Terpenes from *Schisandra sphenanthera*

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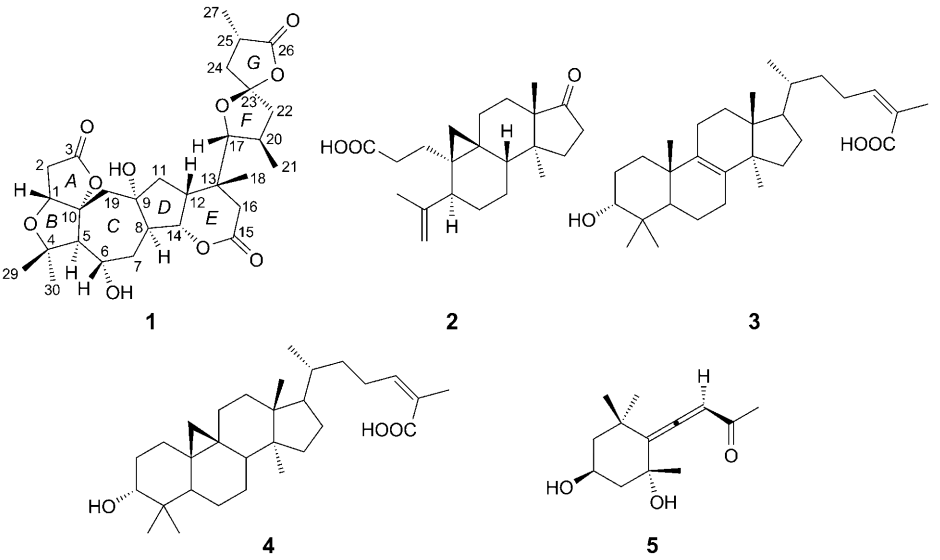
One novel, highly oxygenated nortriterpenoid, schintrilactone C (**1**), and four known compounds, **2**–**5**, were isolated from the rattan of *Schisandra sphenanthera*. Their structures were determined by analysis of 1D- and 2D-NMR spectroscopic data. Schintrilactone C is the third example of wuweiziartane-type nortriterpenoids, bearing a modified five-membered *D* ring, a δ-lactone *E* ring, and a spirocyclic moiety in the side chain at C(13).

Introduction. – The economically and medicinally important family Schisandraceae, a family of climbing plants, contains the genera *Schisandra* and *Kadsura*. There are *ca.* 50 species in total in the world, and there are 29 species in China which are widely used as sedative and tonic agents in Traditional Chinese Medicine (TCM) [1]. The chemical constituents of Schisandraceae plants have been studied extensively. Previous phytochemical investigations on this family revealed lignans, especially dibenzocyclooctadienelignans with antihepatitis, antitumor, and anti-HIV activities [2–4]. In recent years, triterpenoids showing anti-HIV activities, and inhibitory activities toward cholesterol biosynthesis have also been isolated from this family [5].

Schisandra sphenanthera REHDER et E. H. WILSON is a climbing plant that is widely found in Sichuan, Hubei, Shanxi, and Yunnan Provinces. The fruits are used as antitussive and tonic agents under the name of *Nan-wuweizi* in TCM [6]. Phytochemical studies of *S. sphenanthera* fruits led to the isolation of six dibenzocyclooctadiene lignans, five other lignans, and three triterpenes, including schizandronic acid (ganwuweizic acid), anwuweizic acid, and schisanol [7]. Nine novel schisanartane-type nortriterpenoids, sphenadilactones A–C and sphenalactones A–D, a norcycloartane triterpenoid, sphenasin A, and a new phenolic glycoside have been isolated from the leaves and stems of *S. sphenanthera* [6][8]. In the course of our investigation on this genus, a novel highly oxygenated nortriterpenoid, schintrilactone C (**1**), together with four known compounds, micranoic acid B (**2**) [9], anwuweizic acid (**3**) [10], isoschizandrolic acid (**4**) [11], and grasshopper ketone (**5**) [12] were isolated from the rattan of *S. sphenanthera* by normal-phase and reversed-phase silica-gel column chromatography. Their structures were identified by spectroscopic methods, especially 2D-NMR techniques.



Result and Discussion. – Schintrilactone C (**1**) was obtained as an amorphous white powder. Its empirical formula, $C_{29}H_{40}O_{10}$, was deduced, from HR-ESI-MS (*m/z* 571.2510 ($[M+Na]^+$; calc. 571.2519) and ^{13}C-NMR data, indicating ten degrees of unsaturation. Evident in the ^{1}H-NMR spectrum were five Me signals due to three tertiary Me groups at δ(H) 0.92 (*s*), 1.28 (*s*), and 1.50 (*s*), and two secondary Me groups at δ(H) 0.71 (*d*, $J = 5.7$) and 0.92 (*d*, $J = 6.6$). Signals of four O-bearing CH groups appeared at δ(H) 3.41 (*d*, $J = 9.3$), 4.02–4.13 (*m*), 4.20 (*d*, $J = 4.5$), and 5.05 (*t*, $J = 8.7$). The ^{13}C-NMR and DEPT spectra of **1** exhibited signals for 29 C-atoms, including those of three ester groups (δ(C) 175.0, 169.5, and 178.6), five quaternary C-atoms (of three O-bearing C-atoms at δ(C) 85.7, 77.5, and 96.8 and one O–C–O group at δ(C) 112.1), nine CH groups (four O-bearing CH groups at δ(C) 82.1, 65.0, 83.5 and 90.6), seven CH_2 groups, and five Me groups. These features revealed that compound **1** was a highly oxygenated nortriterpene containing seven rings. Careful analysis of the ^{1}H- and ^{13}C-NMR data (*Table*) revealed that **1** is similar to schintrilactone A, isolated before from *Schisandra chinensis* [13].

The structure of **1** was elucidated by analyzing the 2D-NMR data and by comparing these results with the NMR data reported for schintrilactone A. All these data revealed that **1** possesses a structure quite similar to that of schintrilactone A. However, different C- and H-atom chemical shifts were observed, indicating that the structures of **1** and schintrilactone A differ in ring *C* and the side chain at C(13). Specifically, the CH_2 group at C(6) in ring *C* of schintrilactone A was replaced by an O-bearing CH group in **1** with a downfield chemical shift at δ(C) 65.0. In the HMBC spectrum, correlations (*Table*) observed from Me(29) (δ(H) 1.28 (*s*)) to C(4) (δ(C) 85.7), C(5) (δ(C) 66.8), and C(30) (δ(C) 30.6), from Me(30) (δ(H) 1.50 (*s*)) to C(4) (δ(C) 85.7), C(5) (δ(C) 66.8), and C(29) (δ(C) 20.7), and from CH_2(7) (δ(H) 2.37–2.43 (*m*)) to C(5) (δ(C) 66.8), C(6) (δ(C) 65.0), and C(8) (δ(C) 56.2), as well as the ^{1}H,^{1}H-COSY spin system H–C(5)/H–C(6)/CH_2(7)/H–C(8), suggested that the OH group is located at C(6).

Table. *^{1}H- and ^{13}C-NMR, and HMBC Data* (measured in C_5D_5N) *of Compound* **1**. δ in ppm, J in Hz.

	δ(H)	δ(C)	HMBC (H → C)
H–C(1)	4.20 (*d*, $J = 4.5$)	82.1	C(2), C(3), C(10), C(19)
CH_2(2)	2.60 (overlapped, H_α), 2.74 (*d*, $J = 17.8$, H_β)	35.3	
C(3)		175.0	
C(4)		85.7	
H–C(5)	2.30–2.50 (*m*)	66.8	
H–C(6)	4.02–4.13 (*m*)	65.0	
CH_2(7)	2.37–2.43 (*m*)	36.1	C(5), C(6), C(8), C(14)
H–C(8)	2.40–2.45 (*m*)	56.2	C(6), C(7), C(9), C(11)
C(9)		77.5	
C(10)		96.8	
CH_2(11)	2.14–2.20 (*m*, H_α), 1.98–2.06 (*m*, H_β)	43.7	
H–C(12)	2.38–2.45 (*m*)	39.3	C(17)
C(13)		38.2	
H–C(14)	5.05 (*t*, $J = 8.7$)	83.5	C(15)
C(15)		169.5	
CH_2(16)	2.51 (*d*, $J = 14.4$, H_α), 2.27 (*d*, $J = 13.5$, H_β)	35.6	H_α: C(15)
H–C(17)	3.41 (*d*, $J = 9.3$)	90.6	C(13), C(20), C(21), C(23), C(12)
Me(18)	0.92 (*s*)	14.9	C(12), C(13), C(16), C(17)
CH_2(19)	2.16–2.20 (*m*)	41.6	
H–C(20)	2.15–2.20 (*m*)	32.1	C(22), C(23)
Me(21)	0.71 (*d*, $J = 5.7$)	18.1	C(17), C(20), C(22)
CH_2(22)	2.04–2.10 (*m*, H_α), 1.40–1.45 (*m*, H_β)	45.9	H_α: C(23), C(24)
C(23)		112.1	
CH_2(24)	1.73 (*t*-like, $J = 11.7$, H_α), 2.20–2.25 (*m*, H_β)	40.3	C(22), C(23), C(25), C(26)
H–C(25)	2.64–2.71 (*m*)	35.1	C(24), C(26)
C(26)		178.6	
Me(27)	0.92 (*d*, $J = 6.6$)	18.7	C(24), C(25), C(26)
Me(29)	1.28 (*s*)	20.7	C(4), C(5)
Me(30)	1.50 (*s*)	30.6	C(4), C(5)

Further comparison of ^{1}H- and ^{13}C-NMR data with those of schintrilactone A, and analysis of 2D-NMR of **1** (*Table*) allowed us to identify rings *A* –*E*, leading to the establishment of partial structure **1a** (*Fig. 1*). A secondary Me resonance at δ(H) 0.71 (*d*, $J = 5.7$) corresponding to Me(21) showed HMBC cross-peaks (*Table*) with H–C(20) (δ(C) 32.1), and with H–C(17) (δ(C) 90.6) and CH_2(22) (δ(C) 45.9), which required that C(17) and C(22) are both attached to C(20) bearing the Me group. This was confirmed by the HMBCs between the O-bearing CH group at δ(H) 3.41 (*d*, $J =$ 9.3), corresponding to C(17), C(20), and C(21) (δ(C) 18.1). Furthermore, ^{1}H,^{1}H-COSY correlations, H–C(17)/H–C(20)/H–C(22) gave rise to partial structure **1b** (*Fig. 1*). HMBC Cross-peaks (*Table*) observed from Me(27) (δ(H) 0.92 (*d*, $J = 6.6$)) to C(25) (δ(C) 35.1), and to CH_2(24) (δ(C) 40.3) and C(26) (δ(C) 178.6), from H–C(25) (δ(H) 2.64–2.71 (*m*)) to C(24) and C(26), and from H–C(24) (δ(H) 2.20–2.25 (*m*)) to C(23) (δ(C) 112.1), C(25), C(22) (δ(C) 45.9), and C(26), along with the H-atom spin system deduced from ^{1}H,^{1}H-COSY correlations H–C(24)/H–C(25)/Me(27) established the partial structure **1c** (*Fig. 1*).

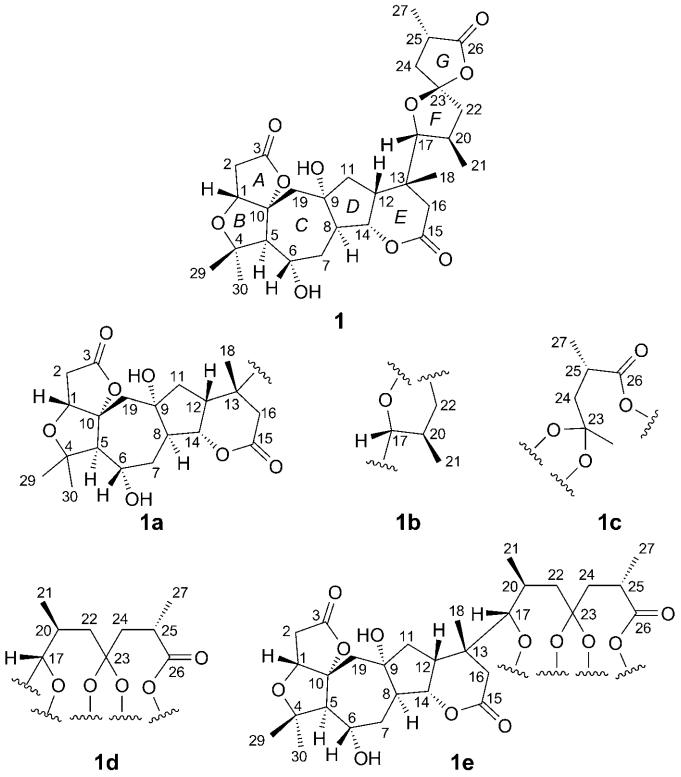


Fig. 1. *The structure and structural fragments of* **1**

Furthermore, HMBCs (*Table*) from CH_2(24) to C(22) and C(23), and from H–C(20) to C(22) and C(23) indicated direct connection of C(22) with C(23) and suggested fragment **1d** (*Fig. 1*). HMBCs (*Table*) from H–C(17) (δ(H) 3.41 (*d*, J = 9.3)) to C(13) (δ(C) 38.2) and from H–C(12) (δ(H) 2.38–2.45 (*m*)) to C(17) (δ(C) 90.6) required direct connection of C(13) with C(17) and permitted fragments **1a** and **1d** to be joined to provide **1e** (*Fig. 1*). On account of C(23), (δ(C) 112.1) which was an O-bearing quaternary C-atom, connected with two O-atoms, at the two O-bridges between C(17)/C(23) and C(23)/C(26) were established.

The relative configuration of **1** was established from the ROESY spectrum (see *Fig. 2*). The relative configuration of rings *A*, *B*, and *E* of **1** was deduced to be the same as that in schintrilactone A from the similar C- and H-atom chemical shifts and ROESY correlations found in the spectra of both compounds. In the ROESY spectrum of **1**, correlations from Me(29) to H–C(1) and H–C(6), and from Me(30) to H–C(5) were observed, indicating that H–C(1) and H–C(6) are β-oriented, while H–C(5) and HO–C(6) possess α-orientation. In addition, the cross-peaks H–C(17)/Me(21), H–C(12), and H_β–C(24), Me(18)/H–C(12), and Me(27)/H_α–C(24) in the ROESY spectrum demonstrated that H–C(12), Me(18), H–C(17), and Me(21) are β-oriented, while Me(27) possesses α-orientation. From these data, the structure and relative configuration of **1** was elucidated, and it was named as schintrilactone C (*Fig. 2*).

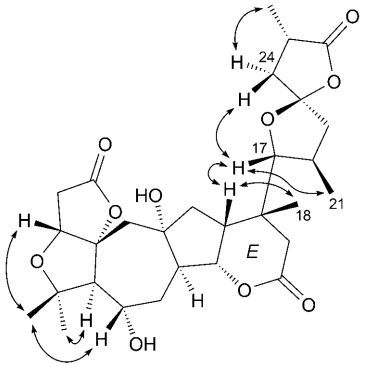


Fig. 2. *ROESY Correlations of compound* **1**

In the present study, a novel nortriterpene, schintrilactone C (**1**), which presented an unprecedented highly oxidized, rearranged cycloartane skeleton, was isolated from *Schisandra sphenanthera*. A series of recently isolated novel nortriterpenoids from the Schisandraceae family can be considered to derive biosynthetically from cycloartane, and further divided into schiartane [14], 18(13–14)-*abeo*-schiartane [15], schisanartane [16], preschisanartane [17], wuweiziartane [13], and kadlongilactone types [18]. Schintrilactone C (**1**) isolated from *S. sphenanthera*, and schintrilactones A and B, isolated from *S. chinensis*, are the only representatives of wuweiziartane-type nortriterpenoids, bearing a modified five-membered *D* ring and a δ-lactone *E* ring, but the distinguishing feature between schintrilactone C (**1**) and schintrilactones A is a spirocyclic moiety in the side chain at C(13) of **1**. The four known compounds were identified as micranoic acid B, anwuweizic acid, isoschizandrolic acid, and grasshopper ketone by comparison of their spectroscopic data with those reported in the literature. Notably, all of the above compounds were isolated from *S. sphenanthera* for the first time.

Experimental Part

General. Chromatography: Silica gel *60 H* and pre-coated silica gel *GF254* plates from *Qingdao Haiyang Chemical Group Co.*, P. R. China; C_{18} reversed-phase (RP) silica gel from *YMC CO., LTD.*, Japan. Optical rotations: *Jasco-DIP-181* polarimeter; HPLC: *Ultimate 3000* HPLC system; *Ultimate 3000* pump; *Ultimate 3000* variable wavelength; column, *Waters 5C_{18}-MS-II* (10 × 250 mm). ^{1}H- and ^{13}C-NMR spectra: *Bruker AM-400* instrument; δ in ppm rel. to Me_4Si as internal standard (= 0 ppm), *J* in Hz. ESI- and HR-ESI-MS: *Finnigan* LCQ-*Deca* and *Waters/Micromass Q-Tof-Ultima* mass spectrometers, resp., in *m*/*z* (rel. int).

Plant Material. The rattans of *S. sphenanthera* were collected from Shen Long Jia county, Hubei Province, P. R. China, and identified by Prof. *M.-C. Liao*. A voucher specimen was deposited with the Herbarium of College of Pharmacy, South Central University for Nationalities.

Extraction and Isolation. The dried rattans (8.6 kg) of *S. sphenanthera* were powdered and extracted three times with MeOH at r.t., and the MeOH extract (685 g) was extracted successively with petroleum ether (PE), AcOEt, and BuOH. The AcOEt extract (92.7 g) was chromatographed (SiO_2; PE/acetone 9:1, 8:2, 7:3, 1:1, 3:7, 0:1) to afford seven main fractions, *Frs. 1–7.* Colorless needles (90 mg) were crystallized from *Fr. 3:* the colorless needles were purified by semi-prep. HPLC (MeOH/H_2O 90:10, 3 ml/min): *anwuweizic acid* (**3**; 8.3 mg) at t_R 42.7 min and *isoschizandrolic acid* (**4**; 8.1 mg) at t_R 45.3 min.

Fr. 3 (20 g) was subjected to CC (SiO_2; cyclohexane/$CHCl_3$/MeOH 9:1:0, 8:2:0, 7:3:0, 1:1:0, 0:1:0, 0:98:2): *Frs. 3.1–3.4. Fr. 3.3* (4.4 g) was subjected to CC (*ODS*; H_2O/MeOH 7:3 → 0:1): *Frs. 3.3.1–3.3.7. Micranoic acid B* (**2**; 17.3 mg) was crystallized from *Fr. 3.3.5. Fr. 5* (13.7 g) was subjected to CC (SiO_2; cyclohexane/$CHCl_3$/acetone 2:8:0, 1:9:0, 0:1:0, 0:95:5, 0:9:1, 0:8:2, 0:6:4): *Frs. 5.1–Fr. 5.9. Fr. 5.6* (5.0 g) was subjected to CC (*ODS*, H_2O/MeOH 7:3 → 0:1): *Frs. 5.6.1–5.6.9. Fr. 5.6.2* (439 mg) was subjected to CC (SiO_2; cyclohexane/$CHCl_3$/MeOH 3:7:0, 2:8:0, 1:9:0, 0:1:0, 0:98:2, 0:95:5, 0:9:1, 0:8:2, 0:7:3, 0:0:1): *Frs. 5.6.2.1–5.6.2.9. Fr. 5.6.2.3* was purified by semi-prep. HPLC (MeOH/H_2O 30:70, 3ml/min) to give *grasshopper ketone* (**5**; 8.9 mg) at t_R 25 min. *Fr. 5.6.5* (369 mg) was subjected to CC (SiO_2; cyclohexane/$CHCl_3$/MeOH 3:7:0, 2:8:0, 1:9:0, 0:1:0, 0:98:2, 0:95:5, 0:9:1, 0:8:2, 0:7:3, 0:0:1): *schintrilactone C* (**1**; 11.6 mg).

Schintrilactone C (= rel-*(3a*R,*5a*S,*6*S,*7a*R,*7b*S,*11*R,*11a*R,*12a*S,*13a*R*)-11-[(2*R,*3*R,*5*R,*8*S*)-3,8-Dimethyl-7-oxo-1,6-dioxaspiro[4.4]non-2-yl]-tetradecahydro-6,12a-dihydroxy-5,5,11-trimethyl-2*H,*9*H-*furo[3'',2'':2',3']furo[3',4':5,6]azuleno[1,2-*b*]pyran-2,9-dione*; **1**). Amorphous white powder. $[\alpha]_D = +55$ ($c = 0.125$, pyridine). ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-MS: 571.2510 ($C_{29}H_{40}NaO_{10}^+$; calc. 571.2519).

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REFERENCES

[1] W.-L. Xiao, R.-T. Li, S.-X. Huang, J.-X. Pu, H.-D. Sun, *Nat. Prod. Rep.* **2008**, *25*, 871.
[2] H.-Y. Min, E.-J. Park, J.-Y. Hong, Y.-J. Kang, S.-J. Kim, H.-J. Chung, E. R. Woo, T. M. Hung. U. F. Houn, U. J. Youn, Y. S. Kim, S. S. Kang, K. H. Bae, S. K. Lee, *Bioorg. Med. Chem.* **2008**, *18*, 523.
[3] D.-F. Chen, S.-X. Zhang, M. Kozuka, Q.-Z. Sun, J. Feng, Q. Wang, T. Mukainaka, Y. Nobukuni, H. Tokuda, H. Nishino. H. K. Wang, S. L. Morris-Natschke, K.-H. Lee, *J. Nat. Prod.* **2002**, *65*, 1242.
[4] X.-M. Gao, J.-X. Pu, S.-X. Huang, L.-M. Yang, H. Huang, W.-L. Xiao, Y.-T. Zheng, H.-D. Sun, *J. Nat. Prod.* **2008**, *71*, 558.
[5] W.-L. Xiao, R.-R. Tian, J.-X. Pu, X. Li, L. Wu, Y. Lu, S.-H. Li, R.-T. Li, Y.-T. Zheng, Q.-T. Zheng, H.-D. Sun, *J. Nat. Prod.* **2006**, *69*, 277.
[6] R. T. Li, Z. Y. Weng, J. X. Pu, H. D. Sun, *Chin. Chem. Lett.* **2008**, *19*, 696.
[7] Y. Lu, D.-F. Chen, *J. Chromatogr. A* **2009**, *1216*, 1980.
[8] W.-L. Xiao, S.-X. Huang, R.-R. Wang, J.-L. Zhong, X.-M. Gao, F. He, J.-X. Pu, Y. Lu, Y.-T. Zheng, Q.-T. Zheng, H.-D. Sun, *Phytochemistry* **2008**, *69*, 2862.
[9] R.-T. Li, Q.-B. Han, A.-H. Zhao, H.-D. Sun, *Chem. Pharm. Bull.* **2003**, *51*, 1174.
[10] J.-S. Liu, M.-F. Huang, *Acta. Chim. Sin.* **1984**, *42*, 264.
[11] J. S. Liu, W. G. Wang, *Chin. Tradit. Herbal Drugs* **1990**, *21*, 294.
[12] T. Miyase, A. Ueno, N. Takizawa, H. Kobayashi, H. Karasawa, *Chem. Pharm. Bull.* **1987**, *35*, 1109.
[13] S.-X. Huang, J. Yang, H. Huang, L.-M. Li, W.-L. Xiao, R.-T. Li, H.-D. Sun, *Org. Lett.* **2007**, *9*, 4175.
[14] C. Lei, S.-X. Huang, J.-J. Chen, L.-B. Yang, W.-L. Xiao, Y. Chang, Y. Lu, H. Huang, J.-X. Pu, H.-D. Sun, *J. Nat. Prod.* **2008**, *71*, 1228.
[15] C. Lei, J.-X. Pu, S.-X. Huang, J.-J. Chen, J.-P. Liu, L.-B. Yang, Y.-B. Ma, W.-L. Xiao, X.-N. Li, H.-D. Sun, *Tetrahedron* **2009**, *65*, 164.
[16] C. Lei, S.-X. Huang, J.-J. Chen, J.-X. Pu, L.-M. Li, W.-L. Xiao, J.-P. Liu, L.-B. Yang, H.-D. Sun, *Helv. Chim. Acta* **2007**, *90*, 1399.
[17] S.-X. Huang, R.-T. Li, J.-P. Liu, Y. Lu, Y. Chang, C. Lei, W.-L. Xiao, L.-B. Yang, Q.-T. Zheng, H.-D. Sun, *Org. Lett.* **2007**, *9*, 2079.
[18] J.-X. Pu, R.-T. Li, W.-L. Xiao, N.-B. Gong, S.-X. Huang, Y. Lu, Q.-T. Zheng, L.-G. Lou, H.-D. Sun, *Tetrahedron* **2006**, *62*, 6073.

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