_{18} HPLC (30% CH_3CN/0.03 M $(NH_4)_2CO_3$) to afford cephalocyclidin A (**1**, 6 mg, 0.0006% yield) together with cephalotaxine (**2**, 20 mg, 0.002%) and drupacine (138 mg, 0.014%) as colorless solids.

Cephalocyclidin A (1): colorless plates; $[\alpha]_D$ −36° (*c* 0.8, CH_3OH); mp 205 °C dec (benzene−MeOH); IR (neat) ν_{max} 3450, 2920, 1480, and 1260 cm^{-1}; UV (MeOH) λ_{max} 293 (ϵ 1700) and 241 nm (1300); ^1H and ^{13}C NMR data (Table 1); FABMS *m*/*z* 318 $(M + H)^+$; HRFABMS *m*/*z* 318.1316 (M + H; calcd for $C_{17}H_{20}NO_5$, 318.1342).

Crystal Data of Cephalocyclidin A (1) Hydrochloride. Cephalocyclidin A (**1**) hydrochloride was crystallized from benzene−MeOH to give colorless plates (mp 205 °C dec). Crystal data: $C_{17.50}H_{22}ClNO_{5.50}$, Mr = 369.82, crystal dimensions 0.25 × 0.03 × 0.02 mm, tetragonal, space group *I*4 (no. 79), a = 20.879 (3) Å, c = 7.5073(5) Å, V = 3272.8(6) $Å^3$, Z = 8, D_{calc} = 1.501 g/cm^3. All measurements were made on a Rigaku RAXIS−RAPID imaging plate diffractometer with graphite monochromated Cu Kα radiation (λ = 1.54178 Å). The data were collected at −160 ± 1 °C to a maximum 2θ value of 136.1°. A total of 56 images, corresponding to 900.0° oscillation angles, were collected with five different goniometer settings. Exposure time was 4.50 min per degree. The camera radius was 127.40 mm. Readout was performed in the 0.100 mm pixel mode. Data were processed by the PROCESS-AUTO program package. Of the 11 355 reflections that were collected, 1543 were unique (R_{int} = 0.079); equivalent reflections were merged. The linear absorption coefficient, m, for Cu Kα radiation is 23.7 cm^{-1}. A symmetry-related absorption correction using the program ABSCOR was applied that resulted in transmission factors ranging from 0.85 to 0.95. The data were corrected for Lorentz and polarization effects.

The structure was solved by SIR97 and expanded using Fourier techniques. One of two MeOH molecules, C-26−O-27,

Scheme 1



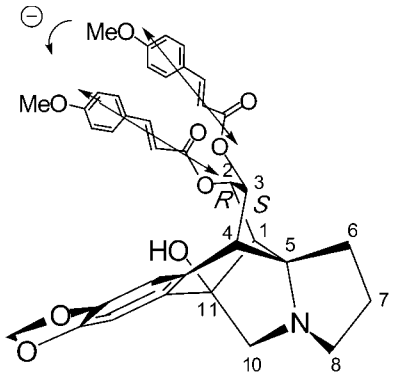


Figure 5. Stereostructure of 2,3-*O*-bis-*p*-methoxycinnamate (**3**) of cephalocyclidin A (**1**). Arrows denote the electric transition dipole between the two chromophores.

lies on a 4-fold axis in the crystal. C-24 of another one lies on a 2-fold axis and O-25 is disordered around the axis. Chlorine atom is disordered at the locations represented by Cl-28 and Cl-29 with an occupancy ratio of 0.3:0.7. Non-hydrogen atoms, excluding those of the disordered portions, were refined anisotropically. Hydrogen atoms of the solvent molecules were not located. The final cycle of full-matrix least-squares refinement was based on 2717 observed reflections and 239 variable parameters and converged with unweighted and weighted agreement factors of $R = 0.138$, Rw = 0.174, and R1 = 0.071 [for $I > 2.0\sigma(I)$ data]. All calculations were performed using the teXsan crystallographic software package of Molecular Structure Corp.

2,3-*O*-Bis-*p*-Methoxycinnamate (3) of Cephalocyclidin A (1). To a solution of **1** (0.8 mg) in pyridine (150 μL) was added *p*-methoxycinnamoyl chloride (9.0 mg) and *N*,*N*-(dimethylamino)pyridine (1.6 mg). The mixture was allowed to stand at 50 °C for 17 h. The residue was dissolved in $CHCl_3$ and washed with H_2O. After evaporation of solvent, the residue was applied to C_{18} HPLC (40% CH_3CN/0.1% TFA) to give compound **3** (1.2 mg); $[\alpha]_D$ −63° (*c* 0.5, CH_3OH); FABMS *m*/*z* 638 $(M + H)^+$; HRFABMS *m*/*z* 638.2396 (M + H; calcd for $C_{37}H_{36}NO_9$, 638.2390); IR (neat) ν_{max} 3340, 2930, 1715, and 1255 cm^{-1}; UV (MeOH) λ_{max} 307 (ϵ 33 000), 296 (ϵ 36 000), 227 (ϵ 21 000); CD (MeOH) $\Delta\epsilon_{325}$ −6.6 and $\Delta\epsilon_{283}$ 5.8; ^{1}H NMR (CD_3-OD) δ 3.28 (1H, d, 7.4, H-1), 5.77 (1H, t, 7.4, H-2), 5.37 (1H, dd, 4.7, 7.4, H-3), 3.60 (1H, d, 4.7, H-4), 2.17 (1H, m, H-6a), 2.31 (1H, m, H-6b), 2.44 (2H, m, H-7), 3.35 (1H, m, H-8a), 3.60 (1H, m, H-8b), 3.45 (1H, d, 10.8, H-10a), 3.69 (1H, d, 10.8, H-10b), 6.69 (1H, s, H-14), 7.17 (1H, s, H-17), 6.00 (1H, s, H-18a), 6.01 (1H, s, H-18b), 6.06 (1H, d, 16.0), 6.10 (1H, d, 16.0), 7.06 (1H, d, 16.0), 7.24 (1H, d, 16.0), 6.74 (2H, d, 8.7), 6.86 (2H, d, 8.7), 7.22 (2H, d, 8.7), and 7.27 (2H, d, 8.7); ^{13}C NMR (CD_3OD) δ 55.99 (C-1), 75.24 (C-2 and C-3), 55.89 (C-4), 81.27 (C-5), 34.55 (C-6), 25.32 (C-7), 53.15 (C-8), 69.49 (C-10), 83.55 (C-11), 135.30 (C-12), 127.76 (C-13), 111.14 (C-14), 148.85 (C-15), 149.89 (C-16), 104.99 (C-17), 102.91 (C-18), 167.45, 166.93, 125.80 × 2, 147.39, 147.08, 129.13 × 2, 131.04 × 4, 114.86 × 4, 163.29, 163.21, and 54.96 × 2.

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Supporting Information Available: 1D and 2D NMR spectra and X-ray crystallographic data of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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