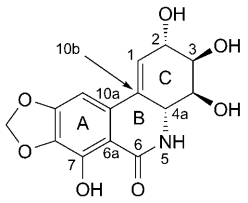
Biological Evaluation of *ent*-Narciclasine, *ent*-Lycoricidine, and Certain Enantiomerically-Related Congeners

by **Maria Matveenko**[a]), **Martin G. Banwell***[a]), **Max Joffe**[b]), **Soosan Wan**[b]), and **Emmanuelle Fantino**[b])

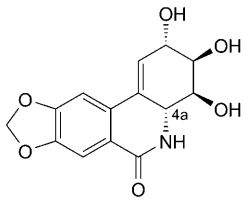
[a]) Research School of Chemistry, Institute of Advanced Studies, The Australian National University, Canberra, ACT 0200, Australia
(phone: +61-2-6125-8202; fax: +61-2-6125-8114; e-mail: mgb@rsc.anu.edu.au)
[b]) Cytopia Pty Ltd, 576 Swan Street, Richmond, VIC 3121, Australia

The non-natural enantiomeric forms of narciclasine and lycoricidine ((−)-**1** and (−)-**2**, respectively), as well as congeners **3**–**6** are available through chemoenzymatic synthesis. Accordingly, they have now been tested for their cytotoxic effects in a 13-member human cancer cell-line panel and found to be only weakly active. In contrast, an authentic sample of the natural enantiomeric form of narciclasine ((+)-**1**) was found to be highly active in the same screens.

1. Introduction. – While it has been estimated that only 15% of the world's plants have been screened for their therapeutic potential [1], the daffodil (*a.k.a. narcissus*) has been the subject of extensive study and proven to be a rich source of biologically active compounds[1]). In particular, and partly as a result of ethnobotanical studies [2], the isocarbostyril-type natural product narciclasine ((+)-**1**) was isolated from a variety of *Narcissus* bulbs [3], as well as *Lycoris radiata* [4], and shown to possess potent antimitotic properties [3]. Its structure was established by single-crystal X-ray analysis [5]. The 7-deoxy analogue (+)-**2**, also known as lycoricidine, was isolated at around the same time from *L. radiata* [4] and *L. sanguinea* [6], as well as from *Pancratium littorale* [7] and *Haemanthus kalbreyeri* [8]. Like congener (+)-**1**, lycoricidine ((+)-**2**) exhibits nanomolar growth-inhibitory potencies against a range of human cancer cell lines [9]. As a consequence, an extraordinary effort has been devoted to the development of syntheses of compounds (+)-**1** and (+)-**2** as well as a range of congeners [2][2]).



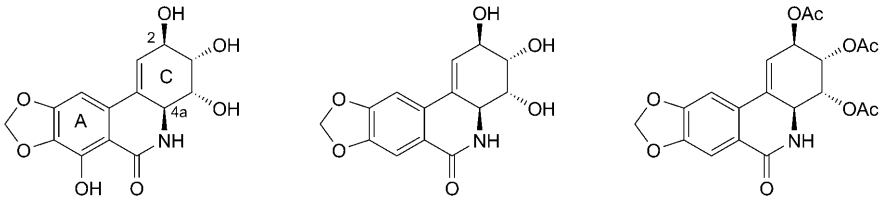
(+)-**1**



(+)-**2**

[1]) For an excellent review of this topic, see [2] and refs. cit. therein.
[2]) For relevant reviews, see [10]. For recent synthetic efforts in this area that have not been covered by the aforementioned reviews, see [11].

As part of our continuing interest in developing new approaches to this fascinating class of compounds[3]), we have recently reported efficient, chemoenzymatic total syntheses of the non-natural enantiomeric forms of narciclasine [13] and lycoricidine [14] ((−)-**1** and (−)-**2**, resp.) as well as congeners **3**–**6** [14]. These vary in terms of the nature and degree of substitution on the A- and C-rings as well as in configuration of certain of the substituents. A constant feature within this series is that each member possesses the non-natural configuration at C(2) and C(4a). Such work provided the first total synthesis of (−)-narciclasine ((−)-**1**) and only the second synthesis of (−)-lycoricidine ((−)-**2**)[4]). Given that nothing has been reported about the cyctotoxic profiles of the non-natural enantiomeric forms of narciclasine and lycoricidine, we have subjected compounds (−)-**1**, (−)-**2**, and **3**–**6**, as well as (+)-**1**, to relevant screening regimes and now report the outcomes of such studies herein.



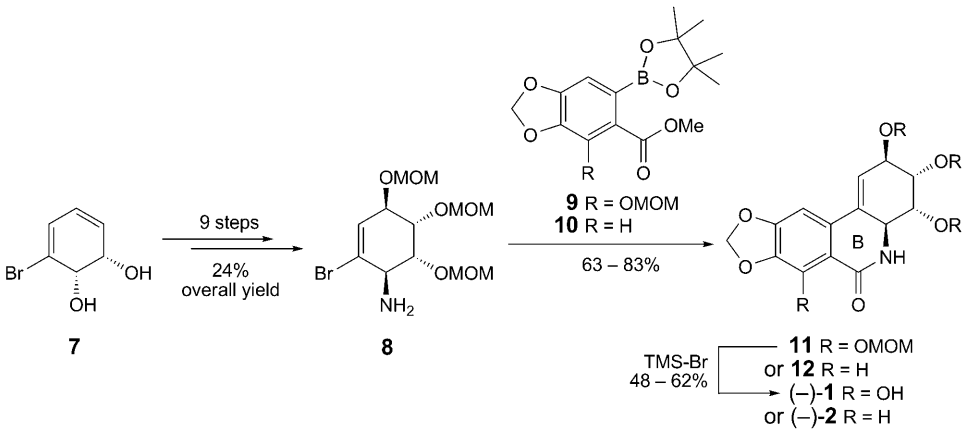
2. Results and Discussion. – For the sake of completeness, and before detailing the outcomes of the screening of the abovementioned compounds, it is appropriate to provide a short commentary on the synthetic approach used in obtaining compounds (−)-**1** and (−)-**2** [13][14]. This is presented immediately below and is followed by a brief section detailing the physical and spectroscopic similarities between compound (−)-**1** and an authentic sample of naturally occurring narciclasine, *viz*. (+)-**1**.

2.1. *Synthetic Studies.* A convergent strategy was employed in obtaining targets (−)-**1** and (−)-**2** (*Scheme*) [13][14]. Specifically, the progenitor, **8**, to the C-ring of each of these compounds was prepared by elaboration of the enantiomerically pure and enzymatically-derived *cis*-1,2-dihydrocatechol **7**[5]) using relatively conventional chemical transformations and an *Overman* rearrangement process[6]) to introduce the pivotal

[3]) For a key earlier publication arising from our efforts in this area, see [12].
[4]) The first synthesis of (−)-lycoricidine was reported by *Keck et al.* [15].
[5]) For reviews on the production and general synthetic utility of these types of metabolites, see [16].
[6]) For a discussion of the application of this type of process in this context, see [17].

amino group. Subjection of the amino-conduritol **8** to *Suzuki–Miyaura* cross-coupling [18] with either the aryl boronate ester **9** [13] or its deoxy counterpart **10** [14] then gave the fully MOM-protected derivative of (−)-narciclasine ((−)-**1**) and (−)-lycoricidine ((−)-**2**) in 63 and 83% yield, respectively. The precise order of events involved in these conversions has not been confirmed, but it seems likely that the *Suzuki–Miyaura* cross-coupling process precedes a lactamization reaction leading to the formation of the B-ring of compounds **11** and **12**. Exhaustive removal of the MOM groups associated with each of isocarbostyrils **11** and **12** could be accomplished using TMS-Br to give the corresponding targets (−)-**1** and (−)-**2** in 48 and 62% yield, respectively.

Scheme



The structures of compounds (−)-**1** and (−)-**2** were initially established through the acquisition of the usual sets of spectral data and comparisons of these with those reported in the literature[7]). In addition, the triacetate derivative, **3**, of (−)-lycoricidine was prepared by established procedures and then subjected to single-crystal X-ray analysis [14].

After the publication of the details of our synthesis of (−)-narciclasine [13], we were kindly provided with an authentic sample of its natural enantiomer by Dr. *Hank Fales* of the *National Institutes of Health* in the USA. Accordingly, we have been able to make, for the first time, direct spectroscopic comparisons of the two samples. A summary of such comparisons is provided in the following section.

2.2. *Spectroscopic Characterization and Comparison of (+)- and (−)-Narciclasine.* The ^{13}C- and ^{1}H-NMR spectra of naturally derived (+)-narciclasine ((+)-**1**) and synthetically derived (−)-narciclasine ((−)-**1**) were recorded under essentially identical conditions in (D_6)DMSO, and the outcomes of such experiments are presented in *Table 1*. As is readily discerned from inspection of the tabulated chemical shifts, the two sets of data are identical within the limits of error of the NMR experiments.

---

[7]) In the case of (−)-narciclasine ((−)-**1**), the initial comparisons were with those data reported for (+)-narciclasine ((+)-**1**); see [15] and [19].

Table 1. *Comparison of the ^{13}C- and ^{1}H-NMR Data Recorded in (D_6)DMSO for Naturally Derived (+)-Narciclasine* ((+)-**1**) *and Synthetically Derived (−)-Narciclasine* ((−)-**1**[a]). δ in ppm, J in Hz.

δ(C)[b])		δ(H)[c])	
(+)-**1**	(−)-**1**	(+)-**1**	(−)-**1**
169.0	169.0	13.26 (*s*, 1 H)	13.26 (*s*, 1 H)
152.4	152.4	7.91 (*s*, 1 H)	7.92 (*s*, 1 H)
144.8	144.8	6.86 (*s*, 1 H)	6.86 (*s*, 1 H)
133.5	133.5	6.18–6.13 (*m*, 1 H)	6.17–6.14 (*m*, 1 H)
132.2	132.2	6.10–6.07 (*m*, 2 H)	6.10–6.07 (*m*, 2 H)
129.3	129.3	5.24–5.15 (*m*, 2 OH)	5.23–5.17 (*m*, 2 OH)
124.8	124.8	5.06–5.00 (*m*, OH)	5.04 (*d*, *J*=3.0, OH)
105.6	105.6	4.19 (*d*, *J*=8.0, 1 H)	4.19 (*d*, *J*=8.0, 1 H)
102.1	102.2	4.04–3.98 (*m*, 1 H)	4.04–3.98 (*m*, 1 H)
95.9	95.9	3.82–3.76 (*m*, 1 H)	3.82–3.76 (*m*, 1 H)
72.4	72.4	3.72–3.67 (*m*, 1 H)	3.72–3.68 (*m*, 1 H)
69.2	69.2		
68.8	68.8		
52.9	52.9		

[a]) The central peak (δ 39.5) of the signals due to (D_6)DMSO was used as the internal reference for ^{13}C-NMR spectra, while the signal (δ 2.50) due to residual protio-DMSO was used as the internal reference for ^{1}H-NMR spectra. [b]) At 75 MHz. [c]) At 500 MHz.

The ESI mass spectra of (+)- and (−)-narciclasine were each recorded in positive-ionization mode, and they proved to be superimposable as did the corresponding IR spectra. In keeping with expectations, the specific rotation of the naturally occurring enantiomer ($[\alpha]_D$ +116 (c=0.19, MeOH)) was of the same magnitude but opposite sign to that of *ent*-narciclasine ($[\alpha]_D$ −116 (c=0.21, MeOH)). The enantiomeric relationship between the two compounds was reinforced through the acquisition of the CD spectra for the two samples. These are shown together in the *Figure* which reveals their near-complementary relationship. The slight distortion in the CD spectrum of (−)-narciclasine ((−)-**1**) observed at *ca*. 250 nm is attributed to the presence of trace amounts of an unidentified impurity displaying an intense absorption at this wavelength.

The naturally occurring (+)-enantiomer had no sharp melting point but began to color intensely at 205° and decomposed slowly above that temperature. Synthetic (−)-narciclasine, on the other hand, colored intensely from 214° and decomposed slowly above this temperature. It changed from white to pale-yellow at 180°. Such a change was not observed for the naturally occurring sample as it was a tan-colored material to begin with.

2.3. *Biological Screening of Compounds (+)-***1**, *(−)-***1**, *(−)-***2**, *and* **3**–**6** *against Certain Human Cancer Cell Lines.* Each of the synthetically derived compounds (−)-**1**, (−)-**2**, and **3**–**6** was screened, at 2 μM concentrations, against a panel of 13 human cancer cell lines as listed in *Table 2* (details of the protocols used are presented in the *Exper. Part*). An authentic sample of (+)-narciclasine ((+)-**1**) was also tested against the same panel. As a consequence, it became clear that the synthetic compounds, which possess the non-natural configuration at C(2) and C(4a), are all one or two orders of

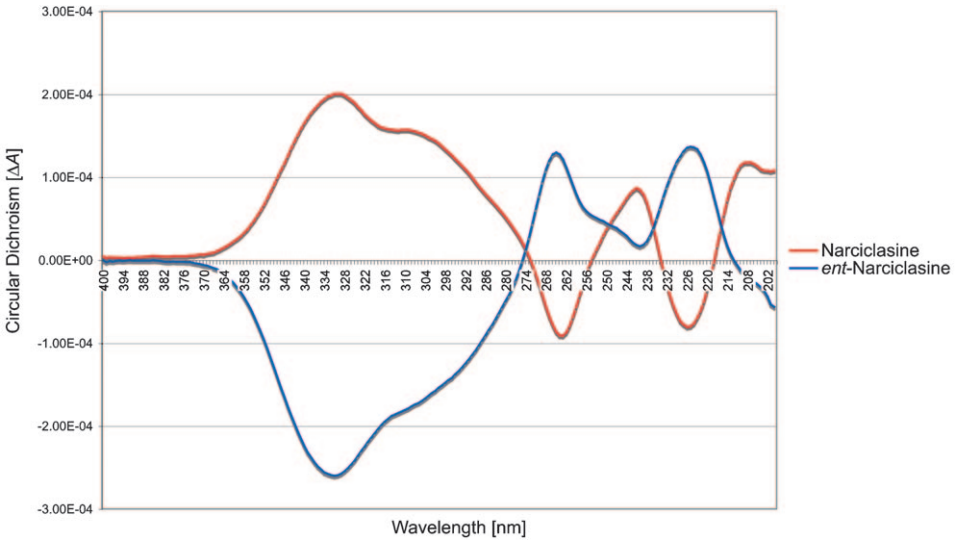


Figure. *Comparison of the CD spectral data of naturally derived (+)-narciclasine* ((+)-**1**; red trace) *and synthetically derived (−)-narciclasine* ((−)-**1**; blue trace). Spectra were recorded on 1 mg samples of (+)- or (−)-**1** dissolved in 2.0 ml of HPLC-grade EtOH using an *Applied Photophysic Chirascan* instrument operating at 18°.

Table 2. *Inhibition of Cell Growth* [%][a]) *Caused by 2 μM Samples of Compounds (+)*-**1**, *(−)*-**1**, *(−)*-**2**, *and* **3**–**6** *in Cytotoxicity Screens Using 13 Human Cancer Cell Lines*[b])

Cell line[c])	(+)-**1**	(−)-**1**	(−)-**2**	**3**	**4**	**5**	**6**
A-375	103	2	−2	3	4	3	6
A-431	100	1	1	2	0	5	6
HCT-15	105	1	5	12	5	6	9
K-562	102	−15	−3	−4	5	−1	7
MDA MB-231	118	3	8	11	6	14	12
A-549	105	−2	−6	2	−6	5	6
PA-1	107	−1	−2	−4	−5	−2	−6
HT-1376	135	−21	−21	−10	−10	−14	−7
HTB-178	151	4	8	14	7	15	18
MES-SA	116	12	1	13	16	5	17
HepG2	155	15	6	17	8	15	14
MCF-7	135	8	3	12	5	10	15
DU-145	104	−8	−20	−20	−10	−9	−3

[a]) Percentage-inhibition values were calculated by comparison with vehicle-treated cells. The numbers shown are as calculated. Since cell growth has some inherent variability, the values shown are approximate measurements of inhibition of cellular proliferation, and those identified as >100% and <0% simply represent complete inhibition and no inhibition of cell growth, respectively. [b]) DMSO was used as solvent in these screens. [c]) See *Exper. Part* for details.

magnitude less cytotoxic than the parent compound and can thus be regarded as essentially inactive materials. Such results seem consistent with the observation that *ent*-7-deoxypancratistatin (an 'alkene hydrated' form of (−)-lycoricidine) exhibits cytotoxic potencies that are tenfold lower than those of the corresponding natural enantiomer [20].

Conclusions. – The differences in the cytotoxic properties of naturally derived (+)-narciclasine ((+)-**1**) and its synthetically derived optical antipode (−)-**1** are particularly noteworthy. These results are consistent with earlier findings [2][20] that high levels of carcinostatic activity are only observed in compounds possessing the full structural framework of the lycorine alkaloids and incorporating the natural configurational relationships of functional groups in the C-ring.

We thank the *Australian Research Council* and *The Institute of Advanced Studies* at the *Australian National University* for generous financial support. Dr. *Hank Fales* (*National Institutes of Health*, Bethesda, Maryland USA) is warmly acknowledged for providing an authentic sample of (+)-narciclasine ((+)-**1**). We are also grateful to Mr. *Paul Gugger* (*Research School of Chemistry, ANU*) for assisting with the acquisition of the CD spectra of compounds (+)-**1** and (−)-**1**. Dr. *Chris Burns* (*Cytopia Pty Ltd.*) is thanked for facilitating the biological testing described herein.

Experimental Part

General. M.p.: Reichert hot-stage microscope apparatus; uncorrected. Optical rotations: at 18° with a *Perkin-Elmer 241* polarimeter at the sodium-D line (589 nm) and the concentrations (*c*) (g/100 ml) indicated using spectroscopic grade solvents; measurements carried out in a cell with a path length (l) of 1 dm. CD Spectra: *Applied Photophysics Spectroscan* instrument. IR Spectra ($\tilde{\nu}_{max}$): *Perkin-Elmer 1800* Series FTIR Spectrometer; as KBr disks. ^{1}H- and ^{13}C-NMR spectra: at 18° in (D_6)DMSO on a *Varian Inova 500* or *300* spectrometer operating at 500 MHz for ^{1}H and 75 MHz for ^{13}C nuclei. Low- and high-resolution (LR and HR, resp.) electrospray (ESI) MS: *VG Quattro II* triple quadrupole MS instrument operating in positive-ionization mode.

Sources of Compounds and Spectral Properties. Samples of compounds (−)-**1**, (−)-**2**, and **3**–**6** required for biological testing were prepared according to previously described procedures [13][14].

An authentic sample of *(+)-narciclasine* ((+)-**1**) (308.0768 ($[M+H]^+$, $C_{14}H_{13}NO_7^+$; calc. 308.0770) was kindly provided by Dr. *Hank Fales* of the *National Institutes of Health*, Bethesda, Maryland, USA. This material was subjected, as obtained, to ^{1}H- and ^{13}C-NMR, as well as CD spectral analyses, the results of which are presented in *Table 1* and the *Figure*, resp. IR (KBr): 3437, 3323, 1674, 1652, 1640, 1477, 1437, 1408, 1377, 1281, 1229, 1097, 1088, 1034, 1019, 803. ESI-MS: 330 (50, $[M+Na]^+$), 308 (100, $[M+H]^+$).

Cytotoxicity Testing. Stock solns. of the test compounds were prepared at 20 mM in neat DMSO and diluted in tissue culture medium to a concentration of 20 μM. Compounds were screened against ATCC cell lines at a final concentration of 2 μM in flat-bottom TC plates. The cells were incubated for 72 h at 37° in a 5% CO_2 incubator. Inhibition of cell proliferation by the test compounds was determined by measuring conversion of *Alamar-Blue* fluorescent dye at excitation/emission 544/590 nm with a *BMG FluoStar* plate reader.

The ATCC cell lines used were: A-375 (CRL-1619), human malignant melanoma; A-431 (CRL-1555), human epidermoid carcinoma; HCT-15 (CCL-225), human colon adenocarcinoma; K-562 (CCL-243), human chronic myelogenous leukaemia; MDA-MB231 (HTB-26), human breast adenocarcinoma; A-549 (CCL-185), human lung carcinoma; PA-1 (CRL-1572), human ovarian teratocarcinoma; HT-1376 (CRL-1472), human bladder carcinoma; HTB-178 (NCI-H82), human small-cell lung carcinoma; MES-SA (CRL-1976), human uterine sarcoma; HepG2/C3A (CRL-10741), human hepatoblastoma; MCF-7 (HTB-22), human breast adenocarcinoma; and DU-145, human prostate carcinoma.

REFERENCES

[1] G. Scott, *Veld Flora* **1993**, *79*, 84.
[2] A. Kornienko, A. Evidente, *Chem. Rev.* **2008**, *108*, 1982.
[3] G. Ceriotti, *Nature* **1967**, *213*, 595.
[4] T. Okamoto, Y. Torii, Y. Isogai, *Chem. Pharm. Bull.* **1968**, *16*, 1860.
[5] A. Immirzi, C. Fuganti, *J. Chem. Soc., Chem. Commun.* **1972**, 240.
[6] S. Takagi, M. Yamaki, *Yakugaku Zasshi* **1974**, *94*, 617.
[7] G. R. Pettit, V. Gaddamidi, D. L. Herald, S. B. Singh, G. M. Cragg, J. M. Schmidt, F. E. Boettner, M. Williams, Y. Sagawa, *J. Nat. Prod.* **1986**, *49*, 995.
[8] S. Ghosal, S. Singh, Y. Kumar, R. S. Srivastava, *Phytochemistry* **1989**, *28*, 611.
[9] G. R. Pettit, G. R. Pettit III, R. A. Backhaus, M. R. Boyd, A. W. Meerow, *J. Nat. Prod.* **1993**, *56*, 1682.
[10] T. Hudlicky, *J. Heterocycl. Chem.* **2000**, *37*, 535; U. Rinner, T. Hudlicky, *Synlett* **2005**, 365; Y. Chapleur, F. Chrétien, S. Ibn Ahmed, M. Khaldi, *Curr. Org. Synth.* **2006**, *3*, 341; M. Manpadi, A. Kornienko, *Org. Prep. Proced. Int.* **2008**, *40*, 107.
[11] H. Zhang, A. Padwa, *Synlett* **2006**, 2317; H. Zhang, A. Padwa, *Org. Lett.* **2006**, *8*, 247; K. H. Shukla, D. J. Boehmler, S. Bogacyzk, B. R. Duvall, W. A. Peterson, W. T. McElroy, P. DeShong, *Org. Lett.* **2006**, *8*, 4183; M. Li, A. Wu, P. Zhou, *Tetrahedron Lett.* **2006**, *47*, 3707; D. Crich, V. Krishnamurthy, *Tetrahedron* **2006**, *62*, 6830; I.-J. Shin, E.-S. Choi, C.-G. Cho, *Angew. Chem., Int. Ed.* **2007**, *46*, 2303; A. Padwa, H. Zhang, *J. Org. Chem.* **2007**, *72*, 2570.
[12] M. G. Banwell, C. J. Cowden, R. W. Gable, *J. Chem. Soc., Perkin Trans. 1* **1994**, 3515.
[13] M. Matveenko, M. G. Banwell, A. C. Willis, *Tetrahedron* **2008**, *64*, 4817.
[14] M. Matveenko, O. J. Kokas, M. G. Banwell, A. C. Willis, *Org. Lett.* **2007**, *9*, 3683.
[15] G. E. Keck, T. T. Wager, J. F. Duarte Rodriquez, *J. Am. Chem. Soc.* **1999**, *121*, 5176.
[16] T. Hudlicky, D. Gonzalez, D. T. Gibson, *Aldrichimica Acta* **1999**, *32*, 35; M. G. Banwell, A. J. Edwards, G. J. Harfoot, K. A. Jolliffe, M. D. McLeod, K. J. McRae, S. G. Stewart, M. Vögtle, *Pure Appl. Chem.* **2003**, *75*, 223; R. A. Johnson, *Org. React.* **2004**, *63*, 117; K. A. B. Austin, M. Matveenko, T. A. Reekie, M. G. Banwell, *Chem. Aust.* **2008**, *75*, 3.
[17] M. Matveenko, M. G. Banwell, A. C. Willis, *Aust. J. Chem.* **2009**, *62*, 64.
[18] N. Miyaura, A. Suzuki, *Chem. Rev.* **1995**, *95*, 2457.
[19] Z. Trimino Ayllon, I. Ramos Martinez, C. Iglesias Perez, I. Spenglers Salabarria, H. Velez Castro, *Rev. Cubana Farm.* **1989**, *23*, 155; A. Evidente, *Planta Med.* **1991**, *57*, 293.
[20] T. Hudlicky, U. Rinner, D. Gonzalez, H. Akgun, S. Schilling, P. Siengalewicz, T. A. Martinot, G. R. Pettit, *J. Org. Chem.* **2002**, *67*, 8726.

Received October 29, 2008