



## Synthesis and evaluation of novel podophyllotoxin analogs

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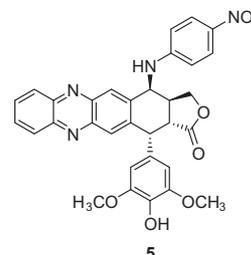
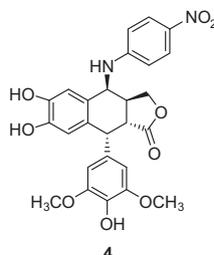
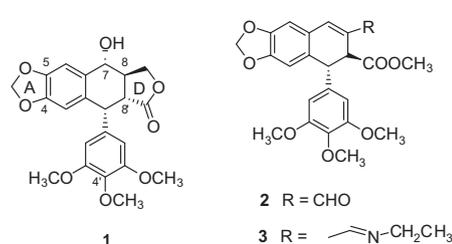
Cytotoxicity

### ABSTRACT

Because prior studies have shown inconsistency between structure–activity relationships for podophyllotoxin derivatives as topoisomerase II inhibitors and cytotoxic agents, eight novel podophyllotoxin analogs were synthesized to further explore the effects of structural variations on both A and D rings on activity. The new compounds contain a 4,5-dimethoxy substituted A ring and opened D-ring variants and were prepared by appropriate functional and stereochemical operations at the methylenedioxy group, C7, C8, and C8'. Four compounds (**15**, **18**, **21** and **22**) demonstrated noticeable inhibitory activity against A549, DU145, KB and KBvin tumor cells, and the most active compound **18** showed IC<sub>50</sub> values less than 10 μg/mL.

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The natural lignan podophyllotoxin (**1**) has been the focus of extensive chemical modification and biological investigation in recent decades. In particular, the discovery of the semi-synthetic anticancer drugs etoposide and teniposide has stimulated prolonged research interest in this structural phenotype.



believed to be essential for Topo II inhibition.<sup>1</sup> However, podophyllotoxin derivatives **2** and **3**, which lack the *trans*-lactone D ring, showed significant cytotoxicity against various tumor cell lines.<sup>2,3</sup> Furthermore, although the A-ring modified derivatives **4** and **5** were only weak inhibitors of Topo II catalytic activity, they

Two alternative molecular mechanisms are generally involved in the antineoplastic activity of podophyllotoxin analogs: preventing the assembly of tubulin into microtubules and inhibiting the catalytic activity of DNA topoisomerase II (Topo II). As the primary mechanism for therapeutically useful podophyllotoxin analogs, Topo II inhibition has been the major focus for previous structure–activity relationship (SAR) studies, and intact A/D rings are

inhibited KB cell growth at sub-micromolar concentrations.<sup>4</sup> These results implied conflicting SAR for Topo II inhibition and cytotoxicity, and supported further SAR exploration on various molecular areas of the structural phenotype, particularly the A and D rings.

Accordingly, we synthesized a series of novel podophyllotoxin analogs with structural variations on both A and D rings (**15–22**). These new analogs feature 4,5-dimethoxy substitution as well as structural alterations at C7, C8, and C8'. To investigate the effects of C8' stereochemistry on cytotoxicity, D-ring variants with opposite chirality at C8' were deliberately incorporated. We report

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herein the synthesis,<sup>5</sup> structural characterization, and preliminary biological evaluation of these novel podophyllotoxin analogs.

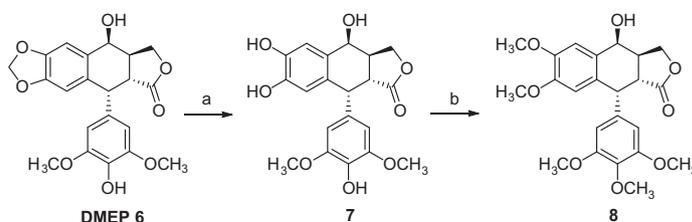
The key intermediate **8** was synthesized from 4'-demethylepipodophyllotoxin (**DMEP**, **6**) in two steps with a protocol modified from the literature method (Scheme 1).<sup>6</sup>

As illustrated in Scheme 2, five analogs **15–19** with  $\alpha$ -configuration at C8' were prepared from **8**. Oxidation with pyridinium dichromate (PDC) and subsequent acid-catalyzed methanolysis gave the D-ring opened variant **10**. Stereoselective reduction of the 7-carboxyl in **10** afforded compound **11**. The stereoselectivity in the reduction of **10** should be attributed to the asymmetric environment of the *Re* and *Si* faces of the 7-carbonyl. The bulky aromatic group and carboxylate substitution preclude hydrogen addition from the rear face (i.e., the *Re* face), therefore, reduction from the front face (i.e., the *Si* face) would be dominant. Under Swern conditions, oxidation and dehydration of **11** occurred simultaneously to yield the unsaturated aldehyde **12**.<sup>2,7</sup> Compound **12** was reacted with the appropriate acetophenones in the presence of *p*-toluenesulfonic acid (*p*-TsOH) as catalyst to

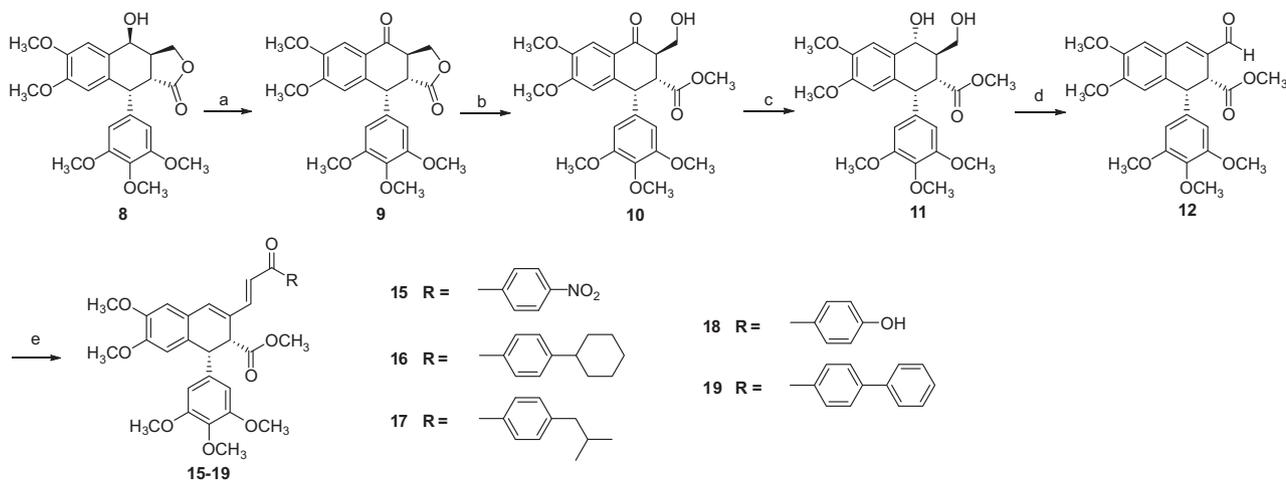
provide analogs **15–19**. The *trans*- $\Delta$ <sup>9,10</sup> stereochemistry in compounds **15–19** was confirmed by the measured  $J_{9,10}$  values (around 15.0 Hz).

Another three analogs **20–22** with  $\beta$ -configuration at C8' were also prepared from **8** (Scheme 3). Aminolysis of **8** under reflux with pyrrolidine as both reactant and solvent provided the dihydroxyamide **13** in good yield. Swern oxidation of **13** afforded the aldehyde–amide **14**, and subsequent aldol condensation of **14** with the corresponding acetophenones produced compounds **20–22**. The C8' $\beta$ -configuration in analogs **20–22** was achieved with a basic reaction milieu and supported by the <sup>1</sup>H NMR data. It has been well recognized that C8' epimerization occurs readily under even mildly basic conditions. In fact, C8' epimerization was previously observed in the presence of 0.1 M piperidine.<sup>8</sup> Furthermore, the chemical shifts of H-7' in **20–22** were upfield from those in **15–19** (~4.34 vs ~4.56), which is consistent with the previous observation for *etoposide* and its C8' $\beta$ -isomer.<sup>9</sup>

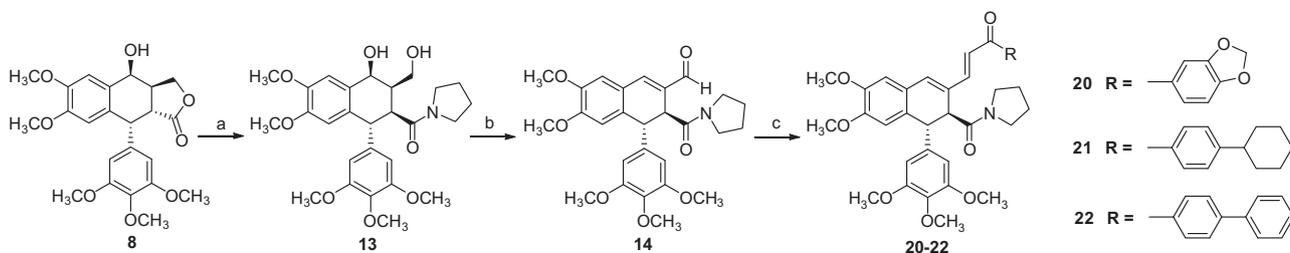
Analog **15–22** were evaluated for their inhibitory activity against the growth of tumor cell lines with an SRB assay. Four



**Scheme 1.** Reagents conditions: (a): (i)  $\text{BCl}_3/\text{CH}_2\text{Cl}_2$ , 0 °C, 6 h; (ii) acetone–water– $\text{CaCO}_3$ , reflux, 3 h; (b):  $\text{CH}_3\text{I}$ ,  $\text{K}_2\text{CO}_3$ ,  $\text{Et}_4\text{NF}$ , acetone, rt, 24 h; (yield 82% for two steps).



**Scheme 2.** Reagents and conditions: (a): PDC/ $\text{CH}_2\text{Cl}_2$ , rt, 2 h, 51%; (b):  $\text{H}_2\text{SO}_4/\text{CH}_3\text{OH}$ , reflux, 2 h, 55%; (c):  $\text{NaBH}_4/\text{CH}_3\text{OH}$ , rt, 0.5 h, 90%; (d):  $(\text{COCl})_2$ , DMSO,  $\text{Et}_3\text{N}$ ,  $\text{CH}_2\text{Cl}_2$ , –65 to –70 °C, 74%; (e): corresponding acetophenones, *p*-TsOH, DCM, reflux, 2–5 d.



**Scheme 3.** Reagents and conditions: (a): pyrrolidine, reflux, 2 h; (b):  $(\text{COCl})_2$ , DMSO,  $\text{Et}_3\text{N}$ ,  $\text{CH}_2\text{Cl}_2$ , –65 to –70 °C, 66%; (c): corresponding acetophenones, *p*-TsOH, DCM, reflux, 2–5 d, 20–50%.

**Table 1**

Inhibitory activity of selected analogs against A549, DU145, KB and KBvin tumor cell lines

Compound	IC <sub>50</sub> (μg/mL)			
	A549	DU145	KB	KBvin
<b>15</b>	16.8 ± 1.32	20.3 ± 0.61	16.0 ± 1.66	23.0 ± 0.35
<b>18</b>	6.79 ± 0.76	5.84 ± 0.91	5.90 ± 1.13	5.17 ± 0.96
<b>21</b>	12.0 ± 0.92	12.8 ± 2.15	12.28 ± 1.84	12.29 ± 2.36
<b>22</b>	24.6 ± 2.02	15.46 ± 1.19	16.6 ± 3.87	16.9 ± 0.71
GL-331*	0.113 ± 0.014	0.800 ± 0.056	0.819 ± 0.162	2.10 ± 0.378

\* GL-331 is a podophyllotoxin analog that previously reached clinical trials.<sup>1</sup>

compounds (**15**, **18**, **21**, and **22**) demonstrated noticeable inhibitory activity against A549, DU145, KB and KBvin tumor cells, and the most active compound **18** exhibited IC<sub>50</sub> values less than 10 μg/mL (Table 1).

In summary, a series of novel podophyllotoxin analogs featuring 4,5-dimethoxy substitution and an opened D ring were synthesized and evaluated for cytotoxic activity. In contrast to previous SAR deduced from Topo II inhibition, which requires intact A and D rings for retention of activity, analogs with modified A and D rings, such as **18**, exhibited evident in vitro anticancer activity.

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### References and notes

- Cragg, G. M.; Kingston, D. G. I.; Newman, D. J. *Anticancer Agents from Natural Products*; CRC Press Taylor & Francis Group: London UK, 2005. 1<sup>st</sup> ed., pp. 71.
- Gordaliza, M.; Castro, M. A.; Miguel del Corral, J. M.; López-Vázquez, M. L.; García, P. A.; Feliciano, A. S.; García-Grávalos, M. D.; Broughton, H. *Tetrahedron* **1997**, *53*, 15743.
- Castro, M. A.; MigueldelCorral, J. M.; Gordaliza, M.; García, P.; Gómez-Zurita, M. A.; García-Grávalos, M. D.; de la Iglesia-Vincente, J.; Gajate, C.; An, F.; Mollinedo, F.; Feliciano, A. S. *J. Med. Chem.* **2004**, *47*, 1214.
- Cho, S. J.; Kashiwada, Y.; Bastow, K. F.; Cheng, Y. C.; Lee, K. H. *J. Med. Chem.* **1996**, *39*, 1396.
- General Preparation of Compounds 15–22*: To a solution of **12** or **14** (0.25 mmol) in 15 mL of anhydrous CH<sub>2</sub>Cl<sub>2</sub> were added *p*-TsOH (0.15 mmol) and the corresponding acetophenones (0.5 mmol). The reaction mixture was stirred at room temperature for 2 to 5 days and then washed with 5% NaHCO<sub>3</sub> and saturated aqueous NaCl. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The residue was chromatographed on silica gel and afforded the

corresponding target molecules. **Compound 15**: Yield: 16.9%; mp: 100–102 °C; [α]<sub>D</sub><sup>20</sup> +322.4 (c, 0.55, CHCl<sub>3</sub>); HR-ESI-MS: *m/z* 590.2011 [M+H]<sup>+</sup> (calcd 590.2021); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 8.31 (d, 2H, *J* = 8.4 Hz, –ArH), 8.08 (d, 2H, *J* = 8.4 Hz, –ArH), 7.68 (d, 1H, *J* = 15.6 Hz, 9-H), 7.10 (d, 1H, *J* = 15.6 Hz, 10-H), 7.07 (s, 1H, 7-H), 6.85 (s, 1H, 6-H), 6.62 (s, 1H, 3-H), 6.59 (s, 2H, 2', 6'-H), 4.57 (d, 1H, *J* = 6.9 Hz, 7'-H), 3.73–3.93 (16H, 5×OCH<sub>3</sub>, 8'-H), 3.50 (s, 3H, –COOCH<sub>3</sub>). **Compound 16**: Yield: 55.1%; mp: 107–109 °C; [α]<sub>D</sub><sup>20</sup> +265.2 (c, 0.7, CHCl<sub>3</sub>); HR-ESI-MS: *m/z* 627.2928 [M+H]<sup>+</sup> (calcd 627.2952); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.90 (d, 2H, *J* = 8.1 Hz, –ArH), 7.62 (d, 1H, *J* = 15.6 Hz, 9-H), 7.29 (d, 2H, *J* = 8.1 Hz, –ArH), 7.17 (d, 1H, *J* = 15.3 Hz, 10-H), 6.99 (s, 1H, 7-H), 6.83 (s, 1H, 6-H), 6.60 (s, 3H, 2', 6'-H, 3-H), 4.56 (d, 1H, *J* = 7.5 Hz, 7'-H), 3.72–3.93 (16H, 5×OCH<sub>3</sub>, 8'-H), 3.49 (s, 3H, –COOCH<sub>3</sub>), 2.56 (m, 1H, –CH–), 1.26–1.86 (m, 10H, –(CH<sub>2</sub>)<sub>5</sub>–). **Compound 17**: Yield: 9.2%; mp: 95–97 °C; [α]<sub>D</sub><sup>20</sup> +260 (c, 0.5, CHCl<sub>3</sub>); HR-ESI-MS: *m/z* 601.2780 [M+H]<sup>+</sup> (calcd 601.2796); <sup>1</sup>H NMR (300 MHz, acetone-*d*<sub>6</sub>): δ 7.91 (d, 2H, *J* = 8.1 Hz, –ArH), 7.60 (d, 1H, *J* = 15.3 Hz, 9-H), 7.33 (d, 2H, *J* = 8.1 Hz, –ArH), 7.24 (d, 1H, *J* = 15.3 Hz, 10-H), 7.16 (s, 1H, 7-H), 7.02 (s, 1H, 6-H), 6.76 (s, 2H, 2', 6'-H), 6.67 (s, 1H, 3-H), 4.56 (d, 1H, *J* = 7.1 Hz, 7'-H), 3.99 (d, 1H, *J* = 7.1 Hz, 8'-H), 3.66–3.85 (15H, 5×OCH<sub>3</sub>), 3.32 (s, 3H, –COOCH<sub>3</sub>), 2.56 (d, 1H, *J* = 7.1 Hz, –CH–), 1.91 (m, 1H, –CH–), 0.99 (s, 3H, CH<sub>3</sub>), 0.96 (s, 3H, CH<sub>3</sub>). **Compound 18**: Yield: 41.7%; mp: 126–129 °C; [α]<sub>D</sub><sup>20</sup> +260.9 (c, 0.7, CHCl<sub>3</sub>); HR-ESI-MS: *m/z* 561.2132 [M+H]<sup>+</sup> (calcd. 561.2119); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.93 (d, 2H, *J* = 8.4 Hz, –ArH), 7.62 (d, 1H, *J* = 15.3 Hz, 9-H), 7.16 (d, 1H, *J* = 15.6 Hz, 10-H), 6.99 (s, 1H, 7-H), 6.88 (d, 2H, *J* = 9.0 Hz, –ArH), 6.83 (s, 1H, 6-H), 6.60 (s, 3H, 2', 6'-H, 3-H), 4.56 (d, *J* = 7.2 Hz, 1H, 7'-H), 3.87 (s, 1H, 8'-H), 3.72–3.92 (15H, 5×OCH<sub>3</sub>), 3.49 (s, 3H, –COOCH<sub>3</sub>). **Compound 19**: Yield: 13.7%; mp: 124–126 °C; [α]<sub>D</sub><sup>20</sup> +311.1 (c, 0.3, CHCl<sub>3</sub>); HR-ESI-MS: *m/z* 621.2471 [M+H]<sup>+</sup> (calcd 621.2483); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 8.05 (d, 2H, *J* = 8.4 Hz, –ArH), 7.45–7.69 (m, 5H, –ArH), 7.63 (d, 2H, *J* = 8.7 Hz, –ArH), 7.47 (d, 1H, *J* = 14.4 Hz, 9-H), 7.22 (d, 1H, *J* = 15.0 Hz, 10-H), 7.03 (s, 1H, 7-H), 6.85 (s, 1H, 6-H), 6.61 (s, 3H, 2', 6'-H, 3-H), 4.57 (d, 1H, *J* = 6.9 Hz, 7'-H), 3.73–3.93 (16H, 5×OCH<sub>3</sub>, 8'-H), 3.51 (s, 3H, –COOCH<sub>3</sub>). **Compound 20**: Yield: 10.4%; mp: 110–113 °C; [α]<sub>D</sub><sup>20</sup> –244.4 (c, 0.6, CHCl<sub>3</sub>); HR-ESI-MS: *m/z* 628.2564 [M+H]<sup>+</sup> (calcd. 649.2541); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.55 (d, 1H, *J* = 15.6 Hz, 9-H), 7.43 (dd, 1H, *J* = 8.4; 1.5 Hz, –ArH), 7.36 (d, 1H, *J* = 1.5 Hz, –ArH), 7.08 (s, 1H, 7-H), 6.82 (d, 1H, *J* = 9.0 Hz, –ArH), 6.81 (s, 1H, 6-H), 6.74 (d, 1H, *J* = 15.6 Hz, 10-H), 6.55 (s, 1H, 3-H), 6.46 (s, 2H, 2', 6'-H), 6.04 (s, 2H, –OCH<sub>2</sub>O–), 4.34 (d, 1H, *J* = 6.3 Hz, 7'-H), 3.96 (d, 1H, *J* = 6.3 Hz, 8'-H), 3.78–3.91 (15H, 5×OCH<sub>3</sub>), 3.32–3.56 (m, 4H, –N(CH<sub>2</sub>)<sub>2</sub>–), 1.87–2.04 (m, 4H, –(CH<sub>2</sub>)<sub>2</sub>–). **Compound 21**: Yield: 10.0%; mp: 113–115 °C; [α]<sub>D</sub><sup>20</sup> –488.9 (c, 0.3, CHCl<sub>3</sub>); HR-ESI-MS: *m/z* 666.3406 [M+H]<sup>+</sup> (calcd 666.3425); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.77 (d, 2H, *J* = 8.4 Hz, –ArH), 7.56 (d, 1H, *J* = 15.9 Hz, 9-H), 7.27 (d, 2H, *J* = 8.1 Hz, –ArH), 7.08 (s, 1H, 7-H), 6.81 (s, 1H, 6-H), 6.78 (d, 1H, *J* = 16.2 Hz, 10-H), 6.56 (s, 1H, 3-H), 6.46 (s, 2H, 2', 6'-H), 4.33 (d, 1H, *J* = 6.0 Hz, 7'-H), 3.97 (d, 1H, *J* = 6.0 Hz, 8'-H), 3.78–3.91 (15H, 5×OCH<sub>3</sub>), 3.31–3.59 (m, 4H, –N(CH<sub>2</sub>)<sub>2</sub>–), 2.56 (m, 1H, –CH(CH<sub>2</sub>)<sub>2</sub>–), 1.79–1.99 (m, 8H, –(CH<sub>2</sub>)<sub>2</sub>–; –CH(CH<sub>2</sub>)<sub>2</sub>–), 1.33–1.41 (m, 6H, –(CH<sub>2</sub>)<sub>3</sub>–). **Compound 22**: Yield: 21.2%; mp: 115–117 °C; [α]<sub>D</sub><sup>20</sup> –358.8 (c, 0.55, CHCl<sub>3</sub>); HR-ESI-MS: *m/z* 660.2937 [M+H]<sup>+</sup> (calcd 660.2956); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.92 (d, 2H, *J* = 8.4 Hz, –ArH), 7.66 (d, 2H, *J* = 8.1 Hz, –ArH), 7.42–7.64 (m, 5H, –ArH), 7.47 (d, 1H, *J* = 14.4 Hz, 9-H), 7.11 (s, 1H, 7-H), 6.82 (d, 1H, *J* = 15.9 Hz, 10-H), 6.82 (s, 1H, 6-H), 6.56 (s, 1H, 3-H), 6.47 (s, 2H, 2', 6'-H), 4.35 (d, 1H, *J* = 6.0 Hz, 7'-H), 3.99 (d, 1H, *J* = 6.3 Hz, 8'-H), 3.79–3.91 (15H, 5×OCH<sub>3</sub>), 3.34–3.60 (m, 4H, –N(CH<sub>2</sub>)<sub>2</sub>–); 1.84–2.00 (m, 4H, –(CH<sub>2</sub>)<sub>2</sub>–).

- Wang, Z. Q.; Hu, H.; Chen, H. X.; Cheng, Y. C.; Lee, K. H. *J. Med. Chem.* **1992**, *35*, 871.
- Mancuso, A. J.; Swern, D. *Synthesis* **1981**, 165.
- Gensler, W. J.; Gatsons, C. D. *J. Org. Chem.* **1966**, *31*, 3224.
- Aso, Y.; Hayashi, Y.; Yoshioka, S.; Takeda, Y.; Kita, Y.; Nishimura, Y.; Arata, Y. *Chem. Pharm. Bull.* **1989**, *37*, 422.