ORGANIC
LETTERS

2007
Vol. 9, No. 5
903−906

Chloraholides A and B, Two Potent and Selective Blockers of the Potassium Channel Isolated from *Chloranthus holostegius*

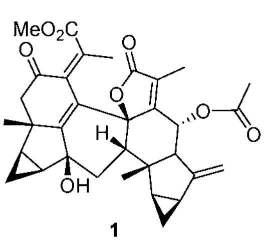
Sheng-Ping Yang, Zhao-Bing Gao, Fang-Dao Wang, Shang-Gao Liao, Hua-Dong Chen, Chuan-Rui Zhang, Guo-Yuan Hu, and Jian-Min Yue*

State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica,
Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Zhangjiang Hi-Tech Park,
Shanghai, 201203, PRC

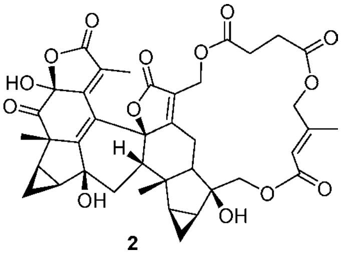
jmyue@mail.shcnc.ac.cn

Received January 10, 2007

ABSTRACT



1



2

Chloraholides A (1) and B (2), two highly complex sesquiterpenoid dimers, were isolated from *Chloranthus holostegius*. Their structures and absolute configurations were established by NMR spectroscopy, X-ray crystallography, and CD. Chloraholides A (1) and B (2) exhibited potent and selective inhibition on the delayed rectifier (I_K) K^+ current, with an IC_{50} of 10.9 and 18.6 μM, respectively.

Potassium (K^+) channels play crucial roles in the regulation of a variety of physiological processes in both electrically excitable and nonexcitable cells and have been implicated in the pathogenesis of severe human diseases, such as long-QT syndromes, atrial fibrillation, epilepsy, Alzheimer's disease, and neuromuscular disorders.[1] Potassium channels have become attractive targets for rational drug design, and a great number of organic modulators of potassium channels have been developed as new drugs or medicinal candidates.[1,2]

The plants of the *Chloranthus* genus (Chloranthaceae) are mainly distributed in the east of Asia,[3] with 13 species and 5 varieties occurring in China.[4] Most plants of this genus have been used in Chinese folk medicine for the treatment of bone fractures.[5] Phytochemical investigations on this genus have led to the isolation of a number of sesquiterpenoids[6] and sesquiterpenoid oligomers.[7] A quite recent study showed that the dimeric sesquiterpenoids shizukaol B, cycloshizykaol

---

(1) Shieh, C. C.; Coghlan, M.; Sullivan, J. P.; Gopalakrishnan, M. *Pharmacol. Rev.* **2000**, *52*, 557−593.

(2) Liu, H.; Li, Y.; Song, M. K.; Tan, X. J.; Cheng, F.; Zheng, S. X.; Sheng, J. H.; Luo, X. M.; Ji, L. Y.; Yue, J. M.; Hu, G. Y.; Jiang, H. L.; Chen, K. X. *Chem. Biol.* **2003**, *10*, 1103−1113.

(3) Zhou, Z. K. *Yunnan Zhiwu Yanjiu* **1993**, *15*, 321−331.

(4) Chen, Y. Q.; Cheng, D. Z.; Wu, G. F.; Cheng, P. S.; Zhu, P. Z. In *Chinese Flora* (*Zhongguo Zhiwu Zhi*); Science: Beijing, 1982; Vol. 20 (1), pp 80−96.

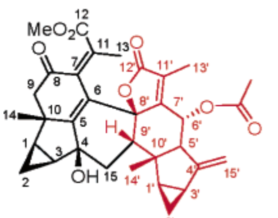
(5) Editorial Committee (over 300 members) of the Administration Bureau of Traditional Chinese Medicine. In *Chinese Materia Medica (Zhonghua Benchao)*; Shanghai Science & Technology: Shanghai, 1998; Vol. 3, pp 451−452.

(6) (a) Kawabata, J.; Mizutani, J. *Agric. Biol. Chem.* **1989**, *53*, 203−207. (b) Kawabata, J.; Mizutani, J. *Agric. Biol. Chem.* **1988**, *52*, 2965−2966. (c) Kawabata, J.; Fukushi, Y.; Tahara, S.; Mizutani, J. *Agric. Biol. Chem.* **1985**, *49*, 1479−1485.

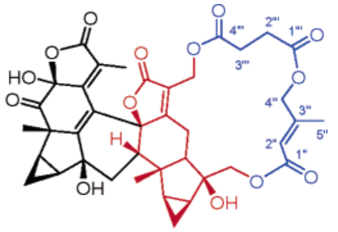
(7) (a) Kawabata, J.; Fukushi, E.; Mizutani, J. *Phytochemistry* **1998**, *47*, 231−235. (b) Kawabata, J.; Fukushi, E.; Mizutani, J. *Phytochemistry* **1995**, *39*, 121−125. (c) Yang, S. P.; Yue, J. M. *Tetrahedron Lett.* **2006**, *47*, 1129−1132. (d) Kawabata, J.; Fukushi, Y.; Tahara, S.; Mizutani, J. *Phytochemistry* **1990**, *29*, 2332−2334.

10.1021/ol0700759 CCC: $37.00

A, and shizukaol F isolated from *C. japonicus* inhibited the expression of cell adhesion molecules.[8] Several sterols and coumarins and one lindenane sesquiterpenoid have been isolated from *Chloranthus holostegius* (Hand.-Mazz.) Pei et Shan in an early report.[9] In the current study, two novel sesquiterpenoid dimers, chlorahololides A (**1**) and B (**2**), were isolated from this plant. Both **1** and **2** exhibited potent and selective inhibition on the delayed rectifier (I_K) K$^+$ current with an IC$_{50}$ of 10.9 and 18.6 μM, respectively, and were 56- and 96-fold more potent than the positive control, tetraethylammonium chloride (IC$_{50}$ = 1.05 mM), a classical blocker of the delayed rectifier (I_K) K$^+$ current. Details of isolation, structural elucidation, and the potassium channel blocking activity of compounds **1** and **2** are presented below.



1



2

The plants of *C. holostegius*, which are called "si-kuai-wa" by the local residents, were collected from Mengla County of the Yunnan Province. The air-dried powder of a whole plant was percolated with 95% EtOH, and the crude extract was partitioned between EtOAc and water. The EtOAc-soluble portion was subjected to MCI gel chromatography using aqueous CH$_3$OH in gradient (50−100%, v/v) to obtain fractions 1−7. Fraction 4 was chromatographed over a silica gel column (CHCl$_3$/MeOH, 75:1), and the major component was purified by using a C-18 silica gel column (aqueous CH$_3$OH, 70%) to yield **2** (0.001%). Fraction 6 was separated on a silica gel column (CHCl$_3$/MeOH, 100:0 to 50:1) and further purified on a Sephadex LH-20 column (CH$_3$OH) to afford **1** (0.008%).

Chlorahololide A (**1**)[10] was obtained as prisms (from CH$_3$-OH) with an optical rotation of $[\alpha]^{20}_{D}$ −180. The molecular formula of **1**, as determined by HREIMS at *m/z* 560.2430 [M]$^+$, was C$_{33}$H$_{36}$O$_8$ with 16 double bond equivalents. This was supported by the ESIMS at *m/z* 583 [M + Na]$^+$ (positive mode) and at *m/z* 559 [M − H]$^-$ (negative mode). The IR absorptions revealed the presence of carbonyl (1740 and 1703 cm^{-1}) and hydroxyl (3485 cm^{-1}) functionalities. In the ^{1}H NMR spectrum (Supporting Information S3), a broad singlet at δ 2.48 (ca. 1H), which did not show any correlations in the HSQC spectrum, was indicative of the presence of an exchangeable proton, such as a hydroxyl. In accord with the molecular formula, 33 carbon signals were resolved in the ^{13}C NMR spectrum (Supporting Information S3) and categorized by DEPT experiments as four carbonyls (δ_C 200.9, 172.6, 171.3, and 170.4), six methyls, one exocyclic and three persubstituted double bonds, four sp^3 methylenes, seven sp^3 methines, and four sp^3 quaternary carbons. Of the six methyl goups, two were attributable to a methoxyl (δ_C 52.3, δ_H 3.70) and an acetoxyl (δ_C 20.6, δ_H 2.13), the latter being confirmed by the HMBC correlations (Figure 1). Through biogenetic reasoning and the occurrence of dimeric sesquiterpenoids within this plant genus,[7d] the remaining 30 carbons suggested that compound **1** was a sesquiterpenoid dimer. The functionalities distinguished above accounted for eight degrees of unsaturation, and the remaining eight double bond equivalents required an octacyclic core in **1**.

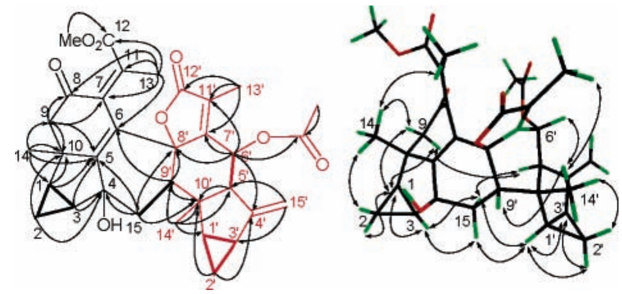


Figure 1. ^{1}H−^{1}H COSY (−), selected HMBC correlations (H → C), and key ROESY correlations (↔) of **1**.

The scaffold of **1** was constructed by a comprehensive analysis of the 1D and 2D NMR spectra. The ^{1}H and ^{13}C NMR and HMQC spectra allowed the assignment of all protons to the bonding carbons. Four proton-bearing, spin coupling units as drawn with bold bonds (Figure 1) were then established by the ^{1}H−^{1}H COSY spectrum. The connectivity of the four structural subunits and most of the other functional groups was achieved via the observed J^2 and J^3 HMBC correlations (Figure 1), leaving only the linkages of C-6/C-7 and C-8′/C-12′ in the polycyclic core of **1** unsettled. Fortunately, an apparent J^4 HMBC correlation observed between H$_3$-13 and C-6 linked the C-6 to C-7. The chemical shift of C-8′ at δ 86.6 implied a linkage between C-8′ and C-12′ via an oxygen atom to form an α,β-unsaturated γ-lactone, as in chloramultilide A.[7c] The methoxyl and acetoxyl groups were assigned to C-12 and C-6′, respectively, on the basis of the HMBC correlations of OMe/C-12 and H-6′/carbonyl of Ac. The oxygenated quaternary carbon at δ 77.9 was attributed to the C-4 bearing a hydroxyl, as judged by the HMBC correlations of H-3 and H-15 to C-4. The complete spectral analysis described above defined chlorahololide A (**1**) as a lindenane sesquiterpenoid dimer.

In the ROESY spectrum (Figure 1), the correlations of H-1/H-3, H-1/H-2α, H-1′/H-3′, H-1′/H-2′α, H-3′/H-2′α, H-5′/

---

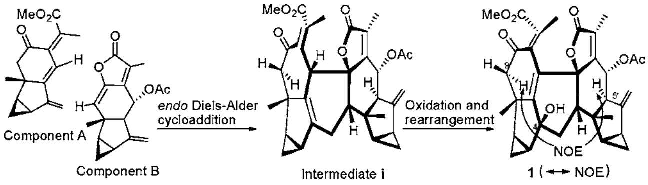
(8) Kwon, O. E.; Lee, H. S.; Lee, S. W.; Bae, K.; Kim, K.; Hayashi, M.; Rho, M. C.; Kim, Y. K. *J. Ethnopharmacol.* **2006**, *104*, 270−277.

(9) Gao, C. W.; Chen, Y. S.; Xie, J. M.; Zhao, S. N. *Gaodeng Xuexiao Huaxue Xuebao* **1987**, *8*, 141−142.

(10) **Chlorahololide A (1):** prisms; mp 223−225 °C; $[\alpha]^{20}_{D}$ = −180 (c = 0.115 in MeOH); UV (MeOH) λ_{max} (log ϵ) = 224 (4.23), 206 nm (4.22); CD (MeOH) λ_{max} ($\Delta\epsilon$) = 330 (−5.85), 258 (+20.6), 220 nm (−14.5); IR (KBr) ν = 3485, 3008, 2940, 1740, 1703, 1612, 1439, 1375, 1290, 1221, 1128, 1022, 966 cm^{-1}; for ^{1}H and ^{13}C NMR data see Supporting Information S3; EIMS (70 eV) *m/z* (%) 560 [M]$^+$ (2), 518 (3), 500 (9), 486 (11), 453 (12), 425 (12), 395 (100), 379 (36), 351 (19), 303 (13), 257 (10), 225 (30), 221 (9), 183 (7), 141 (10), 128 (11), 91 (23), 55 (10); ESI MS (positive) *m/z* 583 [M + Na]$^+$; ESIMS (negative) *m/z* 559 [M − H]$^-$; HREIMS (*m/z*) calcd for C$_{33}$H$_{36}$O$_8$ 560.2410, found 560.2430 [M]$^+$.

H-9α, and H-5′/H-15α indicated they were cofacial and were arbitrarily assigned to be α-oriented. In consequence, the ROESY cross-peaks of H-2β/H_3-14, H_3-14′/H-2′β, and H-6′/H_3-14′ revealed that CH_3-14, CH_3-14′, and H-6′ were in a β-configuration, respectively. The ROESY correlation of H-3α/H_2-15 suggested that the 4-OH was β-oriented. A biogenetic hypothesis (Scheme 1) was proposed for **1** via an enzymatic Diels−Alder cycloaddition[11] of two molecular lindenane-type sesquiterpenoids (components A and B), following the stereoselectivity of Diels−Alder cycloaddition based on the cis and endo rules.[12] A key ROESY correlation of H-9α/H-5′ indicated that **1** was a typical endo cycloaddition product. Accordingly, the C-8′−O bond and H-9′ were theoretically cofacial and β-oriented, and this was substantiated by the ROESY correlation of CH_3-14′(β)/H-9′. The geometry of the $\Delta^{7(11)}$ double bond was left unassigned by the available spectral data. The single-crystal X-ray diffraction of **1** (Figure 2) enabled a determination of a 7Z-geometry for the $\Delta^{7(11)}$ double bond and verified the structure as **1**.

Scheme 1. Biogenetic Pathway Proposed for Chloraholide A (**1**).



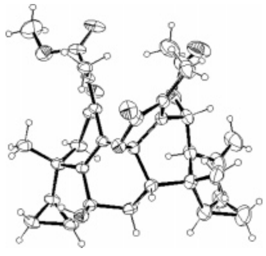


Figure 2. ORTEP drawing of the crystal structure of **1**.

Chloraholide B (**2**)[13] was determined to have a molecular formula of $C_{39}H_{40}O_{14}$ by HR ESIMS at m/z 755.2279 [M + Na]$^+$ (calcd for $C_{39}H_{40}O_{14}Na$, 755.2316 amu), which was supported by the negative mode ESIMS at m/z 731 [M − H]$^-$. The ^1H and ^{13}C NMR data of **2** (Table 1, Supporting Information) showed high similarity with those of **1** in the dimeric core, suggesting that both compounds were structurally related. The presence of a downfield shifted carbon signal at δ 147.8 (C-7) and an upfield shifted carbon signal at δ 94.0 (C-8) and the absence of a methoxyl at C-12 in the ^1H and/or ^{13}C NMR spectra of **2** suggested that an α,β-unsaturated γ-lactone was formed via an ether linkage between C-8 and C-12, and a hemi-ketal at C-8 was therefore established. This conclusion was further confirmed by the HMBC correlation (J^4) between H_3-13 and C-8 (Figure 3).

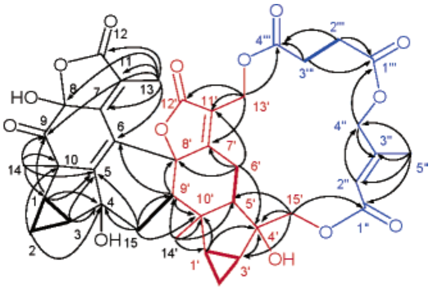


Figure 3. $^1H-^1H$ COSY (−) and key HMBC correlations (H → C) of **2**.

A carbon signal at δ 200.5 was attributed to the C-9 ketone group by the strong HMBC correlation (J^3) between H_3-14 and C-9. In a further comparison with **1**, the ^1H and ^{13}C NMR data of **2** indicated the presence of a C-6′ methylene, the protons of which resonated at δ_H 3.17 and 2.35, and showed HMBC correlations to C-4′, C-5′, C-7′, C-8′, and C-11′ (Figure 3). In the HMBC spectrum, an oxygenated quaternary carbon at δ_C 77.0 correlating with H-1′, H-3′, and H_2-6′ was assigned to C-4′ bearing a hydroxyl. The oxygenated carbon signals at δ_C 53.5 and 72.9 for two methylenes were assigned to C-13′ and C-15′, respectively, on the basis of HMBC correlations of H_2-13′ to C-11′, C-12′, and C-7′ and of H_2-15′ to C-3′, C-4′, and C-5′. The NMR data of **2** also revealed the presence of a succinoxyl and a 4-hydroxyl-3-methyl-2-butenoxyl group, and this was confirmed by the HMBC spectrum (Figure 3). The succinoxyl and 4-hydroxyl-3-methyl-2-butenoxyl groups were connected via an ester linkage between C-4″ and C-1‴ as judged from the HMBC correlation of H_2-4″/C-1‴. Finally, the strong HMBC correlations of H_2-13′/C-4‴ and H_2-15′/C-1″ allowed the linkages between C-13′ and C-4‴ and between C-15′ and C-1″ via ether bonds, respectively, to form a unique 18-membered macrocyclic trilactone ring.

The relative configurations of most stereocenters in **2** were assigned by the ROESY spectrum (Supporting Information S5, S26), leaving only those of C-8, C-4′, and the $\Delta^{2''}$ double bond undetermined. The pyridine-induced solvent shifts[14] and

---

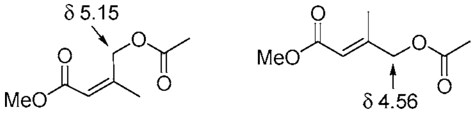
[11] Oikawa, H.; Suzuki, Y.; Naya, A.; Katayama, K.; Ichihara, A. *J. Am. Chem. Soc.* **1994**, *116*, 3605−3606.

[12] Oppolzer, W. In *Comprehensive Organic Synthesis*; Trost, B. M., Fleming, I., Eds.; Pergamon: Oxford, 1991; Vol. 5, pp 315−399.

[13] **Chloraholide B (2):** White powder; mp 200−202 °C; $[\alpha]^{20}_D = +5.9$ ($c = 0.304$ in MeOH); UV (MeOH) λ_{max} (log ε) = 216 nm (4.33); CD (MeOH) λ_{max} (Δε) = 280 (+7.43), 265 (+4.95), 252 (+10.1), 227 (−22.0), 211 nm (−6.62); IR (KBr) ν = 3466, 2928, 1740, 1670, 1367, 1329, 1227, 1161, 1022, 970 cm^{-1}; for ^1H and ^{13}C NMR data see Supporting Information S3; ESI MS (positive) m/z 755 [M + Na]$^+$; ESI MS (negative) m/z 731 [M − H]$^-$; HRESIMS (m/z) calcd for $C_{39}H_{40}O_{14}Na$ 755.2316, found 755.2279 [M + Na]$^+$.

[14] Demacro, P. V.; Farkas, E.; Doddrell, D.; Mylari, B. L.; Wenkert, E. *J. Am. Chem. Soc.* **1968**, *90*, 5480−5486.

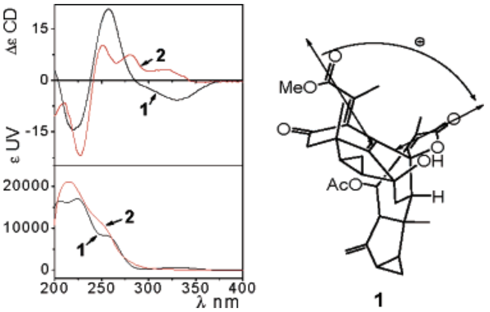
chemical shift correlations with model compounds were therefore applied to assign their stereochemistry. The significant pyridine-induced solvent shifts (Supporting Information S3, S5) for H-5′ ($\Delta\delta$ −0.79) from 8-OH revealed that they were cofacial, and 8-OH was thus assigned as an α-configuration. Similarly, the substantial pyridine-induced solvent shifts from 4′-OH to H_3-14′ ($\Delta\delta$ −0.19) and H-2′β ($\Delta\delta$ −0.28) indicated that 4′-OH possessed a β-configuration. The geometry of the double bond on the macrocylic ester ring could not be established by ROESY correlations due largely to the flexibility of this ring system. To settle this matter, one pair of the synthetic stereoisomers of 4-acetoxyl-3-methyl-2-butenoate reported in the literature[15] was thus applied as the model compounds (Figure 4). The chemical shifts of two H_2-4″ protons at δ 4.77 and 4.27 (centered at δ 4.52) of compound **2** perfectly matched the *E*-isomer (δ_H 4.56) of the model compounds, suggesting that the double bond on the macrocyclic ester ring possessed an *E*-geometry. The structure of compound **2** was therefore established as a highly complex, decacyclic lindenane-type sesquiterpenoid dimer with a unique 18-membered macrocyclic trilactone ring.



Z-form *E*-form

Figure 4. Key chemical shifts of the model compounds.

The exciton chirality method[16] was applied to determine the absolute configuration of chloraholides A (**1**) and B (**2**). The UV spectrum of **1** showed a strong absorption at λ_{max} 224 nm (log ϵ 4.23) corresponding to the α,β-unsaturated γ-lactone moiety (C-7′, C-11′, and C-12′; Woodward's rule gave ca. 227 nm)[17] and a shoulder absorption at λ 250−280 nm corresponding to the twisted π-electron system (C-5−C-8 and C-11−C-12). The split pattern of the CD spectrum (Figure 5) of **1** agreed well with that reported for two different chromophores at the range of λ_{max} 230−260 nm.[18] The positive Cotton effect at λ_{max} 258 nm ($\Delta\epsilon$ +20.6) and the negative Cotton effect at λ_{max} 220 nm ($\Delta\epsilon$ −14.5) arising from the exciton coupling of two different chromophores of the α,β-unsaturated γ-lactone and the twisted π-electron system indicated a positive chirality of **1** (Figure 5). The absolute configuration of **1** was thus established as depicted.



1

Figure 5. CD and UV spectra of **1** and **2** (in MeOH). The stereoview of **1**: arrows denote the electric transition dipole of the chromophores.

The CD spectrum of **2** was very similar to that of **1** in the range of 210−260 nm (Figure 5), where the first positive Cotton effect ($\Delta\epsilon$ +10.1 at λ_{max} 252 nm) and the second negative Cotton effect ($\Delta\epsilon$ −22.0 at λ_{max} 227 nm) of **2** were observed. The absolute configuration of **2** was therefore defined as depicted.

The effects of chloraholides A (**1**) and B (**2**) on voltage-gated potassium (K^+) channels were examined using whole-cell voltage-clamp recording in rat dissociated hippocampal neurons, where tetraethylammonium chloride was used as the positive control.[2] Compounds **1** and **2** exerted potent inhibition on the delayed rectifier K^+ current (I_K), with negligible effect on the fast transient K^+ current (I_A) (Supporting Information S6). The IC_{50} values of chloraholides A (**1**) and B (**2**) on the delayed rectifier K^+ current (I_K) were 10.9 ± 12.3 and 18.6 ± 2.5 μM, respectively. It is noteworthy that compounds **1** and **2** are 56−96-fold more potent than the positive control tetraethylammonium chloride (IC_{50} = 1.05 ± 0.21 mM), a classical blocker of the delayed rectifier K^+ current.

Acknowledgment. Financial support of the Key Project of the National Natural Science Foundation (Grant No. 30630072) and of the foundation from the Ministry of Science and Technology (Grant No. 2002CB512807) of P. R. China is gratefully acknowledged. We thank Prof. You-Kai Xu of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, for the collection and identification of this plant material.

Supporting Information Available: Experimental Section; ^{1}H, ^{13}C, and 2D NMR (HSQC, HMBC, and ROESY); EI MS, ESI MS, and IR spectra for chloraholides A (**1**) and B (**2**); and the X-ray data and the CIF file for chloraholide A (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

OL0700759

---

(15) Tully, L. E.; Carson, M. S.; McMurry, T. B. H. *Tetrahedron* **1987**, *28*, 5925−5928.

(16) Harada, N.; Nakanishi, K.; Tatsuoka, S. *J. Am. Chem. Soc.* **1969**, *91*, 5896−5898.

(17) Pretsch, E.; Bühlmann, P.; Affolter, C. In *Structure Determination of Organic Compounds: Tables of Spectral Data*, Chinese Version; Rong, G. B., Zhu, S. Z., Trans., East China University of Science and Technology: Shanghai, 2002; pp 388−389.

(18) Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy: Exciton Coupling in Organic Stereochemistry*, 1st ed.; Oxford University: Oxford, 1983; pp 85−87.