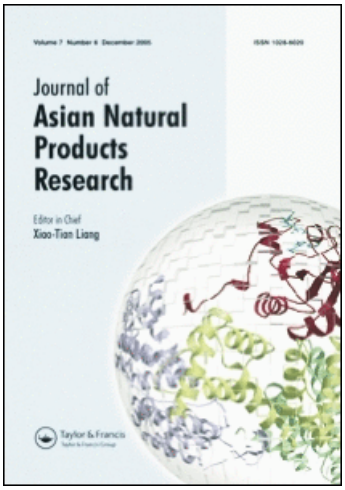



**Journal of Asian Natural Products Research**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

# Cyclohexyl-ethanol derivatives from the roots of *Incarvillea mairei*

Zheng-Sheng Huang[ab]; Yue-Hu Pei[b]; Yun-Heng Shen[a]; Sheng Lin[a]; Chun-Mei Liu[a]; Min Lu[c]; Wei-Dong Zhang[ad]

[a] Department of Natural Medicinal Chemistry, School of Pharmacy, Second Military Medical University, Shanghai, China [b] Department of Natural Medicinal Chemistry, School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang, China [c] Department of Medical Informatics, Second Military Medical University, Shanghai, China [d] School of Pharmacy, Shanghai Jiaotong University, Shanghai, China

**To cite this Article** Huang, Zheng-Sheng , Pei, Yue-Hu , Shen, Yun-Heng , Lin, Sheng , Liu, Chun-Mei , Lu, Min and Zhang, Wei-Dong(2009) 'Cyclohexyl-ethanol derivatives from the roots of *Incarvillea mairei*', Journal of Asian Natural Products Research, 11: 6, 523 — 528
**To link to this Article: DOI:** 10.1080/10286020902927872
**URL:** http://dx.doi.org/10.1080/10286020902927872

*Journal of Asian Natural Products Research*
Vol. 11, No. 6, June 2009, 523–528



# Cyclohexyl-ethanol derivatives from the roots of *Incarvillea mairei*

Zheng-Sheng Huang[ac], Yue-Hu Pei[c], Yun-Heng Shen[a], Sheng Lin[a], Chun-Mei Liu[a], Min Lu[d] and Wei-Dong Zhang[ab]*

[a]*Department of Natural Medicinal Chemistry, School of Pharmacy, Second Military Medical University, Shanghai, China;* [b]*School of Pharmacy, Shanghai Jiaotong University, Shanghai, China;* [c]*Department of Natural Medicinal Chemistry, School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang, China;* [d]*Department of Medical Informatics, Second Military Medical University, Shanghai, China*

(*Received 24 February 2009; final version received 27 March 2009*)

Two new cyclohexyl-ethanol derivatives, incarvmareins A (**1**) and B (**2**), together with two known derivatives, **3** and **4**, were isolated from the ethanolic extract of the roots of *Incarvillea mairei*. The structures of the new compounds were elucidated primarily on the basis of analysis of spectroscopic data.

**Keywords:** *Incarvillea mairei*; Bignoniaceae; cyclohexyl-ethanol derivative; incarvmareins



## 1. Introduction

*Incarvillea mairei* (Bignoniaceae) is mainly distributed in Tibet, Yunnan, and Sichuan Provinces, the roots of which are widely used in folk medicine for the treatments of dizziness, insufficiency of vital energy and blood, languor, and oligogalactia [1,2]. Previous investigations reported that monoterpenoid alkaloids [3–7], macrocyclic spermine alkaloids [8–10], iridoids [11,12], and others [13–15] were isolated from the genus *Incarvillea*, possessing interesting bioactivities. A further chemical study of the active fraction led to the isolation of two new cyclohexyl-ethanol derivatives **1** and **2**, and two known cyclohexyl-ethanol derivatives, **3** and **4** [16–19]. This paper deals with the isolation and structural elucidation of the two new compounds (**1** and **2**).

## 2. Results and discussion

The 80% ethanolic extract of the roots of *I. mairei* was suspended in water and then successively extracted with petroleum ether, $CHCl_3$, EtOAc, and *n*-butanol. The $CHCl_3$ fraction was subjected to silica gel column chromatography and Sephadex LH-20 to afford four compounds (**1**–**4**; Figure 1).

Compound **1** was obtained as a colorless oil. The molecular formula of $C_{17}H_{24}O_7$, with six degrees of unsaturation, was indicated by the positive mode HR-ESI-MS ($[M+Na]^+$ at *m/z* 363.1412). IR absorption bands showed the presence of carbonyl group (1720 $cm^{-1}$) and ether linkages (1022 and 877 $cm^{-1}$). The $^1H$ NMR spectrum (Table 1) of **1** displayed signals characteristic of two AA′XX′ systems at $\delta_H$ 3.97 (m, $H_a$-2) and 3.93 (m, $H_b$-2), $\delta_H$ 2.50 (m, $H_a$-3) and 2.14

*Corresponding author. Email: wdzhangy@hotmail.com

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020902927872
http://www.informaworld.com

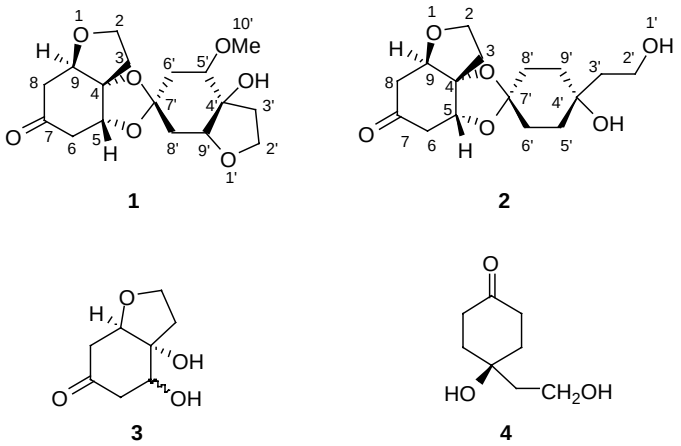



Figure 1. Chemical structures of compounds **1**–**4**.



(m, $H_b$-3), $\delta_H$ 3.96 (m, $H_a$-2′) and 3.94 (m, $H_b$-2′), $\delta_H$ 2.24 (m, $H_a$-3′) and 2.05 (m, $H_b$-3′) of two sequences: —$CH_2$—$CH_2O$—. Its $^{13}$C NMR and DEPT spectral data revealed signals of four quaternary carbons ($\delta_C$ 206.1, 109.9, 83.3, 77.4), four oxygenated methines ($\delta_C$ 80.1, 79.7, 78.9, 75.4), and eight methylenes ($\delta_C$ 66.2, 65.7, 38.6, 37.8, 36.9, 36.1, 35.4, 34.0; Table 1). The detailed analyses of the NMR spectral data and MS data suggested that **1** might contain two moieties of **3** (Figure 1) [16], except for losing a carbonyl group ($\delta_C$ 206.1), which was confirmed by the $^1$H–$^1$H COSY and HMBC spectra.

On the basis of 2D NMR spectroscopic analysis, together with the chemical values of the carbons of **1**, the linkage of the two moieties and the location of the methoxyl were established (Figures 2–4). Since the signals of C-4 and C-5 were downfield shifted from $\delta_C$ 78.1 in **3** to 83.3 in **1** (5.2 ppm) and from $\delta_C$ 70.3 in **3** to 79.7 in **1** (9.4 ppm), respectively, and the signal of C-7′ exhibited at $\delta_C$ 109.9, **1** might be formed by two oxygen bridges between C-5 and C-7′ and between C-4 and C-7′. The HMBC correlations of H-5 with the carbons at $\delta_C$ 206.1 (C-7), 109.9 (C-7′), 83.3 (C-4), 78.9 (C-9), and 36.1 (C-3) were observed (Figure 4). These evidences further confirmed the conclusion. The HMBC correlation between H-5′ and C-10′ was observed (Figure 4), indicating that a methoxyl was placed at C-5′.

The relative configuration of **1** was elucidated by NOESY experiments. Biogenetically, H-9 and 4-OH of **3** were α-oriented [20,21]. The NOESY spectrum of **1** exhibited the cross-peaks of H-3 with H-5 and H-5 with H-8′, indicating that H-5 and H-8′ were β-oriented, which had been established by constructing molecular models (Figure 2). Total assignments of $^1$H and $^{13}$C NMR signals were also confirmed by the $^1$H–$^1$H COSY, HMQC, and HMBC spectra. Accordingly, **1** was established to be a ketal of methyl-substituted **3**, and named incarvmarein A (Figure 1).

Compound **2** was obtained as a colorless oil, and IR absorption bands showed the presence of hydroxyl (3342 $cm^{-1}$), carbonyl (1718 $cm^{-1}$), and ether linkages (1033 and 873 $cm^{-1}$). The molecular formula $C_{16}H_{24}O_6$, with five degrees of unsaturation, was deduced by the positive mode HR-ESI-MS at *m/z* 335.1321 $[M+Na]^+$. Accurate analysis of spectral data including $^1$H and $^{13}$C NMR spectra (Table 1) demonstrated that the structure of compound **2** was similar to that of compound **1**. The only difference

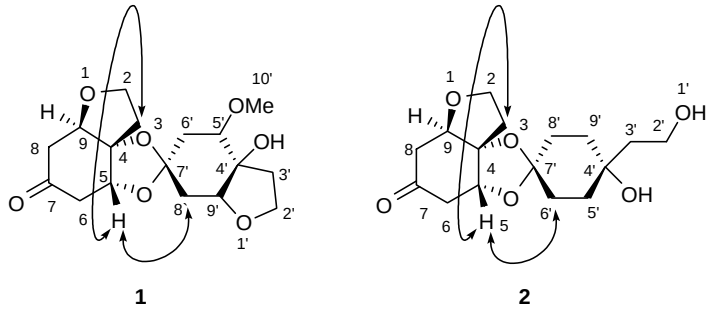
Table 1. $^{1}H$ and $^{13}C$ NMR spectral data of compounds **1** and **2**.

| Position | **1** | | **2** | |
|---|---|---|---|---|
| | $\delta_H$[a] | $\delta_C$[b] | $\delta_H$[c] | $\delta_C$[d] |
| 2a | 3.97 (m) | 65.7 | 3.87 (m) | 66.4 |
| 2b | 3.93 (m) | | 3.84 (m) | |
| 3a | 2.50 (m) | 36.1 | 2.43 (m) | 36.3 |
| 3b | 2.14 (m) | | 2.10 (m) | |
| 4 | | 83.3 | | 83.9 |
| 5 | 3.92 (m) | 79.7 | 4.44 (t, 3.0) | 78.8 |
| 6a | 2.70 (brd, 3.0) | 38.6 | 2.86 (dd, 18.0, 3.0) | 38.4 |
| 6b | 2.70 (brd, 3.0) | | 2.75 (dd, 18.0, 3.0) | |
| 7 | | 206.1 | | 206.1 |
| 8a | 2.88 (dd, 18.0, 3.0) | 37.8 | 2.94 (dd, 18.0, 3.6) | 39.4 |
| 8b | 2.66 (dd, 18.0, 3.0) | | 2.81 (dd, 18.0, 3.0) | |
| 9 | 4.40 (m) | 78.9 | 3.96 (t, 3.0) | 80.5 |
| 2′a | 3.96 (m) | 66.2 | 4.22 (t, 6.6) | 58.8 |
| 2′b | 3.94 (m) | | | |
| 3′a | 2.24 (m) | 36.9 | 1.94 (t, 6.6) | 44.6 |
| 3′b | 2.05 (m) | | | |
| 4′ | | 77.4 | | 70.0 |
| 5′a | 3.49 (m) | 75.4 | 2.21 (dt, 12.6, 4.8) | 30.9 |
| 5′b | | | 1.85 (m) | |
| 6′a | 2.12 (m) | 34.0 | 2.00 (m) | 35.7 |
| 6′b | 1.99 (m) | | 1.87 (m) | |
| 7′ | | 109.9 | | 109.9 |
| 8′a | 2.04 (m) | 35.4 | 2.04 (m) | 32.9 |
| 8′b | 1.97 (m) | | 1.68 (m) | |
| 9′a | 3.92 (m) | 80.1 | 2.40 (dt, 12.6, 4.8) | 35.3 |
| 9′b | | | 1.93 (m) | |
| 10′ | 3.45 (s) | 57.5 | | |

Chemical shifts ($\delta$) are in ppm relative to TMS. The spin coupling ($J$) is given in parentheses (Hz). All assignments are based on $^{1}H$–$^{1}H$ COSY, HMQC, and HMBC experiments.
[a] Measured at 600 MHz in $CDCl_3$.
[b] At 150 MHz in $CDCl_3$.
[c] Measured at 600 MHz in pyridine-$d_5$.
[d] At 150 MHz in pyridine-$d_5$.



in the $^{1}H$ NMR spectrum was the presence of two methylene groups ($\delta_H$ 2.40 and 1.93, $\delta_H$ 2.21 and 1.85) in B ring of **2**, instead of two oxygenated methine groups ($\delta_H$ 3.49 and 3.92) in B ring of **1** (Figure 3). Simultaneously, the signal of a methoxyl at $\delta_H$ 3.45 (3H, s) disappeared. The $^{13}C$ NMR spectral data (Table 1)




**1** **2**

Figure 2. Key NOESY correlations (H ↔ H) of **1** and **2**.

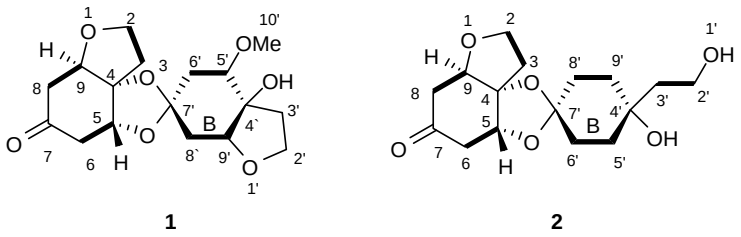


Figure 3. Key $^{1}H-^{1}H$ COSY correlations (H—H) of **1** and **2**.

revealed that the signals of C-2′, C-5′, and C-9′ were upfield shifted from $\delta_C$ 66.2 in **2** to 58.8 in **1** (7.4 ppm), from $\delta_C$ 75.4 in **2** to 30.9 in **1** (44.5 ppm), and from $\delta_C$ 80.1 in **2** to 35.3 in **1** (44.8 ppm), respectively. These observations indicated that **2** might be an adduct of **3** and **4** (Figure 1) [1–4]. The correlations of $^{1}H-^{1}H$ COSY, HMQC, HMBC, and ESI-MS data of **2** further confirmed the conclusion (Figures 3 and 4). Biogenetically, H-9 and 4-OH of **3** were α-oriented [20,21]. The NOESY spectrum of **2** exhibited the cross-peaks of H-3 with H-5 and H-5 with H-6′, indicating that H-5 and H-6′ were β-oriented, which had been established by constructing molecular models. Therefore, the structure of **2** was established to be a ketal of **3** and **4** [16–19], and named incarvmarein B (Figure 2).

## 3. Experimental

### 3.1 General experimental procedures

Optical rotations were acquired with Perkin-Elmer 341 polarimeter. IR spectra were recorded on a Bruker Vector-22 spectrometer with KBr pellets. 1D and 2D NMR spectra were recorded on a Bruker Avance 600 NMR spectrometer in $CDCl_3$ or pyridine-$d_5$ with TMS as an internal standard. ESI-MS and HR-ESI-MS were measured on an Agilent LC/MSD Trap XCT and a Q-TOF micro-mass spectrometer (Waters, Milford, MA, USA), respectively. Materials for column chromatography were silica gel (100–200, 300–400 mesh, and 10–40 μm; Huiyou Silical Gel Development Co., Ltd, Yantai, China) and Sephadex LH-20 (40–70 μm; GE Healthcare Biosciences AB, Uppsala, Sweden). Preparative TLC (0.4–0.5 mm) was conducted with glass precoated silica gel $GF_{254}$ (Huiyou Silical Gel Development Co., Ltd). Spots were visualized under UV light (254 nm) or by spraying with 5% $H_2SO_4$ in 95% EtOH followed by heating.

### 3.2 Plant material

The roots of *I. marei* were collected in Gaopo County, Yunnan Province, China, in June 2007. The plant was identified by Prof. Bao-Zhong Duan (College of Da Li). A voucher specimen (No. 07061008) is deposited in School of Pharmacy, Second Military Medical University.

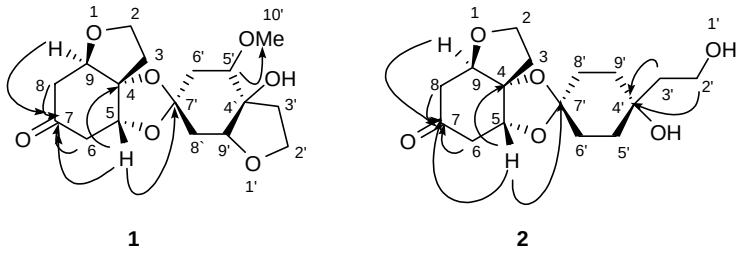


Figure 4. Key HMBC correlations (H → C) of **1** and **2**.

### 3.3 Extraction and isolation

The roots of *I. marei* (2.5 kg) were powdered and extracted thrice with 80% EtOH for 4 h. After removal of the solvent under reduced pressure, the extract was suspended in water and partitioned with petroleum ether, $CHCl_3$, EtOAc, *n*-BuOH, successively. The $CHCl_3$-soluble part (67.5 g) was subjected to silica gel column chromatography (Ø 5 × 60 cm, 200–300 mesh, 300 g), eluting with $CHCl_3$–MeOH (100:3 → 100:50) to afford 16 fractions (fractions 1–16) on the basis of TLC analysis. Fraction 8 was re-chromatographed over a silica gel column eluting with gradient petroleum ether–acetone (3:1) to afford six subfractions (fractions 8.1–8.6). Fraction 8.1 was further purified by preparative TLC with petroleum ether–EtOAc (1:1) to afford **1** (5.8 mg). Fraction 9 (370 mg) was re-chromatographed over a silica gel column and eluted with gradient $CHCl_3$–MeOH (30:1) to afford nine subfractions (fractions 9.1–9.9). Fraction 9.7 was subjected to column chromatography over Sephadex LH-20, using the elution of petroleum ether–$CHCl_3$–MeOH (5:5:1) to afford fractions 9.7.1–9.7.8. Fraction 9.7.8 was further purified by preparative TLC with petroleum ether–EtOAC (1:5) to afford **2** (6.3 mg). Fraction 9.8 was subjected to column chromatography over Sephadex LH-20 (petroleum ether–$CHCl_3$–MeOH (5:5:1)), and then further purified by preparative TLC with $CHCl_3$–acetone (1:1) to afford **3** (9.6 mg). Fraction 10 (152 mg) was subjected to column chromatography over Sephadex LH-20 (petroleum ether–$CHCl_3$–MeOH (5:5:1)) to afford three subfractions, and the third subfraction was further purified by preparative TLC with petroleum ether–acetone (5:1) to yield **4** (17 mg).

#### 3.3.1 Incarvmarein A (**1**)

A colorless oil; $C_{17}H_{24}O_7$; $[\alpha]_D^{22}$ +1.00 ($c = 0.15$, MeOH); IR $\nu_{max}$ (KBr) 2930, 2882, 1720, 1453, 1368, 1085, 1022, 877, 607 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 600 MHz) and $^{13}C$ NMR ($CDCl_3$, 150 MHz) spectral data, see Table 1; ESI-MS (positive) *m/z*: 363 $[M+Na]^+$ and 703 $[2M+Na]^+$, HR-ESI-MS (positive) *m/z*: 363.1412 $[M+Na]^+$ (calcd for $C_{17}H_{24}O_7Na$, 363.1420).

#### 3.3.2 Incarvmarein B (**2**)

A colorless oil; $C_{16}H_{24}O_6$; $[\alpha]_D^{22}$ +1.00 ($c = 0.29$, MeOH); IR $\nu_{max}$ (KBr) 3342, 2927, 2873, 1718, 1384, 1267, 1128, 1078, 1033, 968, 938, 873, 695 $cm^{-1}$; $^1H$ NMR (pyridine-$d_5$, 600 MHz) and $^{13}C$ NMR (pyridine-$d_5$, 150 MHz) spectral data, see Table 1; ESI-MS (positive) *m/z*: 335 $[M+Na]^+$, ESI-MS (negative) *m/z*: 311 $[M-H]^-$, and HR-ESI-MS (positive) *m/z*: 335.1321 $[M+Na]^+$ (calcd for $C_{16}H_{24}O_6Na$, 335.1471).

## Acknowledgements

The work was partially supported by the National Natural Science Foundation of China (30725045), National 863 Program (2006AA02Z338), '973' Program of China (2007CB507400), Shanghai Leading Academic Discipline Project (B906), and in part by the Scientific Foundation of Shanghai China (07DZ19728, 06DZ19717, and 06DZ19005). We acknowledge the Pharmaceutical Analysis Center of Second Military Medical University for measuring the IR and NMR spectra. We also thank Professor Bao-Zhong Duan of College of Da Li for collection and identification of the plant.

## References

[1] W.C. Wang, *Flora of China* (Science Press, Beijing, 1990), Vol. 69, p. 45.
[2] Editorial Committee of Zhong Hua Ben Cao, *Zhong Hua Ben Cao* (Shanghai Science and Technology Press, Shanghai, 1999), Vol. 20, p. 426.
[3] M. Nakumara, Y.M. Chi, J. Kinjo, W.M. Yan, and T. Nohara, *Phytochemistry* **51**, 595 (1999).
[4] Y.M. Chi, F. Hashimoto, W.M. Yan, and T. Nohara, *Phytochemistry* **40**, 353 (1995).
[5] Y.M. Chi, W.M. Yan, C.D. Chang, H. Noguchi, Y. Iitaka, and U. Sankawa, *Phytochemistry* **31**, 2930 (1992).

[6] Y.M. Chi, W.M. Yan, and J.S. Li, *Phytochemistry* **29**, 2376 (1990).
[7] Y.M. Chi, M. Nakamura, X.Y. Zhao, T. Yoshizawa, W.M. Yan, F. Hashimoto, J. Kinjo, and T. Nohara, *Chem. Pharm. Bull.* **53**, 1178 (2005).
[8] K. Drandarov and M. Hesse, *Tetrahedron Lett.* **43**, 5025 (2002).
[9] Y.M. Chi, F. Hashimoto, W.M. Yan, and T. Nohara, *Tetrahedron Lett.* **38**, 2713 (1997).
[10] Y.M. Chi, M. Nakamura, X.Y. Zhao, T. Yoshizawa, W.M. Yan, F. Hashimoto, Y.C. Chi, J. Kinjo, and T. Nohara, *J. Asian Nat. Prod. Res.* **9**, 115 (2007).
[11] M.S. Maksudov, R.U. Umarova, B. Tashkhodzhaev, Z. Saatov, and N.D. Abdullaev, *Chem. Nat. Compd* **31**, 48 (1995).
[12] T. Lu, W.D. Zhang, Y.H. Pei, C. Zhang, J.C. Li, and Y.H. Shen, *Chin. Chem. Lett.* **19**, 829 (2008).
[13] Y.Q. Su, W.D. Zhang, C. Zhang, R.H. Liu, and Y.H. Shen, *Chin. Chem. Lett.* **7**, 829 (2008).
[14] Y.M. Chi, N. Motoyuki, X.Y. Zhao, Y. Toyokichi, W.M. Yan, H. Fumio, N. Toshihiro, and S. Shinobu, *Nat. Prod. Res. Dev.* **17**, 362 (2005).
[15] T.F. Ji and X.Z. Fen, *Chin. Tradit. Herb. Drugs* **33**, 967 (2002).
[16] J. Tian, S.Q. Zhao, H.J. Zhang, Z.W. Lin, and H.D. Sun, *J. Nat. Prod.* **60**, 766 (1997).
[17] H. Abdullahi, E. Nyandat, C. Galeffi, I. Messana, M. Nicoletti, and G.B. Marini Bettolo, *Phytochemistry* **25**, 2821 (1986).
[18] E. Navarro, J. Trujillo, J.L. Breton, and J. Boada, *Phytochemistry* **25**, 1990 (1986).
[19] K. Endo and H. Hikino, *Can. J. Chem.* **62**, 2011 (1982).
[20] T. Hase, K. Ohtani, R. Kasai, K. Yamasaki, and C. Picheansoonthon, *Phytochemistry* **39**, 235 (1995).
[21] S. Barradas, C.M. Carreo, L.M. Gonzlez, A. Latorre, and A. Urbano, *Org. Lett.* **9**, 5019 (2007).