

## An Efficient Synthesis of 5-Bromo-2-methoxy-6-methylaminopyridine-3-carboxylic Acid

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Received April 26, 2000; accepted September 9, 2000

An efficient synthesis of 5-bromo-2-methoxy-6-methylaminopyridine-3-carboxylic acid (**1**), a carboxylic acid moiety of a potent dopamine D<sub>2</sub> and D<sub>3</sub> and serotonin-3 (5-HT<sub>3</sub>) receptors antagonist, (*R*)-5-bromo-*N*-(1-ethyl-4-methylhexahydro-1,4-diazepin-6-yl)-2-methoxy-6-methylaminopyridine-3-carboxamide, is described. Reaction of methyl 2,6-difluoropyridine-3-carboxylate (**12**) with methylamine in EtOH at –25 °C gave a mixture of methyl 2-fluoro-6-methylaminopyridine-3-carboxylate (**13**) and the regioisomer **14** in a ratio of 57 : 43. On the other hand, reaction of **12** and methyl 2,6-dichloropyridine-3-carboxylate (**16**) with sodium methoxide in tetrahydrofuran (THF) and CH<sub>2</sub>Cl<sub>2</sub> provided the 2-methoxypyridine-3-carboxylic esters **20** and **23**, respectively, as main products. Similar reaction of **16** in *N,N*-dimethylformamide (DMF) and MeOH proved to be highly regioselective for the 6-position. A much greater regioselectivity for substitution at the 6-position (>97%) was observed when **16** was treated with 4-methylbenzenethiolate anion in DMF (quantitative yield). After methoxylation of methyl 2-chloro-6-(4-methylbenzenethio)pyridine-3-carboxylate (**25b**) and successive oxidation of the 6-benzenethio moiety, nucleophilic substitution of the sulfoxide derivative **28** with methylamine gave the 6-methylamino derivative **8**. Finally, bromination of **8** and alkaline hydrolysis produced the desired product **1** in an overall yield of 67%.

**Key words** serotonin 5-HT<sub>3</sub> receptor; dopamine D<sub>2</sub>; dopamine D<sub>3</sub>; antiemetic agent; regioselective synthesis

Potent and selective serotonin-3 (5-HT<sub>3</sub>) receptor antagonists, such as DAT-582<sup>1)</sup> [(*R*)-*N*-[1-methyl-4-(3-methylbenzyl)hexahydro-1,4-diazepin-6-yl]-1*H*-indazole-3-carboxamide dihydrochloride], discovered in our laboratories, granisetron and ondansetron are effective in the control of emesis induced by cancer chemotherapeutic agents.<sup>2)</sup> The traditional antiemetic agent domperidone, a peripheral dopamine D<sub>2</sub> receptor antagonist, has been shown to be effective in the prevention of nausea and vomiting resulting from a variety of causes.<sup>3)</sup> However, domperidone is only minimally effective against chemotherapy-induced nausea and vomiting.<sup>4)</sup> In the course of our studies on the structure–activity relationships (SARs) of DAT-582,<sup>5)</sup> benzamides with an alkyl group on the nitrogen atom in the hexahydro-1,4-diazepine ring, such as the 1-ethyl-4-methylhexahydro-1,4-diazepine ring were found to show dopamine D<sub>2</sub> receptor antagonistic activity along with a potent 5-HT<sub>3</sub> receptor antagonistic activity. This finding suggested that these compounds could be broad antiemetic agents, and led us to modify the benzene ring and prepare the optically active 6-aminohexahydro-1,4-diazepine ring, resulting in the discovery of (*R*)-5-bromo-*N*-(1-ethyl-4-methylhexahydro-1,4-diazepin-6-yl)-2-methoxy-6-methylaminopyridine-3-carboxamide difumarate (originally AS-8112) with a potent dopamine D<sub>2</sub> and D<sub>3</sub> and 5-HT<sub>3</sub> receptors antagonistic activity. AS-8112 was finally selected as a promising broad antiemetic agent.<sup>6)</sup> In order to obtain a large amount of AS-8112, an efficient synthesis of 5-bromo-2-methoxy-6-methylaminopyridine-3-carboxylic acid (**1**) was essential. This paper describes the synthetic route to **1** from the 2,6-dichloropyridine-3-carboxylic ester **16**.

### Results and Discussion

**Synthesis of 5-Bromo-2-methoxy-6-methylaminopyridine-3-carboxylic Acid (**1**) from 2,6-Difluoropyridine (**2**)**  
For the success of preparation of **1**, introduction of nitrogen and oxygen functions at the 6- and 2-positions of the pyridine ring, respectively, is an important strategy. Coldwell *et*

*al.* reported the synthesis of 6-amino-5-chloro-2-methoxypyridine-3-carboxylic acid (**3**)<sup>7)</sup> from 2,6-difluoropyridine (**2**) via 2-fluoro-6-pivaloylaminopyridine (**4**). However, the overall yield was poor. In our study we first prepared the key intermediate **8**, methyl 2-methoxy-6-methylaminopyridine-3-carboxylate, from ethyl 2-fluoro-6-(*N*-methyl-*N*-pivaloyl)aminopyridine-3-carboxylate (**6**) according to the method of Coldwell *et al.*<sup>7)</sup> Selective displacement of one fluorine atom of **2** by methylamine in EtOH at *ca.* 140 °C and successive acylation with pivaloyl chloride afforded 2-fluoro-6-(*N*-methyl-*N*-pivaloyl)aminopyridine (**5**) in 65% yield. Unfortunately, ortho-directed lithiation of **5** with *n*-BuLi followed by treatment with ethyl chloroformate did not give the corresponding pyridine-3-carboxylic ester **6** and the starting **5** was recovered. We then examined *N*-methylation of ethyl 2-fluoro-6-pivaloylaminopyridine-3-carboxylate (**7**)<sup>7)</sup> prepared from **4**. Reaction of **7** with MeI in the presence of NaH pro-

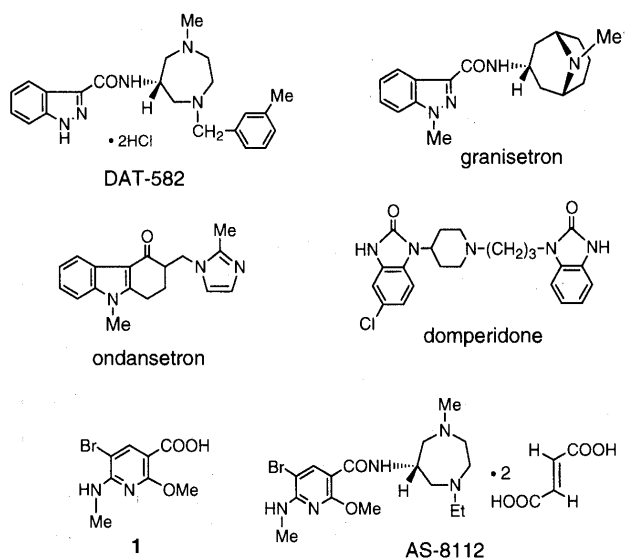
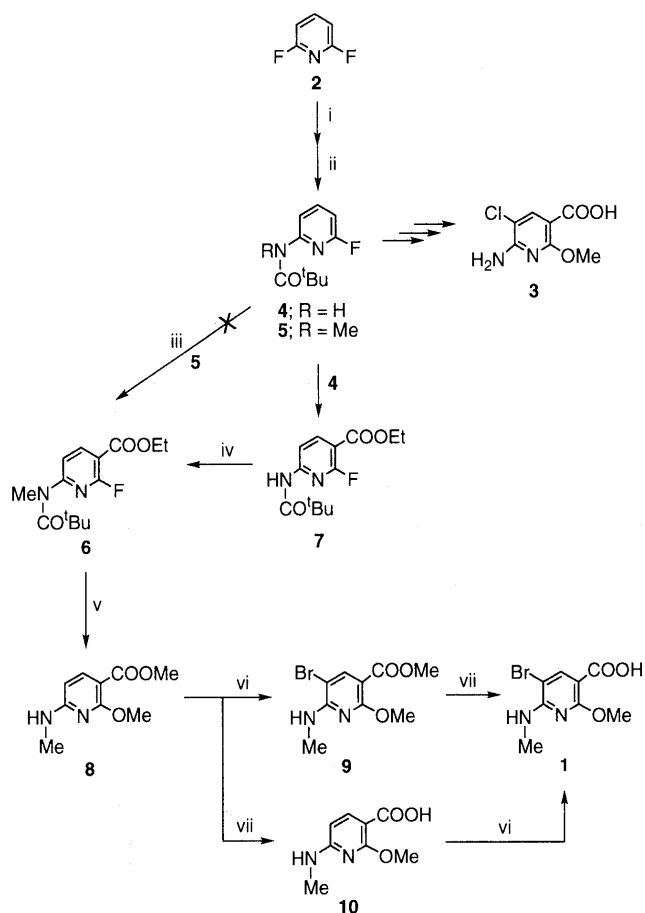


Fig. 1

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Reagents and conditions: i,  $\text{MeNH}_2$ , EtOH,  $140^\circ\text{C}$ ; ii,  $\text{tert-BuCOCl}$ ,  $\text{Et}_3\text{N}$ ,  $\text{CH}_2\text{Cl}_2$ ,  $0^\circ\text{C}$ ; iii,  $n\text{-BuLi}$ ,  $-78^\circ\text{C}$  then  $\text{ClCO}_2\text{Et}$ ; iv,  $\text{NaH}$  then  $\text{MeI}$ , DMF, room temperature; v,  $\text{tert-BuOK}$ , MeOH, reflux; vi, NBS, DMF,  $80^\circ\text{C}$ ; vii, aq.  $\text{NaOH}$ , reflux.

Chart 1

vided the 6-(*N*-methyl-*N*-pivaloyl)aminopyridine-3-carboxylate **6** in only 17% yield. Displacement reaction of the fluorine atom of **6** by methoxide anion produced from potassium *tert*-butoxide and MeOH was accompanied by ester exchange and de-acylation to give **8** in 68% yield. Bromination of **8** with *N*-bromosuccinimide (NBS) followed by alkaline hydrolysis of the resulting **9** produced the desired pyridine-3-carboxylic acid **1** in 96% yield. On the other hand, after alkaline hydrolysis of **8**, bromination of 2-methoxy-6-methylaminopyridine-3-carboxylic acid (**10**) gave the 5-bromopyridine-3-carboxylic acid **1** in 88% yield along with 3,5-dibromo-2-methoxy-6-methylaminopyridine which was thought to be formed *via* decarboxylation of **10** in *ca.* 5% yield (Chart 1).

**Nucleophilic Substitution Reaction of 2,6-Dihalo-3-pyridine-3-carboxylic Esters 12 and 16** In order to improve the overall yield of **1**, nucleophilic substitution reaction of methyl 2,6-difluoropyridine-3-carboxylate (**12**) prepared from 2,6-difluoropyridine-3-carboxylic acid (**11**)<sup>8)</sup> with methylamine was carried out. Treatment of **12** with 20% methylamine in EtOH at  $-25^\circ\text{C}$  gave a mixture of methyl 2-fluoro-6-methylaminopyridine-3-carboxylate (**13**) and the regioisomer of **13**, methyl 6-fluoro-2-methylaminopyridine-3-carboxylate (**14**). The mixture was separated into the less polar compound **14** (42%) as an oil and the more polar compound **13** (55%) as a solid by crystallization and flash chro-

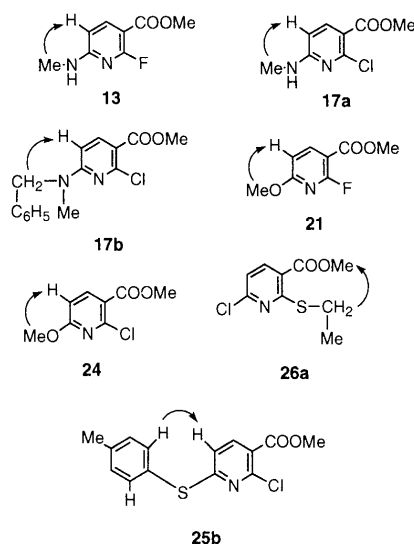
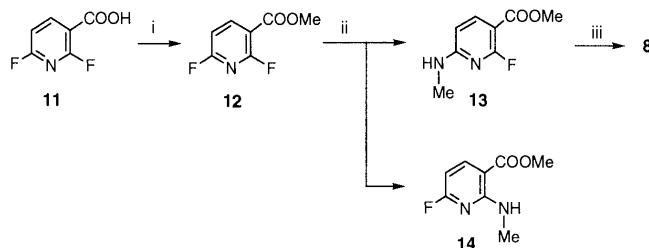


Fig. 2. Significant NOE Signals Observed upon Irradiation of the Methyl Group, Methylene Moiety, or Aromatic Proton of **13**, **17a**, **17b**, **21**, **24**, **25b**, **26a**



Reagents and conditions: i, MeOH,  $\text{H}_2\text{SO}_4$ , reflux; ii,  $\text{MeNH}_2$ , EtOH,  $-25^\circ\text{C}$ ; iii,  $\text{tert-BuOK}$ , MeOH, reflux.

Chart 2

matography on silica gel. The position of the methylamino group of **13** was determined by differential nuclear Overhauser effect (NOE) experiment; irradiation at  $\delta$  2.98 (*N*-Me) enhanced signal intensity of the adjacent pyridine 5-proton ( $\delta$  6.23) (Fig. 2). Treatment of **13** with potassium *tert*-butoxide in MeOH afforded **8** in 97% yield (Chart 2). Next, the effects of solvents and temperature on the reaction of **12** with methylamine were examined (Table 1). The ratio of the mixture of 6- and 2-substituted pyridines **13** and **14** was determined by  $^1\text{H}$ -NMR spectroscopy. The reaction in MeOH at  $5^\circ\text{C}$  for 10 min gave a mixture of **13** and **14** in a ratio of 46:54 in a quantitative yield (run 1). Similar reaction at  $-78^\circ\text{C}$  did not proceed (run 2). The reaction in *N,N*-dimethylformamide (DMF) at  $5^\circ\text{C}$  and lower temperatures ( $-30^\circ\text{C}$ ,  $-60^\circ\text{C}$ ) afforded the 6-methylaminopyridine-3-carboxylic ester **13** as major product (runs 3—5). Using  $\text{CH}_3\text{CN}$ , tetrahydrofuran (THF) and  $\text{CH}_2\text{Cl}_2$  as solvents at  $5^\circ\text{C}$ , the yield of the 6-methylaminopyridine-3-carboxylic ester **13** was poor compared with that observed in the reaction in DMF (runs 6—8). This indicates that the choice of solvent is important for the regioselective substitution reaction.

Similarly, the behavior of the more available methyl 2,6-dichloropyridine-3-carboxylate (**16**)<sup>9)</sup> was then examined (Table 2). Since the reactivity towards a nucleophile of **16** is poor compared with that of **12**, longer reaction time is neces-

sary. Ester **16** prepared by esterification of the commercially available 2,6-dichloropyridine-3-carboxylic acid (**15**) was treated with methylamine in THF and DMF at 5 °C for 3 h to afford the 2-methylaminopyridine-3-carboxylic ester **18a** as main product together with the regioisomer **17a** and the amide derivative **19** (runs 1, 2). In a similar reaction in MeOH, the main product was *N*-methyl-2,6-dichloropyridinecarboxamide (**19**) (run 3). Although the reaction of **16** with the secondary amine, *N*-benzylmethylamine, in THF showed good selectivity, the main product was the undesired 2-substitution product **18b** (run 4). Confirmation of the structure of **17a, b** was provided by differential NOE experiments; irradiation at  $\delta$  2.98 (*N*-Me) of **17a** and  $\delta$  4.82 (the methylene proton of *N*-CH<sub>2</sub>Ph) of **17b** enhanced signal intensity of the pyridine 5-proton ( $\delta$  6.29) of **17a** and ( $\delta$  6.38) of **17b**, respectively (Fig. 2).

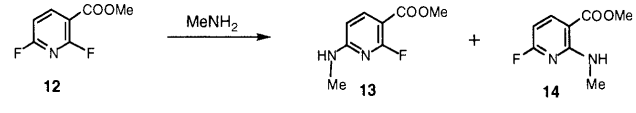
Kawato and Newkome reported that the reaction of 3-cyano-2,6-dichloropyridine and *N,N*-dimethyl-2,6-dichloropyridine-3-carboxamide with electron withdrawing groups at

the 3-position with sodium ethoxide in xylene gives 2-ethoxypyridine derivatives as major products.<sup>9)</sup> As an extension to our nucleophilic substitution reaction of the 2,6-difluoro and 2,6-dichloropyridine-3-carboxylic esters **12** and **16**, the reaction with methoxide anion was examined (Tables 3, 4). The reaction of **12** in CH<sub>3</sub>CN, CH<sub>2</sub>Cl<sub>2</sub> and THF at 5 °C gave the same results described above; regioselectivity for methoxide anion at the 2-position was enriched to give the 2-methoxypyridine-3-carboxylic ester **20** in spite of the reaction temperature (runs 1–5 in Table 3). Treatment in MeOH at 5 °C produced the 6-methoxypyridine-3-carboxylic ester **21** as main product (run 6 in Table 3). The reaction in DMF did not give a good result as the disubstituted product, methyl 2,6-dimethoxypyridine-3-carboxylate (**22**) was obtained as main product (run 7 in Table 3). Confirmation of the structure of **21** was provided by differential NOE experiment; irradiation at  $\delta$  3.92 (OMe) enhanced signal intensity of the pyridine 5-proton ( $\delta$  6.66) (Fig. 2).

Treatment of **16** with sodium methoxide in CH<sub>2</sub>Cl<sub>2</sub> gave a good ratio of **23** and **24** (85 : 15) (run 1 in Table 4). The mixture of **23** and **24** was oil, and both products showed approximately the same *R<sub>f</sub>* value on TLC with common solvent combinations. In a similar reaction in THF, the ratio of the 2-methoxypyridine-3-carboxylic ester **23**, the regioisomer **24** and **22** was 79 : 14 : 7 (run 2 in Table 4). Using DMF and MeOH, however, the 6-methoxypyridine-3-carboxylic ester **24** was obtained as main product (runs 3 and 4 in Table 4). In the reaction conditions of run 4, conversion of the sodium counter cation into potassium cation had no remarkable influence on the reaction (run 5 in Table 4). Confirmation of the structure of **24** was provided by differential NOE experiment; irradiation at  $\delta$  3.91 (OMe) enhanced signal intensity of the pyridine 5-proton ( $\delta$  6.70) (Fig. 2).

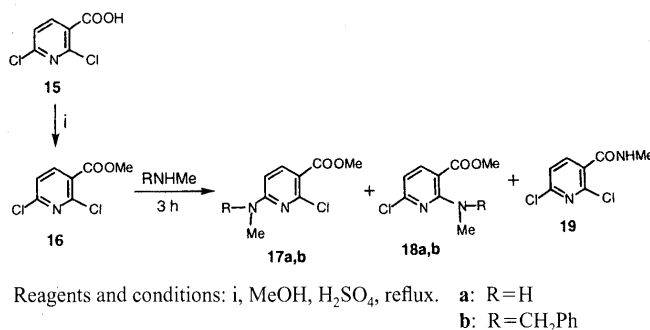
On the basis of the results described above, the reaction of **16** with sulfur nucleophile in DMF was finally investigated (Table 5). Treatment of **16** with ethanethiol in DMF in the presence of NaH at 5 °C gave the 6-ethylthiopyridine derivative **25a** and the regioisomer **26a** in 91 : 9 ratio in a good yield (run 1). Potassium *tert*-butoxide used instead of NaH as

Table 1. Reaction of Methyl 2,6-Difluoropyridine-3-carboxylate **12** with Methylamine

|  |                                                |                         |    |
|------------------------------------------------------------------------------------|------------------------------------------------|-------------------------|----|
| Run                                                                                | Conditions                                     | Ratio (%) <sup>a)</sup> |    |
|                                                                                    |                                                | 13                      | 14 |
| 1                                                                                  | MeOH, 5 °C, 10 min                             | 46                      | 54 |
| 2                                                                                  | MeOH, -78 °C, 3 h                              | No reaction             |    |
| 3                                                                                  | DMF, 5 °C, 10 min                              | 55                      | 45 |
| 4                                                                                  | DMF, -30 °C, 1 h                               | 64                      | 36 |
| 5                                                                                  | DMF, -60 °C, 3 h <sup>b)</sup>                 | 53                      | 32 |
| 6                                                                                  | CH <sub>3</sub> CN, 5 °C, 10 min               | 40                      | 60 |
| 7                                                                                  | THF, 5 °C, 10 min                              | 39                      | 61 |
| 8                                                                                  | CH <sub>2</sub> Cl <sub>2</sub> , 5 °C, 10 min | 25                      | 75 |

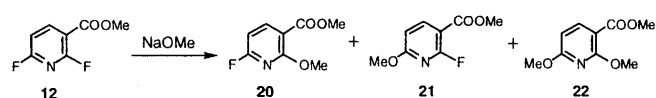
a) An almost quantitative yield was obtained. The ratio was determined by <sup>1</sup>H-NMR spectroscopy. See Experimental section. b) The presence of **12** was detected (15%).

Table 2. Reaction of Methyl 2,6-Dichloropyridine-3-carboxylate **16** with Methylamine



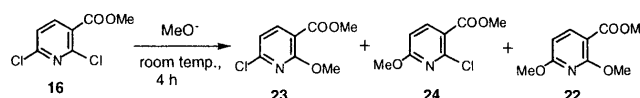
| Run | R                  | Conditions  | Ratio (%) <sup>a)</sup> |        |      |      |
|-----|--------------------|-------------|-------------------------|--------|------|------|
|     |                    |             | 17a, b                  | 18a, b | 19   | 16   |
| 1   | H                  | THF, 5 °C   | 12                      | 49     | 12   | 27   |
| 2   | H                  | DMF, 5 °C   | 32                      | 51     | 7    | 10   |
| 3   | H                  | MeOH, 5 °C  | 4                       | 9      | 37   | 50   |
| 4   | CH <sub>2</sub> Ph | THF, -20 °C | 14                      | 86     | N.D. | N.D. |

a) An almost quantitative yield was obtained. The ratio was determined by <sup>1</sup>H-NMR spectroscopy. See Experimental section. N.D.: Not detected.

Table 3. Reaction of Methyl 2,6-Difluoropyridine-3-carboxylate **12** with Sodium Methoxide

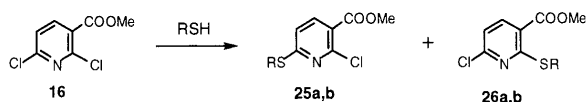
| Run | Conditions                                     | Ratio (%) <sup>a)</sup> |    |      |      |
|-----|------------------------------------------------|-------------------------|----|------|------|
|     |                                                | 20                      | 21 | 22   | 12   |
| 1   | CH <sub>3</sub> CN, 5 °C, 10 min               | 61                      | 19 | 10   | 10   |
| 2   | CH <sub>2</sub> Cl <sub>2</sub> , 5 °C, 10 min | 74                      | 10 | 8    | 8    |
| 3   | THF, 5 °C, 10 min                              | 77                      | 14 | 4.5  | 4.5  |
| 4   | THF, -30 °C, 1.5 h                             | 67                      | 18 | N.D. | 15   |
| 5   | THF, -78 °C, 3 h                               | 15                      | 4  | N.D. | 81   |
| 6   | MeOH, 5 °C, 10 min                             | 18                      | 80 | 2    | N.D. |
| 7   | DMF, 5 °C, 10 min                              | 15                      | 27 | 29   | 2    |

a) An almost quantitative yield was obtained. The ratio was determined by <sup>1</sup>H-NMR spectroscopy. See Experimental section. N.D.: Not detected.

Table 4. Reaction of Methyl 2,6-Dichloropyridine-3-carboxylate **16** with Methoxide Anion

| Run | Conditions                               | Ratio (%) <sup>a)</sup> |    |      |      |
|-----|------------------------------------------|-------------------------|----|------|------|
|     |                                          | 23                      | 24 | 22   | 16   |
| 1   | NaOMe in CH <sub>2</sub> Cl <sub>2</sub> | 85                      | 15 | N.D. | N.D. |
| 2   | NaOMe in THF                             | 79                      | 14 | 7    | N.D. |
| 3   | NaOMe in DMF                             | 28                      | 69 | 3    | N.D. |
| 4   | NaOMe in MeOH                            | 24                      | 74 | 2    | N.D. |
| 5   | <i>tert</i> -BuOK/MeOH                   | 20                      | 72 | N.D. | 8    |

a) An almost quantitative yield was obtained. The ratio was determined by <sup>1</sup>H-NMR spectroscopy. See Experimental section. N.D.: Not detected.

Table 5. Reaction of Methyl 2,6-Dichloropyridine-3-carboxylate **16** with the Thiolate Anions

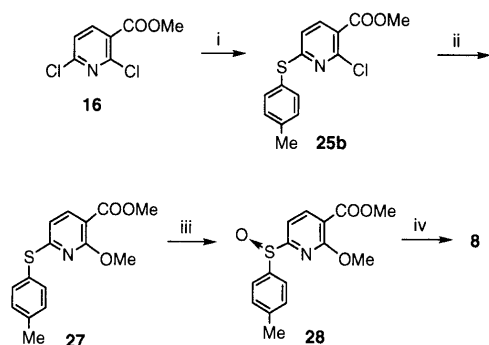
a: R = Et  
b: R = 4-MeC<sub>6</sub>H<sub>4</sub>

| Run | R                                 | Conditions                                 | Ratio (%) <sup>a)</sup> |        |                    |
|-----|-----------------------------------|--------------------------------------------|-------------------------|--------|--------------------|
|     |                                   |                                            | 25a, b                  | 26a, b | 16                 |
| 1   | Et                                | NaH, DMF, 5 °C, 1 h                        | 91                      | 9      | N.D. <sup>b)</sup> |
| 2   | Et                                | <i>tert</i> -BuOK, DMF, -5 °C, 2 h         | 88                      | 12     | N.D.               |
| 3   | Et                                | <i>tert</i> -BuOK, THF, -5 °C, 1 h         | 50                      | 50     | N.D.               |
| 4   | Et                                | <i>tert</i> -BuOK, THF, -30 °C, 1 h        | 50                      | 50     | N.D.               |
| 5   | 4-MeC <sub>6</sub> H <sub>4</sub> | NaH, DMF, room temp., 15 min               | 52                      | 48     | N.D.               |
| 6   | 4-MeC <sub>6</sub> H <sub>4</sub> | <i>tert</i> -BuOK, DMF, room temp., 45 min | 93.7                    | 6.3    | N.D.               |
| 7   | 4-MeC <sub>6</sub> H <sub>4</sub> | <i>tert</i> -BuOK, DMF, 5 °C, 30 min       | 94.1                    | 5.9    | N.D.               |
| 8   | 4-MeC <sub>6</sub> H <sub>4</sub> | <i>tert</i> -BuOK, DMF, -5 °C, 1 h         | 95.2                    | 4.8    | N.D.               |
| 9   | 4-MeC <sub>6</sub> H <sub>4</sub> | <i>tert</i> -BuOK, DMF, -30 °C, 1 h        | 97.0                    | 3.0    | N.D.               |
| 10  | 4-MeC <sub>6</sub> H <sub>4</sub> | <i>tert</i> -BuOK, THF, room temp., 1 h    | 65                      | 22     | 13                 |

a) An almost quantitative yield was obtained. The ratio was determined by <sup>1</sup>H-NMR spectroscopy. See Experimental section. N.D.: Not detected.

a base reduced the ratio of **25a** (run 2). Furthermore, replacement of DMF by THF caused a decrease in the ratio of **25a** regardless of the reaction temperature (runs 3, 4). Next, a similar reaction with more bulky 4-methylbenzenethiol in DMF in the presence of NaH at room temperature furnished approximately the same ratio of the products **25b** and **26b** (run 5). Surprisingly, treatment of **16** with 4-methylbenzenethiol in DMF in the presence of potassium *tert*-butoxide at room temperature gave **25b** and **26b** in a ratio of 93.7 : 6.3 (run 6). Lower temperatures resulted in an increase in the ratio of the product **25b** (runs 7–9). In particular, the reaction at -30 °C produced an excellent ratio of **25b**. Replacement of DMF by THF caused a decrease in the ratio of **25b**, and so did the reaction of **16** with ethanethiol (run 10). Confirmation of the structure of **25b** and **26a** was provided by differential NOE experiments; irradiation at  $\delta$  7.50 (aromatic H) of **25b** and  $\delta$  3.17 (methylene proton of ethyl group) of **26a** enhanced signal intensity of the pyridine 5-proton ( $\delta$  6.68) of **25b** and of the methyl ester group ( $\delta$  3.92) of **26a** (Fig. 2).

As shown in Tables 1–5, when MeOH and DMF were used as solvents, the selectivity for the 6-position of pyridine nucleus was high. On the other hand, in the case of CH<sub>3</sub>CN, CH<sub>2</sub>Cl<sub>2</sub> and THF, the main products were the 2-substituted pyridine derivatives. These interesting results can be explained as follows. The site of the initial nucleophilic attack is considered to be the halogen substituted carbon atom with strong inductive and electric field effects. When the carbon atoms at the 2- and 6-position were compared, the net charge of the carbon atom at the 2-position was more positive than that of the carbon atom at the 6-position. This is independent of the kind of solvent. The five solvents used above can be classified into two groups according to their polarity, namely MeOH, DMF and CH<sub>3</sub>CN have higher dielectric constant,<sup>10)</sup> 32.6, 37.7 and 37.5, respectively, and CH<sub>2</sub>Cl<sub>2</sub> and THF have lower values 8.9 and 7.4, respectively. From their polarity and the reaction conditions shown in Tables 1–5, when solvents with lower polarity were used, the selectivity for the nucleophilic substitution reaction was found to be on the carbon atom at the 2-position. On the other hand, the use of



Reagents and conditions: i, 4-MeC<sub>6</sub>H<sub>4</sub>SH, *tert*-BuOK, DMF, -30 °C; ii, NaOMe, THF, reflux; iii, MCPBA, CH<sub>2</sub>Cl<sub>2</sub>, room temperature; iv, MeNH<sub>2</sub>, DMF, 60 °C.

Chart 3

more polar and better ionizing solvents having higher dielectric constants afforded predominantly the 6-isomers except for CH<sub>3</sub>CN. In general, the more dispersed the charge distribution of anionic transition state in a nucleophilic substitution reaction, the less solvated the transition state is. The result causes a slight decrease in reaction rates for substitution in higher polar solvent.<sup>11)</sup> All reactions described above simultaneously and competitively proceeded and involved the addition at the 2- and 6-position. Because the transition state is more solvated *via* the 6-adducts in more polar solvents compared with the 2-isomers, and the activation energy of the transition state in the reactions for the 6-isomers is lower than that of 2-isomers, the reaction in more polar solvents is considered to give the 6-substituted isomers as main product. In contrast, minor effect on the solvation in less polar solvents predominantly produced the 2-isomers as main products. CH<sub>3</sub>CN behaves as if it was a solvent with low dielectric constant. The detailed mechanism of reactions using CH<sub>3</sub>CN is still unknown. The significant selectivity at the 6-position of the 4-methylbenzenethiol shown in Table 5 was presumed to be the steric effect of nucleophile in addition to the solvent effect.

**Synthesis of Methyl 2-Methoxy-6-methylaminopyridine-3-carboxylate (8) from Methyl 2,6-Dichloropyridine-3-carboxylate (16)** Finally, a large scale synthesis of **8** using the reaction of **16** with 4-methylbenzenethiol was carried out (Chart 3). Treatment of **16** with 4-methylbenzenethiol in the presence of potassium *tert*-butoxide in DMF at -30 °C gave a mixture of **25b** and **26b** in a ratio of >97: <3 in a quantitative yield. The mixture was treated with sodium methoxide to afford the corresponding 2- and 6-methoxypyridine-3-carboxylates, which were purified by recrystallization from AcOEt to give the pure 2-methoxypyridine-3-carboxylate **27** in 87% yield. Oxidation of **27** with *m*-chloroperbenzoic acid (MCPBA) produced the sulfoxide **28** along with the corresponding sulfone derivative in a ratio of 11: 1 in an excellent yield. The ratio of the sulfoxide and sulfone was detected by <sup>1</sup>H-NMR spectroscopy and the mixture was purified by recrystallization to give **28**. Successively, treatment of **28**, which has a 4-methylbenzenesulfinyl group as a leaving group, with methylamine in DMF at *ca.* 60 °C gave methyl 2-methoxy-6-methylaminopyridine-3-carboxylate **8** in 90% yield. The product was identified with samples obtained from **6** or **13**, on the basis of TLC, IR and <sup>1</sup>H-NMR comparisons.

## Conclusion

We examined a large scale synthesis of 5-bromo-2-methoxy-6-methylaminopyridine-3-carboxylic acid (**1**). In order to ascertain the preferred site for nucleophilic attack on the pyridine nucleus, the reaction of the 2,6-difluoro and 2,6-dichloropyridine-3-carboxylic esters **12** and **16** with methylamine and methoxide anion was carried out under widely varied conditions. Although the reaction with methylamine did not result in a good selectivity, the reaction with methoxide anion, however, proved to be very selective as the preferred site for nucleophilic displacement in THF, CH<sub>2</sub>Cl<sub>2</sub> and CH<sub>3</sub>CN was found to be the 2-position. Reaction of **16** with methoxide and thiolate anions in DMF, on the other hand, afforded predominantly the 6-isomer. This indicates that the choice of solvent is very important in this reaction. It can therefore be concluded that the nucleophilic substitution reaction of **16** with 4-methylbenzenethiolate anion in DMF is a simple and efficient method for large scale synthesis of 5-bromo-2-methoxy-6-methylaminopyridine-3-carboxylic acid (**1**) with an overall yield of 67% (**1** from **16** *via* **27**, **28**, **8** and **9**).

## Experimental

All melting points were determined on a Yanagimoto micromelting point apparatus without correction. IR spectra were recorded on a Shimadzu FTIR-8200PC spectrometer with KBr disks. Atmospheric pressure chemical ionization mass spectra (APCI-MS) were obtained on a Hitachi M-1000 spectrometer. <sup>1</sup>H-NMR spectra were recorded on a Varian Gemini-200 (200 MHz) or a JEOL JNM-LA300 (300 MHz) apparatus using dilute solution in CDCl<sub>3</sub> unless otherwise stated. Chemical shifts are expressed as  $\delta$  (ppm) values from tetramethylsilane as an internal standard and coupling constants (*J*) are given in Hz. Organic extracts were dried over anhydrous MgSO<sub>4</sub> unless otherwise specified. Solvents were evaporated under reduced pressure. Flash chromatography was carried out on 60  $\mu$ m mesh silica gel (Fuji Silysia FL60D).

**2-Fluoro-6-(*N*-methyl-*N*-pivaloylamino)pyridine (5)** A mixture of 2,6-difluoropyridine (**2**, 50.0 g, 0.43 mol) and methylamine (240 ml of *ca.* 30% EtOH) was heated at *ca.* 140 °C in a sealed tube for 8 h and cooled to room temperature. After evaporation of all volatiles, aqueous K<sub>2</sub>CO<sub>3</sub> was added the residue, and the mixture was extracted with CHCl<sub>3</sub>. The extract was dried over anhydrous K<sub>2</sub>CO<sub>3</sub> and concentrated to dryness to give 59 g of crude 2-fluoro-6-methylaminopyridine as an oil. Pivaloyl chloride (78.6 g, 0.65 mol) was added dropwise to a solution of crude 2-fluoro-6-methylaminopyridine and Et<sub>3</sub>N (96.8 g, 0.96 mol) in CH<sub>2</sub>Cl<sub>2</sub> (1500 ml) at *ca.* 0 °C. The reaction mixture was stirred at room temperature overnight and then washed successively with water, 2 M aqueous H<sub>2</sub>SO<sub>4</sub>, water and brine. The solvent was evaporated, and the residue was purified by flash chromatography (CHCl<sub>3</sub> to CHCl<sub>3</sub>-MeOH, 10:1) to afford 59.6 g (65%) of **5** as an oil. <sup>1</sup>H-NMR  $\delta$ : 1.15 (9H, s, CMe<sub>3</sub>), 3.33 (3H, s, NMe), 6.85 (1H, dd, *J*<sub>F-3H</sub>=3.0 Hz, *J*<sub>4H-3H</sub>=8.0 Hz, 3-H), 7.18 (1H, dd, *J*<sub>F-5H</sub>=2.0 Hz, *J*<sub>4H-5H</sub>=8.0 Hz, 5-H), 7.83 (1H, ddd, *J*<sub>3H-4H</sub>=8.0 Hz, *J*<sub>F-4H</sub>=8.0 Hz, *J*<sub>5H-4H</sub>=8.0 Hz, 4-H). MS *m/z*: 211 (MH<sup>+</sup>).

**Ethyl 2-Fluoro-6-(*N*-methyl-*N*-pivaloylamino)pyridine-3-carboxylate (6)** NaH (8.1 g of 60% dispersion in oil, 0.20 mol) was added portionwise to a solution of **7** (36.0 g, 0.13 mol), prepared from **4** according to ref. 7 in DMF (360 ml) at *ca.* 0 °C. The mixture was stirred at room temperature for 4 h and then recooled at *ca.* 0 °C. After addition of MeI (17 ml, 0.27 mol), the whole was stirred at room temperature overnight. The mixture was poured into ice-water and extracted with AcOEt. The extract was washed with brine and evaporated, and the residue was purified by flash chromatography (CHCl<sub>3</sub>) to give 6.4 g (17%) of **6** as an oil. <sup>1</sup>H-NMR  $\delta$ : 1.27 (9H, s, CMe<sub>3</sub>), 1.40 (3H, t, *J*=7.0 Hz, CH<sub>2</sub>Me), 3.42 (3H, s, NMe), 4.41 (2H, q, *J*=7.0 Hz, CH<sub>2</sub>Me), 7.37 (1H, dd, *J*<sub>F-5H</sub>=1.5 Hz, *J*<sub>4H-5H</sub>=8.2 Hz, 5-H), 8.33 (1H, dd, *J*<sub>F-4H</sub>=9.5 Hz, *J*<sub>5H-4H</sub>=8.2 Hz, 4-H). MS *m/z*: 283 (MH<sup>+</sup>).

**Methyl 2,6-Difluoropyridine-3-carboxylate (12)** Concentrated H<sub>2</sub>SO<sub>4</sub> (5 ml) was added dropwise to a solution of **11**<sup>8)</sup> (63.0 g, 0.40 mol) in MeOH (700 ml). The mixture was heated to reflux for 20 h and cooled to room temperature. After evaporation of the solvent, the oily residue was added to ice-water and extracted with CHCl<sub>3</sub>. The extract was washed with brine and

concentrated to dryness, and the residue was purified by flash chromatography ( $\text{CHCl}_3$ ) to give 64.0 g (93%) of **12** as an oil.  $^1\text{H-NMR}$   $\delta$ : 3.96 (3H, s,  $\text{CO}_2\text{Me}$ ), 6.93 (1H, ddd,  $J_{2\text{F}-5\text{H}}=3.0$  Hz,  $J_{6\text{F}-5\text{H}}=3.0$  Hz,  $J_{5\text{H}-4\text{H}}=8.2$  Hz, 5-H), 8.52 (1H, ddd,  $J_{2\text{F}-4\text{H}}=8.3$  Hz,  $J_{6\text{F}-4\text{H}}=8.3$  Hz,  $J_{5\text{H}-4\text{H}}=8.2$  Hz, 4-H). MS  $m/z$ : 174 ( $\text{MH}^+$ ).

**Methyl 2-Fluoro-6-methylaminopyridine-3-carboxylate (13) and Methyl 6-Fluoro-2-methylaminopyridine-3-carboxylate (14)** Methylamine (72 g of ca. 20% EtOH solution, 0.46 mol) was added dropwise to a solution of **12** (38.0 g, 0.22 mol) in EtOH (500 ml) at ca.  $-25^\circ\text{C}$ . The mixture was stirred at the same temperature for 5 h and then warmed to room temperature. After evaporation of all volatiles, ice-water was added to the residue. The resulting solid was collected by filtration, washed with water, dried, and recrystallized from diethyl ether–hexane to give 15.7 g (39%) of **13**, mp 156–159  $^\circ\text{C}$ .  $^1\text{H-NMR}$   $\delta$ : 2.98 (3H, d,  $J=5.0$  Hz,  $\text{NHMe}$ ), 3.87 (3H, s,  $\text{CO}_2\text{Me}$ ), 5.50 (1H, br,  $\text{NHMe}$ ), 6.23 (1H, dd,  $J_{\text{F}-5\text{H}}=2.0$  Hz,  $J_{4\text{H}-5\text{H}}=8.5$  Hz, 5-H), 8.09 (1H, dd,  $J_{\text{F}-4\text{H}}=9.5$  Hz,  $J_{5\text{H}-4\text{H}}=8.5$  Hz, 4-H). MS  $m/z$ : 185 ( $\text{MH}^+$ ). IR  $\nu$   $\text{cm}^{-1}$ : 3271, 3134, 1701, 1630, 1319. *Anal.* Calcd for  $\text{C}_8\text{H}_9\text{FN}_2\text{O}_3$ : C, 52.17; H, 4.93; F, 10.32; N, 15.21. Found: C, 52.23; H, 4.89; F, 10.35; N, 15.05.

The filtrate was extracted with  $\text{CHCl}_3$ , and the extract was washed with brine. The solvent was evaporated, and the residue was purified by flash chromatography ( $\text{CHCl}_3$ ) to give 16.9 g (42%) of **14** as a viscous oil and 6.5 g (16%) of **13** as a solid.

**14**; mp 55–56  $^\circ\text{C}$  (hexane).  $^1\text{H-NMR}$   $\delta$ : 3.03 (3H, d,  $J=4.8$  Hz,  $\text{NHMe}$ ), 3.85 (3H, s,  $\text{CO}_2\text{Me}$ ), 6.07 (1H, dd,  $J_{\text{F}-5\text{H}}=2.7$  Hz,  $J_{4\text{H}-5\text{H}}=8.2$  Hz, 5-H), 8.18 (1H, dd,  $J_{\text{F}-4\text{H}}=8.2$  Hz,  $J_{5\text{H}-4\text{H}}=8.2$  Hz, 4-H). MS  $m/z$ : 185 ( $\text{MH}^+$ ). IR  $\nu$   $\text{cm}^{-1}$ : 3373, 1699, 1620, 1583, 1261. *Anal.* Calcd for  $\text{C}_8\text{H}_9\text{FN}_2\text{O}_3$ : C, 52.17; H, 4.93; F, 10.32; N, 15.21. Found: C, 52.48; H, 4.81; F, 10.27; N, 14.88.

**Methyl 2,6-Dichloropyridine-3-carboxylate (16)** A mixture of 2,6-dichloropyridine-3-carboxylic acid (**15**, 400 g, 2.1 mol), MeOH (2000 ml) and concentrated  $\text{H}_2\text{SO}_4$  (40 ml) was heated to reflux for 6 h and cooled to room temperature. The solvent was evaporated to leave an oily residue. After neutralization with saturated aqueous  $\text{NaHCO}_3$ , the resulting solid was collected by filtration, washed with water and dried to give 414 g (96%) of **16**, mp 53–54  $^\circ\text{C}$  (hexane) [lit.<sup>9)</sup> 56.0–56.5  $^\circ\text{C}$  (MeOH)].  $^1\text{H-NMR}$   $\delta$ : 3.96 (3H, s,  $\text{CO}_2\text{Me}$ ), 7.36 (1H, d,  $J=8.1$  Hz, 5-H), 8.16 (1H, dd,  $J=8.1$  Hz, 4-H). MS  $m/z$ : 206 ( $\text{MH}^+$ ). IR  $\nu$   $\text{cm}^{-1}$ : 1732, 1572, 1545, 1418. *Anal.* Calcd for  $\text{C}_7\text{H}_5\text{Cl}_2\text{NO}_2$ : C, 40.81; H, 2.45; Cl, 34.42; N, 6.80. Found: C, 40.63; H, 2.63; Cl, 34.32; N, 6.75.

**Methyl 2-Methoxy-6-(4-methylbenzenethio)pyridine-3-carboxylate (27)** Potassium *tert*-butoxide (30.7 g, 0.27 mol) was added portionwise to a solution of 4-methylbenzenethiol (32.5 g, 0.26 mol) in DMF (150 ml) at room temperature, and the mixture was stirred at the same temperature for 15 min. The resulting reaction mixture was added dropwise to a solution of **16** (51.4 g, 0.25 mol) in DMF (250 ml) at  $-30^\circ\text{C}$ . The whole was stirred at the same temperature for 1 h, poured into ice-water and extracted with a mixture of AcOEt–hexane (2:1). The extract was washed with brine and evaporated to leave an oily residue (73.2 g, quantitative yield) containing methyl 2-chloro-6-(4-methylbenzenethio)pyridine-3-carboxylate **25b** and methyl 6-chloro-2-(4-methylbenzenethio)pyridine-3-carboxylate **26b** in the ratio of  $>97: <3$ .

$\text{NaOMe}$  (50.0 g of ca. 28% MeOH solution, 0.26 mol) was added to a solution of the mixture of **25b** and **26b** (73.2 g, 0.25 mol) in THF (500 ml) at room temperature. The mixture was heated to reflux for 2 h and cooled to room temperature. The resulting deposited material was filtered off and washed with THF. The filtrate was concentrated to dryness, and the residue was dissolved in  $\text{CHCl}_3$  and washed successively with water and brine. The solvent was evaporated to leave a solid (72.0 g), which was recrystallized from AcOEt to give 62.4 g (87%) of **27**, mp 92–94  $^\circ\text{C}$ .  $^1\text{H-NMR}$   $\delta$ : 2.42 (3H, s,  $\text{C}_6\text{H}_4\text{Me}$ ), 3.85 (3H, s, OMe), 3.92 (3H, s,  $\text{CO}_2\text{Me}$ ), 6.45 (1H, d,  $J=8.0$  Hz, 5-H), 7.25 (2H, d,  $J=8.0$  Hz, ArH), 7.50 (2H, d,  $J=8.0$  Hz, ArH), 7.94 (1H, d,  $J=8.0$  Hz, 4-H). MS  $m/z$ : 290 ( $\text{MH}^+$ ). IR  $\nu$   $\text{cm}^{-1}$ : 1726, 1585, 1555, 1460, 1367, 1256. *Anal.* Calcd for  $\text{C}_{15}\text{H}_{15}\text{NO}_3\text{S}$ : C, 62.47; H, 5.23; N, 4.90; S, 10.93. Found: C, 62.26; H, 5.23; N, 4.84; S, 11.08.

**Methyl 2-Methoxy-6-(4-methylbenzenesulfonyl)pyridine-3-carboxylate (28)** Ca. 60% MCPBA (248.5 g, 0.86 mol) was added to a solution of **27** (250 g, 0.87 mol) in  $\text{CH}_2\text{Cl}_2$  (1250 ml) at ca.  $5^\circ\text{C}$ . The mixture was stirred at room temperature for 45 min and washed successively with 5% aqueous  $\text{NaHCO}_3$ , water and brine. The solvent was evaporated to leave a mixture of **28** and methyl 2-methoxy-6-(4-methylbenzenesulfonyl)pyridine-3-carboxylate in a ratio of 11:1 as a solid (257 g, calculated yield of **28** is 89%). The pure **28** was obtained by recrystallization from AcOEt–hexane, mp 106–107  $^\circ\text{C}$ .  $^1\text{H-NMR}$   $\delta$ : 2.37 (3H, s,  $\text{C}_6\text{H}_4\text{Me}$ ), 3.88 (3H, s, OMe), 3.97 (3H, s,  $\text{CO}_2\text{Me}$ ), 7.27 (2H, d,  $J=8.2$  Hz, ArH), 7.67 (2H, d,  $J=8.2$  Hz,

ArH), 7.69 (1H, d,  $J=7.7$  Hz, 5-H), 8.30 (1H, d,  $J=7.7$  Hz, 4-H). MS  $m/z$ : 306 ( $\text{MH}^+$ ). IR  $\nu$   $\text{cm}^{-1}$ : 1709, 1582, 1562, 1466, 1381, 1277. *Anal.* Calcd for  $\text{C}_{15}\text{H}_{15}\text{NO}_4\text{S}$ : C, 58.87; H, 4.92; N, 4.60; S, 10.29. Found: C, 59.00; H, 4.95; N, 4.59; S, 10.50.

**Methyl 2-Methoxy-6-(4-methylbenzenesulfonyl)pyridine-3-carboxylate:**  $^1\text{H-NMR}$   $\delta$ : 2.44 (3H, s,  $\text{C}_6\text{H}_4\text{Me}$ ), 3.90 (3H, s, OMe), 3.96 (3H, s,  $\text{CO}_2\text{Me}$ ), 7.34 (2H, d,  $J=8.1$  Hz, ArH), 7.79 (1H, d,  $J=7.6$  Hz, 5-H), 7.95 (2H, d,  $J=8.1$  Hz, ArH), 8.29 (1H, d,  $J=7.6$  Hz, 4-H). MS  $m/z$ : 322 ( $\text{MH}^+$ ).

**Methyl 2-Methoxy-6-methylaminopyridine-3-carboxylate (8)** a) Potassium *tert*-butoxide (7.3 g, 65 mmol) was added portionwise to a solution of **6** (6.1 g, 22 mmol) in MeOH (250 ml) at ca.  $0^\circ\text{C}$ . The mixture was heated to reflux for 2 h and cooled to room temperature. The solvent was evaporated to leave a residue. After addition of cold aqueous  $\text{NaHCO}_3$ , the resulting precipitates were collected by filtration, washed with water and dried to give 2.9 g (68%) of **8**, mp 120.5–121.5  $^\circ\text{C}$  (diethyl ether–hexane).  $^1\text{H-NMR}$   $\delta$ : 2.97 (3H, d,  $J=5.0$  Hz,  $\text{NHMe}$ ), 3.82 (3H, s, OMe), 3.98 (3H, s,  $\text{CO}_2\text{Me}$ ), 4.83 (1H, br,  $\text{NHMe}$ ), 5.94 (1H, d,  $J=8.5$  Hz, 5-H), 8.01 (1H, d,  $J=8.5$  Hz, 4-H). MS  $m/z$ : 197 ( $\text{MH}^+$ ). IR  $\nu$   $\text{cm}^{-1}$ : 3356, 2949, 1692, 1597, 1267, 1242. *Anal.* Calcd for  $\text{C}_9\text{H}_{12}\text{N}_2\text{O}_3$ : C, 55.09; H, 6.19; N, 14.28. Found: C, 55.05; H, 6.12; N, 14.17.

b) According to the same procedure as described on a), **8** (16.3 g, 97%) was obtained from **13** (15.7 g, 85 mmol), potassium *tert*-butoxide (19.1 g, 0.17 mol) and MeOH (400 ml).

c) A mixture of methylamine (4.2 g of 30% EtOH solution, 41 mmol) and DMF (10 ml) was added dropwise to a solution of **28** (2.5 g, 8.2 mmol) in DMF (30 ml) at room temperature. The mixture was stirred at ca.  $60^\circ\text{C}$  for 1 h, poured into ice-water, and extracted with a mixture of AcOEt–hexane (1:1). The extract was washed successively with 5% aqueous  $\text{NaHCO}_3$ , water and brine and concentrated to give 1.4 g (90%) of **8** as a solid, which was identified with the sample obtained above, on the basis of TLC, IR and  $^1\text{H-NMR}$  comparisons.

**Methyl 5-Bromo-2-methoxy-6-methylaminopyridine-3-carboxylate (9)** A mixture of **8** (7.3 g, 37 mmol), NBS (7.0 g, 39 mmol) and DMF (70 ml) was heated at  $80^\circ\text{C}$  for 4 h. The reaction mixture was poured into ice-water, and the resulting precipitates were collected by filtration, washed with water and dried to give 9.8 g (96%) of **9**, mp 136–138  $^\circ\text{C}$  (diethyl ether–hexane).  $^1\text{H-NMR}$   $\delta$ : 3.08 (3H, d,  $J=5.0$  Hz,  $\text{NHMe}$ ), 3.82 (3H, s, OMe), 4.02 (3H, s,  $\text{CO}_2\text{Me}$ ), 5.47 (1H, br,  $\text{NHMe}$ ), 8.15 (1H, s, 4-H). MS  $m/z$ : 275, 277 ( $\text{MH}^+$ ). IR  $\nu$   $\text{cm}^{-1}$ : 3393, 2949, 1709, 1597, 1566, 1263, 1236. *Anal.* Calcd for  $\text{C}_9\text{H}_{11}\text{BrN}_2\text{O}_3$ : C, 39.29; H, 4.03; Br, 29.05; N, 10.18. Found: C, 39.39; H, 3.96; Br, 29.35; N, 10.00.

**2-Methoxy-6-methylaminopyridine-3-carboxylic Acid (10)** A mixture of **8** (2.4 g, 12 mmol), 2 M aqueous NaOH (6.8 ml, 14 mmol) and MeOH (10 ml) was heated to reflux for 1.5 h and cooled to room temperature. After evaporation of MeOH, the residue was diluted with water (20 ml) and washed with diethyl ether. The aqueous solution was acidified with concentrated HCl, and the resulting precipitates were collected by filtration, washed with water and dried to give 2.1 g (94%) of **10** as a solid, mp 168–168.5  $^\circ\text{C}$  (EtOH).  $^1\text{H-NMR}$  (dimethylsulfoxide- $d_6$ )  $\delta$ : 2.84 (3H, d,  $J=5.0$  Hz,  $\text{NHMe}$ ), 3.87 (3H, s, OMe), 6.03 (1H, d,  $J=8.0$  Hz, 5-H), 7.25 (1H, br q,  $J=5.0$  Hz,  $\text{NHMe}$ ), 7.81 (1H, d,  $J=8.0$  Hz, 4-H), 11.67 (1H, s,  $\text{CO}_2\text{H}$ ). MS  $m/z$ : 183 ( $\text{MH}^+$ ). IR  $\nu$   $\text{cm}^{-1}$ : 3319, 1699, 1601, 1497, 1364. *Anal.* Calcd for  $\text{C}_8\text{H}_{10}\text{N}_2\text{O}_3$ : C, 52.74; H, 5.53; N, 15.38. Found: C, 52.77; H, 5.62; N, 15.47.

**5-Bromo-2-methoxy-6-methylaminopyridine-3-carboxylic Acid (1)** a) A mixture of **9** (20.0 g, 73 mmol), MeOH (100 ml) and aqueous NaOH [NaOH 3.1 g (78 mmol) in  $\text{H}_2\text{O}$  (200 ml)] was heated to reflux for 1.5 h and cooled to room temperature. After evaporation of MeOH, the aqueous solution was acidified with concentrated HCl. The resulting solid was collected by filtration, washed with water, and dried to give 18.9 g (quantitative yield) of **1**, mp 224–225  $^\circ\text{C}$  (EtOH).  $^1\text{H-NMR}$  (DMSO- $d_6$ )  $\delta$ : 2.92 (3H, d,  $J=5.0$  Hz,  $\text{NHMe}$ ), 3.88 (3H, s, OMe), 7.08 (1H, br q,  $J=5$  Hz,  $\text{NHMe}$ ), 7.98 (1H, s, 4-H), 12.08 (1H, s,  $\text{CO}_2\text{H}$ ). MS  $m/z$ : 261, 263 ( $\text{MH}^+$ ). IR  $\nu$   $\text{cm}^{-1}$ : 3412, 1674, 1589, 1560, 1229. *Anal.* Calcd for  $\text{C}_8\text{H}_9\text{BrN}_2\text{O}_3$ : C, 36.80; H, 3.47; Br, 30.61; N, 10.73. Found: C, 36.83; H, 3.43; Br, 30.40; N, 10.68.

b) A solution of NBS (10.8 g, 61 mmol) in DMF (50 ml) was added dropwise to a mixture of **10** (11.0 g, 60 mmol) and DMF (110 ml) kept at ca.  $10^\circ\text{C}$ . The mixture was stirred at the same temperature for 5 min, and cold water (220 ml) was added. The resulting precipitates were collected by filtration, washed with water and dried to give 14.6 g (88%) of **1** containing 3,5-dibromo-2-methoxy-6-methylaminopyridine (ca. 5%) as a pale pink solid.

3,5-Dibromo-2-methoxy-6-methylaminopyridine:  $^1\text{H-NMR}$   $\delta$ : 3.00 (3H, d,  $J=6.0$  Hz,  $\text{NHMe}$ ), 3.96 (3H, s, OMe), 4.9 (1H, br,  $\text{NHMe}$ ), 7.63 (1H, s, 4-H). MS  $m/z$ : 295, 263 ( $\text{MH}^+$ ), 299.

**General Procedure (Tables 1–5): Reaction of 12 or 16 with Methy-**

**lamine, *N*-Benzylmethylamine and Methoxide and Thiolate Anions** Methylamine (30% MeOH solution, 2 mol eq), *N*-benzylmethylamine (2 mol eq), sodium methoxide (ca. 28% MeOH solution, ca. 1 mol eq), potassium *tert*-butoxide (ca. 1 mol eq) in MeOH (10 ml) or ethanethiol and 4-methylbenzenethiol (ca. 1 mol eq) in the presence of a base was added to a solution of **12** or **16** (1.0 g) in an appropriate solvent (10 ml), and the mixture was stirred at an appropriate temperature for an appropriate reaction time. After dilution of the reaction mixture with water, the solution was extracted with AcOEt. The extract was washed with brine and concentrated to dryness. The residue was analyzed using  $^1\text{H}$ -NMR spectra.

Methyl 2-Chloro-6-methylaminopyridine-3-carboxylate (**17a**):  $^1\text{H}$ -NMR  $\delta$ : 2.98 (3H, d,  $J=4.8$  Hz, NHMe), 3.87 (3H, s, CO<sub>2</sub>Me), 5.20 (1H, br), 6.29 (1H, d,  $J=8.7$  Hz, 5-H), 8.04 (1H, d,  $J=8.7$  Hz, 4-H).

Methyl 6-(*N*-Benzyl-*N*-methyl)amino-2-chloropyridine-3-carboxylate (**17b**):  $^1\text{H}$ -NMR  $\delta$ : 3.12 (3H, s, NMe), 3.86 (3H, s, CO<sub>2</sub>Me), 4.82 (2H, s, CH<sub>2</sub>Ph), 6.38 (1H, d,  $J=9.0$  Hz, 5-H), 8.01 (1H, d,  $J=9.0$  Hz, 4-H), 7.2—7.4 (5H, m, ArH).

Methyl 6-Chloro-2-methylaminopyridine-3-carboxylate (**18a**):  $^1\text{H}$ -NMR  $\delta$ : 3.05 (3H, d,  $J=4.8$ , NHMe), 3.96 (3H, s, CO<sub>2</sub>Me), 6.50 (1H, d,  $J=8.7$  Hz, 5-H), 8.01 (1H, d,  $J=8.7$  Hz, 4-H).

Methyl 2-(*N*-Benzyl-*N*-methyl)amino-6-chloropyridine-3-carboxylate (**18b**):  $^1\text{H}$ -NMR  $\delta$ : 2.83 (3H, s, NMe), 3.81 (3H, s, CO<sub>2</sub>Me), 4.79 (2H, s, CH<sub>2</sub>Ph), 6.65 (1H, d,  $J=8.0$  Hz, 5-H), 7.87 (1H, d,  $J=8.0$  Hz, 4-H), 7.2—7.4 (5H, m, ArH).

2,6-Dichloro-*N*-methylpyridine-3-carboxamide (**19**):  $^1\text{H}$ -NMR  $\delta$ : 3.06 (3H, d,  $J=4.8$  Hz, NHMe), 6.56 (1H, br), 7.38 (1H, d,  $J=8.1$  Hz, 5-H), 8.13 (1H, d,  $J=8.1$  Hz, 4-H).

Methyl 6-Fluoro-2-methoxypyridine-3-carboxylate (**20**):  $^1\text{H}$ -NMR  $\delta$ : 3.90 (3H, s, OMe), 4.04 (3H, s, CO<sub>2</sub>Me), 6.53 (1H, dd,  $J_{\text{F-5H}}=3.0$  Hz,  $J_{\text{4H-5H}}=8.1$  Hz, 5-H), 8.31 (1H, dd,  $J_{\text{F-4H}}=8.1$  Hz,  $J_{\text{5H-4H}}=8.1$  Hz, 4-H).

Methyl 2-Fluoro-6-methoxypyridine-3-carboxylate (**21**):  $^1\text{H}$ -NMR  $\delta$ : 3.92 (3H, s, OMe), 3.98 (3H, s, CO<sub>2</sub>Me), 6.66 (1H, dd,  $J_{\text{F-5H}}=1.3$  Hz,  $J_{\text{4H-5H}}=8.4$  Hz, 5-H), 8.24 (1H, dd,  $J_{\text{F-4H}}=9.3$  Hz,  $J_{\text{5H-4H}}=8.4$  Hz, 4-H).

Methyl 2,6-Dimethoxypyridine-3-carboxylate (**22**):  $^1\text{H}$ -NMR  $\delta$ : 3.86 (3H, s, OMe), 3.97 (3H, s, OMe), 4.05 (3H, s, CO<sub>2</sub>Me), 6.32 (1H, d,  $J=8.2$  Hz, 5-H), 8.14 (1H, d,  $J=8.2$  Hz, 4-H).

Methyl 6-Chloro-2-methoxypyridine-3-carboxylate (**23**):  $^1\text{H}$ -NMR  $\delta$ : 3.90 (3H, s, OMe), 4.06 (3H, s, CO<sub>2</sub>Me), 6.96 (1H, d,  $J=7.8$  Hz, 5-H), 8.13 (1H, d,  $J=7.8$  Hz, 4-H).

Methyl 2-Chloro-6-methoxypyridine-3-carboxylate (**24**):  $^1\text{H}$ -NMR  $\delta$ : 3.91 (3H, s, OMe), 4.00 (3H, s, CO<sub>2</sub>Me), 6.70 (1H, d,  $J=8.4$  Hz, 5-H), 8.13 (1H, d,  $J=8.4$  Hz, 4-H).

Methyl 2-Chloro-6-ethylthiopyridine-3-carboxylate (**25a**):  $^1\text{H}$ -NMR  $\delta$ :

1.39 (3H, t,  $J=7.3$  Hz, CH<sub>2</sub>Me), 3.21 (2H, q,  $J=7.3$  Hz, CH<sub>2</sub>Me), 3.92 (3H, s, CO<sub>2</sub>Me), 7.12 (1H, d,  $J=8.3$  Hz, 5-H), 7.97 (1H, d,  $J=8.3$  Hz, 4-H).

Methyl 6-Chloro-2-ethylthiopyridine-3-carboxylate (**26a**):  $^1\text{H}$ -NMR  $\delta$ : 1.37 (3H, t,  $J=7.5$  Hz, CH<sub>2</sub>Me), 3.17 (2H, q,  $J=7.5$  Hz, CH<sub>2</sub>Me), 3.92 (3H, s, CO<sub>2</sub>Me), 7.03 (1H, d,  $J=8.0$  Hz, 5-H), 8.13 (1H, d,  $J=8.0$  Hz, 4-H).

Methyl 2-Chloro-6-(4-methylbenzenethio)pyridine-3-carboxylate (**25b**):  $^1\text{H}$ -NMR  $\delta$ : 2.43 (3H, s, C<sub>6</sub>H<sub>4</sub>Me), 3.92 (3H, s, CO<sub>2</sub>Me), 6.68 (1H, d,  $J=8.0$  Hz, 5-H), 7.29 (2H, d,  $J=8.0$  Hz, ArH), 7.50 (2H, d,  $J=8.0$  Hz, ArH), 7.90 (1H, d,  $J=8.0$  Hz, 4-H). MS  $m/z$ : 294 (MH<sup>+</sup>).

Methyl 6-Chloro-2-(4-methylbenzenethio)pyridine-3-carboxylate (**26b**):  $^1\text{H}$ -NMR  $\delta$ : 2.40 (3H, s, C<sub>6</sub>H<sub>4</sub>Me), 3.97 (3H, s, CO<sub>2</sub>Me), 7.02 (1H, d,  $J=8.0$  Hz, 5-H), 7.23 (2H, d,  $J=8.0$  Hz, ArH), 7.42 (2H, d,  $J=8.0$  Hz, ArH), 8.14 (1H, d,  $J=8.0$  Hz, 4-H).

**Acknowledgments** The authors thank the staff of the Analytical Chemistry Division of Discovery Research Laboratories, Dainippon Pharmaceutical Company, for elemental analyses and spectral measurements.

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# Synthesis of Methoxy-2-quinolones *via* Pummerer-type Cyclization of *N*-Aryl-*N*-methyl-3-(phenylsulfinyl)propionamides

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Received April 27, 2000; accepted August 6, 2000

**The thionium ions 10 generated by Pummerer reaction of *N*-aryl-*N*-methyl-3-(phenylsulfinyl)propionamides 4 caused not only an electrophilic cyclization reaction producing 2-quinolones 8, but also the formation of the vinyl sulfides 5 and 6 in favor of the latter reaction. On the other hand, the treatment of the vinyl sulfides 5 and 6 with *p*-toluenesulfonic acid induced cyclization to afford the 2-quinolones 8 in excellent to moderate yields, depending on the electronic properties of the aromatic ring, thus providing a convenient method for the synthesis of methoxy-2-quinolones.**

**Key word** Pummerer reaction; 2-quinolone; synthesis; trifluoroacetic anhydride; *p*-toluenesulfonic acid

It is well known that the thionium ion formed *in situ*, generated under acidic conditions from a sulfinyl precursor (Pummerer reaction), is a powerful electrophilic group reacting efficiently with nucleophilic carbon species such as alkenes, aromatics and enol ethers.<sup>1)</sup> This reaction was successfully applied for the synthesis of various carbocycles and heterocycles.<sup>2)</sup> Recently, we explored the reaction and used it as the key strategy for the syntheses of 1,2,3,4-tetrahydroisoquinolines,<sup>3)</sup> 1,2,3,4-tetrahydroquinolines,<sup>4)</sup> erythrinan,<sup>5)</sup> isopavine and pavine alkaloids.<sup>6)</sup> In these investigations we found that boron trifluoride diethyl etherate (BF<sub>3</sub>·Et<sub>2</sub>O), when used as an additive, facilitated the cationic cyclization. This efficiency was embodied particularly in the cyclization for a weak nucleophilic aromatic  $\pi$ -bond, which, without the additive, produced no cyclized product.<sup>3a–c,4)</sup> In this paper we describe the Pummerer reaction of *N*-aryl-*N*-methyl-3-(phenylsulfinyl)propionamides 4, leading to the development of a convenient method for the synthesis of methoxy-2-quinolones.

## Results and Discussion

The sulfoxides 4, the precursors of the Pummerer reaction, were prepared from anilines 1 in three steps. Acylation of 1 with 3-(phenylsulfonyl)propionyl chloride yielded the corresponding anilides 2, which on methylation with methyl iodide in the presence of a phase transfer catalyst afforded the *N*-methyl derivatives 3. Oxidation of 3 with sodium metaperiodate (NaIO<sub>4</sub>) in aqueous methanol gave 4 in excellent overall yields. Products 3 and 4 were well characterized by MS, IR, and <sup>1</sup>H- and <sup>13</sup>C-NMR spectral data (See Experimental).

**Pummerer Reaction** A solution of 4 in benzene was treated with trifluoroacetic anhydride (TFAA) at room tem-

perature for 20–72 h under an argon atmosphere. The reaction yielded three products: 5, 6, and 8. The vinyl sulfides 5 and 6 were obtained as major ones in all cases (Table 1). Sulfoxides 4a, b, e, and f, although the nucleophilic aromatic ring is electronically activated by the OMe group positioned at *ortho* and/or *para*, produced methoxy-2-quinolones 8a, b, e, and f in yields of only 17–39%. The reaction of 4c with an *ortho*- and *meta*-OMe group gave 5,8-dimethoxy-2-quinolone 8c in only 7% yield (run 5).

The results were different with those of the Pummerer-type reaction of *N*-aryl-*N*-(3-phenylsulfinylpropyl)formamides 13, which exclusively induced intramolecular cyclization to afford the *N*-formyl-1,2,3,4-tetrahydroquinolines 14 as sole products, though there is an exception of 13d.<sup>4)</sup> Sulfoxides 4d with two OMe groups *meta* to the reaction center, and 4g lacking an OMe group on the benzene ring, as anticipated, produced no cyclized product to any extent. Instead, the *cis*-(5d, g) and *trans*-isomers (6d, g) of the vinyl sulfides were quantitatively produced (runs 7, 13).

The addition of BF<sub>3</sub>·Et<sub>2</sub>O to the reaction mixture accelerates the reaction and in some cases dramatically improves the intramolecular cyclization reaction.<sup>3a–b,4)</sup> A solution of 4 in benzene was treated with TFAA for 30 min at room temperature, then 3 molar equivalents of BF<sub>3</sub>·Et<sub>2</sub>O were added, and the mixture was allowed to react for another 5–24 h, giving rise to four products: 5, 6, 7, and 8. The product ratios varied depending on the electrophilic properties of the aromatic ring. The results are also summarized in Table 1.

The addition of BF<sub>3</sub>·Et<sub>2</sub>O considerably improved the ring closure reaction. However, the vinyl sulfides 5 and 6 always accompanied the reaction product in significant amounts. For example, the reactions of 4c and 4g, which, without the additive, gave poor results in the cyclization as described above,

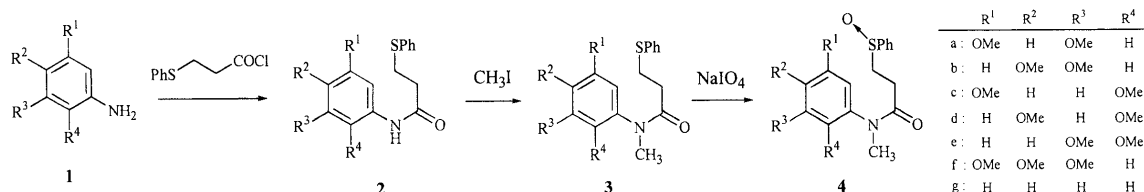


Chart 1

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Table 1. Pummerer Reaction of *N*-Aryl-*N*-methyl-3-phenylsulfinylpropionamides (**4**)

| Run | Substrates | Conditions <sup>a)</sup> |          | Yield (%)                              |    |               |                      |                  |                           |
|-----|------------|--------------------------|----------|----------------------------------------|----|---------------|----------------------|------------------|---------------------------|
|     |            | Reagent <sup>b)</sup>    | Time (h) | Vinyl sulfides ( <b>5</b> , <b>6</b> ) |    |               | 4-SPH-Q ( <b>7</b> ) |                  | 2-Quinolones ( <b>8</b> ) |
| 1   | <b>4a</b>  | A                        | 20       | ( <b>5a</b> )                          | 30 | ( <b>6a</b> ) | 18                   |                  | ( <b>8a</b> ) 32          |
| 2   | <b>4a</b>  | B                        | 5        | ( <b>5a</b> )                          | 10 | ( <b>6a</b> ) | 26                   |                  | ( <b>8a</b> ) 51          |
| 3   | <b>4b</b>  | A                        | 72       | ( <b>5b</b> )                          | 32 | ( <b>6b</b> ) | 44                   |                  | ( <b>8b</b> ) 17          |
| 4   | <b>4b</b>  | B                        | 24       | ( <b>5b</b> )                          | 13 | ( <b>6b</b> ) | 18                   | ( <b>7b</b> ) 15 | ( <b>8b</b> ) 42          |
| 5   | <b>4c</b>  | A                        | 72       | ( <b>5c</b> )                          | 36 | ( <b>6c</b> ) | 53                   |                  | ( <b>8c</b> ) 7           |
| 6   | <b>4c</b>  | B                        | 24       | ( <b>5c</b> )                          | 9  | ( <b>6c</b> ) | 20                   | ( <b>7c</b> ) 46 | ( <b>8c</b> ) 22          |
| 7   | <b>4d</b>  | A                        | 24       | ( <b>5d</b> )                          | 36 | ( <b>6d</b> ) | 63                   |                  |                           |
| 8   | <b>4d</b>  | B                        | 24       | ( <b>5d</b> )                          | 45 | ( <b>6d</b> ) | 52                   |                  |                           |
| 9   | <b>4e</b>  | A                        | 24       | ( <b>5e</b> )                          | 39 | ( <b>6e</b> ) | 34                   |                  | ( <b>8e</b> ) 21          |
| 10  | <b>4e</b>  | B                        | 24       | ( <b>5e</b> )                          | 17 | ( <b>6e</b> ) | 43                   |                  | ( <b>8e</b> ) 40          |
| 11  | <b>4f</b>  | A                        | 24       | ( <b>5f</b> )                          | 32 | ( <b>6f</b> ) | 27                   |                  | ( <b>8f</b> ) 39          |
| 12  | <b>4f</b>  | B                        | 24       | ( <b>5f</b> )                          | 21 | ( <b>6f</b> ) | 26                   |                  | ( <b>8f</b> ) 51          |
| 13  | <b>4g</b>  | A                        | 48       | ( <b>5g</b> )                          | 41 | ( <b>6g</b> ) | 54                   |                  |                           |
| 14  | <b>4g</b>  | B                        | 24       | ( <b>5g</b> )                          | 13 | ( <b>6g</b> ) | 17                   | ( <b>7g</b> ) 43 | ( <b>8g</b> ) 12          |

a) In benzene at room temperature. b) A: TFAA, B: TFAA-BF<sub>3</sub>·Et<sub>2</sub>O.

Table 2. Synthesis of 2-Quinolones (**8**, **17**) by Acid Catalyzed Cyclization of Vinyl Sulfides (**5**, **6**, **15**)

| Run | Substrate  | Conditions <sup>a)</sup>           |        |          | Yield (%) <sup>b)</sup> |    |                |    |           |
|-----|------------|------------------------------------|--------|----------|-------------------------|----|----------------|----|-----------|
|     |            | Acid                               | Temp   | Time (h) | 2-Quinolones            |    | Bis-sulfides   |    | Recovered |
| 1   | <b>5a</b>  | BF <sub>3</sub> ·Et <sub>2</sub> O | 150    | 140      | ( <b>8a</b> )           | 98 |                |    |           |
| 2   | <b>6a</b>  | BF <sub>3</sub> ·Et <sub>2</sub> O | 150    | 140      | ( <b>8a</b> )           | 67 |                |    | 5         |
| 3   | <b>5a</b>  | <i>p</i> -TsOH                     | Reflux | 5        | ( <b>8a</b> )           | 96 |                |    |           |
| 4   | <b>6a</b>  | <i>p</i> -TsOH                     | Reflux | 5        | ( <b>8a</b> )           | 90 |                |    |           |
| 5   | <b>5b</b>  | <i>p</i> -TsOH                     | Reflux | 2        | ( <b>8b</b> )           | 72 |                |    |           |
| 6   | <b>6b</b>  | <i>p</i> -TsOH                     | Reflux | 2        | ( <b>8b</b> )           | 68 |                |    | 12        |
| 7   | <b>5c</b>  | <i>p</i> -TsOH                     | Reflux | 20       | ( <b>8c</b> )           | 55 |                |    | 16        |
| 8   | <b>6c</b>  | <i>p</i> -TsOH                     | Reflux | 20       | ( <b>8c</b> )           | 52 |                |    | 10        |
| 9   | <b>5d</b>  | <i>p</i> -TsOH                     | Reflux | 5        |                         |    |                |    | 56        |
| 10  | <b>6d</b>  | <i>p</i> -TsOH                     | Reflux | 4        |                         |    |                |    | 95        |
| 11  | <b>5g</b>  | <i>p</i> -TsOH                     | Reflux | 19       | ( <b>8g</b> )           | 67 |                |    | 17        |
| 12  | <b>6g</b>  | <i>p</i> -TsOH                     | Reflux | 20       | ( <b>8g</b> )           | 58 |                |    | 34        |
| 13  | <b>5h</b>  | <i>p</i> -TsOH                     | Reflux | 14       | ( <b>8h</b> )           | 55 | ( <b>16h</b> ) | 9  |           |
| 14  | <b>6h</b>  | <i>p</i> -TsOH                     | Reflux | 14       | ( <b>8h</b> )           | 54 | ( <b>16h</b> ) | 8  |           |
| 15  | <b>5i</b>  | <i>p</i> -TsOH                     | Reflux | 4        | ( <b>8i</b> )           | 75 | ( <b>8j</b> )  | 24 |           |
| 16  | <b>6i</b>  | <i>p</i> -TsOH                     | Reflux | 4        | ( <b>8i</b> )           | 73 | ( <b>8j</b> )  | 20 |           |
| 17  | <b>5k</b>  | <i>p</i> -TsOH                     | Reflux | 21       | ( <b>8k</b> )           | 2  | ( <b>16k</b> ) | 21 | 61        |
| 18  | <b>6k</b>  | <i>p</i> -TsOH                     | Reflux | 21       | ( <b>8k</b> )           | 20 | ( <b>16k</b> ) | 24 | 31        |
| 19  | <b>15a</b> | <i>p</i> -TsOH                     | Reflux | 72       | ( <b>17a</b> )          | 68 |                |    |           |
| 20  | <b>15h</b> | <i>p</i> -TsOH                     | Reflux | 14       | ( <b>17h</b> )          | 16 |                |    |           |
| 21  | <b>15i</b> | <i>p</i> -TsOH                     | Reflux | 4        | ( <b>17i</b> )          | 40 | ( <b>17j</b> ) | 10 |           |

a) Toluene was used as the solvent. b) A mixture of **5** and **6**.

afforded the 2-quinolones in considerable yields (runs 6, 14). However, in the case of **4d** with two *meta*-OMe groups, addition of the acid did not improve the cyclization reaction to any extent (run 8).

#### Acid-Induced Cyclization of the Vinyl Sulfides **5** and **6**

As described above, BF<sub>3</sub>·Et<sub>2</sub>O considerably improved the formation of the quinolones **7** and **8**. In order to examine whether BF<sub>3</sub>·Et<sub>2</sub>O induces the cyclization of the vinyl sulfides **5** and **6**, we carried out the reactions under several conditions. For example, the vinyl sulfide **5a**, when treated with 5 molar equivalents of BF<sub>3</sub>·Et<sub>2</sub>O in benzene at room temperature for 24 h, did not induce the cyclization to any extent. The cyclization to quinolones, however, was achieved on heating at an elevated temperature (150 °C) for a long time (7 d) in a sealed tube. Thus, **5a** and **6a** afforded the 2-quinolone **8a** in 98% and 67% yields, respectively (Table 2,

runs 1, 2). *para*-Toluenesulfonic acid (*p*-TsOH) was found to be a more effective reagent for the cyclization. Thus, **5a** and **6a** on the reaction using 3 molar equivalents of *p*-TsOH under refluxing toluene for 5 h produced the 2-quinolone **8a** in excellent yields (runs 3, 4).

The results strongly indicated that the formation of 2-quinolone *via* the Pummerer reaction occurred *via* the *in situ* formed thionium ion **10** and not *via* the vinyl sulfides **5** and **6**. The protonation to **5** or **6** at the amide carbonyl group did not generate the thionium ion **10** but the other thionium, **11**, and/or the immonium ion **12**, which was probably converted into the 2-quinolone **8** as shown in Chart 2.

**Synthesis of Methoxy-2-quinolones** The preparation of **5** and **6** was readily achieved by acylation of the anilines **1** with *cis*-3-(phenylsulfonyl)acryloyl chloride, followed by methylation of the resulting anilides **15** (*cis* and *trans*-geo-

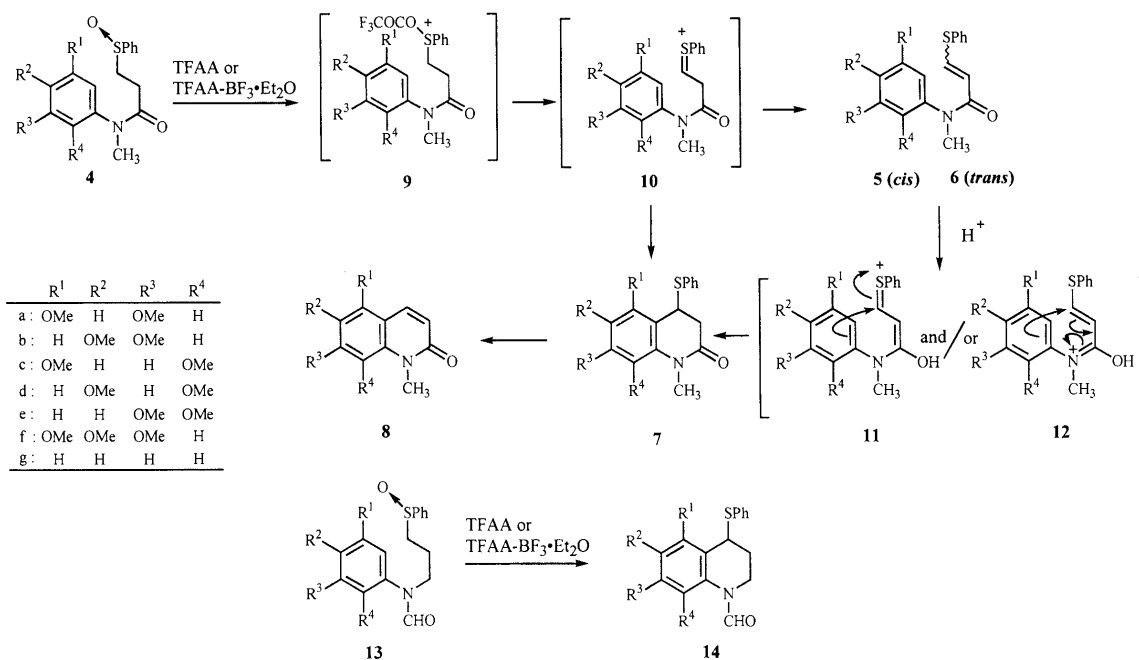


Chart 2

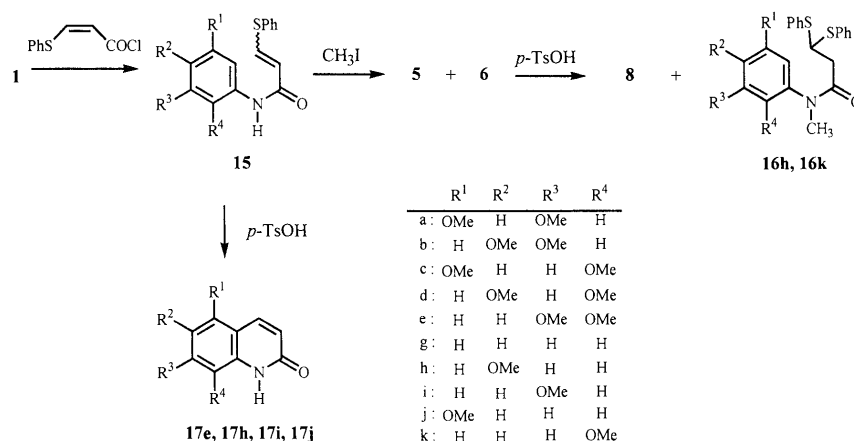


Chart 3

metric mixture) with methyl iodide in the presence of a phase transfer catalyst. During the reactions the geometrical isomerization of the SPh group occurred concomitantly. For example, **1a** afforded **5a** and **6a** in yields of 61% and 29%, respectively. The *cis*-**5** and *trans*-isomer **6** were readily separated by column chromatography (Chart 3).

Treatment involving vinyl sulfides **5** which had at least one OMe group at *para* or *ortho* to the reaction center with *p*-TsOH produced the corresponding 2-quinolones **8** in good to moderate yield, although 3,3-di(phenylsulfonyl)propionamides **16**, a Michael adduct of SPh to the double bond of the vinyl sulfides **5** or **6**, were formed in some cases (Table 2). The Michael addition reaction inhibited the cyclization to 2-quinolone. The cyclization of **5i** competitively occurred at the *para* and *ortho* positions to give 7-methoxy-**8i** and 8-methoxy-2-quinolone **8j** in yields of 75% and 24%, respectively (run 15). Even the reaction of the vinyl sulfide **5g**, which had no OMe group at the nucleophilic aromatic ring, caused the cyclization reaction to produce the 2-quinolone **8g** in good yield (run 11). The vinyl sulfide **5k**, having a *meta*-OMe group, gave poor results, as anticipated as shown in

Table 2 (run 17).

The *trans*-isomers **6** also caused cyclization under conditions similar to those used for the corresponding *cis*-isomers **5**, thus giving rise to the corresponding 2-quinolones **8** in comparative yield. These facts demonstrated that the synthesis of 2-quinolone *via* the acid-induced cyclization of the vinyl sulfides is achieved without separation of the geometric isomers.

The 2-quinolones **17**, NH derivatives of **8**, were also prepared by the acid-induced cyclization of the corresponding vinyl sulfides **15**, although the reaction was less effective than that of the NMe analogs (**5**, **6**), as shown in Table 2 (runs 19, 20, 21).

In summary, the thionium ion **10** generated by Pummerer reaction of *N*-aryl-*N*-methyl-3-(phenylsulfinyl)propionamides **4** caused two reactions: the formation of vinyl sulfides **5** and **6**, and intramolecular cyclization which produced 2-quinolones **8**, in favor of the former reaction. The treatment of vinyl sulfides **5** and **6** under the Pummerer conditions did not induce intramolecular cyclization. In this sense, the Pummerer reaction of **4** is not a suitable method for con-

structing a 2-quinolone ring system. However, the proton-induced cyclization of the vinyl sulfides **5**, **6** and **15** produced the 2-quinolones **8** and **17** in excellent-to-moderate yields. Thus, this reaction provides a convenient method for the synthesis of 2-quinolones, since the vinyl sulfides can be readily prepared in short steps from commercially available compounds.

## Experimental

**General Notes** Unless otherwise noted, the following procedures were adopted. Melting points were taken on a Yanagimoto SP-M1 hot-stage melting point apparatus and are uncorrected. IR spectra were obtained as films for oils and gums, and as KBr disks for solids, using a JASCO FT/IR-5000 spectrometer, and are given in  $\text{cm}^{-1}$ . UV spectra were recorded on a Hitachi U-3200 spectrophotometer in methanol, and values are given in  $\lambda_{\text{max}}$  nm ( $\epsilon$ ). NMR spectra were measured on a JEOL JNM-AL 300 ( $^1\text{H}$ , 300 MHz;  $^{13}\text{C}$ , 75 MHz) NMR spectrometer in  $\text{CDCl}_3$ , with tetramethylsilane as an internal standard, at room temperature, and the chemical shifts are given in  $\delta$  values. Low-resolution (LR)-MS were taken on JMS-AM20, and high resolution MS (HR-MS) on a JEOL JMS-D300 spectrometer at 70 eV [electron ionization MS (EI-MS)] or at 270 eV [chemical ionization (CI)-MS], reactant gas: isobutane using direct or GC/MS inlet systems. Elemental analyses were recorded on a Yanaco-CHN-corder MT-3. Thin layer chromatography (TLC) was performed on Merck pre-coated Silica gel 60  $\text{F}_{254}$  plates (Merck). Column chromatography was carried out with silica gel (Wakogel C-200). The organic extract from each reaction mixture was washed with brine, dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and concentrated *in vacuo* to dryness.

**Preparation of 3-(Phenylsulfanyl)propionic Acid** A solution of 3-bromopropionic acid (34.5 g, 226 mmol) in EtOH (150 ml) was slowly added to a solution of KOH (34.3 g, 520 mmol) and benzenethiol (25 g, 226 mmol) in EtOH (300 ml) at r.t. under an argon atmosphere, and the mixture was refluxed for 2 h. After removal of inorganic precipitates by filtration, the filtrate was concentrated *in vacuo*, and the residue was diluted with water and extracted with  $\text{CHCl}_3$ . Flash chromatography with benzene and recrystallization of the eluate with ether-hexane gave 3-(phenylsulfanyl)propionic acid (37.3 g, 91%) as colorless prisms from hexane, mp 55–59 °C (lit.<sup>7</sup>) mp 56–56.5 °C. IR: 1696, 1584.  $^1\text{H}$ -NMR: 2.5–2.8 (2H, m,  $\text{PhSCH}_2\text{CH}_2$ ), 3.1–3.4 (2H, m,  $\text{PhSCH}_2\text{CH}_2$ ), 7.1–7.6 (5H, m, SPh), 8.82 (1H, br s, OH).  $^{13}\text{C}$ -NMR: 28.8 (t), 34.2 (t), 126.8 (d), 129.1 (d $\times$ 2), 130.4 (d $\times$ 2), 134.9 (s), 177.7 (s). LR-MS  $m/z$ : 182 ( $\text{M}^+$ , base peak).

**Preparation of *N*-(3,5-Dimethoxyphenyl)-3-(phenylsulfanyl)propionamide (2a). Typical Procedure** A solution of 3-(phenylsulfanyl)propionic acid (3.0 g, 16.5 mmol) and oxalyl chloride (6.3 g, 49.6 mmol) was stirred at r.t. for 2 h. Removal of excess oxalyl chloride by repeated evaporation under reduced pressure gave an oily material. To a solution of this acid chloride in benzene (30 ml) was slowly added a solution of 3,4-dimethoxyaniline (**1a**) (2.1 g, 13.7 mmol) and triethylamine (1.7 g, 16.8 mmol) in benzene (100 ml) at r.t., and the mixture was stirred for 17 h. The residual oil was chromatographed, eluted with ethyl acetate-hexane (1:3) to give **2a** (3.69 g, 71%) as colorless needles, mp 104–106 °C from Et<sub>2</sub>O-hexane. IR: 3364, 1707, 1624, 1605, 1551.  $^1\text{H}$ -NMR: 2.63 (2H, t,  $J=7$  Hz,  $\text{PhSCH}_2\text{CH}_2$ ), 3.28 (2H, t,  $J=7$  Hz,  $\text{PhSCH}_2\text{CH}_2$ ), 3.77 (6H, s,  $\text{OCH}_3\times 2$ ), 6.24 (1H, t,  $J=2$  Hz, ArH), 6.73 (2H, d,  $J=2$  Hz, ArH), 7.2–7.4 (5H, m, SPh).  $^{13}\text{C}$ -NMR: 29.2 (t), 37.1 (t), 55.3 (q $\times$ 2), 96.9 (d), 98.2 (d $\times$ 2), 126.5 (d), 129.1 (d $\times$ 2), 129.7 (d $\times$ 2), 135.2 (s), 139.4 (s), 161.0 (s $\times$ 2), 169.3 (s). LR-MS  $m/z$ : 317 ( $\text{M}^+$ , base peak). HR-MS  $m/z$  ( $\text{M}^+$ ): Calcd for  $\text{C}_{17}\text{H}_{19}\text{NO}_3\text{S}$ : 317.1083. Found: 317.1075. Anal. Calcd for  $\text{C}_{17}\text{H}_{19}\text{NO}_3\text{S}$ : C, 64.33; H, 6.03; N, 4.41. Found: C, 64.18; H, 6.01; N, 4.58.

***N*-(3,4-Dimethoxyphenyl)-3-(phenylsulfanyl)propionamide (2b)** (3.68 g, 89%) was obtained from **1b** (2.0 g, 13.0 mmol) as colorless needles from ethyl acetate-hexane, mp 103–106 °C. IR: 3259, 1655, 1603, 1516.  $^1\text{H}$ -NMR: 2.63 (2H, t,  $J=7$  Hz,  $\text{PhSCH}_2\text{CH}_2$ ), 3.30 (2H, t,  $J=7$  Hz,  $\text{PhSCH}_2\text{CH}_2$ ), 3.85, 3.87 (each 3H, s,  $\text{OCH}_3\times 2$ ), 6.79, 6.81 (each 1H, s, ArH), 7.1–7.4 (6H, m, ArH and SPh), 7.85 (1H, br s, NH).  $^{13}\text{C}$ -NMR: 29.3 (t), 37.0 (t), 55.8 (q), 56.1 (q), 105.1 (d), 111.4 (d), 112.0 (d), 126.4 (d), 129.0 (d $\times$ 2), 129.6 (d $\times$ 2), 131.3 (s), 135.3 (s), 145.9 (s), 149.0 (s), 169.0 (s). LR-MS  $m/z$ : 317 ( $\text{M}^+$ , base peak). HR-MS  $m/z$  ( $\text{M}^+$ ): Calcd for  $\text{C}_{17}\text{H}_{19}\text{NO}_3\text{S}$ : 317.1086. Found: 317.1099. Anal. Calcd for  $\text{C}_{17}\text{H}_{19}\text{NO}_3\text{S}$ : C, 64.33; H, 6.03; N, 4.41. Found: C, 64.09; H, 6.05; N, 4.19.

***N*-(2,5-Dimethoxyphenyl)-3-(phenylsulfanyl)propionamide (2c)** (6.16 g, 99%) was obtained from **1c** (3.0 g, 19.6 mmol) as a reddish orange gum. IR: 3410, 1685, 1601, 1533.  $^1\text{H}$ -NMR: 2.68 (2H, t,  $J=7$  Hz,  $\text{PhSCH}_2\text{CH}_2$ ), 3.30

(2H, t,  $J=7$  Hz,  $\text{PhSCH}_2\text{CH}_2$ ), 3.77, 3.82 (each 3H, s,  $\text{OCH}_3\times 2$ ), 6.3–6.9 (3H, m, ArH), 7.2–7.5 (5H, m, SPh), 7.85 (1H, br s, NH).  $^{13}\text{C}$ -NMR: 29.3 (t), 37.4 (t), 55.7 (q), 56.2 (q), 106.0 (d), 108.8 (d), 110.8 (d), 126.4 (d), 128.1 (s), 129.0 (d $\times$ 2), 129.9 (d $\times$ 2), 135.3 (s), 142.0 (s), 153.9 (s), 168.9 (s). LR-MS  $m/z$ : 317 ( $\text{M}^+$ ), 138 (base peak). HR-MS  $m/z$  ( $\text{M}^+$ ): Calcd for  $\text{C}_{17}\text{H}_{19}\text{NO}_3\text{S}$ : 317.1083. Found: 317.1072.

***N*-(2,4-Dimethoxyphenyl)-3-(phenylsulfanyl)propionamide (2d)** (5.92 g, 95%) was obtained from **1d** (3.0 g, 19.6 mmol) as colorless needles from ethyl acetate-hexane, mp 92–95 °C. IR: 3400, 1674, 1614, 1533.  $^1\text{H}$ -NMR: 2.65 (2H, t,  $J=7$  Hz,  $\text{PhSCH}_2\text{CH}_2$ ), 3.30 (2H, t,  $J=7$  Hz,  $\text{PhSCH}_2\text{CH}_2$ ), 3.79, 3.83 (each 3H, s,  $\text{OCH}_3\times 2$ ), 6.4–6.6 (2H, m, ArH), 7.2–7.5 (5H, m, SPh), 7.60 (1H, br s, NH), 8.21 (1H, d,  $J=9$  Hz, ArH).  $^{13}\text{C}$ -NMR: 29.3 (t), 37.2 (t), 55.4 (q), 55.5 (q), 98.5 (d), 103.7 (d), 120.7 (d), 121.0 (s), 126.3 (d), 128.9 (d $\times$ 2), 129.7 (d $\times$ 2), 135.4 (s), 149.1 (s), 156.4 (s), 168.4 (s). LR-MS  $m/z$ : 317 ( $\text{M}^+$ ), 153 (base peak). HR-MS  $m/z$  ( $\text{M}^+$ ): Calcd for  $\text{C}_{17}\text{H}_{19}\text{NO}_3\text{S}$ : 317.1083. Found: 317.1077. Anal. Calcd for  $\text{C}_{17}\text{H}_{19}\text{NO}_3\text{S}$ : C, 64.33; H, 6.03; N, 4.41. Found: C, 64.31 H, 6.04; N, 4.29.

***N*-(2,3-Dimethoxyphenyl)-3-(phenylsulfanyl)propionamide (2e)** (6.20 g, 100%) was obtained from **1e** (3.0 g, 19.6 mmol) as a yellowish gum. IR: 3329, 1685, 1604, 1529.  $^1\text{H}$ -NMR: 2.69 (2H, t,  $J=7$  Hz,  $\text{PhSCH}_2\text{CH}_2$ ), 3.30 (2H, t,  $J=7$  Hz,  $\text{PhSCH}_2\text{CH}_2$ ), 3.86 (6H, s,  $\text{OCH}_3\times 2$ ), 6.66 (1H, dd,  $J=8$ , 1 Hz, ArH), 7.02 (1H, t,  $J=8$  Hz, ArH), 7.2–7.5 (5H, m, SPh), 7.93 (1H, dd,  $J=8$ , 1 Hz, ArH).  $^{13}\text{C}$ -NMR: 29.5 (t), 37.4 (t), 55.8 (q), 60.7 (q), 107.8 (d), 112.7 (d), 124.1 (d), 126.5 (d), 129.1 (d $\times$ 2), 129.9 (d $\times$ 2), 131.9 (s), 135.2 (s), 137.5 (s), 151.9 (s), 169.1 (s). LR-MS  $m/z$ : 317 ( $\text{M}^+$ ), 153 (base peak). HR-MS  $m/z$  ( $\text{M}^+$ ): Calcd for  $\text{C}_{17}\text{H}_{19}\text{NO}_3\text{S}$ : 317.1085. Found: 317.1120.

***N*-(3,4,5-Trimethoxyphenyl)-3-(phenylsulfanyl)propionamide (2f)** (5.47 g, 96%) was obtained from **1f** (3.0 g, 19.6 mmol) as an orange gum. IR: 3338, 1689, 1664, 1610, 1547, 1508.  $^1\text{H}$ -NMR: 2.64 (2H, t,  $J=7$  Hz,  $\text{PhSCH}_2\text{CH}_2$ ), 3.29 (2H, t,  $J=7$  Hz,  $\text{PhSCH}_2\text{CH}_2$ ), 3.80 (3H, s,  $\text{OCH}_3$ ), 3.82 (6H, s,  $\text{OCH}_3\times 2$ ), 6.80 (2H, s, ArH), 7.2–7.4 (5H, m, SPh), 7.85 (1H, br s, NH).  $^{13}\text{C}$ -NMR: 29.1 (t), 36.9 (q), 55.9 (q $\times$ 2), 60.8 (q), 97.6 (d $\times$ 2), 126.3 (d), 129.0 (d $\times$ 2), 129.4 (d $\times$ 2), 134.0 (s), 134.4 (s), 135.3 (s), 153.1 (s $\times$ 2), 169.3 (s). LR-MS  $m/z$ : 347 ( $\text{M}^+$ ), 56 (base peak). HR-MS  $m/z$  ( $\text{M}^+$ ): Calcd for  $\text{C}_{18}\text{H}_{21}\text{NO}_4\text{S}$ : 347.1188. Found: 347.1166.

***N*-Phenyl-3-(phenylsulfanyl)propionamide (2g)** (13.4 g, 97%) was obtained from **1g** (5.0 g, 53.7 mmol) as colorless prisms from ethyl acetate-hexane, mp 78–82 °C. IR: 3319, 1668, 1597, 1523.  $^1\text{H}$ -NMR: 2.61 (2H, t,  $J=7$  Hz,  $\text{PhSCH}_2\text{CH}_2$ ), 3.25 (2H, t,  $J=7$  Hz,  $\text{PhSCH}_2\text{CH}_2$ ), 7.0–7.7 (10H, m, ArH, SPh).  $^{13}\text{C}$ -NMR: 29.4 (t), 37.1 (t), 120.0 (d $\times$ 2), 124.5 (d), 126.5 (d), 129.0 (d $\times$ 2), 129.1 (d $\times$ 2), 129.8 (d $\times$ 2), 135.2 (s), 137.6 (s), 169.3 (s). LR-MS  $m/z$ : 257 ( $\text{M}^+$ ), 93 (base peak). HR-MS  $m/z$  ( $\text{M}^+$ ): Calcd for  $\text{C}_{15}\text{H}_{15}\text{NOS}$ : 257.0875. Found: 257.0896. Anal. Calcd for  $\text{C}_{15}\text{H}_{15}\text{NOS}$ : C, 70.01; H, 5.87; N, 5.44. Found: C, 70.01; H, 5.92; N, 5.28.

**Preparation of *N*-(3,5-dimethoxyphenyl)-*N*-methyl-3-(phenylsulfanyl)propionamide (3a). Typical Procedure** A solution of **2a** (500 mg, 1.56 mmol), methyl iodide (2.24 g, 15.6 mmol), KOH (1.04 g, 15.8 mmol), and tetrabutylammonium bromide (255 mg, 0.79 mmol) in THF (40 ml) was stirred at r.t. for 5.5 h. After removal of inorganic precipitates by filtration, the filtrate was concentrated *in vacuo*, and the residue was diluted with water and extracted with  $\text{CHCl}_3$ . The residual oil was chromatographed, eluted with ethyl acetate-hexane (1:2) to give **3a** (522 mg, 100%) as a pale yellow oil. IR: 1657, 1605.  $^1\text{H}$ -NMR: 2.48 (2H, t,  $J=7$  Hz,  $\text{PhSCH}_2\text{CH}_2$ ), 3.1–3.3 (2H, m,  $\text{PhSCH}_2\text{CH}_2$ ), 3.23 (3H, s,  $\text{NCH}_3$ ), 3.75 (6H, s,  $\text{OCH}_3\times 2$ ), 6.27 (2H, d,  $J=2$  Hz, ArH), 6.41 (1H, t,  $J=2$  Hz, ArH), 7.1–7.3 (5H, m, SPh).  $^{13}\text{C}$ -NMR: 29.1 (t), 33.6 (t), 36.9 (q), 55.2 (q $\times$ 2), 99.6 (d), 105.3 (d $\times$ 2), 125.6 (d), 128.6 (d $\times$ 2), 128.7 (d $\times$ 2), 135.9 (s), 145.0 (s), 161.3 (s $\times$ 2), 170.5 (s). LR-MS  $m/z$ : 331 ( $\text{M}^+$ ), 221 (base peak). HR-MS  $m/z$  ( $\text{M}^+$ ): Calcd for  $\text{C}_{18}\text{H}_{21}\text{NO}_3\text{S}$ : 331.1240. Found: 331.1215.

***N*-(3,4-Dimethoxyphenyl)-*N*-methyl-3-(phenylsulfanyl)propionamide (3b)** (2.61 g, 93%) was obtained from **2b** (2.7 g, 8.2 mmol) as a pale yellow gum. IR: 1655, 1595, 1510.  $^1\text{H}$ -NMR: 2.42 (2H, t,  $J=7$  Hz,  $\text{PhSCH}_2\text{CH}_2$ ), 3.18 (2H, t,  $J=7$  Hz,  $\text{PhSCH}_2\text{CH}_2$ ), 3.24 (3H, s,  $\text{NCH}_3$ ), 3.81, 3.89 (each 3H, s,  $\text{OCH}_3\times 2$ ), 6.6–6.8 (3H, m, ArH), 7.1–7.3 (5H, m, SPh).  $^{13}\text{C}$ -NMR: 29.2 (t), 33.7 (t), 37.4 (q), 55.9 (q $\times$ 2), 110.4 (d), 111.5 (d), 119.3 (d), 125.8 (d), 128.7 (d $\times$ 2), 128.8 (d $\times$ 2), 136.0 (s), 136.5 (s), 148.6 (s), 149.6 (s), 171.1 (s). LR-MS  $m/z$ : 331 ( $\text{M}^+$ ), 152 (base peak). HR-MS  $m/z$  ( $\text{M}^+$ ): Calcd for  $\text{C}_{18}\text{H}_{21}\text{NO}_3\text{S}$ : 331.1240. Found: 331.1279.

***N*-(2,5-Dimethoxyphenyl)-*N*-methyl-3-(phenylsulfanyl)propionamide (3c)** (5.74 g, 100%) was obtained from **2c** (5.5 g, 17.4 mmol) as a pale yellow gum. IR: 1655, 1610, 1585, 1508.  $^1\text{H}$ -NMR: 2.35 (2H, t,  $J=7$  Hz,  $\text{PhSCH}_2\text{CH}_2$ ), 3.17 (2H, t,  $J=7$  Hz,  $\text{PhSCH}_2\text{CH}_2$ ), 3.17 (3H, s,  $\text{NCH}_3$ ), 3.72, 3.74 (each 3H, s,  $\text{OCH}_3\times 2$ ), 6.68 (1H, t,  $J=2$  Hz, ArH), 6.84 (2H, d,

$J=2$  Hz, ArH), 7.1–7.3 (5H, m, SPh).  $^{13}\text{C}$ -NMR: 29.0 (t), 33.3 (t), 35.9 (q), 55.6 (q), 55.7 (q), 112.7 (d), 113.9 (d), 114.9 (d), 125.6 (d), 128.7 (d $\times 2$ ), 128.7 (d $\times 2$ ), 132.3 (s), 136.1 (s), 149.0 (s), 153.7 (s), 171.5 (s). LR-MS  $m/z$ : 331 ( $\text{M}^+$ ), 152 (base peak). HR-MS  $m/z$  ( $\text{M}^+$ ): Calcd for  $\text{C}_{18}\text{H}_{21}\text{NO}_3\text{S}$ : 331.1239. Found: 331.1222.

*N*-(2,4-Dimethoxyphenyl)-*N*-methyl-3-(phenylsulfanyl)propionamide (**3d**): (4.18 g, 100%) was obtained from **2d** (4.0 g, 12.6 mmol) as a pale yellow gum. IR: 1655, 1585, 1512.  $^1\text{H}$ -NMR: 2.2–2.5 (2H, m,  $\text{PhSCH}_2\text{CH}_2-$ ), 3.0–3.3 (2H, m,  $\text{PhSCH}_2\text{CH}_2-$ ), 3.14 (3H, s,  $>\text{NCH}_3$ ), 3.73, 3.82 (each 3H, s,  $\text{OCH}_3\times 2$ ), 6.3–6.5 (2H, m, ArH), 6.9–7.2 (6H, m, ArH and SPh).  $^{13}\text{C}$ -NMR: 29.1 (t), 33.4 (t), 36.2 (q), 55.4 (q), 55.5 (q), 99.6 (d), 104.6 (d), 125.2 (s), 125.7 (d), 128.7 (d $\times 4$ ), 129.3 (d), 136.3 (s), 155.8 (s), 160.5 (s), 172.0 (s). LR-MS  $m/z$ : 331 ( $\text{M}^+$ ), 151 (base peak). HR-MS  $m/z$  ( $\text{M}^+$ ): Calcd for  $\text{C}_{18}\text{H}_{21}\text{NO}_3\text{S}$ : 331.1240. Found: 331.1225.

*N*-(2,3-Dimethoxyphenyl)-*N*-methyl-3-(phenylsulfanyl)propionamide (**3e**): (5.88 g, 99%) was obtained from **2e** (5.7 g, 18.0 mmol) as a yellowish gum. IR: 1660, 1618, 1589.  $^1\text{H}$ -NMR: 2.40 (2H, t,  $J=7$  Hz,  $\text{PhSCH}_2\text{CH}_2-$ ), 3.19 (2H, t,  $J=7$  Hz,  $\text{PhSCH}_2\text{CH}_2-$ ), 3.23 (3H, s,  $>\text{NCH}_3$ ), 3.79, 3.88 (each 3H, s,  $\text{OCH}_3\times 2$ ), 6.6–7.3 (8H, m, ArH and SPh).  $^{13}\text{C}$ -NMR: 29.1 (t), 35.5 (t), 36.8 (q), 56.0 (q), 60.9 (q), 112.2 (d), 120.4 (d), 124.2 (d), 125.7 (d), 128.8 (d $\times 4$ ), 135.2 (s), 137.1 (s), 145.2 (s), 153.8 (s), 171.5 (s). LR-MS  $m/z$ : 331 ( $\text{M}^+$ ), 152 (base peak). HR-MS  $m/z$  ( $\text{M}^+$ ): Calcd for  $\text{C}_{18}\text{H}_{21}\text{NO}_3\text{S}$ : 331.1240. Found: 331.1195.

*N*-(3,4,5-Trimethoxyphenyl)-*N*-methyl-3-(phenylsulfanyl)propionamide (**3f**): (5.20 g, 100%) was obtained from **2f** (5.0 g, 14.4 mmol) as a yellow gum. IR: 1655, 1593, 1502.  $^1\text{H}$ -NMR: 2.47 (2H, t,  $J=7$  Hz,  $\text{PhSCH}_2\text{CH}_2-$ ), 3.20 (2H, t,  $J=7$  Hz,  $\text{PhSCH}_2\text{CH}_2-$ ), 3.24 (3H, s,  $>\text{NCH}_3$ ), 3.78 (6H, s,  $\text{OCH}_3\times 2$ ), 3.85 (3H, s,  $\text{OCH}_3$ ), 6.34 (2H, s, ArH), 7.1–7.3 (5H, m, SPh), 7.85 (1H, br s,  $>\text{N-H}$ ).  $^{13}\text{C}$ -NMR: 29.4 (t), 33.7 (t), 37.3 (q), 56.2 (q $\times 2$ ), 60.9 (q), 104.6 (d $\times 2$ ), 126.0 (d), 128.8 (d $\times 2$ ), 129.0 (d $\times 2$ ), 136.0 (s), 137.7 (s), 139.2 (s), 153.8 (s $\times 2$ ), 170.9 (s). LR-MS  $m/z$ : 361 ( $\text{M}^+$ ), 182 (base peak). HR-MS  $m/z$  ( $\text{M}^+$ ): Calcd for  $\text{C}_{19}\text{H}_{23}\text{NO}_4\text{S}$ : 361.1348. Found: 361.1360.

*N*-Methyl-*N*-phenyl-3-(phenylsulfanyl)propionamide (**3g**): (10.54 g, 100%) was obtained from **2g** (10.0 g, 38.9 mmol) as a yellow gum. IR: 1657, 1595.  $^1\text{H}$ -NMR: 2.40 (2H, t,  $J=7$  Hz,  $\text{PhSCH}_2\text{CH}_2-$ ), 3.18 (2H, t,  $J=7$  Hz,  $\text{PhSCH}_2\text{CH}_2-$ ), 3.27 (3H, s,  $>\text{NCH}_3$ ), 7.0–7.4 (10H, m, ArH, SPh).  $^{13}\text{C}$ -NMR: 29.2 (t), 33.9 (t), 37.3 (q), 125.8 (d), 127.1 (d $\times 2$ ), 127.8 (d), 128.8 (d $\times 2$ ), 128.9 (d $\times 2$ ), 129.7 (d $\times 2$ ), 136.0 (s), 143.6 (s), 170.9 (s). LR-MS  $m/z$ : 271 ( $\text{M}^+$ ), 51 (base peak). HR-MS  $m/z$  ( $\text{M}^+$ ): Calcd for  $\text{C}_{16}\text{H}_{17}\text{NOS}$ : 271.1028. Found: 271.1010.

**Preparation of *N*-(3,5-Dimethoxyphenyl)-*N*-methyl-3-(phenylsulfanyl)propionamide (4a). Typical Procedure** To a solution of **3a** (1.0 g, 3.02 mmol) in MeOH (100 ml) was added a solution of  $\text{NaIO}_4$  (0.97 g, 4.54 mmol) in  $\text{H}_2\text{O}$  (20 ml), and the mixture was stirred at r.t. for 6 h. After removal of inorganic precipitates by filtration, the filtrate was concentrated *in vacuo*. The residue was extracted with  $\text{CHCl}_3$ . The product was chromatographed, eluted with ethyl acetate to give **4a** (1.05 g, 100%) as a pale yellow oil. IR: 1657, 1605, 1046.  $^1\text{H}$ -NMR: 2.4–3.2 (4H, m), 3.22 (3H, s,  $>\text{NCH}_3$ ), 3.80 (6H, s,  $\text{OCH}_3\times 2$ ), 6.29 (2H, d,  $J=2$  Hz, ArH), 6.44 (1H, t,  $J=2$  Hz, ArH), 7.4–7.7 (5H, m, SPh).  $^{13}\text{C}$ -NMR: 26.9 (t), 37.2 (q), 52.8 (t), 55.5 (q $\times 2$ ), 100.0 (d), 105.4 (d $\times 2$ ), 123.9 (d), 129.1 (d $\times 2$ ), 130.9 (d $\times 2$ ), 143.7 (s), 144.8 (s), 161.6 (s $\times 2$ ), 169.9 (s). LR-MS  $m/z$ : 347 ( $\text{M}^+$ ), 222 (base peak). HR-MS  $m/z$  ( $\text{M}^+$ ): Calcd for  $\text{C}_{18}\text{H}_{21}\text{NO}_4\text{S}$ : 347.1369. Found: 347.1406.

*N*-(3,4-Dimethoxyphenyl)-*N*-methyl-3-(phenylsulfanyl)propionamide (**4b**): (2.49 g, 95%) was obtained from **3b** (2.5 g, 7.5 mmol) as a yellow gum. IR: 1655, 1595, 1512, 1026.  $^1\text{H}$ -NMR: 2.2–3.4 (4H, m), 3.23 (3H, s,  $>\text{NCH}_3$ ), 3.87, 3.91 (each 3H, s,  $\text{OCH}_3\times 2$ ), 6.6–7.0 (3H, m, ArH), 7.3–7.7 (5H, m, SPh).  $^{13}\text{C}$ -NMR: 26.9 (t), 37.5 (q), 52.8 (t), 56.0 (q $\times 2$ ), 110.4 (d), 111.7 (d), 119.4 (d), 123.9 (d $\times 2$ ), 129.0 (d $\times 2$ ), 130.8 (d), 136.1 (s), 143.8 (s), 148.9 (s), 149.8 (s), 170.3 (s). LR-MS  $m/z$ : 347 ( $\text{M}^+$ ), 56 (base peak). HR-MS  $m/z$  ( $\text{M}^+$ ): Calcd for  $\text{C}_{18}\text{H}_{21}\text{NO}_4\text{S}$ : 347.1192. Found: 347.1203.

*N*-(2,5-Dimethoxyphenyl)-*N*-methyl-3-(phenylsulfanyl)propionamide (**4c**): (5.38 g, 99%) was obtained from **3c** (5.2 g, 15.6 mmol) as a yellow gum. IR: 1655, 1610, 1508, 1043.  $^1\text{H}$ -NMR: 2.2–3.4 (4H, m), 3.15, 3.16 (total 3H, s,  $>\text{NCH}_3$ ), 3.70, 3.76, 3.79, 3.81 (total 6H, s,  $\text{OCH}_3\times 2$ ), 6.6–6.9 (3H, m, ArH), 7.4–7.7 (5H, m, SPh).  $^{13}\text{C}$ -NMR: 26.2, 26.3 (each t), 36.1 (q), 52.8 (t $\times 2$ ), 55.7 (q), 55.8 (q), 112.7, 112.8 (each d), 114.0, 114.2 (each d), 114.8 (d), 123.8 (d $\times 2$ ), 128.9 (d $\times 2$ ), 130.7 (d), 131.8 (s), 143.7 (s), 148.8, 148.9 (s), 153.7, 153.8 (s), 170.6 (s). LR-MS  $m/z$ : 347 ( $\text{M}^+$ ), 56 (base peak). HR-MS  $m/z$  ( $\text{M}^+$ ): Calcd for  $\text{C}_{18}\text{H}_{21}\text{NO}_4\text{S}$ : 347.1190. Found: 347.1195.

*N*-(2,4-Dimethoxyphenyl)-*N*-methyl-3-(phenylsulfanyl)propionamide

(**4d**): (5.63 g, 98%) was obtained from **3d** (5.5 g, 16.5 mmol) as a pale yellow gum. IR: 1655, 1512, 1039.  $^1\text{H}$ -NMR: 2.2–3.4 (4H, m), 3.13 (3H, s,  $>\text{NCH}_3$ ), 3.72, 3.84 (each 3H, s,  $\text{OCH}_3\times 2$ ), 6.4–6.6 (2H, m, ArH), 6.9–7.2 (1H, m, ArH), 7.4–7.7 (5H, m, SPh).  $^{13}\text{C}$ -NMR: 26.1, 26.2 (each t), 36.2 (q), 52.8, 52.9 (each t), 55.2 (q), 55.4 (q), 99.5 (d), 104.7 (d), 123.7 (d $\times 2$ ), 124.5 (s), 128.9 (d $\times 2$ ), 129.2 (d), 130.6 (d), 143.7 (s), 155.5, 155.6 (s), 160.5 (s), 170.9 (s). CI-MS  $m/z$ : 348 ( $\text{MH}^+$ ), 141 (base peak).

*N*-(2,3-Dimethoxyphenyl)-*N*-methyl-3-(phenylsulfanyl)propionamide (**4e**): (3.10 g, 98%) was obtained from **3e** (3.0 g, 9.0 mmol) as a pale yellow gum. IR: 1658, 1589, 1045.  $^1\text{H}$ -NMR: 2.3–3.2 (4H, m), 3.20, 3.23 (total 3H, s,  $>\text{NCH}_3$ ), 3.76, 3.86, 3.89, 3.90 (total 6H, s,  $\text{OCH}_3\times 2$ ), 6.6–7.2 (3H, m, ArH), 7.4–7.7 (5H, m, SPh).  $^{13}\text{C}$ -NMR: 26.5, 26.8 (each t), 36.8, 36.8 (each q), 52.9, 53.1 (each t), 56.0 (q), 60.9 (q), 112.5 (d), 120.1, 120.3 (each d), 123.9 (d $\times 2$ ), 124.2, 124.4 (each d), 129.0 (d $\times 2$ ), 130.8 (d), 136.6 (s), 143.8, 144.0 (s), 145.0 (s), 153.6, 153.7 (s), 170.4, 170.6 (s). LR-MS  $m/z$ : 347 ( $\text{M}^+$ ), 190 (base peak). HR-MS  $m/z$  ( $\text{M}^+$ ): Calcd for  $\text{C}_{18}\text{H}_{21}\text{NO}_4\text{S}$ : 347.1189. Found: 347.1184.

*N*-(3,4,5-Trimethoxyphenyl)-*N*-methyl-3-(phenylsulfanyl)propionamide (**4f**): (4.89 g, 98%) was obtained from **3f** (4.8 g, 13.2 mmol) as a yellow gum. IR: 1655, 1593, 1508, 1047.  $^1\text{H}$ -NMR: 2.2–3.4 (4H, m), 3.24 (3H, s,  $>\text{NCH}_3$ ), 3.85 (6H, s,  $\text{OCH}_3\times 2$ ), 3.88 (3H, s,  $\text{OCH}_3$ ), 6.37 (2H, s, ArH), 7.4–7.7 (5H, m, SPh).  $^{13}\text{C}$ -NMR: 25.9 (t), 36.6 (q), 51.9 (t), 55.5 (q $\times 2$ ), 60.1 (q), 104.0 (d $\times 2$ ), 123.2 (d $\times 2$ ), 127.6 (d $\times 2$ ), 130.2 (d), 137.2 (s), 138.2 (s), 143.0 (s), 153.2 (s $\times 2$ ), 169.3 (s). LR-MS  $m/z$ : 377 ( $\text{M}^+$ ), 56 (base peak). HR-MS  $m/z$  ( $\text{M}^+$ ): Calcd for  $\text{C}_{19}\text{H}_{23}\text{NO}_5\text{S}$ : 377.1297. Found: 377.1300.

*N*-Methyl-*N*-phenyl-3-(phenylsulfanyl)propionamide (**4g**): (10.51 g, 99%) was obtained from **3g** (10.0 g, 30.0 mmol) as a yellow gum. IR: 1657, 1595, 1043.  $^1\text{H}$ -NMR: 2.2–3.2 (4H, m), 3.25 (3H, s,  $>\text{NCH}_3$ ), 7.1–7.6 (10H, m, ArH and SPh).  $^{13}\text{C}$ -NMR: 26.9 (t), 37.2 (q), 52.6 (t), 123.7 (d $\times 2$ ), 127.0 (d), 127.9 (d), 128.9 (d $\times 2$ ), 129.8 (d $\times 2$ ), 130.7 (d $\times 2$ ), 143.0 (s), 143.6 (s), 169.8 (s). LR-MS  $m/z$ : 287 ( $\text{M}^+$ ), 106 (base peak). HR-MS  $m/z$  ( $\text{M}^+$ ): Calcd for  $\text{C}_{16}\text{H}_{17}\text{NO}_2\text{S}$ : 287.0978. Found: 287.0963.

**Pummerer Reaction of 4a. Typical Procedure** i) Method A. TFAA (303 mg, 1.44 mmol) was added to a solution of **4a** (100 mg, 0.29 mmol) in benzene (50 ml) at r.t., and the mixture was stirred for 20 h under argon atmosphere. After removal of the solvent *in vacuo*, the product was chromatographed with ethyl acetate–hexane (1 : 3) to give **5a** (28 mg, 30%), **6a** (17 mg, 18%), and **8a** (20 mg, 32%).

ii) Method B. TFAA (913 mg, 4.35 mmol) was added to a solution of **4a** (300 mg, 0.87 mmol) in benzene (50 ml) at r.t., and the mixture was stirred for 30 min under argon atmosphere. To this solution,  $\text{BF}_3\cdot\text{Et}_2\text{O}$  (370 mg, 2.61 mmol) was added, and the mixture was stirred at r.t. for 3 h. After removal of the solvent *in vacuo*, the residue was treated with 5%  $\text{NaHCO}_3$  and extracted with  $\text{CHCl}_3$ . The residual oil was chromatographed, eluted with ethyl acetate–hexane (1 : 3) to give **5a** (29 mg, 10%), **6a** (74 mg, 26%), and **8a** (97 mg, 51%).

*cis*-*N*-(3,5-Dimethoxyphenyl)-*N*-methyl-3-(phenylsulfanyl)acrylamide (**5a**): Colorless prisms from  $\text{Et}_2\text{O}$ –hexane, mp 114–118 °C. IR: 1638, 1593.  $^1\text{H}$ -NMR: 3.34 (3H, s,  $>\text{NCH}_3$ ), 3.79 (6H, s,  $\text{OCH}_3\times 2$ ), 5.89 (1H, d,  $J=10$  Hz,  $\text{PhSCH}=\text{CH}-$ ), 6.3–6.5 (3H, m, ArH), 7.01 (1H, d,  $J=10$  Hz,  $\text{PhSCH}=\text{CH}-$ ), 7.2–7.5 (5H, m, SPh).  $^{13}\text{C}$ -NMR: 36.8 (q), 55.5 (q $\times 2$ ), 99.6 (d), 105.6 (d $\times 2$ ), 113.6 (d), 127.7 (d), 129.1 (d $\times 2$ ), 130.9 (d $\times 2$ ), 137.6 (s), 145.4 (s), 146.5 (d), 161.4 (s $\times 2$ ), 166.3 (s). CI-MS  $m/z$ : 330 ( $\text{MH}^+$ ), 57 (base peak). Anal. Calcd for  $\text{C}_{18}\text{H}_{19}\text{NO}_3\text{S}$ : C, 65.63; H, 5.81; N, 4.25. Found: C, 65.41; H, 5.88; N, 4.17.

*trans*-*N*-(3,5-Dimethoxyphenyl)-*N*-methyl-3-(phenylsulfanyl)acrylamide (**6a**): Yellow gum. IR: 1644, 1593.  $^1\text{H}$ -NMR: 3.28 (3H, s,  $>\text{NCH}_3$ ), 3.78 (6H, s,  $\text{OCH}_3\times 2$ ), 5.78 (1H, d,  $J=15$  Hz,  $\text{PhSCH}=\text{CH}-$ ), 6.25 (1H, d,  $J=1$  Hz, ArH), 6.28 (1H, d,  $J=1$  Hz, ArH), 6.3–6.4 (1H, m, ArH), 7.2–7.4 (5H, m, SPh), 7.70 (1H, d,  $J=15$  Hz,  $\text{PhSCH}=\text{CH}-$ ).  $^{13}\text{C}$ -NMR: 37.0 (q), 55.4 (q $\times 2$ ), 99.5 (d), 105.7 (d $\times 2$ ), 117.0 (d), 128.3 (d), 129.2 (d $\times 2$ ), 131.8 (d $\times 2$ ), 132.0 (s), 142.8 (d), 145.0 (s), 161.2 (s $\times 2$ ), 164.4 (s). LR-MS  $m/z$ : 329 ( $\text{M}^+$ ), 206 (base peak). HR-MS  $m/z$  ( $\text{M}^+$ ): Calcd for  $\text{C}_{18}\text{H}_{19}\text{NO}_3\text{S}$ : 329.1082. Found: 329.1074.

5,7-Dimethoxy-1-methyl-2-quinolone (**8a**): Yellow needles from ethyl acetate, mp 117–120 °C. IR: 1684, 1642, 1622, 1582, 1501. UV: 213 (14800), 238 (11400), 257 (4600), 306 (4700), 324 (4200), 338 (2900).  $^1\text{H}$ -NMR: 3.67 (3H, s,  $>\text{NCH}_3$ ), 3.91 (6H, s,  $\text{OCH}_3\times 2$ ), 6.29, 6.37 (each 1H, d,  $J=2$  Hz, 6-H, 8-H), 6.51, 8.02 (each 1H, d,  $J=10$  Hz, 3-H, 4-H).  $^{13}\text{C}$ -NMR: 29.6 (q), 55.4 (q), 55.6 (q), 90.2 (d), 92.5 (d), 105.9 (s), 116.1 (d), 133.1 (d), 142.2 (s), 157.4 (s), 162.6 (s), 163.0 (s). LR-MS  $m/z$ : 219 ( $\text{M}^+$ , base peak). Anal. Calcd for  $\text{C}_{12}\text{H}_{13}\text{NO}_3$ : C, 65.74; H, 5.98; N, 6.39. Found: C, 65.90; H, 5.95; N, 6.44.

**Pummerer Reaction of 4b** i) Method A. **4b** (1.0 g, 2.88 mmol) gave **5b**

(305 mg, 32%), **6b** (416 mg, 44%), and **8b** (108 mg, 17%).

ii) Method B. **4b** (1.0 g, 2.88 mmol) gave **5b** (123 mg, 13%), **6b** (166 mg, 18%), **7b** (139 mg, 15%), and **8b** (268 mg, 42%).

*cis-N*-(3,4-Dimethoxyphenyl)-*N*-methyl-3-(phenylsulfanyl)acrylamide (**5b**): Pale yellow prisms from Et<sub>2</sub>O-hexane, mp 102–104 °C. IR: 1633, 1595, 1566. UV: 296 (24100). <sup>1</sup>H-NMR: 3.34 (3H, s, >NCH<sub>3</sub>), 3.87, 3.90 (each 3H, s, OCH<sub>3</sub>×2), 5.80 (1H, d, *J*=10 Hz, PhSCH=CH–), 6.7–6.9 (3H, m, ArH), 6.99 (1H, d, *J*=10 Hz, PhSCH=CH–), 7.2–7.6 (5H, m, SPh). <sup>13</sup>C-NMR: 37.1 (q), 56.0 (q×2), 110.7 (d), 111.5 (d), 113.6 (d), 119.5 (d), 127.7 (d), 129.1 (d×2), 130.8 (d×2), 136.7 (s), 137.7 (s), 146.2 (d), 148.5 (s), 149.6 (s), 166.5 (s). LR-MS *m/z*: 329 (M<sup>+</sup>), 167 (base peak). *Anal.* Calcd for C<sub>18</sub>H<sub>19</sub>NO<sub>3</sub>S: C, 65.64; H, 5.82; N, 4.25. Found: C, 65.70; H, 5.87; N, 4.11.

*trans-N*-(3,4-Dimethoxyphenyl)-*N*-methyl-3-(phenylsulfanyl)acrylamide (**6b**): Pale yellow prisms from Et<sub>2</sub>O-hexane, mp 99–101 °C. IR: 1633, 1570, 1512. UV: 284 (15500). <sup>1</sup>H-NMR: 3.28 (3H, s, >NCH<sub>3</sub>), 3.85, 3.91 (each 3H, s, OCH<sub>3</sub>×2), 5.70 (1H, d, *J*=15 Hz, PhSCH=CH–), 6.6–6.9 (3H, m, ArH), 7.2–7.5 (5H, m, SPh), 7.69 (1H, d, *J*=15 Hz, PhSCH=CH–). <sup>13</sup>C-NMR: 37.2 (q), 55.93 (q), 55.98 (q), 110.7 (d), 111.3 (d), 116.9 (d), 119.5 (d), 128.2 (d), 129.2 (d×2), 131.8 (d×2), 131.9 (s), 136.3 (s), 142.5 (d), 148.4 (s), 149.4 (s), 164.7 (s). LR-MS *m/z*: 329 (M<sup>+</sup>), 167 (base peak). *Anal.* Calcd for C<sub>18</sub>H<sub>19</sub>NO<sub>3</sub>S: C, 65.64; H, 5.82; N, 4.25. Found: C, 65.55; H, 5.87; N, 4.11.

6,7-Dimethoxy-1-methyl-4-phenylsulfanyl-3,4-dihydro-2-quinolone (**7b**): Colorless needles from ethyl acetate-hexane, mp 147–151 °C. IR: 1666, 1612, 1520. <sup>1</sup>H-NMR: 2.90 (2H, d, *J*=4 Hz, 3-H), 3.30 (3H, s, >NCH<sub>3</sub>), 3.76, 3.90 (each 3H, s, OCH<sub>3</sub>×2), 4.34 (1H, t, *J*=4 Hz, 4-H), 6.54, 6.59 (each 1H, s, 5-H, 8-H), 7.2–7.5 (5H, m, SPh). <sup>13</sup>C-NMR: 29.5 (q), 37.5 (t), 44.9 (d), 56.3 (q×2), 100.5 (d), 112.0 (d), 116.3 (s), 128.4 (d), 128.9 (d×2), 132.7 (s), 134.0 (s), 135.0 (d×2), 144.4 (s), 149.3 (s), 167.1 (s). LR-MS *m/z*: 329 (M<sup>+</sup>), 220 (base peak). *Anal.* Calcd for C<sub>18</sub>H<sub>19</sub>NO<sub>3</sub>S: C, 65.63; H, 5.81; N, 4.25. Found: C, 65.43; H, 5.82; N, 4.09.

6,7-Dimethoxy-1-methyl-2-quinolone (**8b**): Yellow prisms from Et<sub>2</sub>O-hexane, mp 148–152 °C. IR: 1649, 1583, 1562, 1520. UV: 236 (20300), 276 (2600), 286 (2700), 348 (6100), 366 (4300). <sup>1</sup>H-NMR: 3.72 (3H, s, >NCH<sub>3</sub>), 3.93, 4.01 (each 3H, s, OCH<sub>3</sub>×2), 6.59, 7.56 (each 1H, d, *J*=9 Hz, 3-H, 4-H), 6.77, 6.95 (each 1H, s, 5-H, 8-H). <sup>13</sup>C-NMR: 29.6 (q), 56.1 (q), 56.2 (q), 97.2 (d), 109.5 (d), 113.9 (s), 119.0 (d), 135.7 (s), 138.1 (d), 145.0 (s), 152.1 (s), 162.2 (s). LR-MS *m/z*: 219 (M<sup>+</sup>, base peak). HR-MS *m/z* (M<sup>+</sup>): Calcd for C<sub>12</sub>H<sub>13</sub>NO<sub>3</sub>: 219.0893. Found: 219.0795. *Anal.* Calcd for C<sub>12</sub>H<sub>13</sub>NO<sub>3</sub>: C, 65.74; H, 5.98; N, 6.39. Found: C, 65.58; H, 6.08; N, 6.34.

**Pummerer Reaction of 4c** i) Method A. **4c** (1.0 g, 2.88 mmol) gave **5c** (343 mg, 36%), **6c** (498 mg, 53%), and **32c** (44 mg, 7%).

ii) Method B. **4c** (1.0 g, 2.88 mmol) gave **5c** (84 mg, 9%), **6c** (185 mg, 20%), **7c** (434 mg, 46%), and **8c** (137 mg, 22%).

*cis-N*-(2,5-Dimethoxyphenyl)-*N*-methyl-3-(phenylsulfanyl)acrylamide (**5c**): Colorless prisms from Et<sub>2</sub>O-hexane, mp 128–130 °C. IR: 1635, 1568, 1508. UV: 298 (19500). <sup>1</sup>H-NMR: 3.27 (3H, s, >NCH<sub>3</sub>), 3.76, 3.77 (each 3H, s, OCH<sub>3</sub>×2), 5.73 (1H, d, *J*=10 Hz, PhSCH=CH–), 6.7–6.9 (3H, m, ArH), 6.95 (1H, d, *J*=10 Hz, PhSCH=CH–), 7.2–7.6 (5H, m, SPh). <sup>13</sup>C-NMR: 35.5 (q), 55.7 (q), 56.0 (q), 112.9 (d), 113.4 (d), 113.7 (d), 115.2 (d), 127.5 (d), 129.0 (d×2), 130.7 (d×2), 132.4 (s), 137.7 (s), 145.7 (d), 149.4 (s), 153.5 (s), 166.6 (s). LR-MS *m/z*: 329 (M<sup>+</sup>), 167 (base peak). *Anal.* Calcd for C<sub>18</sub>H<sub>19</sub>NO<sub>3</sub>S: C, 65.64; H, 5.82; N, 4.25. Found: C, 65.55; H, 5.92; N, 4.09.

*trans-N*-(2,5-Dimethoxyphenyl)-*N*-methyl-3-(phenylsulfanyl)acrylamide (**6c**): Colorless prisms from Et<sub>2</sub>O-hexane, mp 91–95 °C. IR: 1631, 1583, 1560. UV: 283 (20300). <sup>1</sup>H-NMR: 3.21 (3H, s, >NCH<sub>3</sub>), 3.73, 3.76 (each 3H, s, OCH<sub>3</sub>×2), 5.67 (1H, d, *J*=15 Hz, PhSCH=CH–), 6.65 (1H, t, *J*=2 Hz, ArH), 6.83 (2H, d, *J*=2 Hz, ArH), 7.2–7.3 (5H, m, SPh), 7.69 (1H, d, *J*=15 Hz, PhSCH=CH–). <sup>13</sup>C-NMR: 35.8 (q), 55.7 (q), 56.0 (q), 112.8 (d), 113.7 (d), 115.1 (d), 116.9 (d), 128.0 (d), 129.1 (d×2), 131.6 (d×2), 132.0 (s), 142.2 (d), 149.3 (s), 153.4 (s), 164.8 (s). LR-MS *m/z*: 329 (M<sup>+</sup>), 167 (base peak). *Anal.* Calcd for C<sub>18</sub>H<sub>19</sub>NO<sub>3</sub>S: C, 65.64; H, 5.82; N, 4.25. Found: C, 65.64; H, 5.92; N, 4.06.

5,8-Dimethoxy-1-methyl-4-phenylsulfanyl-3,4-dihydro-2-quinolone (**7c**): Colorless prisms from Et<sub>2</sub>O-hexane, mp 137–141 °C. IR: 1666, 1597. <sup>1</sup>H-NMR: 2.76 (2H, d, *J*=3 Hz, 3-H), 3.34 (3H, s, >NCH<sub>3</sub>), 3.79, 3.79 (each 3H, s, OCH<sub>3</sub>×2), 4.80 (1H, t, *J*=3 Hz, 4-H), 6.60, 6.86 (each 1H, d, *J*=9 Hz, 6-H, 7-H), 7.2–7.6 (5H, m, SPh). <sup>13</sup>C-NMR: 34.4 (q), 37.6 (t), 38.6 (d), 56.1 (q), 56.7 (q), 106.8 (d), 113.8 (d), 117.8 (s), 128.1 (d), 128.7 (d×2), 132.0 (s), 133.1 (s), 134.6 (d×2), 144.3 (s), 150.0 (s), 169.3 (s). LR-MS *m/z*: 329 (M<sup>+</sup>), 220 (base peak). HR-MS *m/z* (M<sup>+</sup>): Calcd for C<sub>18</sub>H<sub>19</sub>NO<sub>3</sub>S: 329.1086. Found: 329.1122. *Anal.* Calcd for C<sub>18</sub>H<sub>19</sub>NO<sub>3</sub>S: C, 65.63; H, 5.81;

N, 4.25. Found: C, 65.47; H, 5.85; N, 4.15.

5,8-Dimethoxy-1-methyl-2-quinolone (**8c**): Yellow needles from Et<sub>2</sub>O-hexane, mp 70–74 °C. IR: 1657, 1597. UV: 213 (25700), 241 (20100), 263 (13100), 302 (8100), 314 (6800), 340 (2300). <sup>1</sup>H-NMR: 3.83 (3H, s, >NCH<sub>3</sub>), 3.88, 3.94 (each 3H, s, OCH<sub>3</sub>×2), 6.59, 7.00 (each 1H, d, *J*=9 Hz, 6-H, 7-H), 6.64, 8.09 (each 1H, d, *J*=10 Hz, 3-H, 4-H). <sup>13</sup>C-NMR: 34.8 (q), 55.9 (q), 57.7 (q), 102.7 (d), 113.4 (s), 115.5 (d), 120.3 (d), 132.6 (s), 133.1 (d), 142.7 (s), 150.5 (s), 163.6 (s). LR-MS *m/z*: 219 (M<sup>+</sup>, base peak). HR-MS *m/z* (M<sup>+</sup>): Calcd for C<sub>12</sub>H<sub>13</sub>NO<sub>3</sub>: 219.0895. Found: 219.0915.

**Pummerer Reaction of 4d** i) Method A. **4d** (1.0 g, 2.88 mmol) gave **5d** (340 mg, 36%) and **6d** (598 mg, 63%).

ii) Method B. **4d** (1.0 g, 2.88 mmol) gave **5d** (427 mg, 45%) and **6d** (493 mg, 52%).

*cis-N*-(2,4-Dimethoxyphenyl)-*N*-methyl-3-phenylsulfanylacrylamide (**5d**): Pale yellow prisms from Et<sub>2</sub>O-hexane, mp 73–75 °C. IR: 1639, 1577, 1512. UV: 290 (20400). <sup>1</sup>H-NMR: 3.23 (3H, s, >NCH<sub>3</sub>), 3.79, 3.82 (each 3H, s, OCH<sub>3</sub>×2), 5.70 (1H, d, *J*=10 Hz, PhSCH=CH–), 6.47 (1H dd, *J*=8, 3 Hz, 5'-H), 6.52 (1H, d, *J*=3 Hz, 3'-H), 6.93 (1H, d, *J*=10 Hz, PhSCH=CH–), 7.07 (1H, d, *J*=8 Hz, 6'-H), 7.2–7.5 (5H, m, SPh). <sup>13</sup>C-NMR: 35.8 (q), 55.49 (q), 55.55 (q), 99.6 (d), 104.4 (d), 113.6 (d), 125.3 (s), 127.5 (d), 129.0 (d×2), 129.7 (d), 130.8 (d×2), 145.3 (d), 156.2 (s), 160.4 (s), 167.1 (s). LR-MS *m/z*: 329 (M<sup>+</sup>), 109 (base peak). *Anal.* Calcd for C<sub>18</sub>H<sub>19</sub>NO<sub>3</sub>S: C, 65.64; H, 5.82; N, 4.25. Found: C, 65.62; H, 5.92; N, 4.11.

*trans-N*-(2,4-Dimethoxyphenyl)-*N*-methyl-3-phenylsulfanylacrylamide (**6d**): Pale yellow prisms from ether-hexane, mp 100–102 °C. IR: 1637, 1608, 1570. UV: 285 (17400). <sup>1</sup>H-NMR: 3.18 (3H, s, >NCH<sub>3</sub>), 3.76, 3.84 (each 3H, s, OCH<sub>3</sub>×2), 5.69 (1H, d, *J*=15 Hz, PhSCH=CH–), 6.43 (1H, dd, *J*=8, 3 Hz, 5'-H), 9.46 (1H, d, *J*=3 Hz, 3'-H), 6.97 (1H, d, *J*=8 Hz, 6'-H), 7.2–7.4 (5H, m, SPh), 7.67 (1H, d, *J*=15 Hz, PhSCH=CH–). <sup>13</sup>C-NMR: 36.0 (q), 55.5 (q×2), 99.5 (d), 104.4 (d), 117.2 (d), 124.9 (s), 127.9 (d), 129.1 (d×2), 129.6 (d), 131.5 (d×2), 132.3 (s), 141.8 (d), 156.0 (s), 160.4 (s), 165.3 (s). LR-MS *m/z*: 329 (M<sup>+</sup>), 167 (base peak). *Anal.* Calcd for C<sub>18</sub>H<sub>19</sub>NO<sub>3</sub>S: C, 65.64; H, 5.82; N, 4.25. Found: C, 65.61; H, 5.92; N, 4.07.

**Pummerer Reaction of 4e** i) Method A. **4e** (1.0 g, 2.88 mmol) gave **5e** (374 mg, 39%), **6e** (321 mg, 34%), and **8e** (127 mg, 21%).

ii) Method B. **4e** (1.0 g, 2.88 mmol) gave **5e** (156 mg, 17%), **6e** (409 mg, 43%), and **8e** (252 mg, 40%).

*cis-N*-(2,3-Dimethoxyphenyl)-*N*-methyl-3-(phenylsulfanyl)acrylamide (**5e**): Yellow gum. IR: 1635, 1585, 1566. <sup>1</sup>H-NMR: 3.32 (3H, s, >NCH<sub>3</sub>), 3.81, 3.89 (each 3H, s, OCH<sub>3</sub>×2), 5.77 (1H, d, *J*=10 Hz, PhSCH=CH–), 6.7–7.1 (3H, m, ArH), 6.98 (1H, d, *J*=10 Hz, PhSCH=CH–), 7.2–7.6 (5H, m, SPh). <sup>13</sup>C-NMR: 36.2 (q), 56.0 (q), 60.9 (q), 112.1 (d), 113.4 (d), 121.0 (d), 124.0 (d), 127.7 (d), 129.1 (d×2), 130.9 (d×2), 137.3 (s), 137.8 (s), 145.5 (s), 146.3 (d), 153.8 (s), 166.6 (s). LR-MS *m/z*: 329 (M<sup>+</sup>), 57 (base peak).

*trans-N*-(2,3-Dimethoxyphenyl)-*N*-methyl-3-(phenylsulfanyl)acrylamide (**6e**): Yellow gum. IR: 1643, 1585. <sup>1</sup>H-NMR: 3.26 (3H, s, >NCH<sub>3</sub>), 3.76, 3.90 (each 3H, s, OCH<sub>3</sub>×2), 5.72 (1H, d, *J*=15 Hz, PhSCH=CH–), 6.6–7.1 (3H, m, ArH), 7.2–7.5 (5H, m, SPh), 7.72 (1H, d, *J*=15 Hz, PhSCH=CH–). <sup>13</sup>C-NMR: 36.3 (q), 56.0 (q), 60.8 (q), 112.1 (d), 116.8 (d), 121.0 (d), 123.9 (d), 128.1 (d), 129.2 (d×2), 131.7 (d×2), 132.1 (s), 136.9 (s), 142.7 (d), 145.4 (s), 153.6 (s), 164.7 (s). LR-MS *m/z*: 329 (M<sup>+</sup>), 153 (base peak).

7,8-Dimethoxy-1-methyl-2-quinolone (**8e**): Yellow prisms from Et<sub>2</sub>O-hexane, mp 92–94 °C. IR: 1653, 1589, 1558, 1502. UV: 240 (36200), 262 (8800), 294 (8800), 328 (6200). <sup>1</sup>H-NMR: 3.81 (3H, s, >NCH<sub>3</sub>), 3.96 (6H, s, OCH<sub>3</sub>×2), 6.53, 7.52 (each 1H, d, *J*=9 Hz, 3-H, 4-H), 6.87, 7.24 (each 1H, d, *J*=9 Hz, 5-H, 6-H). <sup>13</sup>C-NMR: 33.6 (q), 56.3 (q), 61.7 (q), 107.6 (d), 117.1 (s), 119.0 (d), 124.7 (d), 135.0 (s), 137.0 (s), 139.0 (d), 155.3 (s), 163.9 (s). LR-MS *m/z*: 219 (M<sup>+</sup>) 64 (base peak). HR-MS *m/z* (M<sup>+</sup>): Calcd for C<sub>12</sub>H<sub>13</sub>NO<sub>3</sub>: 219.0895. Found: 219.0928. *Anal.* Calcd for C<sub>12</sub>H<sub>13</sub>NO<sub>3</sub>: C, 65.74; H, 5.98; N, 6.39. Found: C, 65.66; H, 6.01; N, 6.25.

**Pummerer Reaction of 4f** i) Method A. **4f** (2.0 g, 5.31 mmol) gave **5f** (609 mg, 32%), **6f** (504 mg, 27%), and **8f** (515 mg, 39%).

ii) Method B. **4f** (2.0 g, 5.31 mmol) gave **5f** (404 mg, 21%), **6f** (485 mg, 26%), and **8f** (677 mg, 51%).

*cis-N*-(3,4,5-Trimethoxyphenyl)-*N*-methyl-3-(phenylsulfanyl)acrylamide (**5f**): Colorless plates from ethyl acetate, mp 159–163 °C. IR: 1643, 1591, 1502. <sup>1</sup>H-NMR: 3.35 (3H, s, >NCH<sub>3</sub>), 3.85 (6H, s, OCH<sub>3</sub>×2), 3.87 (3H, s, OCH<sub>3</sub>), 5.85 (1H, d, *J*=10 Hz, PhSCH=CH–), 6.44 (2H, s, 2'-H, 6'-H), 7.03 (1H, d, *J*=10 Hz, PhSCH=CH–), 7.2–7.6 (5H, m, SPh). <sup>13</sup>C-NMR: 36.8 (q), 56.1 (q×2), 60.7 (q), 104.6 (d×2), 113.4 (d), 127.6 (d), 129.0 (d×2), 130.6 (d×2), 137.3 (s), 137.5 (s), 139.2 (s), 146.2 (d), 153.6 (s×2), 166.1 (s). LR-MS *m/z*: 359 (M<sup>+</sup>), 163 (base peak). *Anal.* Calcd for C<sub>19</sub>H<sub>21</sub>NO<sub>4</sub>S:

C, 63.49; H, 5.89; N, 3.90. Found: C, 63.35; H, 5.91; N, 3.74.

*trans-N*-(3,4,5-Trimethoxyphenyl)-*N*-methyl-3-(phenylsulfanyl)acrylamide (**6f**): Colorless prisms from Et<sub>2</sub>O-hexane, mp 85–90 °C. IR: 1641, 1593, 1504. <sup>1</sup>H-NMR: 3.28 (3H, s, >NCH<sub>3</sub>), 3.82 (6H, s, OCH<sub>3</sub>×2), 3.87 (3H, s, OCH<sub>3</sub>), 5.68 (1H, d, *J*=15 Hz, PhSCH=CH–), 6.31 (2H, s, 2'-H, 6'-H), 7.2–7.4 (5H, m, SPh), 7.71 (1H, d, *J*=15 Hz, PhSCH=CH–). <sup>13</sup>C-NMR: 37.1 (q), 56.2 (q×2), 60.9 (q), 104.8 (d×2), 116.7 (d), 128.5 (d), 129.3 (d×2), 131.6 (s), 132.1 (d×2), 137.5 (s), 139.0 (s), 142.9 (d), 153.6 (s×2), 164.5 (s). *Anal.* Calcd for C<sub>19</sub>H<sub>21</sub>NO<sub>4</sub>S: C, 63.49; H, 5.89; N, 3.90. Found: C, 63.22; H, 5.95; N, 3.79.

5,6,7-Trimethoxy-1-methyl-2-quinolone (**8f**): Reddish orange gum. IR: 1653, 1589, 1560, 1504. UV: 239 (30000), 294 (6700), 337 (7900), 352 (5900). <sup>1</sup>H-NMR: 3.68 (3H, s, >NCH<sub>3</sub>), 3.89, 4.00, 4.03 (each 3H, s, OCH<sub>3</sub>×3), 6.54, 7.93 (each 1H, d, *J*=10 Hz, 3-H, 4-H), 6.54 (1H, s, 8-H). <sup>13</sup>C-NMR: 29.5 (q), 56.0 (q), 60.9 (q), 61.5 (q), 92.6 (d), 109.2 (s), 117.9 (d), 133.0 (d), 136.8 (s), 137.4 (s), 149.5 (s), 156.1 (s), 162.4 (s). LR-MS *m/z*: 249 (M<sup>+</sup>, base peak). HR-MS *m/z* (M<sup>+</sup>): Calcd for C<sub>13</sub>H<sub>15</sub>NO<sub>4</sub>: 249.0999. Found: 249.0996.

**Pummerer Reaction of 4g** i) Method A. **4g** (2.0 g, 6.97 mmol) gave **5g** (769 mg, 41%) and **6g** (1.02 g, 54%).

ii) Method B. **4g** (2.0 g, 6.97 mmol) gave **5g** (243 mg, 13%), **6g** (315 mg, 17%), **7g** (744 mg, 43%), and **8g** (127 mg, 12%).

*cis-N*-Methyl-*N*-phenyl-3-(phenylsulfanyl)acrylamide (**5g**): Colorless needles from Et<sub>2</sub>O-hexane, mp 84–86 °C. IR: 1635, 1585, 1558. UV: 259 (7500), 297 (8100). <sup>1</sup>H-NMR: 3.37 (3H, s, >NCH<sub>3</sub>), 5.79 (1H, d, *J*=10 Hz, PhSCH=CH–), 6.99 (1H, d, *J*=10 Hz, PhSCH=CH–), 7.2–7.6 (10H, m, ArH, SPh). <sup>13</sup>C-NMR: 37.0 (q), 113.6 (d), 127.3 (d×2), 127.5 (d), 127.7 (d), 129.2 (d×2), 129.6 (d×2), 130.9 (d×2), 137.7 (s), 146.5 (d), 166.3 (s). LR-MS *m/z*: 269 (M<sup>+</sup>), 107 (base peak). *Anal.* Calcd for C<sub>16</sub>H<sub>15</sub>NOS: C, 71.36; H, 5.61; N, 5.20. Found: C, 71.28; H, 5.78; N, 5.01.

*trans-N*-Methyl-*N*-phenyl-3-(phenylsulfanyl)acrylamide (**6g**): Yellow gum. IR: 1637, 1595, 1570. UV: 262 (12700), 291 (16200). <sup>1</sup>H-NMR: 3.34 (3H, s, >NCH<sub>3</sub>), 5.73 (1H, d, *J*=15 Hz, PhSCH=CH–), 7.0–7.5 (10H, m, ArH, SPh), 7.75 (1H, d, *J*=15 Hz, PhSCH=CH–). <sup>13</sup>C-NMR: 37.1 (q), 116.8 (d), 127.1 (d×2), 127.3 (d), 128.1 (d), 129.2 (d×2), 129.3 (d×2), 131.7 (d×2), 131.8 (s), 142.8 (d), 143.3 (s), 164.4 (s). LR-MS *m/z*: 269 (M<sup>+</sup>), 163 (base peak).

1-Methyl-4-phenylsulfanyl-3,4-dihydro-2-quinolone (**7g**): Colorless needles from Et<sub>2</sub>O-hexane, mp 97–103 °C. IR: 1660, 1597. <sup>1</sup>H-NMR: 2.92 (2H, d, *J*=4 Hz, 3-H), 3.30 (3H, s, >NCH<sub>3</sub>), 4.42 (1H, t, *J*=4 Hz, 4-H), 6.9–7.5 (9H, m, ArH, SPh). <sup>13</sup>C-NMR: 29.4 (q), 37.3 (t), 44.8 (d), 115.1 (d), 122.8 (d), 124.7 (s), 128.5 (d×2), 129.0 (d×2), 132.4 (s), 134.9 (d×3), 140.1 (s), 167.3 (s). LR-MS *m/z*: 269 (M<sup>+</sup>), 160 (base peak). *Anal.* Calcd for C<sub>16</sub>H<sub>15</sub>NOS: C, 71.34; H, 5.61; N, 5.20. Found: C, 71.22; H, 5.68; N, 4.96.

1-Methyl-2-quinolone (**8g**): Colorless needles from Et<sub>2</sub>O-hexane, mp 68–73 °C. IR: 1647, 1585. UV: 231 (44200), 268 (5100), 277 (5600), 333 (5800), 350 (3700). <sup>1</sup>H-NMR: 3.72 (3H, s, >NCH<sub>3</sub>), 6.70, 7.66 (each 1H, d, *J*=9 Hz, 3-H, 4-H), 7.1–7.7 (4H, m, ArH). <sup>13</sup>C-NMR: 29.4 (q), 114.1 (d), 120.7 (s), 121.8 (d), 122.0 (d), 128.7 (d), 130.6 (d), 138.9 (d), 140.1 (s), 162.3 (s). LR-MS *m/z*: 159 (M<sup>+</sup>), 107 (base peak). HR-MS *m/z* (M<sup>+</sup>): Calcd for C<sub>10</sub>H<sub>9</sub>NO: 159.0682. Found: 159.0656. *Anal.* Calcd for C<sub>10</sub>H<sub>9</sub>NO: C, 75.45; H, 5.70; N, 8.80. Found: C, 75.40; H, 5.81; N, 8.60.

**Preparation of 5a and 6a by Acylation of 1a with *cis*-3-(Phenylsulfanyl)acrylic Acid. A Typical Procedure** A solution of *cis*-3-(phenylsulfanyl)acrylic acid (1.44 g, 7.8 mmol) and oxalyl chloride (2.98 g, 23.5 mmol) was stirred at r.t. for 2 h. Removal of excess oxalyl chloride by repeated evaporation under reduced pressure gave an oily material. To a solution of this acid chloride in benzene (30 ml) was slowly added a solution of **1e** (1.0 g, 6.5 mmol) and triethylamine (0.79 g, 7.8 mmol) in benzene (100 ml) under ice-cooling, and the mixture was stirred at r.t. for 14 h. After removal of the precipitates by filtration, the filtrate was evaporated *in vacuo*. The residue was chromatographed, eluted with ethyl acetate-hexane (1:2) to give *N*-(3,5-dimethoxyphenyl)-3-(phenylsulfanyl)acrylamide (**15a**) as a mixture of *cis*- and *trans*-stereoisomers.

To a solution of **15a** (200 mg, 0.63 mmol) in tetrahydrofuran (THF) (25 ml), CH<sub>3</sub>I (900 mg, 6.34 mmol), KOH (420 mg, 6.36 mmol), and tetrabutylammonium bromide (TBAB) (102 mg, 0.32 mmol) were added at r.t., and the mixture was stirred for 3 h. After removal of the precipitates by filtration, the filtrate was extracted with CHCl<sub>3</sub> and evaporated *in vacuo*. The residual oil was chromatographed with ethyl acetate-hexane (3:1) to give **5a** (128 mg, 61%) and **6a** (60 mg, 29%). The acrylamides (**5b–e**, **g**) and the *trans*-isomers (**6b–e**, **g**) were prepared from the corresponding vinyl sulfides (**15**) in similar yields.

**Preparation of *cis-N*-(4-Methoxyphenyl)-*N*-methyl-3-(phenylsulfanyl)-**

**acrylamide (5h) and the *trans*-Isomer (6h)** From **15h** (2.0 g, 6.99 mmol); column chromatography (CHCl<sub>3</sub>) followed by medium pressure column chromatography (benzene-acetone 20:1) gave **5h** (0.66 g, 31%) and **6h** (0.95 g, 45%).

**5h**: Colorless needles from Et<sub>2</sub>O-hexane, mp 89–91 °C. IR: 1635. UV: 299 (20600). <sup>1</sup>H-NMR: 3.33 (3H, s, NMe), 3.83 (3H, s, OMe), 5.77 (1H, d, *J*=10 Hz, olefinic H), 6.90 (2H, d, *J*=9 Hz, ArH), 7.14 (2H, d, *J*=9 Hz, ArH), 7.2–7.5 (6H, m, SPh and olefinic H). <sup>13</sup>C-NMR: 36.9 (q), 55.3 (q), 113.5 (d), 114.6 (d×2), 127.5 (d), 128.2 (d×2), 129.0 (d×2), 130.6 (d×2), 136.4 (s), 137.6 (s), 145.9 (d), 158.6 (s), 166.4 (s). LR-MS *m/z*: 299 (M<sup>+</sup>), 137 (base peak). HR-MS *m/z* (M<sup>+</sup>): Calcd for C<sub>17</sub>H<sub>17</sub>NO<sub>2</sub>S: 299.0980. Found: 299.0982.

**6h**: Yellow gum. IR (Film): 1635. UV: 289 (16200). <sup>1</sup>H-NMR: 3.26 (3H, s, NMe), 3.80 (3H, s, OMe), 5.69 (1H, d, *J*=15 Hz, olefinic H), 5.83 (2H, d, *J*=9 Hz, ArH), 7.02 (2H, d, *J*=9 Hz, ArH), 7.1–7.4 (5H, m, SPh), 7.68 (1H, d, *J*=15 Hz, olefinic H). <sup>13</sup>C-NMR: 36.9 (q), 55.1 (q), 114.3 (d×2), 116.7 (d), 127.8 (d), 127.9 (d×2), 128.9 (d×2), 131.4 (d×2), 131.6 (s), 135.7 (s), 142.1 (d), 158.3 (s), 164.3 (s). LR-MS *m/z*: 299 (M<sup>+</sup>), 137 (base peak). HR-MS *m/z* (M<sup>+</sup>): Calcd for C<sub>17</sub>H<sub>17</sub>NO<sub>2</sub>S: 299.0980. Found: 299.0997.

**Preparation of *cis-N*-(3-Methoxyphenyl)-*N*-methyl-3-(phenylsulfanyl)-acrylamide (5i) and the *trans*-Isomer (6i)** From **15i** (0.97 g, 3.4 mmol); column chromatography (CHCl<sub>3</sub>) followed by medium pressure column chromatography (benzene-acetone 20:1) gave **5i** (0.66 g, 31%) and **6i** (0.95 g, 45%).

**5i**: Yellow gum. IR (film): 1641. UV: 298 (14900). <sup>1</sup>H-NMR: 3.36 (3H, s, NMe), 3.81 (3H, s, OMe), 5.84 (1H, d, *J*=10 Hz, olefinic H), 6.7–7.1 (3H, m, ArH), 7.2–7.5 (7H, m, ArH, SPh). <sup>13</sup>C-NMR: 36.8 (q), 55.3 (q), 113.0 (d×2), 113.5 (d), 119.3 (d), 127.6 (d), 129.0 (d×2), 130.2 (d), 130.7 (d×2), 137.6 (s), 144.7 (s), 146.2 (d), 160.4 (s), 166.1 (s). LR-MS *m/z*: 299 (M<sup>+</sup>), 163 (base peak). HR-MS *m/z* (M<sup>+</sup>): Calcd for C<sub>17</sub>H<sub>17</sub>NO<sub>2</sub>S: 299.0980. Found: 299.0983.

**6i**: Yellow gum. IR (film): 1633. UV: 380 (200), 280 (16500). <sup>1</sup>H-NMR: 3.29 (3H, s, NMe), 3.79 (3H, s, OMe), 5.74 (1H, d, *J*=15 Hz, olefinic H), 6.6–6.9 (3H, m, ArH), 7.1–7.4 (6H, m, ArH, SPh), 7.71 (1H, d, *J*=15 Hz, olefinic H). <sup>13</sup>C-NMR: 36.5 (q), 54.8 (q), 112.5 (d×2), 116.5 (d), 118.9 (d), 127.8 (d), 128.8 (d×2), 129.6 (d), 131.3 (d×2), 142.1 (d), 144.0 (s×2), 159.9 (s), 163.8 (s). LR-MS *m/z*: 299 (M<sup>+</sup>), 163 (base peak). HR-MS *m/z* (M<sup>+</sup>): Calcd for C<sub>17</sub>H<sub>17</sub>NO<sub>2</sub>S: 299.0977. Found: 299.0952.

**Preparation of *cis-N*-(2-Methoxyphenyl)-*N*-methyl-3-(phenylsulfanyl)-acrylamide (5k) and the *trans*-Isomer (6k)** From **15k** (10.0 g, 35.0 mmol); column chromatography (CHCl<sub>3</sub>) followed by medium pressure column chromatography (benzene-acetone 20:1) gave **5k** (3.56 g, 34%), **6k** (3.56 g, 34%), and the mixture of **5k** and **6k** (2.4 g, 23%).

**5k**: Colorless prisms from Et<sub>2</sub>O-hexane, mp 135–137 °C. IR: 1625. UV: 293 (19900). <sup>1</sup>H-NMR: 3.27 (3H, s, NMe), 3.82 (3H, s, OMe), 5.68 (1H, d, *J*=10 Hz, olefinic H), 6.8–7.5 (10H, m, ArH, SPh and olefinic H). <sup>13</sup>C-NMR: 35.3 (q), 55.3 (q), 111.8 (d), 113.3 (d), 120.6 (d), 127.3 (d), 128.8 (d×2), 129.0 (d×2), 130.4 (d×2), 131.6 (s), 137.5 (s), 145.2 (d), 154.9 (s), 166.4 (s). LR-MS *m/z*: 299 (M<sup>+</sup>), 137 (base peak). HR-MS *m/z* (M<sup>+</sup>): Calcd for C<sub>17</sub>H<sub>17</sub>NO<sub>2</sub>S: 299.0978. Found: 299.0967.

**6k**: Yellow gum. IR (film): 1631. UV: 283 (16400). <sup>1</sup>H-NMR: 3.21 (3H, s, NMe), 3.79 (3H, s, OMe), 5.66 (1H, d, *J*=15 Hz, olefinic H), 6.8–7.4 (10H, m, ArH, SPh), 7.69 (1H, d, *J*=15 Hz, olefinic H). <sup>13</sup>C-NMR: 35.5 (q), 55.1 (q), 111.6 (d), 116.5 (d), 120.5 (d), 127.7 (d), 128.8 (d×4), 131.1 (s), 131.2 (d×2), 131.6 (s), 141.7 (d), 154.6 (s), 164.5 (s). LR-MS *m/z*: 299 (M<sup>+</sup>), 109 (base peak). HR-MS *m/z* (M<sup>+</sup>): Calcd for C<sub>17</sub>H<sub>17</sub>NO<sub>2</sub>S: 299.0979. Found: 299.0979.

**Acid-Induced Cyclization of 5a and 6a. Typical Procedure** i) With BF<sub>3</sub>·Et<sub>2</sub>O: A solution of **5a** or **6a** (each 30 mg, 0.09 mmol) and BF<sub>3</sub>·Et<sub>2</sub>O (66 mg, 0.46 mmol) in toluene (5 ml) was heated at 150 °C in a sealed tube for 150 h. The mixture was extracted with CHCl<sub>3</sub>, and the extract was washed with 5% NaOH and water. The product was chromatographed, eluted with ethyl acetate-hexane (2:1) to give **8a** (18 mg, 98% from **5a** and 12 mg, 67% from **6a**).

ii) With *p*-TsOH: A solution of **5a** or **6a** (each 450 mg, 0.09 mmol) and *p*-TsOH·H<sub>2</sub>O (780 mg, 4.1 mmol) in toluene (80 ml) was refluxed using a Dean-Stark water separator for 5 h. The mixture was extracted with CHCl<sub>3</sub>, and the extract was washed with 5% NaOH and water. The product was chromatographed, eluted with ethyl acetate-hexane (2:1) to give **8a** (288 mg, 96 % from **5a** and 275 mg, 90% from **6a**). Acid-induced cyclization reactions of **5** and **6** using *p*-TsOH were carried out in a similar way, and the results are summarized in Table 2.

6-Methoxy-1-methyl-2-quinolone (**8h**): Colorless needles from Et<sub>2</sub>O-

hexane, mp 75–77 °C. IR: 1646. UV: 356 (6000), 279 (5200), 270 (5600), 232 (25900). <sup>1</sup>H-NMR: 3.70 (3H, s, NMe), 3.86 (3H, s, OMe), 6.70 (1H, d, *J*=9 Hz, 3-H), 6.99 (1H, br d, *J*=2 Hz, 5-H), 7.16 (1H, dd, *J*=9, 2 Hz, 7-H), 7.32 (1H, d, *J*=9 Hz, 8-H), 7.60 (1H, d, *J*=9 Hz, 4-H). <sup>13</sup>C-NMR: 29.1 (q), 55.3 (q), 110.1 (d), 115.0 (d), 118.8 (d), 120.9 (s), 121.8 (d), 134.2 (s), 138.0 (d), 154.3 (s), 161.4 (s). LR-MS *m/z*: 189 (M<sup>+</sup>), 174 (base peak). HR-MS *m/z* (M<sup>+</sup>): Calcd for C<sub>11</sub>H<sub>11</sub>NO<sub>2</sub>: 189.0790. Found: 189.0805.

*N*-(4-Methoxyphenyl)-*N*-methyl-3,3-di(phenylsulfanyl)propionamide (**16h**): Yellow gum. IR (film): 1652. <sup>1</sup>H-NMR: 2.62 (2H, d, *J*=7 Hz, COCH<sub>2</sub>), 3.25 (3H, s, NMe), 3.83 (3H, s, OMe), 5.03 (1H, t, *J*=7 Hz, COCH<sub>2</sub>CH), 6.86 (2H, d, *J*=9 Hz, ArH), 7.05 (2H, d, *J*=9 Hz, ArH), 7.2–7.5 (10H, m, SPh×2). <sup>13</sup>C-NMR: 37.6 (q), 40.1 (t), 53.8 (d), 55.5 (q), 114.9 (d×2), 127.6 (d×2), 128.5 (d×2), 128.9 (d×4), 132.3 (d×4), 134.0 (s×2), 136.1 (s), 159.0 (s), 169.4 (s). CI-MS *m/z*: 409 (MH<sup>+</sup>), 190 (base peak). HR-MS *m/z* (M<sup>+</sup>): Calcd for C<sub>23</sub>H<sub>23</sub>NO<sub>2</sub>S<sub>2</sub>: 409.1167. Found: 409.1150.

7-Methoxy-1-methyl-2-quinolone (**8i**): Colorless needles from acetone–hexane, mp 100–102 °C. IR: 1646. UV: 344 (8500), 329 (12300), 286 (6700), 257 (5800), 229 (39400), 223 (37400). <sup>1</sup>H-NMR: 3.68 (3H, s, NMe), 3.92 (3H, s, OMe), 6.54 (1H, d, *J*=10 Hz, 3-H), 6.7–6.9 (2H, m, 6-H, 8-H), 7.96 (1H, d, *J*=9 Hz, 5-H), 7.59 (1H, d, *J*=10 Hz, 4-H). <sup>13</sup>C-NMR: 29.1 (q), 55.3 (q), 98.4 (d), 109.3 (d), 114.6 (s), 118.1 (d), 129.8 (d), 138.4 (d), 141.4 (s), 161.6 (s), 162.4 (s). LR-MS *m/z*: 189 (M<sup>+</sup>, base peak). HR-MS *m/z* (M<sup>+</sup>): Calcd for C<sub>11</sub>H<sub>11</sub>NO<sub>2</sub>: 189.0787. Found: 189.0776.

5-Methoxy-1-methyl-2-quinolone (**8j**): Pale yellow needles from ethyl acetate–hexane, mp 131–133 °C (lit.<sup>8</sup>) mp 129–130 °C. IR: 1652. <sup>1</sup>H-NMR: 3.70 (3H, s, NMe), 3.94 (3H, s, OMe), 6.64 (1H, d, *J*=9 Hz, 3-H), 6.68 (1H, d, *J*=8 Hz, 6-H), 6.94 (1H, d, *J*=8 Hz, 8-H), 7.48 (1H, t, *J*=8 Hz, 7-H), 8.13 (1H, d, *J*=9 Hz, 4-H). <sup>13</sup>C-NMR: 29.6 (q), 55.8 (q), 102.8 (d), 106.7 (d), 111.3 (s), 119.8 (d), 131.2 (d), 133.1 (d), 141.2 (s), 156.4 (s), 162.4 (s). LR-MS *m/z*: 189 (M<sup>+</sup>), 146 (base peak).

8-Methoxy-1-methyl-2-quinolone (**8k**): Yellow needles from hexane, mp 81–83 °C (lit.<sup>9</sup>) mp 80–81 °C. IR: 1646. UV: 341 (2900), 282 (5700), 257 (15300), 240 (28000), 217 (19600). <sup>1</sup>H-NMR: 3.84 (3H, s, NMe), 3.90 (3H, s, OMe), 6.64 (1H, d, *J*=9 Hz, 3-H), 6.9–7.2 (3H, m, 5-H, 6-H, 7-H), 7.52 (1H, d, *J*=9 Hz, 4-H). <sup>13</sup>C-NMR: 35.2 (q), 56.6 (q), 113.9 (d), 121.6 (d), 121.9 (d), 122.7 (d), 122.9 (s), 131.5 (s), 139.1 (d), 148.6 (s), 163.6 (s). LR-MS *m/z*: 189 (M<sup>+</sup>), 174 (BP). HR-MS *m/z* (M<sup>+</sup>): Calcd for C<sub>11</sub>H<sub>11</sub>NO<sub>2</sub>: 189.0787. Found: 189.0777.

*N*-(2-Methoxyphenyl)-*N*-methyl-3,3-di(phenylsulfanyl)propionamide (**16k**): Yellow gum. IR (film): 1658. <sup>1</sup>H-NMR: 2.57 (2H, d, *J*=7 Hz, COCH<sub>2</sub>), 3.20 (3H, s, NMe), 3.74 (3H, s, OMe), 5.04 (1H, t, *J*=7 Hz, COCH<sub>2</sub>CH), 6.8–7.5 (14H, m, ArH, SPh×2). <sup>13</sup>C-NMR: 36.2 (q), 39.7 (t), 53.5 (d), 55.4 (q), 112.0 (d), 121.2 (d), 127.4 (d), 127.6 (d), 128.8 (d×4), 129.1 (d), 129.5 (d), 131.7 (s), 132.2 (d×2), 132.5 (d×2), 134.0 (s), 134.3 (s), 155.0 (s), 169.8 (s). LR-MS *m/z*: 409 (M<sup>+</sup>), 164 (BP). HR-MS *m/z* (M<sup>+</sup>): Calcd for C<sub>23</sub>H<sub>23</sub>NO<sub>2</sub>S<sub>2</sub>: 409.1170. Found: 409.1177.

**Acid-Induced Cyclization of 15a. Typical Procedure** i) With BF<sub>3</sub>·Et<sub>2</sub>O: A solution of **15a** (130 mg, 0.40 mmol) and BF<sub>3</sub>·Et<sub>2</sub>O (293 mg, 2.06 mmol) in toluene (5 ml) was refluxed for 24 h. The mixture was extracted with CHCl<sub>3</sub>, and the extract was washed with 5% NaOH and water. The product was chromatographed, eluted with ethyl acetate–hexane (2:1) to give **17a** (13 mg, 15%) and the recovered material (55 mg, 42%).

ii) with *p*-TsOH: A solution of **15a** (250 mg, 0.76 mmol) and *p*-TsOH·H<sub>2</sub>O (226 mg, 1.19 mmol) in toluene (50 ml) was refluxed using a Dean–Stark water separator for 72 h. The mixture was extracted with CHCl<sub>3</sub>, and the extract was washed with 5% NaOH and water. The product was chromatographed, eluted with ethyl acetate to give 5,7-dimethoxy-2-quinolone (**17a**) (110 mg, 68%) as yellow needles from MeOH, mp 275–278 °C. IR: 1651, 1632, 1562, 1510. UV: 213 (17900), 237 (15500), 256 (5400), 307 (7600), 322 (7400), 337 (5000). <sup>1</sup>H-NMR: 3.89 (3H, s, OCH<sub>3</sub>),

3.90 (3H, s, OCH<sub>3</sub>), 6.23, 6.29 (each 1H, d, *J*=2 Hz, 6-H and 8-H), 6.44, 8.06 (each 1H, d, *J*=10 Hz, 3-H, 4-H). <sup>13</sup>C-NMR: 55.7 (q), 55.8 (q), 90.3 (d), 93.8 (d), 105.8 (s), 116.4 (d), 135.7 (d), 141.0 (s), 157.2 (s), 162.9 (s), 164.6 (s). LR-MS *m/z*: 205 (M<sup>+</sup>, base peak). *Anal.* Calcd for C<sub>11</sub>H<sub>11</sub>NO<sub>3</sub>: C, 64.38; H, 5.40; N, 6.83. Found: C, 64.14; H, 5.58; N, 6.71. Acid-induced cyclization reactions of **15h** and **15i** were carried out in a similar way and the results are summarized in Table 2.

6-Methoxy-2-quinolone (**17h**): Yellow needles from ethyl acetate–hexane, mp 218–223 °C (lit.<sup>10</sup>) mp 207–208 °C. IR: 3138, 1672. UV: 353 (4300), 278 (4300), 269 (4900), 232 (33100). <sup>1</sup>H-NMR: 3.81 (3H, s, OMe), 6.71 (1H, d, *J*=10 Hz, 4-H), 7.04 (1H, d, *J*=3 Hz, 5-H), 7.14 (1H, dd, *J*=9, 3 Hz, 7-H), 7.38 (1H, d, *J*=9 Hz, 8-H), 7.75 (1H, d, *J*=10 Hz, 3-H). LR-MS *m/z*: 175 (M<sup>+</sup>), 58 (base peak).

7-Methoxy-2-quinolone (**17i**): Pale yellow prisms from ethyl acetate–hexane, mp 179–182 °C (lit.<sup>11</sup>) mp 201 °C. IR: 3400, 1600. UV: 342 (6000), 328 (8500), 285 (5200), 228 (26900), 220 (29800). <sup>1</sup>H-NMR: 3.90 (3H, s, OMe), 6.54 (1H, d, *J*=10 Hz, 3-H), 6.7–6.9 (2H, m, 6-H, 8-H), 7.48 (1H, d, *J*=9 Hz, 5-H), 7.73 (1H, d, *J*=10 Hz, 4-H). <sup>13</sup>C-NMR: 55.7 (q), 98.2 (d), 112.4 (d), 114.2 (s), 118.0 (d), 129.1 (d), 140.3 (s), 140.8 (d), 161.9 (s), 164.8 (s). LR-MS *m/z*: 175 (M<sup>+</sup>, base peak).

5-Methoxy-2-quinolone (**17j**): Pale yellow prisms from ethyl acetate–hexane, mp 170–175 °C (lit.<sup>8</sup>) mp 240 °C. <sup>1</sup>H-NMR: 4.03 (3H, s, OMe), 6.57 (1H, d, *J*=10 Hz), 6.67 (1H, dd, *J*=10, 9 Hz), 6.92 (1H, d, *J*=9 Hz), 7.58 (1H, d, *J*=10 Hz), 7.61 (1H, d, *J*=9 Hz), 8.12 (1H, d, *J*=10 Hz). LR-MS *m/z*: 175 (M<sup>+</sup>, base peak).

**Acknowledgments** This work was supported by a Grant-in-Aid for Scientific Research (No 11672115) from the Ministry of Education, Science, Sports and Culture of Japan.

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## Four New Compounds, Ficusal, Ficusesquilignan A, B, and Ficusolide Diacetate from the Heartwood of *Ficus microcarpa*

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Received May 1, 2000; accepted August 15, 2000

**Three new lignans, ficusal (1) and fusesquilignans A (2), B (3) and one new  $\gamma$ -lactone, fusesolide diacetate (4), were isolated from the wood of *Ficus microcarpa* L.f. Their structures were determined by spectral evidence.**

**Key words** *Ficus microcarpa*; Moraceae; ficusal; fusesquilignan A; fusesquilignan B; fusesolide diacetate

*Ficus microcarpa* L.f. (Moraceae) is a popular ornamental plant in Taiwan. Previous chemical studies on this plant, the previous one has been reported on its leaves,<sup>1)</sup> in which six terpenoids were observed. The strong vitality of the plant, as well as its antiplatelet activity, prompted us to study the chemical components. We early investigated chemical components of the bark and found two new isoflavones together with twenty-eight components,<sup>2,3)</sup> three new compounds from the heartwood,<sup>4,5)</sup> and five new triterpenes from the aerial roots.<sup>6)</sup> Further chemical studies on the heartwood of the plant were undertaken in our laboratory.

The methanol extract of the heartwood of *F. microcarpa* was concentrated to give a residue which was suspended with water. The suspended aqueous solution was partitioned with *n*-hexane, ethyl acetate, and *n*-BuOH successively. The detailed purification of the ethyl acetate extract by Si gel and HPLC resulted in three new lignans, ficusal (1), fusesquilignans A (2) and B (3), and one new lactone, fusesolide diacetate (4). In this paper, we report the structure of these new compounds.

Ficusal (1) was isolated as an oil. It showed positive optical activity and a molecular formula of  $C_{18}H_{18}O_6$  by high-resolution mass (HR-MS) spectroscopy. Its IR spectrum revealed the presence of hydroxyl group ( $3442\text{ cm}^{-1}$ ), a conjugated carbonyl group ( $1679\text{ cm}^{-1}$ ) and an aromatic nucleus ( $1607, 1517\text{ cm}^{-1}$ ). The  $\lambda_{\text{max}}$  at 235 and 284 nm in its UV spectrum can be assigned to the aromatic-carbonyl conjugated system with two oxygenated substituents.<sup>7)</sup> The existence of 18 carbons with 11 directly attached protons was confirmed from  $^{13}\text{C}$ -NMR and distortionless enhancement by polarization transfer (DEPT) experiments, and NMR data indicated the presence of a conjugated aldehyde group [ $\delta_{\text{C}}$  190.9;  $\delta_{\text{H}}$  9.83 (s)]. The chemical shifts (C-1 to C-9, C-2' to C-6') in the  $^{13}\text{C}$ -NMR spectrum of 1 showed similarities to those of balanophonin (5).<sup>8)</sup> The  $^1\text{H}$ -NMR signals due to a methine at  $\delta$  5.69 (d,  $J=6.8\text{ Hz}$ , H-7), a methylene at  $\delta$  3.90—3.94 (2H, m, H-9, overlapped with methoxyl signals), and a methine at  $\delta$  3.68 (m, H-8), whose chemical shifts were similar to a dehydrodiconiferyl alcohol-type lignan.<sup>8)</sup> The stereochemistry between hydroxymethyl and aryl groups was suggested to be *trans*, by the absence of the nuclear Overhauser effect (NOE) correlation between H-8 and H-7. An ABX system [ $\delta_{\text{H}}$  6.82 (1H, d,  $J=8.2\text{ Hz}$ , H-5), 6.88 (1H, dd,  $J=8.2, 2.4\text{ Hz}$ , H-6), 7.05 (1H, d,  $J=2.4\text{ Hz}$ , H-2)] was assigned to phenyl protons, and H-7 ( $\delta_{\text{H}}$  5.69) exhibited nuclear Overhauser enhancement and exchange spectroscopy (NOESY) correlations with H-2 and H-6. Meanwhile, the H-

2 signal has a NOESY correlation with a phenolic methyl group at  $\delta$  3.81. A pair of phenyl proton signals with *meta*-coupling ( $J=1.2\text{ Hz}$ ) occurring at  $\delta_{\text{H}}$  7.42 (H-6') and 7.52 (H-2') showed NOESY correlations with a formyl proton [ $\delta_{\text{H}}$  9.83 (1H, s, H-7')]. All of these data of 1 were similar to those of the balanophonin (5) except for an aldehyde group which replaced a propenal group. The assignment was also supported by heteronuclear multiple-quantum coherence (HMQC) and heteronuclear multiple-bond correlation (HMBC) experiments. Accordingly, 1 is 5-formyl-2-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-7-methoxy-*trans*-dihydrobenzofuran. The configuration of 1 was shown to be 7*R* and 8*S* due to the optical rotation ( $[\alpha]_{\text{D}} +3.0^\circ$ ).<sup>9,10)</sup>

The composition of fusesquilignan A (2), was determined to be  $C_{31}H_{36}O_{11}$  by HR-FAB-MS. The IR spectrum showed hydroxyl ( $3407\text{ cm}^{-1}$ ) and benzene ring ( $1597, 1516\text{ cm}^{-1}$ ) absorption bands. Comparison of  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data of 2 with those of the known buddlenol C (6)<sup>11)</sup> suggested that 2 possesses the same skeletal structure (furofuran and 1-arylglycerol ether linkage) but lacks one phenolic methyl group. The electron impact (EI)-MS (20 eV) of 2 exhibited major fragment peaks at  $m/z$  414, 388, 181, 180, 167, 151 and 137. The peaks at  $m/z$  137 and 151 are likely to arise from a coniferyl residue and those at  $m/z$  167 and 181 from a syringyl (4-OH 3,5-di-OMe phenyl) residue attached to the alicyclic part of a lignan.<sup>11,12)</sup> Accurate measurement of the  $m/z$  180 peak showed that it could be due to coniferyl alcohol ( $C_{10}H_{12}O_3$ ). The mass measurement showed that the peak at  $m/z$  388 had a likely composition of  $C_{21}H_{24}O_7$ , which is the same as furofurans (Chart 1). The  $^1\text{H}$ -NMR spectrum showed signals indicating the presence of four aromatic methoxyl groups and eight aromatic protons. This further increases the possibility that 2 consists of two coniferyl and one syringyl phenylpropanoid residue. The  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR (Table 1) signals of glyceryl moiety [ $\delta_{\text{H}}$  4.98 (H-7''), 4.11 (H-8''), 3.89 (H-9'');  $\delta_{\text{C}}$  72.5 (C-7''), 87.0 (C-8''), 60.5 (C-9'')] indicated that the furofuran unit with phenoxy linked at C-2 of 1-aryl glycerol like buddlenol C (6)<sup>11)</sup> and carinatidiol (7).<sup>8)</sup> The  $^{13}\text{C}$ -NMR of 2 showed signals at  $\delta$  54.0, 54.5, 71.5, 72.1, 85.7, 86.0 which are typical of the furofuran carbons in lignans with 2,6-diequatorial diaryl substitution with different aryl groups.<sup>12)</sup> It therefore seems likely that compound 2 consists of a furofuran ring with aromatic attachments, one of which has a coniferylglycerol residue attached to it *via* an ether bridge. Both the  $^1\text{H}$ - and the  $^{13}\text{C}$ -NMR spectra of 2 showed peaks which were similar to those given by syringaresinol except for the absence of a methoxyl

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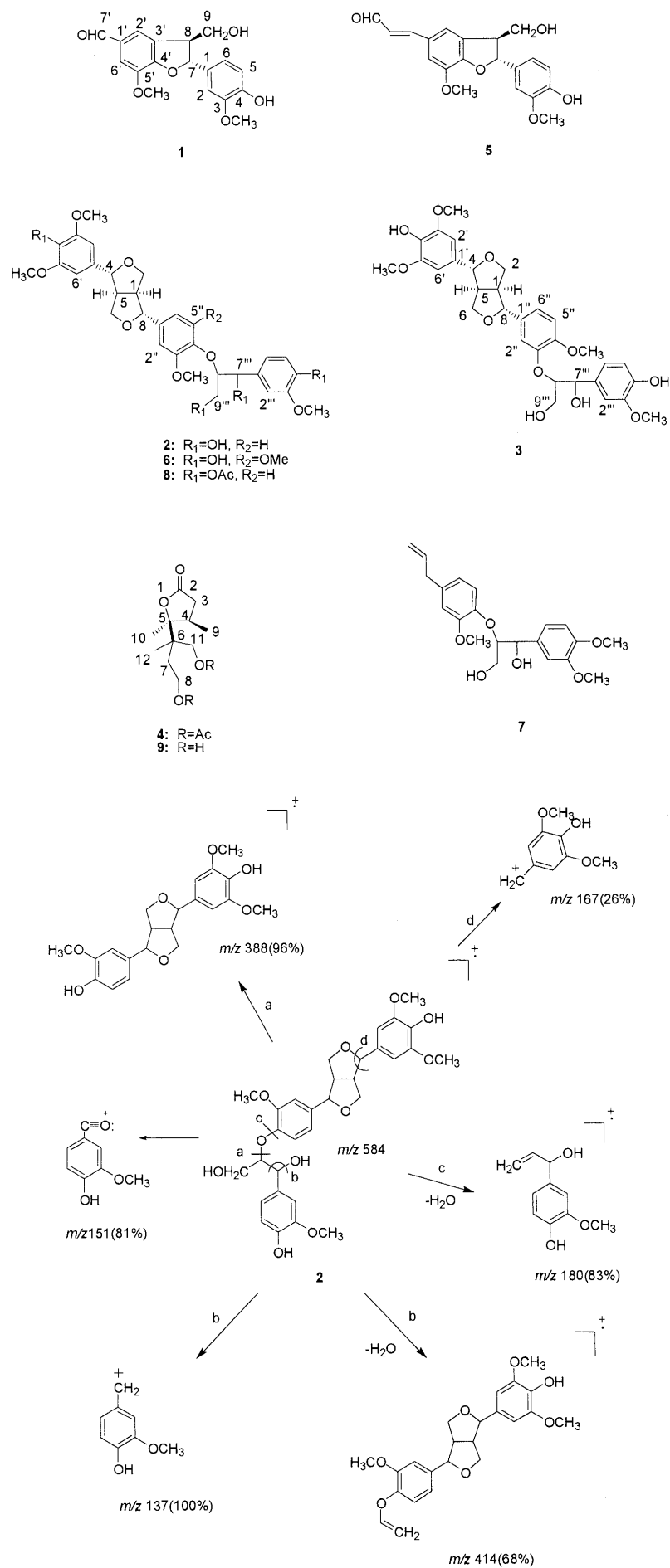


Chart 1

substituent attached to the benzene ring. Thus, the monomethoxyl substituted benzene ring is attached to a coniferylglycerol *via* an ether linkage. Since cleavage upon EI occurs most easily at the ether bridge, it is suggested that the major peaks at  $m/z$  388 and 180 are formed as shown in Chart 1. The IR spectrum of tetraacetylficusesquilignane A (**8**) (prepared by acetylation) showed strong absorptions at 1763 and 1741  $\text{cm}^{-1}$  and the  $^1\text{H}$ -NMR spectrum showed signals of four acetyl groups at  $\delta$  1.96, 2.12, 2.26 and 2.29, along with signals due to four methoxyl groups at  $\delta$  3.73, 3.73, 3.78, and 3.82 and eight aromatic protons at  $\delta$  6.49–7.00. As mentioned above, the  $m/z$  peaks at 180 and 137 suggest that the aryl attached to the glycerol has two substituents, -OH and -OMe. Two sets of ABX system of phenyl protons [H-2''', -5''', -6'''; H-2'', -5'', -6''] were deduced from their coupling patterns, and the signals at  $\delta$  3.82 (3H) and 3.78 (3H) showed NOE correlations with H-2'' and H-2''', respectively. This result confirmed the location of MeO-3'' and MeO-3'''. H-8''' also showed an NOE correlation with H-5'' indicating that the coniferylglycerol is attached to C-4'' *via* an ether linkage. The above evidence suggests that ficus-esquilignan A has the structure **2**. The coupling constant ( $J=3.3$  Hz) observed between H-7''' and H-8''' of the glycerol moiety indicated that it exists as the *erythro* isomer.<sup>10,13)</sup>

Ficusesquilignan B (**3**) exhibited a molecular formula of  $\text{C}_{31}\text{H}_{36}\text{O}_{11}$ , as did **2**, in HR-FAB-MS, and gave an EI-MS (20 eV) spectral fragmentation also similar to that of ficus-esquilignan A (**2**): such as  $m/z$  414, 388, 181, 180, 167, 151 and 137. It also gave similar  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data (Table 1) to those of ficusesquilignan A (**2**). Therefore, it was shown that **3** has the same skeletal structure as **2**, *i.e.*, a furofuran with two aryl substituents, one of which is attached to an arylglycerol. A difference was seen in the NOESY spectrum where H-8''' exhibited an NOE correlation with H-2'' [ $\delta$  6.86 (1H, d,  $J=1.6$  Hz)] as well as the 4''-OMe having a NOESY correlation with H-5'' [ $\delta$  6.89 (1H, d,  $J=8.4$  Hz)]. This indicated that the ether bridge is attached at C-3'', which differs from ficusesquilignan A. Additional proof for structure **3** was obtained using HMBC and HMQC techniques; hence, ficus-esquilignan B was presumed to have this structure. The glycerol moiety possessed *threo* stereochemistry as shown by the coupling constant ( $J=8.7$  Hz) observed between H-7'' and H-8'''<sup>9,13)</sup>

The molecular formula of ficusolide diacetate (**4**) was established as  $\text{C}_{15}\text{H}_{24}\text{O}_6$  by HR-MS mass spectroscopy. The IR spectrum of **4** exhibited absorption bands at 1774 and 1740  $\text{cm}^{-1}$  which suggested the presence of  $\gamma$ -lactone and ester carbonyl groups. The  $^1\text{H}$ -NMR spectrum of **4** showed two aliphatic acetate ( $\delta_{\text{H}}$  2.06, 2.03). Based on the molecular formula  $\text{C}_{15}\text{H}_{24}\text{O}_6$  of **4**, its index of hydrogen deficiency (IHD) is four. Ficusolide diacetate (**4**) has ABXY<sub>3</sub> system signals, corresponding to H $_{\beta}$ -3 [ $\delta$  2.28 (dd,  $J=18.1$ , 11.2 Hz)], H $_{\alpha}$ -3 [ $\delta$  2.69 (dd,  $J=18.1$ , 10.0 Hz)], H-4 [ $\delta$  2.84 (ddd,  $J=11.2$ , 10.0, 6.8 Hz)], and H $_{\gamma}$ -9 [ $\delta$  1.19 (d,  $J=6.8$  Hz)]. With irradiation at  $\delta$  2.84, the signals at  $\delta$  2.28, 2.69 and 1.19 collapsed to d, d, and s, respectively. The carbonyl carbon at  $\delta$  175.0 showed an HMBC correlation with H-3, so that the C-3–C-4–C-9 moiety was connected to a carbonyl. The moiety (C-4–C-5–C-6) was connected to two singlet methyl groups [ $\delta$  1.03 (H $_{\beta}$ -12), 1.02 (H $_{\beta}$ -10)], an acetoxymethyl [ $\delta$  68.7;  $\delta$  3.97, 4.00 (1H each, d,  $J=11.2$  Hz),

Table 1.  $^{13}\text{C}$ -NMR Data for **2** and **3** ( $\text{CDCl}_3$ , 100 MHz)

| C    | 2         | 3         |
|------|-----------|-----------|
| 1    | 54.0 (d)  | 54.5 (d)  |
| 2    | 71.5 (t)  | 72.1 (t)  |
| 3    | —         | —         |
| 4    | 86.0 (d)  | 85.7 (d)  |
| 5    | 54.5 (d)  | 54.0 (d)  |
| 6    | 72.1 (t)  | 71.5 (t)  |
| 7    | —         | —         |
| 8    | 85.7 (d)  | 85.9 (d)  |
| 1'   | 131.2 (s) | 132.6 (s) |
| 2'   | 102.8 (d) | 102.7 (d) |
| 3'   | 153.4 (s) | 153.1 (s) |
| 4'   | 134.2 (s) | 134.6 (s) |
| 5'   | 153.4 (s) | 153.1 (s) |
| 6'   | 102.8 (d) | 102.7 (d) |
| 1''  | 137.8 (s) | 137.9 (s) |
| 2''  | 118.7 (d) | 114.3 (d) |
| 3''  | 146.6 (s) | 145.3 (s) |
| 4''  | 144.8 (s) | 146.4 (s) |
| 5''  | 108.3 (d) | 108.6 (d) |
| 6''  | 114.1 (d) | 118.9 (d) |
| 1''' | 132.7 (s) | 131.9 (s) |
| 2''' | 108.6 (d) | 109.8 (d) |
| 3''' | 146.7 (s) | 146.7 (s) |
| 4''' | 145.3 (s) | 145.4 (s) |
| 5''' | 114.3 (d) | 114.2 (d) |
| 6''' | 118.9 (d) | 120.3 (d) |
| 7''' | 72.5 (d)  | 74.1 (d)  |
| 8''' | 87.0 (d)  | 89.1 (d)  |
| 9''' | 60.5 (t)  | 60.5 (t)  |
| OMe  | 56.2 (q)  | 56.2 (q)  |
|      | 55.9 (q)  | 55.9 (q)  |
|      | 56.2 (q)  | 56.2 (q)  |
|      | 55.9 (q)  | 55.9 (q)  |

and an acetoxymethyl [ $\delta$  29.9, 60.6;  $\delta$  2.10, 2.23 (1H each, dt,  $J=11.6$ , 6.8 Hz, H-7), 4.13, 4.21 (1H each, dt,  $J=10.2$ , 6.8 Hz, H-8)]. Six methylene protons in the acetoxymethyl and acetoxymethyl groups are all nonequivalent as well as expressing HMBC correlations with a quaternary C at  $\delta$  42.5. It is concluded that these two groups are linked to an asymmetric C-6 ( $\delta$  42.5). Three methyl groups protons are also correlated to C-5 ( $\delta$  91.2) in the HMBC spectrum. The signal at  $\delta$  1.03 was assigned as H-12 owing to its correlation to C-11 in the HMBC spectrum. Compound **4** was hydrolyzed with 0.5 M sodium hydroxide to afford a product that was identical to ficusolide (**9**)<sup>5)</sup> by comparing the  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra. Its relative stereochemistry was confirmed by the NOESY technique. According to the above evidence, the structure of ficusolide diacetate is assigned as **4**.

## Experimental

**General Procedures** Melting points were determined with a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 781 spectrophotometer.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra were obtained on a Bruker AM-300 spectrometer. Two dimensional (2D) NMR spectra were run on a Varian Unity 400 spectrometer. EI-MS, HR-MS, and HR-FAB-MS were taken on a Finnigan TSQ-46C, JEOL 5X-10-2A, and JEOL JMS-HX110, respectively, and UV and specific rotations were taken on a Hitachi S-3200 spectrometer and a JASCO DIP-180 digital polarimeter, respectively. Extracts were chromatographed on silica gel (Merck 3374, 70–230 mesh) and purified on a semi-preparative normal-phase HPLC column (250×10 mm, 7  $\mu\text{m}$ , LiChrosorb Si 60).

**Plant Material** The heartwood of *Ficus microcarpa* L.f. was collected

on the campus of the National Taiwan University and was identified by Prof. Shao-Shun Ying, Department of Forestry, National Taiwan University, and a voucher specimen has been deposited at the Herbarium of the Department of Botany, National Taiwan University, Taipei, Taiwan.

**Extraction and Isolation** The heartwood of *F. microcarpa* was crushed to give 7.0 kg (air-dried) of raw material, which was extracted with MeOH (60 l) three times (7 d each time) at room temperature. The combined extracts were evaporated *in vacuo* to give a black residue (58.8 g) that was suspended in water (500 ml). Then, the aqueous solution was partitioned with hexane (500 ml×3), EtOAc (500 ml×4), and *n*-BuOH (500 ml×3), successively. The EtOAc extract (13.3 g) was chromatographed by Si gel column chromatography (hexane–EtOAc and EtOAc–MeOH solvent system). Crude compounds **2**, **3** and **4** were eluted by hexane–EtOAc (1:4), and crude **1** eluted by hexane–EtOAc (2:5). Further purification by HPLC gave **1** (2.5 mg), **2** (2.0 mg), **3** (2.6 mg), and **4** (2.5 mg) using hexane–EtOAc–iso-PrOH (1:1:0.2), acetone–CH<sub>2</sub>Cl<sub>2</sub>–iso-PrOH (1:3:0.2), acetone–CH<sub>2</sub>Cl<sub>2</sub>–iso-PrOH (1:3:0.2), and hexane–EtOAc–CH<sub>2</sub>Cl<sub>2</sub>–iso-PrOH (5:1:2:0.2), respectively. Compound **2** (2.0 mg) was acetylated with Ac<sub>2</sub>O and pyridine in the usual way and purified by HPLC using acetone–CH<sub>2</sub>Cl<sub>2</sub>–iso-PrOH (1:10:0.2) to give tetraacetate (**8**) (1.9 mg).

**Ficusal (1)**: Pale yellow oil.  $[\alpha]_D^{25} = +3.0^\circ$  ( $c=0.18$ , CHCl<sub>3</sub>). UV  $\lambda_{\text{max}}^{\text{MeOH}}$  nm (log  $\epsilon$ ): 235 (4.60), 284 (4.15). IR  $\nu_{\text{max}}^{\text{KBr}}$  cm<sup>-1</sup>: 3442 (OH), 1679 (C=O, conjugated), 1607, 1517 (benzene ring), 1321, 1130. <sup>1</sup>H-NMR (acetone-*d*<sub>6</sub>, 400 MHz), see text. <sup>13</sup>C-NMR (acetone-*d*<sub>6</sub>, 100 MHz)  $\delta$ : 190.9 (s, C-7'), 154.8 (s, C-4'), 148.4 (s, C-4), 147.6 (s, C-3), 145.8 (s, C-5'), 133.3 (s, C-1), 132.3 (s, C-1'), 131.1 (s, C-3'), 121.4 (d, C-2'), 119.8 (d, C-6), 115.7 (d, C-5), 113.3 (d, C-6'), 110.6 (d, C-2), 89.8 (d, C-7), 64.1 (t, C-9), 56.3, 56.2 (3H each, s, OMe), 53.8 (d, C-8). EI-MS (70 eV)  $m/z$  (rel. int. %): 330 (M<sup>+</sup>, 88), 317 (45), 312 (100), 300 (57), 297 (34), 280 (34), 267 (40). HR-EI-MS  $m/z$ : 330.1098 (Calcd for C<sub>18</sub>H<sub>18</sub>O<sub>6</sub>: 330.1103).

**Ficusesquilignan A (2)**: Pale yellow oil.  $[\alpha]_D^{27} = -11.1^\circ$  ( $c=0.17$ , CHCl<sub>3</sub>). UV  $\lambda_{\text{max}}^{\text{MeOH}}$  nm (log  $\epsilon$ ): 235 (3.87), 280 (3.50). IR  $\nu_{\text{max}}^{\text{KBr}}$  cm<sup>-1</sup>: 3407 (OH), 1597, 1516 (benzene ring, C=C), 1453, 1276, 1240, 1122, 1035. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz), see text. <sup>13</sup>C-NMR, see Table I. FAB-MS  $m/z$ : 584 (M<sup>+</sup>). EI-MS (20 eV)  $m/z$  (rel. int. %): 414 (M<sup>+</sup>–C<sub>8</sub>H<sub>10</sub>O<sub>4</sub>, 68), 388 (96), 357 (6), 193 (29), 181 (49), 180 (83), 167 (26), 151 (81), 137 (100). HR-FAB-MS  $m/z$ : 584.2264 (Calcd for C<sub>31</sub>H<sub>36</sub>O<sub>11</sub>: 584.2258).

**Tetraacetylficusesquilignan A (8)** Prepared by Acetylation of **2**: Pale yellow oil.  $[\alpha]_D^{27} = -10.3^\circ$  ( $c=0.20$ , CHCl<sub>3</sub>). UV  $\lambda_{\text{max}}^{\text{MeOH}}$  nm (log  $\epsilon$ ): 225 (sh, 3.85), 272 (3.37). IR  $\nu_{\text{max}}^{\text{KBr}}$  cm<sup>-1</sup>: 1763, 1741 (ester, C=O), 1593, 1509 (benzene ring, C=C), 1464, 1220, 1198, 1125, 1046. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$ : 7.00 (1H, d,  $J=9.2$  Hz, H-5''), 6.98 (1H, d,  $J=2.0$  Hz, H-2''), 6.96 (1H, d,  $J=1.0$  Hz, H-2''), 6.94 (1H, dd,  $J=9.2$ , 1.0 Hz, H-6''), 6.90 (1H, d,  $J=8.0$  Hz, H-5''), 6.87 (1H, dd,  $J=8.0$ , 2.0 Hz, H-6''), 6.49 (2H, s, H-2', 6'), 6.06 (1H, d,  $J=3.2$  Hz, H-7''), 4.79 (1H, d,  $J=5.2$  Hz, H-8), 4.69 (1H, d,  $J=6.4$  Hz, H-4), 4.59 (1H, m, H-8''), 4.46 (1H, dd,  $J=11.4$ , 5.4 Hz, H<sub>a</sub>-9''), 4.27 (1H, dd,  $J=8.8$ , 7.5 Hz, H<sub>a</sub>-6), 4.25 (1H, dd,  $J=11.4$ , 2.4 Hz, H<sub>b</sub>-9''), 4.24 (1H, dd,  $J=8.8$ , 7.3 Hz, H<sub>a</sub>-2), 3.90 (2H, dd,  $J=8.8$ , 6.8 Hz, H<sub>β</sub>-2, H<sub>β</sub>-6), 3.82, 3.78, 3.73, 3.73 (3H each, MeO-3'', 3'', 3', 5'), 3.09 (1H, m, H-1), 3.04 (1H, m, H-5), 2.29, 2.26, 2.12, 1.96 (3H each, OAc). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$ : 170.7, 169.4, 169.0, 168.8 (s, COCH<sub>3</sub>), 153.4 (s, C-3', 5'), 151.3 (s, C-4''), 150.9 (s, C-3''), 140.2 (s, C-1''), 139.6 (s, C-3'''), 139.3 (s, C-4'), 137.3 (s, C-1'), 136.2 (s, C-1''), 135.1 (s, C-4'), 122.8 (d, C-5''), 122.4

(d, C-5'''), 119.4 (d, C-6''), 117.9 (d, C-6'''), 111.8 (d, C-2''), 110.1 (d, C-2'), 103.0 (d, C-2', 6'), 85.9 (d, C-4), 85.5 (d, C-8), 80.8 (d, C-8''), 74.1 (d, C-7''), 72.1 (t, C-6), 71.9 (t, C-2), 54.4 (d, C-1, 5), 62.7 (t, C-9''), 56.1, 56.0, 56.0 (q, CH<sub>3</sub>O-3', 5', 3'', 3''), 21.0, 20.7, 20.7, 20.6 (q, CH<sub>3</sub>CO). FAB-MS  $m/z$ : 752 (M<sup>+</sup>). HR-FAB-MS  $m/z$ : 752.2686 (Calcd for C<sub>39</sub>H<sub>44</sub>O<sub>15</sub>: 752.2680).

**Ficusesquilignan B (3)**: Pale yellow oil.  $[\alpha]_D^{27} = -8.0^\circ$  ( $c=0.24$ , CHCl<sub>3</sub>); UV  $\lambda_{\text{max}}^{\text{MeOH}}$  nm (log  $\epsilon$ ): 240 (3.86), 279 (3.47). IR  $\nu_{\text{max}}^{\text{KBr}}$  cm<sup>-1</sup>: 3436 (OH), 1592, 1516 (benzene ring, C=C), 1462, 1273, 1233, 1124, 1033. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz), see text. <sup>13</sup>C-NMR, see Table I. FAB-MS  $m/z$ : 584 (M<sup>+</sup>). EI-MS (20 eV)  $m/z$  (rel. int. %): 414 (M<sup>+</sup>–C<sub>8</sub>H<sub>10</sub>O<sub>4</sub>, 30), 388 (80), 280 (26), 208 (29), 181 (25), 180 (20), 167 (8), 151 (32), 137 (25), 118 (50), 83 (100). HR-FAB-MS  $m/z$ : 584.2252 (Calcd for C<sub>31</sub>H<sub>36</sub>O<sub>11</sub>: 584.2258).

**Ficusolide Diacetate (4)**: Amorphous.  $[\alpha]_D^{25} = -1.8^\circ$  ( $c=0.23$ , CHCl<sub>3</sub>); IR  $\nu_{\text{max}}^{\text{KBr}}$  cm<sup>-1</sup>: 1774 (C=O), 1740 (C=O), 1421, 1369, 1234, 1040. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz), see text. <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$ : 175.0 (s, C-2), 170.8 (s, 2×CH<sub>2</sub>CO), 91.2 (s, C-5), 68.7 (t, C-11), 60.6 (t, C-8), 42.5 (s, C-6), 37.2 (t, C-3), 33.4 (d, C-4), 29.9 (t, C-7), 20.9 (q, CH<sub>3</sub>CO), 20.9 (q, CH<sub>3</sub>CO), 20.6 (q, C-10), 20.6 (q, C-12), 16.4 (q, C-9). EI-MS (20 eV)  $m/z$  (rel. int. %): 300 (M<sup>+</sup>, 2), 280 (1), 241 (2), 213 (4), 185 (5), 171 (6), 153 (4), 125 (100), 115 (6). HR-EI-MS  $m/z$ : 300.1584 (Calcd for C<sub>15</sub>H<sub>24</sub>O<sub>6</sub>: 300.1573).

**Hydrolysis of 4 in 0.5 M NaOH** Compound **4** (2 mg) was dissolved in 0.5 M NaOH methanolic solution (2 ml) for 2 h under stirring. After removing of methanol, the reaction mixture was extracted with EtOAc (5 ml×3) and purified to afford **9** (1.2 mg, yield 83.3%): Amorphous,  $[\alpha]_D^{20} = +2.5^\circ$  ( $c=0.20$ , CHCl<sub>3</sub>). EI-MS (70 eV)  $m/z$  (rel. int. %): 216 (M<sup>+</sup>, 76), 198 (33), 187 (95), 177 (71), 154 (100), 137 (89), 107 (38). HR-EI-MS  $m/z$ : 216.1358 (Calcd for C<sub>11</sub>H<sub>20</sub>O<sub>4</sub>: 216.1362).

**Acknowledgments** This research was supported by the National Science Council of the Republic of China.

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## Models to Predict Solubility in Ternary Solvents Based on Sub-binary Experimental Data

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**The capability of the extended forms, of two well established cosolvency models, *i.e.* the combined nearly ideal binary solvent/Redlich–Kister equation and the modified Wilson model, used to predict the solute solubility in non-aqueous ternary solvent mixtures is presented. These predictions are based on the measured solubilities of anthracene in binary solvent mixtures. As a result the values of average percent deviations were less than 2% for the anthracene solubility in ternary mixtures.**

**This work was also extended to other cosolvency models, *i.e.* the extended Hildebrand solubility approach and the mixture response surface method, which are also commonly used for correlating solubility data in ternary solvents. The accuracy of the models is compared with each other and also with a published solubility model for ternary mixtures. The results illustrate that all models produced comparable accuracy.**

**Key words** solubility prediction; anthracene; cosolvency; combined nearly ideal binary solvent/Redlich–Kister; modified Wilson model; ternary solvent

Mixing of miscible solvents in order to enhance solubility of poorly soluble compounds is a very common method in chemical and pharmaceutical industries. The cosolvency phenomenon is important in liquid drug formulations, chemical separation, petroleum industry and environmental studies. In most cases, addition of one cosolvent to the main solvent, *e.g.* water in pharmaceutical applications, is not able to dissolve the compound in desired concentrations and one should add the second cosolvent.<sup>2–5)</sup> Using two or more cosolvents in mixed solvent systems improves the solubility of the solute and also decreases the risk of toxicity. In pharmaceutical applications, the concentration of the cosolvents should be kept as low as possible, because of toxicity and cost effects. The often method to optimise the cosolvent concentration in pharmaceutical industries is based on trial and error approach. In order to present a rational method to optimise the cosolvents' concentrations in ternary solvent mixtures based on sub-binary data, anthracene solubility data in mixed solvents have been employed as a model system. It is obvious that one can employ the proposed procedure to calculate drug solubilities in aqueous ternary systems. Anthracene is a polycyclic aromatic hydrocarbon and solubility data of polycyclic aromatic hydrocarbons are becoming increasingly important in the petroleum industry, particularly in light of present trends towards heavier feedstock's and known carcinogenicity and/or mutagenicity of many of the larger polycyclic aromatic compounds.

In addition to the experimental measurements of solute solubility in mixed solvents, there are numerous models for calculating and/or correlating solubility data. We have previously summarised many of these models in and also compared their accuracy.<sup>6)</sup> The final goal of developing cosolvency equations is that it enables researchers to predict the solute solubility in mixed solvents from a minimum number of experiments. Group contribution methods, such as universal functional group activity coefficient (UNIFAC), have proved fairly successful in estimating solid solubility in solvent mixtures from structural information. The predictive ability of the UNIFAC model for biphenyl solubilities in several binary mixed solvents was reported in a previous paper.<sup>7)</sup>

An alternative method to predict the solubility in mixed solvents is to use the correlative cosolvency models after training the models by employing measured solubilities at given cosolvent concentrations and predicting at other cosolvent concentrations by interpolation technique. It has been shown that solubility prediction in binary solvents based on trained models using insufficient experimental data points produced unacceptable errors.<sup>8)</sup> On the other hand, Bustamante and co-workers<sup>9)</sup> employed a modified form of the extended Hildebrand solubility approach to correlate structurally related drug solubility in binary solvent mixtures. The authors used the solute solubility in water and cosolvent, the solute solubility parameter, the Hildebrand solubility parameter of the solvent and the basic solubility parameter of the mixed solvent as independent variables. This approach is a useful solution to predict the solubility of the chemically related drugs which is often used in drug discovery studies where different derivatives of a drug/drug candidate are synthesised and evaluated. In a recent paper,<sup>10)</sup> the applicability of the combined nearly ideal binary solvent/Redlich–Kister model for reproducing solubility data of structurally related drugs in binary solvents was presented. It was also shown that the prediction error of the later model is less than that of the modified form of the extended Hildebrand solubility approach.<sup>10)</sup>

In the present paper, the capability of the combined nearly ideal binary solvent/Redlich–Kister and the modified Wilson models for predicting solute solubility in ternary mixtures based on the model constants calculated by employing solubility data in sub-binary solvents, is presented. Usually, solubility data in binary solvents has been determined and in the case of inefficient solubilization by binary system, a possible solution is to use ternary solvent mixtures. The produced experimental binary data from the early stage can be used to predict solubility in ternary solvents as it has been shown in this report. Also, the published cosolvency models in binary solvents are extended in order to reproduce the solute solubility in ternary mixtures. Accuracy of the models is compared with that of an extended form of the combined nearly ideal binary solvent/Redlich–Kister equation.

**Theoretical Background** The combined nearly ideal bi-

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nary solvent/Redlich–Kister equation was suggested to reproduce the solubility profile in binary solvents, based upon the thermodynamic mixing model.<sup>11)</sup> Our previous reports have shown that the combined nearly ideal binary solvent/Redlich–Kister equation is the most accurate model for calculating and/or correlating solute solubility data in binary solvents.<sup>6,12)</sup> It can be readily extended to describe solute solubility in ternary solvent mixtures. The obtained model represents the thermodynamic extension of the basic mixing model to a quaternary solution. For convenience, the equation is written in terms of the natural logarithm of the mole fraction solubilities of the solute. The fixed values of  $RT$  ( $R$  is the molar gas constant and  $T$  denotes absolute temperature of the solution) at a constant temperature are incorporated into the model constants. The extended form of the model is expressed by Eq. 1:

$$\ln X_m = f_1 \ln X_1 + f_2 \ln X_2 + f_3 \ln X_3 + f_1 f_2 \sum_{i=0}^p W_i (f_1 - f_2)^i + f_1 f_3 \sum_{i=0}^p W'_i (f_1 - f_3)^i + f_2 f_3 \sum_{i=0}^p W''_i (f_2 - f_3)^i \quad (1)$$

Where  $X_m$  is the solute mole fraction solubility in mixed solvent,  $f_1$ ,  $f_2$  and  $f_3$  represent the solute free volume fraction of solvents 1, 2 and 3, respectively,  $X_1$ ,  $X_2$  and  $X_3$  denote the solute mole fraction solubility in pure solvents 1, 2 and 3,  $W_i$ ,  $W'_i$  and  $W''_i$  are binary solvent–solute interaction terms which are calculated by employing binary solubility data *via* a least squares analysis (for details see appendix). In the Eq. 1 the value of  $p$  can be varied from 0–3.

An alternative equation is the modified Wilson model, which has been demonstrated as producing comparable results with the combined nearly ideal binary solvent/Redlich–Kister model.<sup>13)</sup> It is possible to extend it for ternary solvent mixtures, such that:

$$\ln \left( \frac{X_s^i}{X_m} \right) = 1 - \frac{f_1 \left[ 1 - \ln \left( \frac{X_s^i}{X_1} \right) \right]}{f_1 + \lambda_{12} f_2 + \lambda_{13} f_3} - \frac{f_2 \left[ 1 - \ln \left( \frac{X_s^i}{X_2} \right) \right]}{\lambda_{21} f_1 + f_2 + \lambda_{23} f_3} - \frac{f_3 \left[ 1 - \ln \left( \frac{X_s^i}{X_3} \right) \right]}{\lambda_{31} f_1 + \lambda_{32} f_2 + f_3} \quad (2)$$

Where  $X_s^i$  is ideal mole fraction solubility of the solute and  $\lambda_{12}$ ,  $\lambda_{21}$ ,  $\lambda_{13}$ ,  $\lambda_{31}$ ,  $\lambda_{23}$  and  $\lambda_{32}$  are the model constants, which are calculated from sub-binary solubility data.

In order to examine the applicability of these equations to real data, the prediction of anthracene solubility in ternary solvents, employed published solubility data in binary solvents and this allowed the calculation of the binary solvent–solute interaction constants. The binary interaction terms for the combined nearly ideal binary solvent/Redlich–Kister equation and the modified Wilson model were taken from the literature (for reference numbers, see Table 1). From these binary interaction terms, anthracene solubility in ternary solvents was predicted by using Eqs. 1 and 2.

In order to check the best fitness ability of various published models, the experimental data in ternary solvents were fitted to different equations. From this the correlative ability of the combined nearly ideal binary solvent/Redlich–Kister

model was compared with that of the excess free energy model, the extended form of a mixture response surface method and the modified form of the extended Hildebrand solubility model. These equations and the related extensions for correlating solubility data in ternary solvents, are briefly reviewed for the models.

Martin and co-workers<sup>14)</sup> presented the extended form of the Hildebrand solubility approach to correlate experimental solubility of drugs in aqueous mixed solvents. Their equation was also applicable to non-aqueous solvents. The model was presented by Eq. 3:

$$-\ln X_m = -\ln X_s^i + \frac{V_s \phi_m^2 (\delta_m^2 + \delta_s^2 - 2WW)}{RT} \quad (3)$$

where  $V_s$  is the molar volume of the solute,  $\phi_m$  denotes the volume fraction of the solvent in the solution which can be assumed equal to unity,  $\delta_m$  and  $\delta_s$  are mixed solvent and solute's solubility parameters, respectively and  $WW$  is the interaction term and is calculated by:

$$WW = \frac{\ln X_m - \ln X_s^i + \left( \frac{V_s \phi_m^2}{RT} \right) \delta_m^2 + \left( \frac{V_s \phi_m^2}{RT} \right) \delta_s^2}{2 \left( \frac{V_s \phi_m^2}{RT} \right)} \quad (4)$$

The authors correlated the experimental values of the interaction term to a power series of solvent's solubility parameter,  $\delta_m$ :

$$WW = \sum_{i=0}^q A_i \delta_m^i \quad (5)$$

Where  $A_i$  is the curve-fit parameter. The numerical values of  $\delta_m$  for ternary solvent mixtures are calculated by Eq. 6:

$$\delta_m = f_1 \delta_1 + f_2 \delta_2 + f_3 \delta_3 \quad (6)$$

In which  $\delta_1$ ,  $\delta_2$  and  $\delta_3$  are the solubility parameters of pure solvents 1, 2 and 3, respectively.

However, it is possible to use the solute free volume fraction of solvents instead of  $\delta_m$ , to correlate experimental values of  $WW$ <sup>15)</sup>:

$$WW = J_0 + J_1 f_1 + J_2 f_2 + J_3 f_3 + J_4 f_1^2 + J_5 f_2^2 + J_6 f_3^2 + J_7 f_1 f_2 + J_8 f_1 f_3 + J_9 f_2 f_3 \quad (7)$$

Where  $J_0$ – $J_9$  are the curve-fit parameters. Equation 7 was obtained by substitution of  $\delta_m$  values from Eq. 6 into Eq. 5 and then the appropriate rearrangements. This formalism allows us to reproduce solubility data in ternary solvents.

Williams and Amidon<sup>16)</sup> presented the excess free energy approach to calculate the solute solubility in ternary solvent mixtures:

$$\ln X_m = f_1 \ln X_1 + f_2 \ln X_2 + f_3 \ln X_3 - A_{12} f_1 f_2 (2f_1 + 2f_2 - 1)(V_s/V_1) + A_{21} 2f_1 f_2 (f_1 + f_2)(V_s/V_2) - A_{13} f_1 f_3 (2f_1 + 1)(V_s/V_1) + A_{31} 2f_1^2 f_3 (V_s/V_3) - A_{32} f_2 f_3 (2f_3 - 1)(V_s/V_3) + A_{23} 2f_2 f_3^2 (V_s/V_2) - G_{123} V_s f_1 f_2 f_3 + G_{1s2} V_s f_1 f_2 + G_{1s3} V_s f_1 f_3 + G_{s23} V_s f_2 f_3 + K V_s f_1 f_2 f_3 \quad (8)$$

Where  $A$  denotes binary solvent–solvent interaction terms in the absence of the solute, subscripts 1, 2 and 3 refer to solvents 1, 2 and 3, and  $s$  refers to the solute,  $V$  represents the molar volume,  $G$  stands for the binary solvent–solute interac-

tion constant,  $G_{123}$  is the ternary solvent–solvent interaction parameter in the absence of the solute and  $K$  is the ternary solvent–solute interaction constant. The solvent–solvent interaction constants ( $A$ ,  $G_{123}$ ) are obtained from the vapour–liquid equilibrium data. The binary solvent–solute interaction constants ( $G_{1s2}$ ,  $G_{1s3}$ , and  $G_{s23}$  terms) are calculated by employing experimental solubility data in binary solvents (theoretically this may be calculated from one solubility experiment in each binary solvent) and the constant  $K$  is obtained from experimental solubility data in ternary solvents. Because  $A$ ,  $V$ ,  $G$  and  $K$  terms are constant values for a given ternary system, one can rearrange Eq. 8 as:

$$\ln X_m = f_1 \ln X_1 + f_2 \ln X_2 + f_3 \ln X_3 + M_1 f_1 f_2 + M_2 f_1 f_3 + M_3 f_1 f_2 + M_4 f_1 f_3 + M_5 f_1 f_3 + M_6 f_2 f_3 + M_7 f_2 f_3 \quad (9)$$

Where  $M_1$ — $M_7$  are the model constants which are calculated via the least squares analysis by employing solubility data in ternary solvents.

The mixture response surface model is presented for reproducing solute solubility data in binary solvents.<sup>17)</sup> The original five constant term model is expressed by:

$$\ln X_m = S_1 f'_1 + S_2 f'_2 + S_3 \left( \frac{1}{f'_1} \right) + S_4 \left( \frac{1}{f'_2} \right) + S_5 f'_1 f'_2 \quad (10)$$

Where  $f' = 0.96f + 0.02$  and  $S_1$ — $S_5$  are the model constants. One can extend the model to describe solute solubility in ternary solvents as:

$$\ln X_m = B_1 f'_1 + B_2 f'_2 + B_3 f'_3 + B_4 \left( \frac{1}{f'_1} \right) + B_5 \left( \frac{1}{f'_2} \right) + B_6 \left( \frac{1}{f'_3} \right) + B_7 f'_1 f'_2 + B_8 f'_1 f'_3 + B_9 f'_2 f'_3 + B_{10} f'_1 f'_2 f'_3 \quad (11)$$

Where  $B_1$ — $B_{10}$  denote the model constants.

To assess the prediction capability and the best fit ability of the models, we calculated percent deviation, %D, of back-calculated solubilities from experimental values by using Eq. 12:

$$\%D = \frac{100}{N} \sum \left( \frac{|(X_m)_{\text{Calc.}} - (X_m)_{\text{Exp.}}|}{(X_m)_{\text{Exp.}}} \right) \quad (12)$$

Where  $N$  is the number of data points in each set,  $(X_m)_{\text{Calc.}}$  denotes back-calculated data and  $(X_m)_{\text{Exp.}}$  represents experimental solubilities which are taken from published papers.

## Computational Results and Discussion

Tables 1 and 2 show the details of anthracene solubilities in binary and ternary solvent systems collected from our published papers. The employed binary interaction terms and the produced prediction errors (%D) by Eqs. 1 and 2 are shown in Tables 3 and 4, respectively. Careful examination of these tables reveals that both Eqs. 1 and 2 provide accurate predictions for anthracene solubility in ternary solvent mixtures. The mean and standard deviation values of %D for 30 sets studied are  $1.47 \pm 0.47$  and  $1.42 \pm 0.47$ , respectively for Eqs. 1 and 2. The difference between two mean values is not significant ( $t$ -test,  $p > 0.05$ ). Average %D values are less than 2% which is comparable to the experimental uncertainty, which suggests that both models can be employed to predict solute solubility in ternary solvents based on experimental

Table 1. The Details of Anthracene Solubility in Binary Solvent Mixtures

| Solvent 1                              | Solvent 2                   | $\ln X_1$ | $\ln X_2$ | Ref. No. |
|----------------------------------------|-----------------------------|-----------|-----------|----------|
| 1-Butanol                              | 2-Butoxyethanol             | -7.130    | -5.577    | 18       |
| 1-Butanol                              | 1-Propanol                  | -7.130    | -7.434    | 19       |
| 1-Butanol                              | 2-Propanol                  | -7.130    | -7.797    | 19       |
| 2-Butanol                              | 2-Butoxyethanol             | -7.444    | -5.577    | 18       |
| 2-Butanol                              | 1-Propanol                  | -7.444    | -7.434    | 19       |
| 2-Butanol                              | 2-Propanol                  | -7.444    | -7.797    | 19       |
| Cyclohexane                            | 1-Butanol                   | -6.468    | -7.130    | 20       |
| Cyclohexane                            | 2-Butanol                   | -6.468    | -7.444    | 21       |
| Cyclohexane                            | 2-Butoxyethanol             | -6.468    | -5.577    | 22       |
| Cyclohexane                            | 1-Propanol                  | -6.468    | -7.434    | 20       |
| Cyclohexane                            | 2-Propanol                  | -6.468    | -7.797    | 23       |
| Heptane                                | 1-Butanol                   | -6.456    | -7.130    | 20       |
| Heptane                                | 2-Butanol                   | -6.456    | -7.444    | 21       |
| Heptane                                | 2-Butoxyethanol             | -6.456    | -5.577    | 22       |
| Heptane <sup>a)</sup>                  | Cyclohexane <sup>a,b)</sup> | -6.456    | -6.468    | 24       |
| Heptane                                | 1-Propanol                  | -6.456    | -7.434    | 20       |
| Heptane                                | 2-Propanol                  | -6.456    | -7.797    | 23       |
| 1-Propanol                             | 2-Butoxyethanol             | -7.434    | -5.577    | 18       |
| 2-Propanol                             | 2-Butoxyethanol             | -7.797    | -5.577    | 18       |
| 2,2,4-Trimethylpentane                 | 1-Butanol                   | -6.836    | -7.130    | 20       |
| 2,2,4-Trimethylpentane                 | 2-Butanol                   | -6.836    | -7.444    | 21       |
| 2,2,4-Trimethylpentane                 | 2-Butoxyethanol             | -6.836    | -5.577    | 22       |
| 2,2,4-Trimethylpentane <sup>a,b)</sup> | Cyclohexane <sup>a,b)</sup> | -6.836    | -6.468    | 24       |
| 2,2,4-Trimethylpentane                 | 1-Propanol                  | -6.836    | -7.434    | 20       |
| 2,2,4-Trimethylpentane                 | 2-Propanol                  | -6.836    | -7.797    | 23       |

a) The numerical values of the modified Wilson model constants ( $\lambda$  terms) used in Table 4 were computed using an in-house computer program<sup>25)</sup> (For details, see appendix). The other  $\lambda$  values were taken from the references. b) The anthracene solubility data in pure cyclohexane and 2,2,4-trimethylpentane were taken from ref. 22)

solubilities in pure solvents and sub-binary mixtures. From a computational standpoint, Eq. 1 is likely to be preferred because the binary solvent–solute interaction terms can be calculated by a simple least squares analysis which is provided by scientific calculators and commercial software. Equation 2 is an alternative model and the corresponding binary solvent–solute interaction constants can be estimated by a non-linear least squares analysis which is provided by most of the generally available statistical packages. It should be noted that there is a three-dimensional map for several parameter pairs against %D that describes the anthracene solubility within an acceptable error range. Any parameter set having a %D less than about 2% are sufficient for prediction purpose.

The numerical value of  $X_s^i = 0.00984^{22)}$  used in Eqs. 2 and 3 was calculated from the molar enthalpy of fusion,  $\Delta H_s^{\text{fus}}$ , at the normal melting point temperature of the solute,  $T_{\text{mp}}$ :

$$\ln X_s^i = \frac{-\Delta H_s^{\text{fus}}(T_{\text{mp}} - T)}{RTT_{\text{mp}}} \quad (13)$$

Attempts to eliminate  $X_s^i$  from Eq. 2 in favour of a simplified version ( $X_s^i = 1$ ) however proved unsuccessful with the anthracene solubility data in non-aqueous binary solvents.<sup>20)</sup> In this study, the values of  $\lambda_{12}$  and  $\lambda_{21}$  were calculated by assuming  $X_s^i = 1$  for sub-binary mixtures and the obtained %D is compared with that of original modified Wilson model.<sup>13)</sup> There is no significant difference between the two %D values for binary mixtures. However, the predicted values of Eq. 2 for ternary solvent mixtures is more accurate than that of the simplified form of Eq. 2 ( $X_s^i = 1$ ).

Table 5 shows the accuracy of Eqs. 1, 7, 9 and 11 to correlate experimental solubilities in ternary solvents. The results

Table 2. The Details of Solvents and the References

| No. | Solvent 1       | Solvent 2              | Solvent 3              | $\delta_1$ | $\delta_2$ | $\delta_3$ | Ref. No. |
|-----|-----------------|------------------------|------------------------|------------|------------|------------|----------|
| 1   | 1-Butanol       | Heptane                | Cyclohexane            | 11.29      | 7.50       | 8.20       | 26       |
| 2   | 1-Butanol       | 2,2,4-Trimethylpentane | Cyclohexane            | 11.29      | 6.86       | 8.20       | 27       |
| 3   | 2-Butanol       | Heptane                | Cyclohexane            | 11.13      | 7.50       | 8.20       | 26       |
| 4   | 2-Butanol       | 2,2,4-Trimethylpentane | Cyclohexane            | 11.13      | 6.86       | 8.20       | 27       |
| 5   | 2-Butoxyethanol | Cyclohexane            | 1-Propanol             | 9.88       | 8.20       | 11.99      | 28       |
| 6   | 2-Butoxyethanol | Cyclohexane            | 2-Propanol             | 9.88       | 8.20       | 11.50      | 28       |
| 7   | 2-Butoxyethanol | Cyclohexane            | Heptane                | 9.88       | 8.20       | 7.50       | 29       |
| 8   | 2-Butoxyethanol | Cyclohexane            | 2,2,4-Trimethylpentane | 9.88       | 8.20       | 6.86       | 29       |
| 9   | 2-Butoxyethanol | Heptane                | 1-Propanol             | 9.88       | 7.50       | 11.99      | 28       |
| 10  | 2-Butoxyethanol | Heptane                | 2-Propanol             | 9.88       | 7.50       | 11.50      | 28       |
| 11  | 2-Butoxyethanol | 1-Propanol             | 1-Butanol              | 9.88       | 11.99      | 11.29      | 30       |
| 12  | 2-Butoxyethanol | 1-Propanol             | 2-Butanol              | 9.88       | 11.99      | 11.13      | 30       |
| 13  | 2-Butoxyethanol | 2-Propanol             | 1-Butanol              | 9.88       | 11.50      | 11.29      | 30       |
| 14  | 2-Butoxyethanol | 2-Propanol             | 2-Butanol              | 9.88       | 11.50      | 11.13      | 30       |
| 15  | 1-Propanol      | 1-Butanol              | Cyclohexane            | 11.99      | 11.29      | 8.20       | 31       |
| 16  | 1-Propanol      | 1-Butanol              | Heptane                | 11.99      | 11.29      | 7.50       | 32       |
| 17  | 1-Propanol      | 1-Butanol              | 2,2,4-Trimethylpentane | 11.99      | 11.29      | 6.86       | 33       |
| 18  | 1-Propanol      | 2-Butanol              | Cyclohexane            | 11.99      | 11.13      | 8.20       | 31       |
| 19  | 1-Propanol      | 2-Butanol              | Heptane                | 11.99      | 11.13      | 7.50       | 32       |
| 20  | 1-Propanol      | 2-Butanol              | 2,2,4-Trimethylpentane | 11.99      | 11.13      | 6.86       | 33       |
| 21  | 1-Propanol      | Heptane                | Cyclohexane            | 11.99      | 7.50       | 8.20       | 26       |
| 22  | 1-Propanol      | 2,2,4-Trimethylpentane | Cyclohexane            | 11.99      | 6.86       | 8.20       | 27       |
| 23  | 2-Propanol      | 1-Butanol              | Cyclohexane            | 11.50      | 11.29      | 7.30       | 31       |
| 24  | 2-Propanol      | 1-Butanol              | Heptane                | 11.50      | 11.29      | 7.50       | 32       |
| 25  | 2-Propanol      | 1-Butanol              | 2,2,4-Trimethylpentane | 11.50      | 11.29      | 6.86       | 33       |
| 26  | 2-Propanol      | 2-Butanol              | Cyclohexane            | 11.50      | 11.13      | 7.30       | 31       |
| 27  | 2-Propanol      | 2-Butanol              | Heptane                | 11.50      | 11.13      | 7.50       | 32       |
| 28  | 2-Propanol      | 2-Butanol              | 2,2,4-Trimethylpentane | 11.50      | 11.13      | 6.86       | 33       |
| 29  | 2-Propanol      | Heptane                | Cyclohexane            | 11.50      | 7.50       | 7.30       | 26       |
| 30  | 2-Propanol      | 2,2,4-Trimethylpentane | Cyclohexane            | 11.50      | 6.86       | 7.30       | 27       |

Table 3. The %D Values of Eq. 1 for the Sets Studied and the Constants Employed in the Calculations Taken from References

| No. <sup>a)</sup> | %D   | $W_0$ | $W_1$  | $W_2$ | $W'_0$ | $W'_1$ | $W'_2$ | $W''_0$ | $W''_1$ | $W''_2$ |
|-------------------|------|-------|--------|-------|--------|--------|--------|---------|---------|---------|
| 1                 | 1.11 | 0.723 | -0.004 | 0.201 | 0.741  | -0.345 | 0.223  | 0.175   | -0.126  | 0       |
| 2                 | 1.49 | 0.536 | -0.151 | 0.142 | 0.741  | -0.345 | 0.223  | 0.050   | 0       | 0       |
| 3                 | 1.74 | 1.225 | 0.292  | 0     | 1.260  | -0.206 | 0      | 0.175   | -0.126  | 0       |
| 4                 | 2.05 | 1.070 | 0.213  | 0     | 1.260  | -0.206 | 0      | 0.050   | 0       | 0       |
| 5                 | 1.63 | 1.081 | -0.863 | 0.565 | 1.222  | -0.572 | 0.255  | 1.121   | 0.040   | 0.256   |
| 6                 | 1.59 | 1.081 | -0.863 | 0.565 | 1.550  | -0.790 | 0.726  | 1.589   | -0.143  | 0.248   |
| 7                 | 3.22 | 1.081 | -0.863 | 0.565 | 0.928  | -0.488 | 0.123  | 0.175   | 0.126   | 0       |
| 8                 | 1.21 | 1.081 | -0.863 | 0.565 | 0.883  | -0.548 | 0.198  | 0.050   | 0       | 0       |
| 9                 | 1.48 | 0.928 | -0.488 | 0.123 | 1.222  | -0.572 | 0.255  | 1.098   | -0.106  | 0.324   |
| 10                | 1.25 | 0.928 | -0.488 | 0.123 | 1.550  | -0.790 | 0.726  | 1.491   | -0.587  | 0.566   |
| 11                | 1.20 | 1.222 | -0.572 | 0.255 | 0.800  | -0.319 | 0.185  | 0.117   | -0.015  | 0       |
| 12                | 1.69 | 1.222 | -0.572 | 0.255 | 1.155  | -0.609 | 0.267  | 0       | 0       | 0       |
| 13                | 1.42 | 1.550 | -0.790 | 0.726 | 0.800  | -0.319 | 0.185  | 0.243   | 0.011   | 0       |
| 14                | 1.75 | 1.550 | -0.790 | 0.726 | 1.155  | -0.609 | 0.267  | 0.097   | -0.013  | 0       |
| 15                | 1.34 | 0.117 | -0.015 | 0     | 1.121  | -0.040 | 0.256  | 0.741   | -0.345  | 0.223   |
| 16                | 1.36 | 0.117 | -0.015 | 0     | 1.098  | 0.106  | 0.324  | 0.723   | -0.004  | 0.201   |
| 17                | 0.84 | 0.117 | -0.015 | 0     | 0.825  | 0.103  | 0.291  | 0.536   | -0.151  | 0.142   |
| 18                | 1.56 | 0     | 0      | 0     | 1.121  | -0.040 | 0.256  | 1.260   | -0.206  | 0       |
| 19                | 0.94 | 0     | 0      | 0     | 1.098  | 0.106  | 0.324  | 1.225   | 0.292   | 0       |
| 20                | 0.47 | 0     | 0      | 0     | 0.825  | 0.103  | 0.291  | 1.070   | 0.213   | 0       |
| 21                | 1.66 | 1.098 | 0.106  | 0.324 | 1.121  | -0.040 | 0.256  | 0.175   | -0.126  | 0       |
| 22                | 1.34 | 0.825 | 0.103  | 0.291 | 1.121  | -0.040 | 0.256  | 0.050   | 0       | 0       |
| 23                | 1.55 | 0.243 | 0.011  | 0     | 1.589  | 0.143  | 0.248  | 0.741   | -0.345  | 0.223   |
| 24                | 1.72 | 0.243 | 0.011  | 0     | 1.491  | 0.587  | 0.566  | 0.723   | -0.004  | 0.201   |
| 25                | 1.22 | 0.243 | 0.011  | 0     | 1.193  | 0.369  | 0.333  | 0.536   | -0.151  | 0.142   |
| 26                | 1.43 | 0.097 | -0.013 | 0     | 1.589  | 0.143  | 0.248  | 1.260   | -0.206  | 0       |
| 27                | 1.89 | 0.097 | 0.013  | 0     | 1.491  | 0.587  | 0.566  | 1.225   | 0.292   | 0       |
| 28                | 1.38 | 0.097 | -0.013 | 0     | 1.193  | 0.369  | 0.333  | 1.070   | 0.213   | 0       |
| 29                | 0.99 | 1.491 | 0.587  | 0.566 | 1.589  | 0.143  | 0.248  | 0.175   | -0.126  | 0       |
| 30                | 1.62 | 1.193 | 0.369  | 0.333 | 1.589  | 0.143  | 0.248  | 0.050   | 0       | 0       |
| Mean              | 1.47 |       |        |       |        |        |        |         |         |         |
| S.D.              | 0.47 |       |        |       |        |        |        |         |         |         |

a) Numbers are the same as in Table 2.

Table 5. The Accuracy of Models to Correlate Experimental Solubility Data in Ternary Solvents

| No. <sup>a)</sup> | Eq. 1 | Eq. 7 | Eq. 9 | Eq. 11 |
|-------------------|-------|-------|-------|--------|
| 1                 | 0.81  | 0.85  | 0.66  | 0.26   |
| 2                 | 0.29  | 0.34  | 0.27  | 0.27   |
| 3                 | 1.02  | 0.80  | 0.88  | 0.57   |
| 4                 | 0.51  | 0.50  | 0.47  | 0.35   |
| 5                 | 0.98  | 1.30  | 0.90  | 0.90   |
| 6                 | 1.11  | 0.73  | 0.99  | 0.56   |
| 7                 | 1.37  | 0.88  | 1.18  | 0.48   |
| 8                 | 0.90  | 0.89  | 0.72  | 0.45   |
| 9                 | 1.34  | 1.14  | 1.22  | 0.67   |
| 10                | 1.75  | 0.76  | 1.53  | 0.54   |
| 11                | 1.00  | 0.95  | 1.04  | 0.58   |
| 12                | 0.59  | 0.57  | 0.59  | 0.42   |
| 13                | 0.99  | 0.75  | 1.02  | 0.75   |
| 14                | 0.82  | 0.71  | 0.73  | 0.61   |
| 15                | 0.49  | 0.52  | 0.49  | 0.38   |
| 16                | 1.22  | 1.20  | 1.22  | 0.91   |
| 17                | 0.57  | 0.52  | 0.57  | 0.41   |
| 18                | 0.54  | 0.50  | 0.54  | 0.35   |
| 19                | 0.76  | 0.74  | 0.77  | 0.65   |
| 20                | 0.39  | 0.35  | 0.39  | 0.33   |
| 21                | 0.60  | 0.61  | 0.58  | 0.41   |
| 22                | 0.98  | 0.75  | 0.54  | 0.55   |
| 23                | 0.50  | 0.51  | 0.44  | 0.41   |
| 24                | 1.36  | 1.12  | 1.23  | 0.83   |
| 25                | 0.45  | 0.45  | 0.43  | 0.42   |
| 26                | 0.64  | 0.55  | 0.64  | 0.50   |
| 27                | 0.67  | 0.53  | 0.66  | 0.35   |
| 28                | 0.59  | 0.43  | 0.47  | 0.41   |
| 29                | 1.22  | 0.59  | 1.01  | 0.45   |
| 30                | 0.69  | 0.74  | 0.55  | 0.49   |
| Mean              | 0.84  | 0.71  | 0.76  | 0.51   |
| S.D.              | 0.35  | 0.25  | 0.31  | 0.17   |

a) Numbers are the same as in Table 2.

of analysis of variance indicate that the models produced comparable accuracy. It is suggested that this finding is acceptable, because it has been shown that all cosolvency models are identical from a mathematical point of view and one can convert the equations to a general form using simple algebraic manipulations.

## Conclusion

The combined nearly ideal binary solvent/Redlich–Kister and the modified Wilson models are extended for predicting solute solubility in ternary (and higher multi-component) solvent mixtures based on the model constants calculated from solubility data in sub-binary solvents. Average %*D* values less than 2% illustrates that these models have a good prediction capability and one can use the models for solubility prediction in ternary or higher multi-component solvent mixtures in order to find the optimum solvent composition for solubilization or desolubilization of a solute. It is considered that the combined nearly ideal binary solvent/Redlich–Kister equation may be preferred by experimentalists because of its simplicity and availability for calculation by common software and scientific calculators. In the previous work it has been demonstrated that its application can be expanded to prediction of solubility in different temperatures<sup>34)</sup> and solubility of structurally related solutes in mixed solvents.<sup>10)</sup>

## Appendix

The programs written in SPSS and GWBASIC environments for computing the sub-binary interaction terms are given here. It is obvious that, these types of calculations can be done in other software like Excel or Minitab.

The program file to calculate the binary interaction terms for anthracene solubility data in heptane+cyclohexane in SPSS environment is:

```
DATA LIST FREE /F1 XM.
BEGIN DATA.
0.0000 0.001552
0.1542 0.001608
0.3283 0.001642
0.4230 0.001640
0.5250 0.001621
0.7236 0.001605
1.0000 0.001571
END DATA.
COMPUTE X1=0.001571.
COMPUTE X2=0.001552.
COMPUTE F2=1-F1.
COMPUTE X3=X1*(X1).
COMPUTE L1=X1*(X1).
COMPUTE L2=X1*(X2).
COMPUTE Y=L3*X1+L1*X1+L2*X2.
COMPUTE W0=F1*F2
COMPUTE W1=F1*F2*(F1-F2).
COMPUTE W2=F1*F2*(F1-F2)*(F1-F2).
REGRESSION VARIABLES /W0 W1 W2/ORIGIN /DEPENDENT Y /METHOD ENTER
```

The corresponding program file to compute the modified Wilson model constants in GWBASIC environment is:

```

10 REM *** CALCULATION OF MODIFIED WILSON PARAMETERS ***
20 ***** A. Juyayan -Garamaleki *****
30 REM *****
40 COLOR 2,1,4:CLS:COLOR 2,0:KEY OFF:N=7 :AE=1000
50 STEP1=1 :STEP2=1 :MIN1=1 :MINJ=1
60 LOCATE 10,15:INPUT "Enter maximum value of lambda1 (-30):":MAX1
70 LOCATE 12,15:INPUT "Enter maximum value of lambda2 (-4):":MAXJ
80 STEP2=2,1,4:CLS=250:DIM F(1),XM(1),X(1),X2(1),F2(1)
90 FOR Q=1 TO N:READ F1(Q),XM(Q),X1(Q),X2(Q),F2(Q):F1(Q)/NEXT Q
100 COLOR 14,4:LOCATE 6,12:PRINT " Optimum Values : "COLOR 2,0 :LOCATE 13,12:PRINT " Current Values : "
110 COLOR 7,0:LOCATE 19,12:PRINT " First digit optimisation of lambda1 and lambda2 " "COLOR 2,0
120 GOSUB 350
130 COLOR 14,0:LOCATE 19,12:PRINT " Second digit optimisation of lambda1 and lambda2 " "COLOR 2,0
140 GOTO 1,1,4: "G1B-01"G1B"L1 MK=" :G2B-01"G2B"L1
150 MIN1=G1B-AJ:MAX1=G1B-AJ :STEP1=MIN1-MINJ:01MINJ=G2B-MK:MAXJ=G2B-MK:STEP2=(MAXJ-MINJ)/10
160 LOCATE 14,02:PRINT USING "AJ1: MIN1=##### MAX1=##### Step=##### AJ=##### MIN1,MAX1,STEP1,AJ
170 LOCATE 14,21:PRINT USING "lambda2: MIN=##### MAX=##### Step=##### MK=##### MINJ,MAXJ,STEP2,MK
180 NNN=NNNN+1:LOCATE 22,20:PRINT USING "No. of repetition=####",NNN
190 STEP1=1:0001 AND STEP2=2:0001 THEN GOTO 220
200 GOSUB 350
210 GOTO 140

```

```

220 AJ=G1B*.001 MK=G2B*.001 MINJ=G1B-AJ MAXJ=G1B+AJ STEP1=.0001 MINJ=G2B-
MK MAXJ=G2B+MK STEP2=.0001
230 LOCATE 20.2:PRINT USING "lamda1: Min=#### Max=#### Step=#### AJ=#.####" MINJ MAXJ STEP1 AJ
240 LOCATE 21.2:PRINT USING "lamda2: Min=#### Max=#### Step=#### MK=#.####" MINJ MAXJ STEP2 MK
250 COLOR 0.7:LOCATE 19,12:PRINT " Fourth digit optimisation of lamda1 and lamda2 " :COLOR 2,0
260 GOSUB 350
270 CLOSE :BEEP
280 COLOR 2,1,14,CLS:COLOR 2,0:LOCATE 10, 15:PRINT " The optimised modified Wilson model is: "
290 LOCATE 12,15:PRINT " F1(1 -;):PRINT USING "####" F2(1 -;####) " :LOG(1/X1(1)):LOG(1/X2(1))
300 LOCATE 13,15:PRINT " lnXm = 1 -;:STRINGS(17,196) " -;:STRINGS(17,196) "
310 LOCATE 14,15:PRINT " F1 +;:PRINT USING "####F2 "####F1 + F2 " :G1B,G2B
320 END
330 REM -----
340 REM ----- Minimisation of the PAE values -----
350 FOR G1 =MINJ TO MAXJ STEP STEP1
360 FOR G2 =MINJ TO MAXJ STEP STEP2
370 E=0:SE=0:ER=0:SPER=0:QPER=0
380 FOR I=1 TO N
390 Y=1/EXP(1-((F1(I)*(1+LOG(X1(I))))/(F1(I)+F2(I)*G1)) -((F2(I)*(1+LOG(X2(I))))/(F2(I)+F1(I)*G2)))
400 Q=1/EXP(1-((F1(I)*(1+LOG(X1(I))))/(F1(I)+F2(I)*G2)) -((F2(I)*(1+LOG(X2(I))))/(F2(I)+F1(I)*G1)))
410 QER=ABS(100*(Q-XM(I))/XM(I)):QPER=QPER+QER :PER=ABS(100*(Y-XM(I))/XM(I)):SPER=SPER+PER
420 NEXT I
430 E=SPER/N:W=QPER/N
440 IF E<=AE THEN COLOR 14,4:AE=E:G1B=G1:G2B=G2: LOCATE 8,10:PRINT USING " PAE =#### lamda1=####
lamda2=####" AE,G1B,G2B:COLOR 2,0
450 IF W<=AE THEN COLOR 15,4:AE=W:G1B=G2:G2B=G1:LOCATE 9,10:PRINT USING " PAE =#### lamda1=####
lamda2=####" AE,G1B,G2B:COLOR 2,0
460 LOCATE 15,10:PRINT USING " PAE =#### lamda1=#### lamda2=####" E,G1,G2
470 NEXT G2
480 NEXT G1
490 RETURN
500 REM -----
510 DATA 0.0000 , 0.001552, 0.001571, 0.001552
520 DATA 0.1542 , 0.001606, 0.001571, 0.001552
530 DATA 0.3283 , 0.001642, 0.001571, 0.001552
540 DATA 0.4230 , 0.001640, 0.001571, 0.001552
550 DATA 0.5250 , 0.001621, 0.001571, 0.001552
560 DATA 0.7236 , 0.001606, 0.001571, 0.001552
570 DATA 1.0000 , 0.001571, 0.001571, 0.001552

```

After calculating the model constants in binary mixtures, the solubility in ternary mixtures have been predicted by Eqs. 1 and 2. As an example, the solubility of anthracene in 1-butanol+heptane+cyclohexane was predicted using a SPSS program file:

```

DATA LIST FREE/F1 F2 XM.
BEGIN DATA.
.4069 .2468 .001452
.2147 .6053 .001569
.2968 .1822 .001575
.3226 .4014 .00154
.7475 .0707 .001066
.7687 .137 .001071
.1842 .1127 .001625
.7618 .1002 .001075
.6402 .197 .00122
.1232 .1534 .001643
.1437 .6183 .00159
.2331 .073 .001573
.2798 .6005 .001542
.1291 .4223 .001606
.4974 .3564 .001369
.5649 .2993 .001266
.1308 .3221 .001623
.4499 .0721 .001394
.5536 .0713 .001293
END DATA.
COMPUT X1= 0.00080104.
COMPUT X2= 0.001571067.
COMPUT X3= 0.001552949.
COMPUT X2I= 0.00684.
COMPUT L12= 1.291.
COMPUT L21= 1.476.
COMPUT L13= 1.01.
COMPUT L31= 2.377.
COMPUT L23= .541.
COMPUT L32= 2.244.
COMPUT W012= .723.
COMPUT W112= -.004.
COMPUT W212= .201.
COMPUT W013= .741.
COMPUT W113= -.345.
COMPUT W213= .223.
COMPUT W023= 0.175.
COMPUT W123= -.126.
COMPUT W223= 0.
COMPUT F3=1-F1-F2.
COMPUT F1F2=F1*F2.
COMPUT F1F3=F1*F3.
COMPUT F2F3=F2*F3.
COMPUT FF1F2=F1F2*(F1-F2).
COMPUT FF1F3=F1F3*(F1-F3).
COMPUT FF2F3=F2F3*(F2-F3).
COMPUT Q12=(F1-F2).
COMPUT Q13=(F1-F3).
COMPUT Q23=(F2-F3).
COMPUT LXm=LN(Xm).
COMPUT LX2I=LN(X2I).
COMPUT LX1=LN(X1).
COMPUT LX2=LN(X2).
COMPUT LX3=LN(X3).
COMPUT LXMEQ1=F1*LX1+F2*LX2+F3*LX3+F1F2*(W012+W112*Q12+W212*Q12*Q12)
+F1F3*(W013+W113*Q13+W213*Q13*Q13)+F2F3*(W023+W123*Q23+W223*Q23*Q23).
COMPUT XMEQ1=EXP(XMEQ1).
COMPUT PDEQ1=ABS(100*(XMEQ1-XM)/XM).
COMPUT LXMEQ2=1-(F1*(1-LN(X2I/X1)))/(F1+F2*L12+F3*L13)
-(F2*(1-LN(X2I/X2)))/(F1*L21+F2+F3*L23))
-(F3*(1-LN(X2I/X3)))/(F1*L31+F2*L32+F3).
COMPUT XMEQ2=X2I/EXP(LXMEQ2).
COMPUT PDEQ2=ABS(100*(XMEQ2-XM)/XM).
DESCRIPTIVE/VARIABLE PDEQ1 PDEQ2/STATISTICS MEAN.

```

**Acknowledgements** The authors would like to thank the Tabriz University of Medical Sciences, Tabriz, Iran for providing financial support to A. Jouyban-Gharamaleki and ORS committee for the Overseas Research Scholarship to study at the University of Bradford.

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# Synthesis and *in Vitro* Cytotoxic Evaluation of *N*-Substituted Benzo[5,6]cyclohepta[b]indoles

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Received May 15, 2000; accepted July 24, 2000

**A new series of *N*-substituted benzo[5,6]cyclohepta[b]indole derivatives were synthesised and evaluated for *in vitro* cytotoxic activities against L1210 murine leukemia and HT29 cell lines. Among them, several compounds showed potent antitumor activity and blocked cell cycle progression of L1210 cells in G<sub>2</sub>+M phase.**

**Key words** synthesis; indole; seven-membered ring; cytotoxicity; cell cycle progression analysis

The DNA molecule is an important target of many antitumor agents.<sup>1)</sup> Conformational changes in the double helix is the result of the binding of drugs to DNA involving modification in the mechanism of DNA replication and consequently cell killing. Different derivatives such as ellipticine or related structures,<sup>2)</sup> DACA<sup>3)</sup> and TAS-103<sup>4)</sup> (Fig. 1) are highly toxic to tumour cells through their capacity to intercalate into DNA and to poison DNA topoisomerases.

On the basis of these facts, we became interested in the synthesis of related ellipticine skeleton with a seven-membered ring to evaluate their antitumoral potential. First, we reported the synthesis of tetracyclic derivative **1**,<sup>5)</sup> which exhibited potent cytotoxic activity *in vitro* on L1210 murine leukemia cells. Unfortunately, a non specific L1210 cell cycle effect and a toxicity at 50  $\mu$ M were also observed for the latter. From these pharmacological results, we investigated the preparation and the reactivity of 5,6,11,12-tetrahydrobenzo[5,6]cyclohepta[b]indol-6-one **2** or 5,6-dihydrobenzo[5,6]cyclohepta[b]indol-6-one **3** (Fig. 2), with a view to designing potent anticancer agents.<sup>6)</sup>

In the present study, *N*-substituted benzo[5,6]cyclohepta[b]indole derivatives **A** (Fig. 2) were synthesised and evaluated. Three structural modifications were investigated to improve the antitumour activity: 1) length of the amino linker chain, 2) nature of the amino substituents, and 3) nature of the indole substituent in position-5.

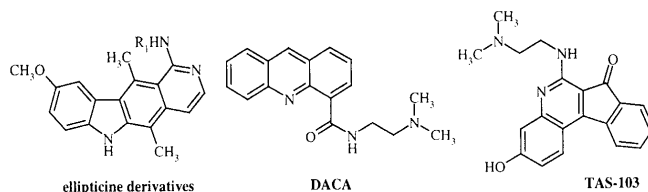


Fig. 1

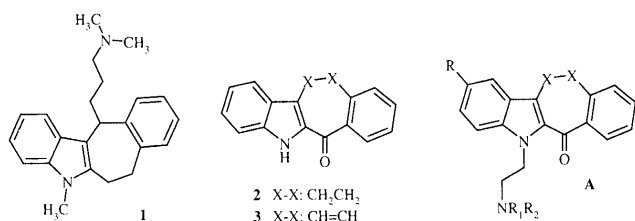


Fig. 2

**Chemistry** Following the procedure used for the preparation of **2**,<sup>6)</sup> we first prepared the fused 5-methoxyindole ring system **9** starting from either 5-methoxy-1-phenylsulfonylindole-3-carboxaldehyde **4a**<sup>7)</sup> or 5-methoxy-1-*tert*-butoxycarbonylindole-3-carboxaldehyde **4b** (Chart 1). A Wittig reaction of **4** with the ylide generated from **5**<sup>8)</sup> gave an *E/Z* mixture of alkenes **6** in 60–65% yield. Compounds **6** were hydrogenated over 10% Pd/C catalyst to afford **7** (96% yield). Saponification of ester **7a** gave the corresponding acid **8a** in 65% yield. The same conditions applied to **7b** afforded the *N*-deprotected acid **8b** (84% yield). Intramolecular acidic cyclisation<sup>9)</sup> of acid **8b** afforded tetracyclic compound **9** in 65% yield.

With **8a**, the cyclisation led to **9** and the unexpected 5-methoxy-6-phenylsulfonyl-5,6,11,12-tetrahydrobenzo[5,6]cyclohepta[b]indol-6-one **10** in 20–30% yield (Chart 2). The structures of **9** and **10** were confirmed by one and two dimensional (1D, 2D) NMR, (NOESY) and (HETCOR) data (Fig. 3). No cyclisation on position-4 of the indole ring was observed.

All derivatives **11–15** and **19**, **20** were synthesised by reaction of an alkyl halide (chloride or bromide) with an indolic anion, which was conveniently prepared from **2**, **3**, **9**, **18** in the presence of sodium hydride or KOH in *N,N*-dimethylformamide (DMF). First, we obtained 5,6,11,12-tetrahydrobenzo derivatives **11** and **12** in 65–73% yield from **2** with 2-(dimethylamino)ethyl chloride or with 3-(dimethylamino)propyl chloride; then, the 5,6-dihydrobenzo derivatives **13** and **14** were similarly obtained from **3** in 60–70% yield. *N*-Alkylation of **3** with an excess of 1,3-dibromopropane afforded **15** which was treated by 2-aminoethanol to lead to the derivative **16** in 46% yield (Chart 3).

A number of reports have discussed the synthesis of dimeric compounds designed as potential bis-intercalating agents.<sup>10)</sup> Treatment of **3** with an appropriate amount of 1,6-dibromohexane gave the dimeric form **17** in 60% yield (Chart 4). Unfortunately, the latter has not been tested due to its low solubility in solvents.

*N*-Alkylation of **9** was performed to give a mixture of 5-methoxy-5,6-dihydrobenzo and 5-methoxy-5,6,11,12-tetrahydrobenzo derivatives not separable by column chromatography. To solve this problem, only 5-methoxy-5,6-dihydrobenzo derivatives were prepared as a pure form. The unsaturated ketone **18** was obtained in 90% yield by treatment of **9** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)

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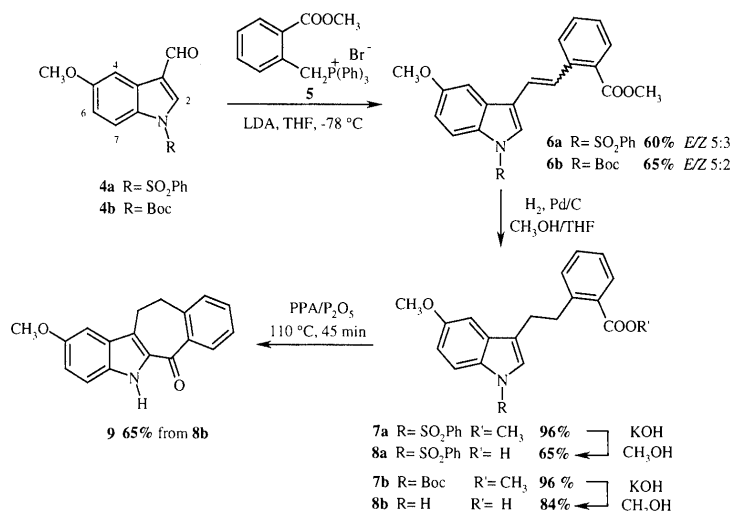


Chart 1

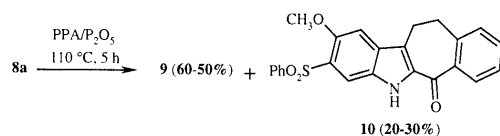


Chart 2

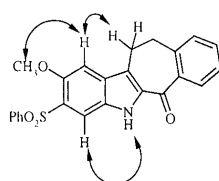


Fig. 3. Correlations in the NOESY Spectrum of 10

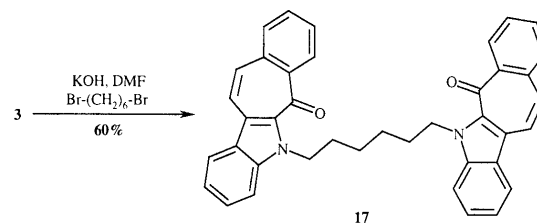


Chart 4

pressed as IC<sub>50</sub> (concentration reducing the cell proliferation by 50%). Similarly, to examine the effect of the new derivatives on cell cycle progression, flow cytometric analysis was performed. All pharmacological results are reported in Table 1.

## Results and Discussion

The 5,6,11,12-tetrahydrobenzo[5,6]cyclohepta[b]indol-6-one derivative **11** was first evaluated. The calculated IC<sub>50</sub> value with the murine L1210 leukemia cell line was 16.8 μM. The unsaturated compound **13** is significantly more active (IC<sub>50</sub>=3.4 μM) than its saturated counterpart **11**. Next, we investigated the effect of the amino alkyl chain on the cytotoxic profile. Insertion of one methylene in the alkyl chain as in **14** or modification of the terminal amino part as **16** slightly improves the cytotoxicity (**14**: IC<sub>50</sub>=2.9 μM, **16**: IC<sub>50</sub>=1.9 μM), although it's inferior to that measured with the tumour-active drug adriamycin. Similarly, compound **14** shows the same overall activity against HT29 cells (IC<sub>50</sub>=2.4 μM). In the 5-methoxy series, compound **19** has the same potency against L1210 or HT29 cell lines as **14**.

Compounds **13**, **14**, **16**, and **19** induce an accumulation of L1210 cells in the G<sub>2</sub>+M phase of the cell cycle. Among them, compound **13** causes a massive G<sub>2</sub>+M arrest at a concentration of 2.5–5 μM.

These preliminary biological results show that *N*-substituted 5,6-dihydrobenzo-[5,6]cyclohepta[b]indol-6-one derivatives are an interesting new class of cytotoxic compounds worthy of further development.

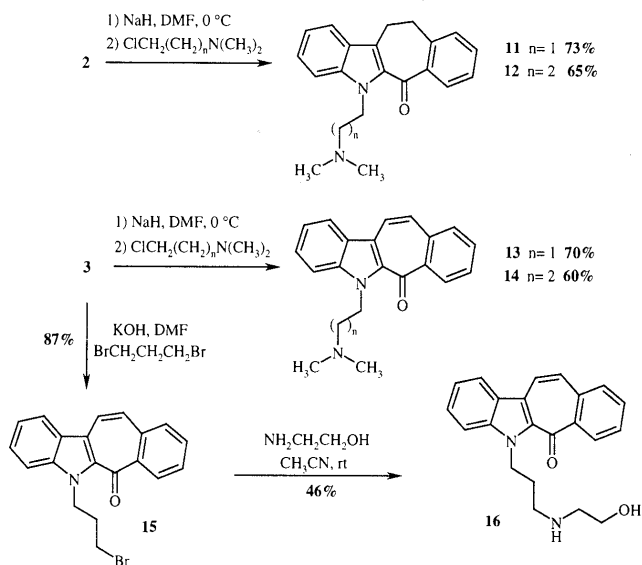
## Experimental

**Chemistry** Melting points were determined using a Büchi capillary instrument and are uncorrected. The infrared (IR) spectra of compounds were

in dioxane. Final alkylation led to **19** and **20** in fair yields (Chart 5).

**Pharmacology** The cytotoxic properties of only five compounds **11**, **13**, **14**, **16** and **19** were assessed by a cell growth inhibition assay using the murine L1210 leukemia and HT29 (colon cancer) cell lines.<sup>11)</sup> The results are ex-

Chart 3



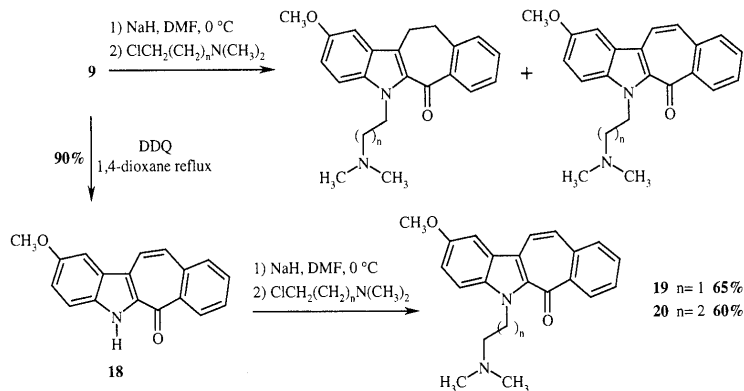


Table 1. Inhibition of L1210 Cell Proliferation and L1210 Cell Cycle Progression by Compounds **11**, **13**, **14**, **16** and **19**

| Compound    | IC <sub>50</sub> (μM) |                  | % of L1210 cells <sup>a)</sup><br>in the G <sub>2</sub> +M phase (μM) |
|-------------|-----------------------|------------------|-----------------------------------------------------------------------|
|             | L1210                 | HT29             |                                                                       |
| Adriamycine | 0.024                 | 0.066            | +++ <sup>c)</sup> (0.1 μM)                                            |
| <b>11</b>   | 16.8                  | NT <sup>b)</sup> | + (50 μM)                                                             |
| <b>13</b>   | 3.4                   | NT               | +++ (2.5–5 μM)                                                        |
| <b>14</b>   | 2.9                   | 3.1              | ++ (5 μM)                                                             |
| <b>16</b>   | 1.9                   | NT <sup>b)</sup> | ++ (5 μM)                                                             |
| <b>19</b>   | 2.9                   | 2.4              | + (10 μM)                                                             |

a) 24% of untreated control cells were in the G<sub>2</sub>+M phase of the cell cycle. b) Not tested. c) +30–50%, ++ 50–70%, +++ >70%.

recorded on a Perkin Elmer FT-IR paragon 1000 spectrometer. NMR spectra were recorded at 300 °K in CDCl<sub>3</sub> on a Bruker Avance DPX 250. Chemical shifts are expressed in parts per million relative to tetramethylsilane (TMS), and spin multiplicities are given as s (singlet), d (doublet), dd (double doublet), t (triplet) and m (multiplet). Mass spectra were recorded on Perkin-Elmer SCIEX API 300 using ionspray methodology. The elemental compositions of the compounds agreed to within 0.4% of the calculated value. Thin-layer chromatography (TLC) was run on precoated silica gel plates (Merck 60F<sub>254</sub>) and the spots visualised using an ultraviolet lamp. Flash chromatography was carried out on a column using Merck Silica gel 60 (40–63 μm) as the stationary phase. Tetrahydrofuran (THF) was distilled under nitrogen from sodium and benzophenone before use. All reactions requiring anhydrous conditions were conducted in flame-dried apparatus.

**5-Methoxy-1-*tert*-butoxycarbonylindole-3-carboxaldehyde (4b)** A solution of 5-methoxyindole-3-carboxaldehyde<sup>7)</sup> (1.0 g, 5.7 mmol), di-*tert*-butyldicarbonate (1.50 g, 6.9 mmol) and 4-dimethylaminopyridine (100 mg, 0.82 mmol) in anhydrous acetonitrile (100 ml) was stirred at 40 °C for 12 h. After cooling, the solvent was evaporated *in vacuo*. The residue was partitioned between CH<sub>2</sub>Cl<sub>2</sub> (50 ml) and 10% aqueous HCl (50 ml), then the aqueous phase was separated and extracted with CH<sub>2</sub>Cl<sub>2</sub> (2×30 ml). The organic layer was dried over anhydrous MgSO<sub>4</sub> and evaporated *in vacuo* to give **4b** (1.54 g, 98%). mp 134–135 °C (EtOAc). IR (KBr) cm<sup>-1</sup>: 1734, 1676 (CO). <sup>1</sup>H-NMR (250 MHz, CDCl<sub>3</sub>) δ: 1.70 (s, 9H, CH<sub>3</sub>), 3.89 (3H, s, CH<sub>3</sub>), 7.01 (1H, dd, *J*=2.5, 9.1 Hz, H<sub>6</sub>), 7.76 (1H, d, *J*=2.5 Hz, H<sub>4</sub>), 8.01 (1H, d, *J*=9.1 Hz, H<sub>7</sub>), 8.19 (1H, s, H<sub>2</sub>), 10.06 (1H, s, CHO). <sup>13</sup>C-NMR (62.90 MHz, CDCl<sub>3</sub>) δ: 28.0 (3 CH<sub>3</sub>), 55.7 (CH<sub>3</sub>), 85.5 (C), 103.7 (CH), 115.5 (CH), 115.9 (CH), 121.3 (C), 126.9 (C), 130.4 (C), 136.7 (CH), 148.1 (C), 157.2 (CO), 185.8 (CO). Ionspray-MS *m/z*: 276 (M<sup>+</sup>+1). Anal. Calcd for C<sub>15</sub>H<sub>17</sub>NO<sub>4</sub>: C, 65.44; H, 6.22; N, 5.09. Found: C, 65.77; H, 6.09; N, 4.98.

**2-[(*E/Z*)-2-[5-Methoxy-1-(phenylsulfonyl)-1*H*-3-indolyl]-1-ethenyl]-benzoic Acid Methyl Ester (6a)** To a suspension of (2-carbomethoxybenzyl)triphenyl phosphonium bromide **5**<sup>8)</sup> (9.36 g, 19 mmol) in anhydrous THF (120 ml), 2*M* lithium diisopropylamide (LDA) in heptane (9.6 ml, 19 mmol) was added dropwise at –78 °C. After 30 min, a solution of **4a** (2.0 g, 6.34 mmol) in THF (20 ml) was added dropwise with vigorous stirring at –78 °C. The mixture was stirred at –78 °C for 1 h, then for 1.5 h at room temperature and THF was evaporated *in vacuo*. The residue was partitioned between EtOAc (30 ml) and 10% aqueous HCl (30 ml), the aqueous phase was

separated and extracted with EtOAc (2×30 ml). The combined organic layer was dried over anhydrous MgSO<sub>4</sub> and evaporated *in vacuo*. The crude oil was purified by column chromatography (eluent petroleum ether/CH<sub>2</sub>Cl<sub>2</sub> 1 : 1) to afford **6a** as two isomers in 60% yield (1.7 g, *E/Z* ratio 5:3, *E* isomer was first eluted).

*E*-Isomer: mp 139–140 °C (MeOH). IR (KBr) cm<sup>-1</sup>: 1714 (CO). <sup>1</sup>H-NMR (250 MHz, CDCl<sub>3</sub>) δ: 3.91 (6H, s, CH<sub>3</sub>), 6.99 (1H, dd, *J*=2.6, 9.1 Hz, H<sub>6</sub>), 7.08 (1H, d, *J*=16.6 Hz, =CH), 7.28–7.53 (6H, m, H<sub>4</sub>+H<sub>Ar</sub>), 7.68 (1H, s, H<sub>2</sub>), 7.74 (1H, br d, *J*=8.0 Hz, H<sub>Ar</sub>), 7.86–7.97 (4H, m, H<sub>7</sub>+H<sub>Ar</sub>), 8.15 (1H, d, *J*=16.6 Hz, =CH). <sup>13</sup>C-NMR (62.90 MHz, CDCl<sub>3</sub>) δ: 52.0 (CH<sub>3</sub>), 55.6 (CH<sub>3</sub>), 103.3 (CH), 114.2 (CH), 114.5 (CH), 121.2 (C), 121.8 (CH), 125.3 (CH), 126.2 (CH), 126.6 (2 CH), 127.1 (CH), 128.1 (C), 128.2 (CH), 129.2 (2 CH), 129.9 (C), 130.2 (C), 130.7 (CH), 132.1 (CH), 133.7 (CH), 137.8 (C), 139.1 (C), 156.8 (C), 167.6 (CO). IS-MS *m/z*: 448 (M<sup>+</sup>+1). Anal. Calcd for C<sub>25</sub>H<sub>21</sub>NO<sub>5</sub>S: C, 67.10; H, 4.73; N, 3.13. Found: C, 66.89; H, 4.91; N, 2.99.

*Z*-Isomer: mp 152–154 °C (MeOH). IR (KBr) cm<sup>-1</sup>: 1714 (CO). <sup>1</sup>H-NMR (250 MHz, CDCl<sub>3</sub>) δ: 3.67 (3H, s, CH<sub>3</sub>), 3.86 (3H, s, CH<sub>3</sub>), 6.61 (1H, d, *J*=12.0 Hz, =CH), 6.71 (1H, d, *J*=2.2 Hz, H<sub>4</sub>), 6.86 (1H, dd, *J*=2.2, 9.5 Hz, H<sub>6</sub>), 7.01 (1H, s, H<sub>2</sub>), 7.14–7.53 (7H, m, =CH+H<sub>Ar</sub>), 7.68 (2H, d, *J*=7.0 Hz, H<sub>Ar</sub>), 7.80 (1H, d, *J*=9.0 Hz, H<sub>7</sub>), 8.01 (1H, dd, *J*=1.2, 8.0 Hz, H<sub>Ar</sub>). <sup>13</sup>C-NMR (62.90 MHz, CDCl<sub>3</sub>) δ: 52.0 (CH<sub>3</sub>), 55.4 (CH<sub>3</sub>), 102.1 (CH), 114.2 (CH), 114.4 (CH), 118.9 (CH), 119.1 (C), 125.1 (CH), 126.7 (2 CH), 127.4 (CH), 129.0 (C), 129.1 (2 CH), 129.3 (CH), 130.5 (C), 130.6 (C), 131.0 (CH), 132 (CH), 132.1 (CH), 133.6 (CH), 138.0 (C), 139.6 (C), 156.4 (C), 167.4 (CO). IS-MS *m/z*: 448 (M<sup>+</sup>+1). Anal. Calcd for C<sub>25</sub>H<sub>21</sub>NO<sub>5</sub>S: C, 67.10; H, 4.73; N, 3.13. Found: C, 67.02; H, 4.62; N, 3.00.

**2-[(*E/Z*)-2-[5-Methoxy-1-(*tert*-butoxycarbonyl)-1*H*-3-indolyl]-1-ethenyl]benzoic Acid Methyl Ester (6b)** With the same methodology but using **4b** as starting material, **6b** was isolated after column chromatography (eluent petroleum ether/CH<sub>2</sub>Cl<sub>2</sub> 1 : 1) in 65% yield as a yellow oil (*E/Z* ratio 5 : 2, *E* isomer was first eluted).

*E*-Isomer: IR (film) cm<sup>-1</sup>: 1721, 1675 (CO). <sup>1</sup>H-NMR (250 MHz, CDCl<sub>3</sub>) δ: 1.72 (9H, s, CH<sub>3</sub>), 3.95 (3H, s, CH<sub>3</sub>), 3.99 (3H, s, CH<sub>3</sub>), 7.03 (1H, dd, *J*=2.5, 9.1 Hz, H<sub>6</sub>), 7.16 (1H, d, *J*=16.4 Hz, =CH), 7.30 (1H, t, *J*=8.0 Hz, H<sub>Ar</sub>), 7.51 (1H, t, *J*=8.0 Hz, H<sub>Ar</sub>), 7.61 (1H, d, *J*=2.5 Hz, H<sub>4</sub>), 7.75 (1H, s, H<sub>2</sub>), 7.77 (1H, d, *J*=8.2 Hz, H<sub>Ar</sub>), 7.97 (1H, dd, *J*=1.0, 7.7 Hz, H<sub>Ar</sub>), 8.13 (1H, br d, *J*=9.1 Hz, H<sub>7</sub>), 8.20 (1H, d, *J*=16.4 Hz, =CH). <sup>13</sup>C-NMR (62.90 MHz, CDCl<sub>3</sub>) δ: 27.8 (3 CH<sub>3</sub>), 51.8 (CH<sub>3</sub>), 55.4 (CH<sub>3</sub>), 83.5 (C), 102.9 (CH), 113.3 (CH), 115.8 (CH), 118.9 (Cq), 122.6 (CH), 125.1 (CH), 125.9 (CH), 126.6 (CH), 126.9 (CH), 127.8 (C), 129.2 (C), 130.5 (C), 130.5 (CH), 131.9 (CH), 139.1 (C), 149.2 (C), 156.1 (CO), 167.5 (CO). IS-MS *m/z*: 408 (M<sup>+</sup>+1). Anal. Calcd for C<sub>24</sub>H<sub>25</sub>NO<sub>5</sub>: C, 70.75; H, 6.18; N, 3.44. Found: C, 70.93; H, 6.30; N, 3.25.

*Z*-Isomer: IR (film) cm<sup>-1</sup>: 1721, 1675 (CO). <sup>1</sup>H-NMR (250 MHz, CDCl<sub>3</sub>) δ: 1.60 (9H, s, CH<sub>3</sub>), 3.69 (3H, s, CH<sub>3</sub>), 3.91 (3H, s, CH<sub>3</sub>), 6.72–6.77 (2H, m, =CH+H<sub>4</sub>), 6.90 (1H, dd, *J*=2.5, 8.5 Hz, H<sub>6</sub>), 7.14–7.20 (2H, m, H<sub>Ar</sub>), 7.27–7.41 (3H, m, =CH+H<sub>Ar</sub>), 7.95–8.07 (2H, m, H<sub>7</sub>+H<sub>Ar</sub>). <sup>13</sup>C-NMR (62.90 MHz, CDCl<sub>3</sub>) δ: 27.6 (3 CH<sub>3</sub>), 52.2 (CH<sub>3</sub>), 55.3 (CH<sub>3</sub>), 83.2 (C), 103.1 (CH), 113.3 (CH), 115.7 (CH), 118.5 (C), 123.5 (CH), 125.1 (CH), 125.7 (CH), 126.7 (CH), 126.9 (CH), 127.6 (C), 129.4 (C), 130.5 (C), 130.5 (CH), 133.0 (CH), 139.3 (C), 149.9 (C), 156.2 (CO), 168.5 (CO). IS-MS *m/z*: 408 (M<sup>+</sup>+1). Anal. Calcd for C<sub>24</sub>H<sub>25</sub>NO<sub>5</sub>: C, 70.75; H, 6.18; N, 3.44. Found: C, 70.97; H, 6.25; N, 3.37.

**2-2-[5-Methoxy-1-phenylsulfonyl-1*H*-3-indolyl]ethyl]benzoic Acid**

**Methyl Ester (7a)** A mixture of **6a** (1.42 g, 3.18 mmol) and 10% Pd/C (150 mg) in MeOH/THF (30 ml, 3/1 v/v) was shaken in a Parr apparatus under 45 psi of hydrogen at room temperature for 2 h. The catalyst was removed by filtration, and solvents evaporated. The crude residue was purified by column chromatography (eluent petroleum ether/CH<sub>2</sub>Cl<sub>2</sub> 1:1) to give **7a** (1.37 g, 96%) as a white solid. mp 121–122 °C (MeOH). IR (KBr) cm<sup>-1</sup>: 1724 (CO). <sup>1</sup>H-NMR (250 MHz, CDCl<sub>3</sub>) δ: 2.92 (2H, t, *J*=7.5 Hz, CH<sub>2</sub>), 3.29 (2H, t, *J*=7.5 Hz, CH<sub>2</sub>), 3.84 (6H, s, CH<sub>3</sub>), 6.91 (1H, dd, *J*=2.5, 9.0 Hz, H<sub>6</sub>), 7.08 (1H, d, *J*=2.5 Hz, H<sub>4</sub>), 7.12 (1H, dd, *J*=1.2, 8.0 Hz, H<sub>Ar</sub>), 7.25–7.54 (6H, m, H<sub>2</sub>+H<sub>Ar</sub>), 7.77–7.92 (4H, m, H<sub>7</sub>+H<sub>Ar</sub>). <sup>13</sup>C-NMR (62.90 MHz, CDCl<sub>3</sub>) δ: 27.1 (CH<sub>3</sub>), 34.2 (CH<sub>3</sub>), 51.8 (CH<sub>3</sub>), 55.6 (CH<sub>3</sub>), 102.2 (CH), 113.6 (CH), 114.5 (CH), 123.1 (C), 123.6 (CH), 126.2 (CH), 126.6 (2 CH), 129.0 (2 CH), 129.3 (C), 129.9 (C), 130.7 (CH), 131.2 (CH), 132.0 (CH), 132.1 (C), 133.5 (CH), 138.2 (C), 143.2 (C), 156.3 (CO), 167.7 (CO). IS-MS *m/z*: 450 (M<sup>+</sup>+1). *Anal.* Calcd for C<sub>25</sub>H<sub>23</sub>NO<sub>5</sub>S: C, 66.80; H, 5.16; N, 3.12. Found: C, 66.56; H, 4.98; N, 3.01.

**2-{2-[5-Methoxy-1-(*tert*-butoxycarbonyl)-1*H*-3-indolyl]ethyl}benzoic Acid Methyl Ester (7b)** Using the same procedure and the same eluent for the final purification, **7b** was prepared from **6b** in 96% yield as an oil. IR (film) cm<sup>-1</sup>: 1724, 1674 (CO). <sup>1</sup>H-NMR (250 MHz, CDCl<sub>3</sub>) δ: 1.72 (9H, s, CH<sub>3</sub>), 3.02 (2H, t, *J*=8.0 Hz, CH<sub>2</sub>), 3.41 (2H, t, *J*=8.0 Hz, CH<sub>2</sub>), 3.93 (3H, s, CH<sub>3</sub>), 3.95 (3H, s, CH<sub>3</sub>), 7.00 (1H, dd, *J*=2.5, 9.1 Hz, H<sub>6</sub>), 7.26 (1H, d, *J*=2.5 Hz, H<sub>4</sub>), 7.29–7.35 (2H, m, H<sub>2</sub>+H<sub>Ar</sub>), 7.44–7.51 (2H, m, H<sub>Ar</sub>), 7.97 (1H, dd, *J*=1.4, 8.0 Hz, H<sub>Ar</sub>), 8.09 (1H, brd, *J*=9.1 Hz, H<sub>7</sub>). <sup>13</sup>C-NMR (62.90 MHz, CDCl<sub>3</sub>) δ: 27.3 (CH<sub>2</sub>), 28.0 (3CH<sub>3</sub>), 34.4 (CH<sub>2</sub>), 51.7 (CH<sub>3</sub>), 55.4 (CH<sub>3</sub>), 82.8 (C), 101.8 (CH), 112.7 (CH), 115.6 (CH), 120.5 (C), 122.9 (CH), 123.1 (C), 125.9 (CH), 129.2 (C), 130.5 (CH), 131.1 (CH), 131.4 (C), 131.9 (CH), 143.5 (C), 149.6 (C), 155.6 (CO), 167.6 (CO). IS-MS *m/z*: 410 (M<sup>+</sup>+1). *Anal.* Calcd for C<sub>24</sub>H<sub>27</sub>NO<sub>5</sub>: C, 70.40; H, 6.65; N, 3.42. Found: C, 70.12; H, 6.77; N, 3.40.

**2-{2-[5-Methoxy-1-(phenylsulfonyl)-1*H*-3-indolyl]ethyl}benzoic Acid (8a)** A solution of ester **7a** (500 mg, 1.1 mmol) in MeOH (95% (20 ml) and potassium hydroxide (997 mg, 17.6 mmol) was stirred at reflux for 60 h. The solvent was removed *in vacuo*, water (20 ml) was added to the residue and the pH was adjusted to 1 by careful addition of 10% aqueous HCl. After extraction with CH<sub>2</sub>Cl<sub>2</sub> (2×20 ml), the organic layer was dried over anhydrous MgSO<sub>4</sub> and evaporated. The residue was separated by column chromatography (eluent CH<sub>2</sub>Cl<sub>2</sub>) to give **8a** (315 mg, 65%) as crystals. mp 127–129 °C (MeOH). IR (KBr) cm<sup>-1</sup>: 3300–2400 (OH), 1700 (CO). <sup>1</sup>H-NMR (250 MHz, CDCl<sub>3</sub>+D<sub>2</sub>O) δ: 2.96 (2H, t, *J*=7.5 Hz, CH<sub>2</sub>), 3.37 (2H, t, *J*=7.5 Hz, CH<sub>2</sub>), 3.79 (3H, s, CH<sub>3</sub>), 6.89 (1H, dd, *J*=2.5, 9.0 Hz, H<sub>6</sub>), 6.95 (1H, d, *J*=2.5 Hz, H<sub>4</sub>), 7.12 (1H, d, *J*=8.0 Hz, H<sub>Ar</sub>), 7.25–7.51 (6H, m, H<sub>2</sub>+H<sub>Ar</sub>), 7.79 (2H, d, *J*=8.0 Hz, H<sub>Ar</sub>), 7.87 (1H, d, *J*=9.0 Hz, H<sub>7</sub>), 8.06 (1H, dd, *J*=1.0, 8.0 Hz, H<sub>Ar</sub>). <sup>13</sup>C-NMR (62.90 MHz, CDCl<sub>3</sub>) δ: 27.0 (CH<sub>2</sub>), 34.4 (CH<sub>2</sub>), 55.6 (CH<sub>3</sub>), 102.1 (CH), 113.4 (CH), 114.6 (CH), 123.0 (C), 123.8 (CH), 126.4 (CH), 126.6 (2 CH), 128.0 (C), 129.1 (2CH), 129.9 (C), 131.5 (CH), 131.8 (CH), 132.1 (C), 132.9 (CH), 133.5 (CH), 138.2 (C), 144.2 (C), 156.3 (C), 172.2 (CO). IS-MS *m/z*: 436 (M<sup>+</sup>+1). *Anal.* Calcd for C<sub>24</sub>H<sub>21</sub>NO<sub>5</sub>S: C, 66.19; H, 4.86; N, 3.22. Found: C, 65.93; H, 4.97; N, 3.36.

**2-[2-(5-Methoxy-1*H*-3-indolyl)ethyl]benzoic Acid (8b)** Following the same methodology, compound **8b** was obtained from **7b** in 84% yield as crystals. mp 143–145 °C (MeOH). IR (KBr) cm<sup>-1</sup>: 3403 (NH), 3300–2400 (OH), 1708 (CO). <sup>1</sup>H-NMR (250 MHz, CDCl<sub>3</sub>+D<sub>2</sub>O) δ: 3.05 (2H, t, *J*=8.0 Hz, CH<sub>2</sub>), 3.43 (2H, t, *J*=8.0 Hz, CH<sub>2</sub>), 3.86 (3H, s, CH<sub>3</sub>), 6.85 (1H, dd, *J*=2.5, 9.0 Hz, H<sub>6</sub>), 6.89 (1H, d, *J*=3.5 Hz, H<sub>2</sub>), 7.11 (1H, d, *J*=2.5 Hz, H<sub>4</sub>), 7.22 (1H, d, *J*=9.0 Hz, H<sub>7</sub>), 7.31 (2H, t, *J*=8.0 Hz, H<sub>Ar</sub>), 7.47 (1H, t, *J*=8.0 Hz, H<sub>Ar</sub>), 7.71 (1H, brs, NH), 8.06 (1H, brd, *J*=8.1 Hz, H<sub>Ar</sub>). <sup>13</sup>C-NMR (62.90 MHz, CDCl<sub>3</sub>) δ: 27.5 (CH<sub>2</sub>), 35.1 (CH<sub>2</sub>), 55.8 (CH<sub>3</sub>), 100.8 (CH), 111.6 (CH), 112.0 (CH), 115.8 (C), 122.2 (CH), 125.8 (CH), 127.8 (C), 129.6 (C), 130.4 (CH), 131.1 (CH), 131.4 (C), 131.8 (C), 144.0 (C), 153.7 (C), 168.1 (CO). IS-MS *m/z*: 296 (M<sup>+</sup>+1). *Anal.* Calcd for C<sub>18</sub>H<sub>17</sub>NO<sub>5</sub>: C, 73.20; H, 5.80; N, 4.74. Found: C, 73.53; H, 5.91; N, 4.63.

**2-Methoxy-5,6,11,12-tetrahydrobenzo[5,6]cyclohepta[b]indol-6-one (9)** Finely powdered **8b** (300 mg, 1.02 mmol) was added to polyphosphoric acid (PPA, 1.7 g) and phosphorus pentoxide (233 mg) with stirring at 90 °C. After the addition was complete, the mixture was stirred at 90 °C for 1 h, then cooled, ice was added, and the mixture was neutralised with saturated aqueous NaHCO<sub>3</sub>, and extracted with CH<sub>2</sub>Cl<sub>2</sub> (2×15 ml). The organic layer was dried over anhydrous MgSO<sub>4</sub> and evaporated. The crude residue was purified by column chromatography (eluent CH<sub>2</sub>Cl<sub>2</sub>) to afford **9** (184 mg, 65%) as crystals. mp 171–173 °C (EtOAc–petroleum ether). IR (KBr) cm<sup>-1</sup>: 3314 (NH), 1613 (CO). <sup>1</sup>H-NMR (250 MHz, CDCl<sub>3</sub>) δ: 3.15–3.21 (2H, m, CH<sub>2</sub>), 3.26–3.30 (2H, m, CH<sub>2</sub>), 3.88 (3H, s, CH<sub>3</sub>), 7.00–7.07 (2H, m, H<sub>Ar</sub>), 7.31–7.52 (4H, m, H<sub>Ar</sub>), 8.13 (1H, dd, *J*=1.6, 8.2 Hz, H<sub>Ar</sub>), 9.33

(1H, brs, NH). <sup>13</sup>C-NMR (62.90 MHz, CDCl<sub>3</sub>) δ: 24.6 (CH<sub>2</sub>), 36.2 (CH<sub>2</sub>), 55.9 (CH<sub>3</sub>), 101.2 (CH), 113.3 (CH), 118.8 (CH), 125.7 (C), 127.1 (CH), 127.5 (C), 130.3 (2CH), 132.5 (C), 132.7 (CH), 133.9 (C), 137.5 (C), 141.2 (C), 154.5 (C), 184.3 (CO). IS-MS *m/z*: 278 (M<sup>+</sup>+1). *Anal.* Calcd for C<sub>18</sub>H<sub>15</sub>NO<sub>2</sub>: C, 77.96; H, 5.45; N, 5.05. Found: C, 78.24; H, 5.41; N, 4.87.

**2-Methoxy-3-phenylsulfonyl-5,6,11,12-tetrahydrobenzo[5,6]cyclohepta[b]indol-6-one (10)** Following the same methodology, compound **8a** afforded **9** and **10**, respectively, in 50–60% and 20–30% yields. Compound **10**: mp 231–233 °C (MeOH); IR (KBr) cm<sup>-1</sup>: 3310 (NH), 1611 (CO). <sup>1</sup>H-NMR (250 MHz, CDCl<sub>3</sub>) δ: 3.06–3.23 (4H, m, CH<sub>2</sub>), 3.69 (3H, s, CH<sub>3</sub>), 7.32 (1H, s, H<sub>Ar</sub>), 7.40–7.46 (2H, m, H<sub>Ar</sub>), 7.52–7.66 (4H, m, H<sub>Ar</sub>), 7.89 (2H, d, *J*=7.2 Hz, H<sub>Ar</sub>), 7.95 (1H, d, *J*=7.5 Hz, H<sub>Ar</sub>), 8.24 (1H, s, H<sub>Ar</sub>), 12.03 (1H, brs, NH). <sup>13</sup>C-NMR (62.90 MHz, DMSO-*d*<sub>6</sub>) δ: 23.8 (CH<sub>2</sub>), 34.9 (CH<sub>2</sub>), 56.2 (CH<sub>3</sub>), 103.4 (CH), 114.6 (CH), 123.9 (C), 127.0 (CH), 127.8 (2 CH), 128.3 (CH), 128.9 (2 CH), 129.7 (CH), 130.4 (CH), 130.5 (C), 130.7 (C), 133.0 (CH), 133.2 (CH), 136.2 (C), 137.0 (C), 141.1 (C), 141.3 (C), 150.2 (C), 184.0 (CO). IS-MS *m/z*: 418 (M<sup>+</sup>+1). *Anal.* Calcd for C<sub>24</sub>H<sub>19</sub>NO<sub>4</sub>S: C, 69.05; H, 4.59; N, 3.36. Found: C, 68.79; H, 4.40; N, 3.19.

**5-[3-(Dimethylamino)ethyl]-5,6,11,12-tetrahydrobenzo[5,6]cyclohepta[b]indol-6-one (11)** To a solution of **2** (200 mg, 0.81 mmol) in anhydrous DMF (10 ml) was added portionwise sodium hydride (30 mg, 1.50 mmol, 60% oil dispersion) at 0 °C. The mixture was stirred for 15 min at 0 °C, then 2-(dimethylamino)ethyl chloride (0.18 ml, 1.6 mmol) diluted in anhydrous DMF (5 ml) was added and the solution was stirred at 90 °C overnight. After cooling, the solvent was removed *in vacuo* and the residue was partitioned between water and CH<sub>2</sub>Cl<sub>2</sub> and extracted. The organic layer was dried over anhydrous MgSO<sub>4</sub> and concentrated *in vacuo*. The crude residue was purified by column chromatography (eluent CH<sub>2</sub>Cl<sub>2</sub>/MeOH 98:2) to give **11** (188 mg, 73%) as yellow crystals. mp 96–97 °C (CH<sub>2</sub>Cl<sub>2</sub>–petroleum ether). IR (KBr) cm<sup>-1</sup>: 1621 (CO). <sup>1</sup>H-NMR (250 MHz, CDCl<sub>3</sub>) δ: 2.41 (6H, s, CH<sub>3</sub>), 2.71 (2H, t, *J*=7.2 Hz, CH<sub>2</sub>), 3.18–3.27 (4H, m, CH<sub>2</sub>), 4.75 (2H, t, *J*=7.2 Hz, CH<sub>2</sub>), 7.15 (1H, t, *J*=8.0 Hz, H<sub>Ar</sub>), 7.26–7.48 (5H, m, H<sub>Ar</sub>), 7.66 (1H, d, *J*=8.0 Hz, H<sub>Ar</sub>), 7.92 (1H, dd, *J*=1.6, 8.0 Hz, H<sub>Ar</sub>). <sup>13</sup>C-NMR (62.90 MHz, CDCl<sub>3</sub>) δ: 25.5 (CH<sub>2</sub>), 35.2 (CH<sub>2</sub>), 44.0 (CH<sub>2</sub>), 46.0 (2 CH<sub>3</sub>), 59.2 (CH<sub>2</sub>), 110.5 (CH), 120.4 (CH), 121.1 (CH), 126.2 (C), 126.8 (CH), 127.0 (CH), 127.4 (C), 129.1 (CH), 129.9 (CH), 131.8 (CH), 132.4 (C), 139.3 (C), 139.7 (C), 140.6 (C), 187.4 (CO). IS-MS *m/z*: 319 (M<sup>+</sup>+1). *Anal.* Calcd for C<sub>21</sub>H<sub>22</sub>N<sub>2</sub>O: C, 79.21; H, 6.96; N, 8.80. Found: C, 78.89; H, 7.07; N, 8.97.

**5-[3-(Dimethylamino)propyl]-5,6,11,12-tetrahydrobenzo[5,6]cyclohepta[b]indol-6-one (12)** Following the same methodology, compound **12** was obtained from **2** with 2-(dimethylamino)propyl chloride in 65% yield as an oil. IR (film) cm<sup>-1</sup>: 1605 (CO). <sup>1</sup>H-NMR (250 MHz, CDCl<sub>3</sub>) δ: 1.99–2.10 (2H, m, CH<sub>2</sub>), 2.31 (6H, s, CH<sub>3</sub>), 2.45 (2H, t, *J*=7.0 Hz, CH<sub>2</sub>), 3.17–3.27 (4H, m, CH<sub>2</sub>), 4.66 (2H, t, *J*=7.0 Hz, CH<sub>2</sub>), 7.12 (1H, t, *J*=8.0 Hz, H<sub>Ar</sub>), 7.23–7.47 (5H, m, H<sub>Ar</sub>), 7.65 (1H, brd, *J*=8.0 Hz, H<sub>Ar</sub>), 7.89 (1H, dd, *J*=1.5, 8.0 Hz, H<sub>Ar</sub>). <sup>13</sup>C-NMR (62.90 MHz, CDCl<sub>3</sub>) δ: 24.5 (CH<sub>2</sub>), 28.5 (CH<sub>2</sub>), 35.0 (CH<sub>2</sub>), 43.7 (CH<sub>2</sub>), 45.2 (2CH<sub>3</sub>), 56.8 (CH<sub>2</sub>), 110.5 (CH), 120.1 (CH), 120.9 (CH), 125.9 (C), 126.6 (CH), 126.9 (CH), 127.4 (C), 129.9 (CH), 129.8 (CH), 131.6 (CH), 132.2 (C), 139.2 (C), 139.5 (C), 140.5 (C), 187.4 (CO). IS-MS *m/z*: 333 (M<sup>+</sup>+1). *Anal.* Calcd for C<sub>22</sub>H<sub>24</sub>N<sub>2</sub>O: C, 79.48; H, 7.28; N, 8.43. Found: C, 79.75; H, 7.35; N, 8.32.

**5-[3-(Dimethylamino)ethyl]-5,6-dihydrobenzo[5,6]cyclohepta[b]indol-6-one (13)** Following the same methodology, compound **13** was obtained from **3** with 2-(dimethylamino)ethyl chloride in 70% yield. mp 88–89 °C (CH<sub>2</sub>Cl<sub>2</sub>–petroleum ether). IR (KBr) cm<sup>-1</sup>: 1635 (CO). <sup>1</sup>H-NMR (250 MHz, CDCl<sub>3</sub>) δ: 2.40 (6H, s, CH<sub>3</sub>), 2.81 (2H, t, *J*=7.2 Hz, CH<sub>2</sub>), 4.96 (2H, t, *J*=7.2 Hz, CH<sub>2</sub>), 7.26 (1H, d, *J*=12.0 Hz, =CH), 7.34 (1H, t, *J*=8.0 Hz, H<sub>Ar</sub>), 7.51–7.77 (6H, m, =CH+H<sub>Ar</sub>), 8.07 (1H, d, *J*=8.0 Hz, H<sub>Ar</sub>), 8.76 (1H, dd, *J*=1.6, 8.0 Hz, H<sub>Ar</sub>). <sup>13</sup>C-NMR (62.90 MHz, CDCl<sub>3</sub>) δ: 44.1 (CH<sub>2</sub>), 46.0 (2CH<sub>3</sub>), 59.3 (CH<sub>2</sub>), 110.6 (CH), 120.9 (CH), 121.3 (CH), 121.5 (CH), 122.0 (C), 125.1 (C), 127.3 (CH), 128.3 (CH), 129.2 (CH), 130.9 (CH), 131.5 (CH), 133.0 (CH), 136.1 (C), 136.2 (C), 137.6 (C), 139.8 (C), 181.3 (CO). IS-MS *m/z*: 317 (M<sup>+</sup>+1). *Anal.* Calcd for C<sub>21</sub>H<sub>20</sub>N<sub>2</sub>O: C, 79.72; H, 6.37; N, 8.85. Found: C, 79.87; H, 6.18; N, 8.72.

**5-[3-(Dimethylamino)propyl]-5,6-dihydrobenzo[5,6]cyclohepta[b]indol-6-one (14)** Following the same methodology, compound **14** was obtained from **3** with 2-(dimethylamino)propyl chloride in 60% yield. mp 102–103 °C (CH<sub>2</sub>Cl<sub>2</sub>–MeOH). IR (KBr) cm<sup>-1</sup>: 1605 (CO). <sup>1</sup>H-NMR (250 MHz, CDCl<sub>3</sub>) δ: 2.27–2.39 (2H, m, CH<sub>2</sub>), 2.49 (6H, s, CH<sub>3</sub>), 2.78 (2H, t, *J*=7.0 Hz, CH<sub>2</sub>), 4.91 (2H, t, *J*=7.0 Hz, CH<sub>2</sub>), 7.30 (1H, d, *J*=11.5 Hz, =CH), 7.35 (1H, t, *J*=8.0 Hz, H<sub>Ar</sub>), 7.53–7.79 (6H, m, =CH+H<sub>Ar</sub>), 8.08 (1H, d, *J*=8.0 Hz, H<sub>Ar</sub>), 7.89 (1H, d, *J*=8.0 Hz, H<sub>Ar</sub>). <sup>13</sup>C-NMR (62.90 MHz, CDCl<sub>3</sub>) δ: 28.5 (CH<sub>2</sub>), 43.9 (CH<sub>2</sub>), 45.2 (2 CH<sub>3</sub>), 56.7 (CH<sub>2</sub>), 110.8 (CH),

121.2 (CH), 121.5 (2CH), 122.1 (C), 124.9 (C), 127.2 (CH), 128.2 (CH), 129.0 (CH), 130.8 (CH), 131.4 (CH), 132.8 (CH), 135.9 (C), 136.0 (C), 137.5 (C), 139.7 (C), 181.3 (CO). IS-MS  $m/z$ : 331 ( $M^+ + 1$ ). *Anal.* Calcd for  $C_{22}H_{22}N_2O$ : C, 79.97; H, 6.71; N, 8.48. Found: C, 80.33; H, 6.66; N, 8.57.

**5-[3-(Bromopropyl)-5,6-dihydrobenzo[5,6]cyclohepta[b]indol-6-one (15)** To a solution of **3** (200 mg, 0.81 mmol) and KOH (100 mg, 1.78 mmol) in anhydrous DMF (10 ml) was added a solution of 1,3-dibromopropane (0.51 ml, 2.56 mmol) diluted in anhydrous DMF (2 ml) at 0 °C. The mixture was stirred at room temperature overnight. After cooling, the solvent was removed *in vacuo*. The residue was partitioned between water (10 ml) and EtOAc (10 ml) and extracted. The organic layer was dried over anhydrous  $MgSO_4$  and concentrated *in vacuo*, and the crude residue was purified by column chromatography (eluent petroleum ether/ $CH_2Cl_2$  2:8) to give **15** (130 mg, 87%) as yellow crystals. mp 150–152 °C (EtOAc–petroleum ether). IR (KBr)  $cm^{-1}$ : 1603 (CO).  $^1H$ -NMR (250 MHz,  $CDCl_3$ )  $\delta$ : 2.50–2.59 (2H, m,  $CH_2$ ), 3.50 (2H, t,  $J=7.0$  Hz,  $CH_2$ ), 4.99 (2H, t,  $J=7.0$  Hz,  $CH_2$ ), 7.28 (1H, d,  $J=11.8$  Hz, =CH), 7.36 (1H, t,  $J=8.0$  Hz,  $H_{Ar}$ ), 7.54–7.78 (6H, m, =CH+ $H_{Ar}$ ), 8.10 (1H, d,  $J=8.0$  Hz,  $H_{Ar}$ ), 8.76 (1H, d,  $J=8.0$  Hz,  $H_{Ar}$ ).  $^{13}C$ -NMR (62.90 MHz,  $CDCl_3$ )  $\delta$ : 31.2 ( $CH_2$ ), 34.0 ( $CH_2$ ), 44.6 ( $CH_2$ ), 110.7 (CH), 121.0 (CH), 121.5 (2CH), 122.5 (C), 125.9 (C), 127.5 (CH), 128.4 (CH), 129.4 (CH), 130.9 (CH), 131.6 (CH), 133.2 (CH), 135.9 (C), 136.2 (C), 137.4 (C), 139.8 (C), 181.1 (CO); IS-MS  $m/z$ : 366 ( $M^+ + 1$ ,  $^{79}Br$ ), 368 ( $M^+ + 1$ ,  $^{81}Br$ ). *Anal.* Calcd for  $C_{20}H_{16}BrNO$ : C, 65.59; H, 4.40; N, 3.82. Found: C, 65.88; H, 4.23; N, 3.63.

**5-[2-[(2-Hydroxyethyl)amino]ethyl]-5,6-dihydrobenzo[5,6]-cyclohepta[b]indol-6-one (16)** A solution of **15** (190 mg, 0.52 mmol) and 2-aminoethanol (0.08 ml, 1.32 mmol) in acetonitrile (7 ml) was stirred at room temperature for 72 h. After evaporation, the crude residue was purified by column chromatography (eluent  $CH_2Cl_2$ /MeOH/ $NH_4OH$  100:10:1) to give **16** (83 mg, 46%) as yellow crystals. mp 114–115 °C ( $CH_2Cl_2$ –MeOH). IR (KBr)  $cm^{-1}$ : 3014 (NH), 1606 (CO).  $^1H$ -NMR (250 MHz,  $CDCl_3$ )  $\delta$ : 2.07–2.18 (2H, m,  $CH_2$ ), 2.73 (2H, t,  $J=7.0$  Hz,  $CH_2$ ), 2.77 (2H, t,  $J=5.1$  Hz,  $CH_2$ ), 2.82 (2H, brs, OH+NH), 3.66 (2H, t,  $J=5.1$  Hz,  $CH_2$ ), 4.88 (2H, t,  $J=7.0$  Hz,  $CH_2$ ), 7.23 (1H, d,  $J=11.6$  Hz, =CH), 7.36 (1H, brt,  $J=8.0$  Hz,  $H_{Ar}$ ), 7.48–7.74 (6H, m, =CH+ $H_{Ar}$ ), 8.04 (1H, d,  $J=8.0$  Hz,  $H_{Ar}$ ), 8.72 (1H, dd,  $J=1.7$ , 8.0 Hz,  $H_{Ar}$ ).  $^{13}C$ -NMR (62.90 MHz,  $CDCl_3$ )  $\delta$ : 30.5 ( $CH_2$ ), 43.7 ( $CH_2$ ), 46.5 ( $CH_2$ ), 51.2 ( $CH_2$ ), 60.4 ( $CH_2$ ), 110.7 (CH), 120.9 (CH), 121.4 (CH), 121.5 (CH), 122.5 (C), 125.0 (C), 127.5 (CH), 128.4 (CH), 129.3 (CH), 130.8 (CH), 131.6 (CH), 133.0 (CH), 136.0 (C), 136.1 (C), 137.2 (C), 139.7 (C), 181.2 (CO). IS-MS  $m/z$ : 347 ( $M^+ + 1$ ). *Anal.* Calcd for  $C_{22}H_{22}N_2O_2$ : C, 76.28; H, 6.40; N, 8.09. Found: C, 75.96; H, 6.28; N, 8.23.

**5-[4-(6-Oxo-5,6-dihydrobenzo[5,6]cyclohepta[b]indol-5-yl)hexyl]-5,6-dihydrobenzo[5,6]cyclohepta[b]indol-6-one (17)** To a solution of **3** (200 mg, 0.82 mmol) and KOH (50 mg, 0.89 mmol) in anhydrous DMF (10 ml) was added a solution of 1,6-dibromohexane (0.06 ml, 0.41 mmol) diluted in anhydrous DMF (1 ml) at 0 °C. The mixture was stirred at room temperature overnight. The solvent was removed *in vacuo*. The residue was partitioned between water (10 ml) and EtOAc (10 ml) and extracted. The organic layer was dried over anhydrous  $MgSO_4$  and concentrated *in vacuo*. The crude residue was purified by column chromatography (eluent petroleum ether/ $CH_2Cl_2$  2:8) to give **17** (104 mg, 60%) as yellow crystals. mp 219–220 °C (MeOH). IR (KBr)  $cm^{-1}$ : 1617 (CO).  $^1H$ -NMR (250 MHz,  $DMSO-d_6$ )  $\delta$ : 1.48–1.58 (4H, m,  $CH_2$ ), 1.85–2.00 (4H, m,  $CH_2$ ), 4.87 (4H, t,  $J=7.0$  Hz,  $CH_2$ ), 7.28 (2H, d,  $J=10.5$  Hz, =CH), 7.32–7.37 (2H, m,  $H_{Ar}$ ), 7.51–7.55 (4H, m,  $H_{Ar}$ ), 7.63 (2H, t,  $J=8.0$  Hz,  $H_{Ar}$ ), 7.69–7.79 (6H, m, =CH+ $H_{Ar}$ ), 8.10 (2H, d,  $J=8.0$  Hz,  $H_{Ar}$ ), 8.76 (2H, d,  $J=8.0$  Hz,  $H_{Ar}$ ). The very low solubility of compound **17** did not allow us to perform  $^{13}C$ -NMR. IS-MS  $m/z$ : 573 ( $M^+ + 1$ ). *Anal.* Calcd for  $C_{40}H_{32}N_2O_2$ : C, 83.89; H, 5.63; N, 4.89. Found: C, 84.21; H, 6.78; N, 4.75.

**2-Methoxy-5,6-dihydrobenzo[5,6]cyclohepta[b]indol-6-one (18)** A solution of **9** (500 mg, 2.02 mmol) and DDQ (1.38 g, 6.08 mmol) in anhydrous dioxane (20 ml) was stirred under argon at reflux overnight. After cooling, the solution was diluted with  $CH_2Cl_2$  (10 ml), then washed successively with 10% aqueous NaOH two times and water once. The organic layer was dried over anhydrous  $MgSO_4$  and evaporated. The crude residue was purified by column chromatography (eluent  $CH_2Cl_2$ ) to afford **18** (446 mg, 90%) as yellow crystals. mp >245 °C (EtOAc); IR (KBr)  $cm^{-1}$ : 3245 (NH), 1583 (CO).  $^1H$ -NMR (250 MHz,  $DMSO-d_6$ )  $\delta$ : 3.89 (3H, s,  $CH_3$ ), 7.17 (1H, dd,  $J=2.2$ , 9.0 Hz,  $H_{Ar}$ ), 7.46 (1H, d,  $J=11.6$  Hz, =CH), 7.58 (1H, d,  $J=9.0$  Hz,  $H_{Ar}$ ), 7.71–7.90 (3H, m,  $H_{Ar}$ ), 8.01 (1H, d,  $J=11.6$  Hz, =CH), 8.04 (1H, d,  $J=8.2$  Hz,  $H_{Ar}$ ), 8.87 (1H, dd,  $J=1.2$ , 8.2 Hz,  $H_{Ar}$ ), 12.37 (1H, brs, NH).  $^{13}C$ -NMR (62.90 MHz,  $CDCl_3$ )  $\delta$ : 55.6 ( $CH_3$ ), 101.7 (CH), 113.8 (CH), 118.6 (CH), 119.8 (C), 123.0 (CH), 126.0 (2C), 128.2 (CH), 130.1 (CH), 132.2 (CH), 133.0 (C), 133.9 (C), 134.4 (CH), 136.8 (C), 137.3 (C),

154.7 (C), 177.1 (CO). IS-MS  $m/z$ : 276 ( $M^+ + 1$ ). *Anal.* Calcd for  $C_{18}H_{13}NO_2$ : C, 78.53; H, 4.76; N, 5.09. Found: C, 78.15; H, 4.94; N, 4.96.

**5-[3-(Dimethylamino)ethyl]-2-methoxy-5,6-dihydrobenzo[5,6]cyclohepta[b]indol-6-one (19)** Following the methodology used for the preparation of **11**, compound **19** was obtained from **18** with 2-(dimethylamino)-ethyl chloride in 65% yield. mp 90–91 °C (petroleum ether); IR (KBr)  $cm^{-1}$ : 1619 (CO).  $^1H$ -NMR (250 MHz,  $CDCl_3$ )  $\delta$ : 2.36 (6H, s,  $CH_3$ ), 2.75 (2H, t,  $J=7.5$  Hz,  $CH_2$ ), 3.87 (3H, s,  $CH_3$ ), 4.81 (2H, t,  $J=7.5$  Hz,  $CH_2$ ), 7.06 (1H, d,  $J=11.5$  Hz, =CH), 7.14 (1H, dd,  $J=2.2$ , 9.0 Hz,  $H_{Ar}$ ), 7.30 (1H, d,  $J=2.2$  Hz,  $H_{Ar}$ ), 7.41 (1H, d,  $J=9.0$  Hz,  $H_{Ar}$ ), 7.47 (1H, d,  $J=11.5$  Hz, =CH), 7.52–7.64 (3H, m,  $H_{Ar}$ ), 8.71 (1H, dd,  $J=1.6$ , 7.9 Hz,  $H_{Ar}$ ).  $^{13}C$ -NMR (62.90 MHz,  $CDCl_3$ )  $\delta$ : 44.3 ( $CH_2$ ), 46.0 (2  $CH_3$ ), 56.0 ( $CH_2$ ), 59.5 ( $CH_3$ ), 101.3 (CH), 111.7 (CH), 118.6 (CH), 121.7 (2 CH), 125.4 (Cq), 128.2 (CH), 128.5 (CH), 130.9 (CH), 131.5 (CH), 132.9 (CH), 135.2 (C), 136.2 (C), 136.4 (C), 137.4 (C), 155.3 (C), 181.2 (CO). IS-MS  $m/z$ : 347 ( $M^+ + 1$ ). *Anal.* Calcd for  $C_{22}H_{22}N_2O_2$ : C, 76.28; H, 6.40; N, 8.09. Found: C, 76.55; H, 6.45; N, 8.06.

**5-[3-(Dimethylamino)propyl]-2-methoxy-5,6-dihydrobenzo[5,6]cyclohepta[b]indol-6-one (20)** Following the methodology used for the preparation of **11**, compound **20** was obtained from **18** with 2-(dimethylamino)-propyl chloride in 60% yield as an oil. IR (film)  $cm^{-1}$ : 1619 (CO).  $^1H$ -NMR (250 MHz,  $CDCl_3$ )  $\delta$ : 2.03–2.15 (2H, m,  $CH_2$ ), 2.26 (6H, s,  $CH_3$ ), 2.40 (2H, t,  $J=7.2$  Hz,  $CH_2$ ), 3.92 (3H, s,  $CH_3$ ), 4.84 (2H, t,  $J=7.2$  Hz,  $CH_2$ ), 7.17 (1H, d,  $J=11.6$  Hz, =CH), 7.17 (1H, dd,  $J=2.2$ , 9.0 Hz,  $H_{Ar}$ ), 7.40 (1H, d,  $J=2.2$  Hz,  $H_{Ar}$ ), 7.52 (1H, d,  $J=9.0$  Hz,  $H_{Ar}$ ), 7.58–7.72 (4H, m, =CH+ $H_{Ar}$ ), 8.73 (1H, dd,  $J=1.6$ , 7.8 Hz,  $H_{Ar}$ ).  $^{13}C$ -NMR (62.90 MHz,  $CDCl_3$ )  $\delta$ : 28.8 ( $CH_2$ ), 44.1 ( $CH_2$ ), 45.3 (2 $CH_3$ ), 55.9 ( $CH_2$ ), 56.7 ( $CH_3$ ), 101.0 (CH), 111.9 (CH), 118.5 (CH), 121.6 (2CH), 125.2 (C), 128.0 (CH), 128.3 (CH), 130.8 (CH), 131.4 (CH), 132.9 (CH), 135.2 (C), 136.1 (C), 136.2 (C), 137.3 (C), 155.2 (C), 181.1 (CO). IS-MS  $m/z$ : 361 ( $M^+ + 1$ ). *Anal.* Calcd for  $C_{23}H_{24}N_2O_2$ : C, 76.64; H, 6.71; N, 7.77. Found: C, 76.37; H, 6.91; N, 7.89.

**Antiproliferative Activity** L1210 Murine leukemia cells provided by the NCI (Frederick, MD U.S.A.) were cultivated in RPMI 1640 medium (Gibco) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 units/ml penicillin, 100 mg/ml streptomycin and 10 mM HEPES buffer (pH=7.4). The cytotoxicity was measured by microculture tetrazolium assay as described in ref. 11. Cells were exposed for 48 h to graded concentrations in triplicate of the test drug compounds. Results are expressed as  $IC_{50}$  (mean,  $n=3$ ), which is defined as the drug concentration inhibiting the absorbance by 50% with respect to that of untreated cells.

**Flow Cytometric Analysis** L1210 cells ( $2.5 \times 10^5$  cells/ml) were incubated for 21 h with various concentrations of the compounds, then fixed by 70% ethanol (v/v), washed and incubated in phosphate buffer saline (PBS) containing 100 mg/ml RNase and 24 mg/ml propidium iodide for 30 min at 20 °C. For each sample,  $1 \times 10^4$  cells were analysed on an ATC3000 flow cytometer (Bruker) using an argon laser (Spectra-Physics) emitting 400 mW at 488 nm. The fluorescence of propidium iodide was collected through a 615 nm long-pass filter. Data are displayed as the percentage of cells found in the  $G_2 + M$  phase of the cell cycle.

**Acknowledgments** The authors gratefully acknowledge the technical assistance of Mrs Cyril Rousseau and Franck Amblard.

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## Development of Spherical Crystals of Acetylsalicylic Acid for Direct Tablet-making

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Received May 23, 2000; accepted August 8, 2000

**The production of spherical crystals has recently gained great attention due to the fact that the crystal habit (form, surface, size, etc.) can be modified during the crystallization process. Spherical crystals of ASA were developed by non-typical and typical spherical crystallization techniques. The non-typical spherical crystallization process (conventional stirred tank method) resulted in few monocrystals and non-spherical crystal agglomerates. The typical spherical crystallization process was carried out by the three solvent-system (ethanol–water–carbon tetrachloride). The products were qualified by morphological study, NIR investigation, salicylic acid content, dissolution rate, studies on flowability, compactibility, cohesivity and tablettability. The results demonstrate that only typical spherical crystallization can be recommended for the production of spherical crystals of ASA. Only product made by this technique shows excellent flow properties and favourable compactibility, cohesiveness and tablettability values.**

**Key words** acetylsalicylic acid; spherical crystallization; flowability; roundness; diffuse reflectance

Direct compression is the most efficient process used in tablet manufacturing because it is the fastest, simplest and least expensive tablet-compression procedure.<sup>1)</sup> However, although this technique seems quite simple, it requires that different properties be observed simultaneously:

- good flowability of the materials,
- a suitable bulk density of the powder, in order that the correct amount of drug may be fed into a die cavity,
- appropriate particle size distribution, and
- good compressibility of the powder.

Many drug crystals do not exhibit these properties: they have poor flowability and compressibility.<sup>2)</sup> In tablet making from these materials, possible solutions may be as follows:

- the use of wet granulation (if this is possible with regard to the drug stability),
- the use of direct tablet making with a good excipient, or
- the use of direct pressing with spherical crystals or crystal agglomerates of drug material with good flowability and compactibility properties.

The production of spherical crystals has recently gained great attention and importance, due to the fact that the crystal habit (form, surface, size and particle size distribution) can be modified during the crystallization process. In consequence of such modifications in the crystal habit, certain parameters of materials can also be changed: bulk density, flow property, compactibility, dissolution rate, stability,<sup>3)</sup> etc.

“Spherical crystallization” was defined by Kawashima as “an agglomeration technique that transforms crystals directly into a compacted spherical form during the crystallization process.”<sup>4)</sup> A few methods are to be found in the literature. The typical spherical crystallization process employs three solvents: one is the substance dissolution medium, another is a medium which partially dissolves the substance, and the third is the wetting solvent for the substance. Of course, the traditional crystallization processes (salting-out precipitation,

cooling crystallization, crystallization from the melting, etc.) can also be used to produce spherical crystal agglomerates.<sup>5)</sup> The traditional crystallization process is carried out by controlling the physical and chemical properties and can be called the non-typical spherical crystallization process.

Many drugs which do not have suitable technological properties have been transformed successfully into spherical agglomerates with good flowability and compressibility during manufacturing (salicylic acid, tolbutamide, dibasic calcium phosphate anhydrous, norfloxacin, DL-methionine, phenytoin, ferrous sulfate, etc.).<sup>6–13)</sup> Other materials are currently awaiting such transformation.

A few years ago Aoki *et al.* (Japan) and Deshpande *et al.* (India) were employed in making spherical ASA crystals from a mixture of heptane–pentane–methanol<sup>14)</sup> and a mixture of methanol–chloroform–acid buffer.<sup>15)</sup> The commercial ASA crystals are tetragonal and prism-shaped, with different sizes and particle size distribution. Their flow property is poor due to the crystal habit and the electrostatic charge. Since ASA crystals can be compressed to tablets (e.g., effervescent or chewable) only *via* direct tablet-making methods (stability!), good flowability, cohesivity and tablettability of the crystals are very important.

Therefore in the work reported here, ASA crystals were developed by means of non-typical and typical spherical crystallization processes by different solvent mixtures.

### Experimental

**Development of Spherical ASA Crystals** Three different samples were produced by variation of the experimental conditions. The experiments were carried out in a mechanically stirred tank with a volume of 500 ml. A Julabo thermostat with computer control was used for the cooling process. A non-typical spherical crystallization process was used for the samples ASA 1 and ASA 2. ASA 3 was developed, however, by the typical spherical crystallization procedure.

ASA 1: 160 g of ASA was dissolved in 500 ml of ethanol (40% v/v) at 60 °C, followed by cooling at 1 °C/min to 20 °C with stirring (200 rpm).

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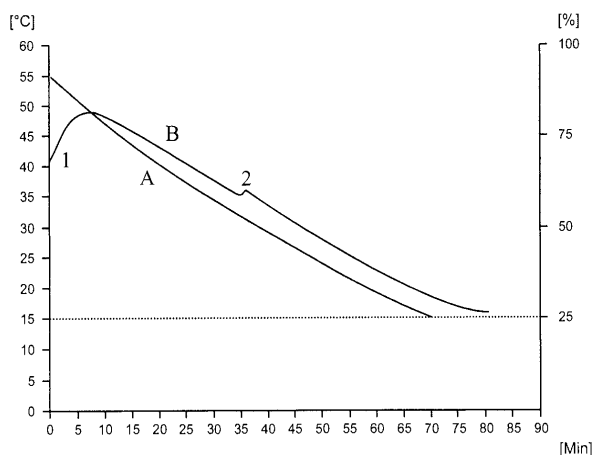


Fig. 1. Cooling Curves of Crystallization Process of ASA3

A, cooling curve of equipment; B, cooling curve of solution; 1, dissolution of ASA crystals with addition of a carbon tetrachloride–water mixture (4% w/v) to the solution; 2, appearance of ASA crystals.

ASA 2: 160 g of ASA was dissolved in 500 ml of ethanol (40% v/v) at 60 °C, followed by cooling at 0.5 °C/min to 20 °C with stirring (200 rpm).

ASA 3: 121.6 g of ASA was dissolved in 225 ml of ethanol (40% v/v) at 50 °C, and a carbon tetrachloride–water mixture (4% w/v) was added to the solution, followed by cooling at 0.6 °C/min to 20 °C with stirring (200 rpm). The ASA 3 crystallization process can be followed in Fig. 1.

**Particle Size Analysis** Determination of the particle size (length, breadth and roundness) was carried out with a Laborlux S light microscope and a Quantimet 500MC (Q500MC) image processing and analysis system (LEICA Cambridge, Ltd., U.K.).

Roundness is a shape factor that provides information on the circularity of particles. It is calculated by software according to the following formula:

$$\text{roundness} = \frac{\text{perimeter}^2}{4\pi \cdot \text{area}} \cdot 1.064 \quad (1)$$

The perimeter was calculated from the horizontal and vertical projections, with an allowance for the number of corners. An adjustment factor of 1.064 corrected the perimeter for the effect of the corners produced by digitization of the image. When roundness value is close to one, the particles are nearly spherical.

**Morphological Study** The ASA crystals were observed with a scanning electron microscope (Hitachi Scientific Instrument, Ltd., Tokyo, Japan). A Polaron sputter coating apparatus (Polaron Equipment, Ltd., Greenhill, U.K.) was applied to induce electric conductivity on the surface of the sample. The air pressure was 1.3–13 mPa.

**Friability Test** ASA3 sample was investigated by Heberlein apparatus (Flisa, Le Locle, Switzerland). Five grams of sieved sample (<0.32 mm) was put into the apparatus and 100 revolutions were performed. After this process the fine particles were removed by sieve (<0.32 mm) and the percentage of weight loss was calculated.

**Determination of Salicylic Acid (SA) Contents of the ASA Samples** The SA contents of the samples were controlled by a spectrophotometer (Spectromom, MOM, Budapest, Hungary); 0.250 g of sample was dissolved in 25 ml of ethanol (96%) and diluted with water up to 100 ml. After additional dilution (5 ml aliquot+95 ml water), the absorbance was read at 297 nm for SA. The testing was done with a near-infrared spectroscopy.

**Dissolution Test** The investigations were performed with the USP dissolution method (paddle). The medium was artificial gastric juice (pH=1.2) at a temperature of 37±0.5 °C. The paddle speed was 50 rpm. The samples were analyzed spectrophotometrically (Spectromom) at 276 nm. The dissolution rate of ASA was determined from the capsules, and the hard gelatin capsules (No. 1) were filled with 100 mg of ASA crystals.

**Study of Flow Properties** The flow time was determined with a powder testing instrument (PTG-51, Pharma Test Apparatebau GmbH, Hamburg). Poured and tapped densities were measured with a Stampfvolumeter 2003 (J. Engelsmann AG Apparatebau, Ludwigshafen, Germany). The Carr index was calculated from the bulk densities<sup>16)</sup>.

$$\text{Carr index (\%)} = \frac{\text{tapped density} - \text{poured density}}{\text{tapped density}} \cdot 100 \quad (2)$$

The Carr index reflects the compactibility of a powder, and there is a correlation between this index and the flowability of the crystals.<sup>17)</sup>

**Compactibility and Cohesiveness** The Stampfvolumeter measurements allow calculation of the compactibility and cohesiveness values via the modified Kawakita equation<sup>18,19)</sup>:

$$\frac{N}{C} = \frac{1}{a} \cdot N + \frac{1}{ab} \quad (3)$$

where  $N$  is the number of taps,  $C$  is the degree of volume reduction, and  $a$  and  $b$  are constants:  $a$  describes the degree of volume reduction at the limit of tapping and is called the compactibility;  $1/b$  is considered to be a constant related to cohesion and is called the cohesiveness.  $C$  is calculated via the following equation, and graphs of  $N/C$  vs.  $N$  are plotted.

$$C = \frac{V_0 - V}{V_0} \quad (4)$$

The compactibility  $a$  and cohesiveness  $1/b$  are obtained from the slope ( $1/a$ ) and the intercept ( $1/ab$ ) of the plot of the modified Kawakita equation.

**Recording Diffuse-Reflectance Spectra** Near-infrared spectroscopy furnishes useful information for qualitative and quantitative analysis of materials without invasive sample preparation. It can be used for the control of differences among the particle forms of the samples.<sup>20)</sup> Diffuse reflectance was measured by a Hitachi (Japan) U-3501 UV/VIS/NIR spectrophotometer equipped with integrating sphere ( $d=60$  mm) and PbS detector. The reflectance ( $R\%$ ) of samples was determined in the wavelength range 200–2500 nm using a 5 mm layered cell

$$R\% = (I_R/I_0) \cdot 100 \quad (5)$$

where  $I_R$  is the intensity of the diffusely reflected light collected by the integrating sphere and  $I_0$  is the intensity of the incident light.<sup>21)</sup>

**Production of ASA Tablets** The materials without any excipients were pressed into tablets by a KORSCH EKO single punch tablet machine (Emil Korsch Maschinenfabrik, Berlin, Germany). The compression tools were single flat punches 10 mm in diameter. The rate of compression was 30 tablets/min with a pressure force of 18 kN, at an air temperature 24 °C and an air relative humidity of 30%.

**Investigation of ASA Tablets** Investigations were carried out 24 h after pressing. Tablet weight was measured to the order of 0.1 mg. The relative standard deviation (RSD) was calculated from 20 data points and breaking hardness was determined using a Heberlein apparatus (Flisa, Le Locle, Switzerland) (20 tablets).

**Statistical Calculation** The standard deviation and the regression analysis were carried out with the Statgraphics package (copyright STSC Inc. and Statistical Graphics Co. U.S.A.); the confidence limit was 95%.

## Results and Discussion

The ASA1 crystals were tetragonal monocrystals with plane boundaries (Fig. 2). The cooling rate of 1 °C/min resulted in formation of a great number of seeds and did not act favourably either on crystal growth or on seed build-up.

Decreasing the cooling rate (0.5 °C/min) (ASA2) led to the appearance of a small number of crystal agglomerates beside the monocrystals (Fig. 3). Due to the slow cooling, the monocrystals were very large and well-developed. Agglomerates were formed by the accidental encounter of 4–5 crystal seeds. Crystals built up at one point have a habit characteristic of monocrystals.

Further reduction of the cooling rate and changes in the other parameters of crystallization did not cause many changes in the formation of agglomerates either. For example the use of higher stirring speed decreased the possibility of the agglomeration of ASA2 monocrystals but increased the danger of crystal breakage. Let us remark here that commercially available ASA products have a habit similar to that of ASA1 and ASA2 products.

During the formation of ASA3, the crystals were transformed into spherical agglomerates (Fig. 4). The process was

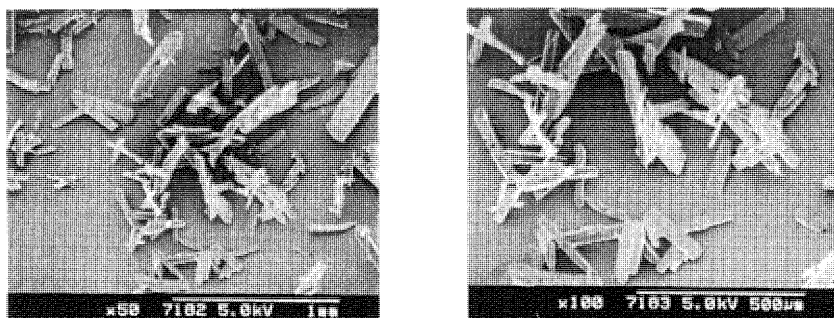


Fig. 2. Crystals of ASA1

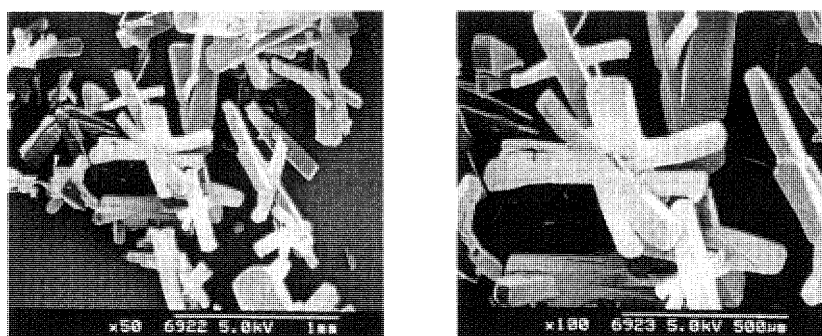


Fig. 3. Crystals of ASA2

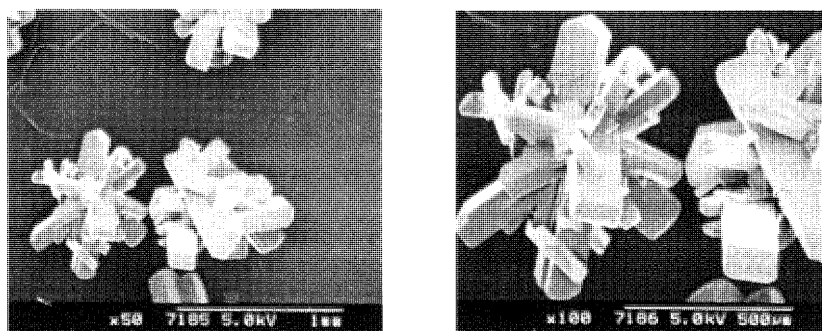


Fig. 4. Crystals of ASA3

the typical spherical crystallization (agglomeration) with three solvents (ethanol–water–carbon tetrachloride). In the three-solvent system the dissolved ASA was carried in the emulsion drops. Due to the effect of diffusion, the ethanol molecules left the emulsion drop and entered the aqueous phase through the emulsion film. The drop became oversaturated with respect to the ASA, and the *in situ* seed-formation started at 37 °C (see Fig.1), followed by crystal formation, which was also influenced by the cooling and mixing rates. Thus, in this process it was not the accidental encounter of the crystals which resulted in the formation of crystal agglomerates. As a matter of fact, crystallization in emulsion drops was the case here, where the driving force was oversaturation caused by solvent diffusion. Although the built-up ASA crystals had a habit characteristic of monocrystals, the size of the agglomerates did not exceed the size of monocrystals and crystal agglomerates produced by non-typical spherical crystallization. ASA3 agglomerates were very hard, open structures. Their friability, which is very important during further processing, was very favorable. The fri-

Table 1. Particle Size Data of ASA Samples

| Sample |         | Length<br>( $\mu\text{m}$ ) | Breadth<br>( $\mu\text{m}$ ) | Roundness |
|--------|---------|-----------------------------|------------------------------|-----------|
| ASA1   | Average | 273.85                      | 72.09                        | 2.54      |
|        | S.D.    | 61.93                       | 13.33                        | 0.29      |
|        | Max.    | 367.91                      | 95.73                        | 3.28      |
|        | Min.    | 168.52                      | 44.02                        | 2.19      |
| ASA2   | Average | 667.94                      | 314.69                       | 1.72      |
|        | S.D.    | 173.36                      | 96.74                        | 0.25      |
|        | Max.    | 1200.00                     | 747.69                       | 2.38      |
|        | Min.    | 391.54                      | 179.23                       | 1.26      |
| ASA3   | Average | 521.72                      | 332.01                       | 1.40      |
|        | S.D.    | 143.16                      | 98.65                        | 0.13      |
|        | Max.    | 913.85                      | 690.77                       | 1.66      |
|        | Min.    | 300.31                      | 136.61                       | 1.15      |

ability of the ASA3 sample was found to be  $6.24 \pm 0.45\%$ , while the maximum friability of granules is usually limited to 20% in pharmacopoeias.

The length, breadth and roundness of the samples are de-

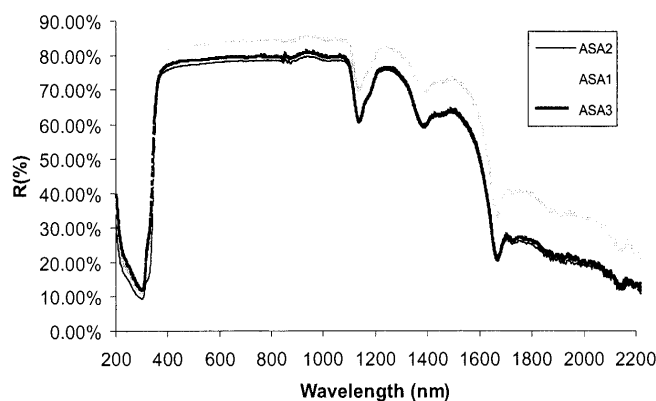


Fig. 5. Diffuse-Reflectance Spectra of ASA Samples

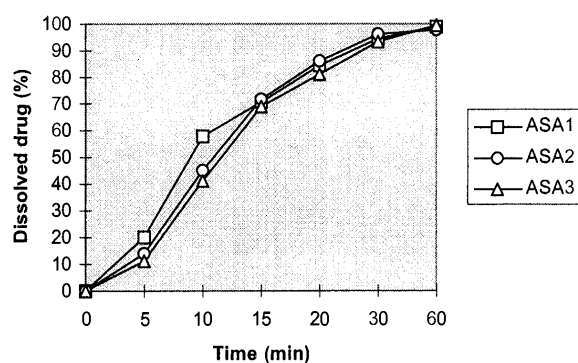


Fig. 6. Dissolution Rate of ASA Samples

tailed in Table 1. The different crystallization processes strongly influenced the habit of the crystals or their agglomerates. A faster cooling process resulted in very small crystals (ASA1) with an unfavorable roundness value. Slower cooling helped the build-up of crystal agglomerates, but they were few in number and the structure was built up only partly (ASA2). Table 1 shows that ASA3 had the best roundness value.

The ASA and salicylic acid content of the samples were qualified by NIR method. The diffuse-reflectance spectra of the ASA1 and ASA3 samples in the wavelength range of 200–350 nm was the same, which can be attributed to the uniform ASA content of the samples (Fig. 5). In this range only the ASA2 sample showed a small peak for the salicylic acid as a decomposition product. The amount of the salicylic acid determined by spectrophotometer was 1.02%. In spite of the higher temperature, the decomposition of ASA2 was low because the crystallization time was only about 1 hour. The data for the slower cooling rate (0.1 °C/min) and the longer process time (about 5 h) showed higher salicylic acid content in the sample (about 10%). Between 350–2500 nm ASA2 and ASA3 samples with larger particle sizes and the best values of roundness showed smaller diffuse reflectances than that of ASA1 with a smaller particle size and a unfavourable roundness value.

As the dissolution rate of the samples was basically influenced by particle size, this was also checked. It is clearly shown in Fig. 6 that the dissolution rate of the samples was almost the same, and only the ASA1 crystals, which have a small particle size and consequently a great surface, showed more rapid dissolution at the beginning of the examination.

Table 2. Parameters of Powder Rheological Investigation of ASA Samples

| Sample | Flow time<br>(s/100 ml) | Poured density<br>(g/ml) | Tapped density<br>(g/ml) | Carr index<br>(%) |
|--------|-------------------------|--------------------------|--------------------------|-------------------|
| ASA1   | no flow                 | 0.3613                   | 0.4517                   | 19.99             |
| ASA2   | 12.1                    | 0.3730                   | 0.4025                   | 7.33              |
| ASA3   | 8.4                     | 0.4337                   | 0.4613                   | 5.99              |

Table 3. Compactibility ( $a$ ) and Cohesiveness ( $1/b$ ) Values of ASA Crystals by Kawakita Model

| Sample | $1/ab$<br>intercept | $1/a$<br>slope | $a$    | $1/b$ | $r^2$  |
|--------|---------------------|----------------|--------|-------|--------|
| ASA1   | 91.75               | 3.97           | 0.2519 | 23.10 | 0.9872 |
| ASA2   | 210.64              | 9.83           | 0.1017 | 21.42 | 0.9515 |
| ASA3   | 131.34              | 9.55           | 0.1017 | 13.76 | 0.9764 |

Table 4. Physical Parameters of ASA Tablets

| Material | Pressing force<br>(kN) | Average height<br>(mm)  | Average weight<br>(g) | Weight variation<br>(R.S.D.)<br>(%) | Breaking hardness<br>(N) |
|----------|------------------------|-------------------------|-----------------------|-------------------------------------|--------------------------|
| ASA1     | 10–15                  | 2.8923<br>(S.D.=0.0153) | 0.2757                | 2.5025<br>(S.D.=3.5339)             | 75.4                     |
| ASA2     | 10±1.5                 | 3.0394<br>(S.D.=0.060)  | 0.2948                | 2.3258<br>(S.D.=2.6998)             | 66.8                     |
| ASA3     | 10±0.5                 | 3.1323<br>(S.D.=0.0427) | 0.3054                | 1.4833<br>(S.D.=1.6576)             | 71.4                     |

S.D.=Standard Deviation, R.S.D.=Relative Standard Deviation.

A very important fact to be pointed out is that the spherical crystal agglomerates of ASA3 did not exert unfavourable influence on the dissolution rate.

The change in the habit of the crystals was reflected in the flowability of the samples. The spherical ASA3 had 'excellent' flow properties, as shown by the flow time and the Carr index (5–15%) (Table 2). The flow time of the prism-shaped monocrystals of ASA1 could not be determined with the Pharmatest apparatus because the substance did not flow from the funnel of the apparatus. The Carr index of ASA1 was very high (19.99%). This means 'fair to passable' (18–21%) flow properties.

The data from the modified Kawakita equation can be seen in Table 3. The compactibilities ( $a$ ) of ASA2 and ASA3 hardly differed; the compactibility of ASA1 was poorer. The cohesiveness ( $1/b$ ) of ASA3 was outstanding, while those of the other two samples were higher and more disadvantageous. ASA3, which contained spherical crystals, had the best flowability, compactibility and cohesivity properties. ASA1, which did not form spherical agglomerates, gave the worst results. Hence, the transformation of commercial ASA crystals into spherical agglomerates led to improved flowability and compactibility. For ASA which did not undergo complete agglomeration, investigation revealed worse technological parameters.

As ASA samples were developed for direct tableting, the preformulation results to date were completed with tablettability studies. The samples were compressed to tablets without excipient and the physical parameters of the tablets were determined (Table 4). During the pressing of the ASA1 crys-

tals great friction was indicated by a powerful machine sound. The die cavity was filled unevenly due to the unfavourable habit of the crystals and their electrostatic charge. In consequence of this a high degree of pressure force variation could be observed, which also influenced the other parameters, thus, *e.g.*, deviation from the theoretical mass was high and weight variation exceeded 2%. The ASA2 sample, which has a greater particle size and is only partially spherical, exhibited better properties of compressibility. The machine sound caused by friction became less intense but the weight variation value of the tablet was still over 2%, which is related to the great cohesivity value of the crystals. The latter can be attributed not only to the shape and size parameters of the crystals but also to their surface properties. The results obtained with tablets compressed from ASA3 were in harmony with the values of flow property, compactibility and cohesivity of the sample. At the same time the importance of spherical crystallization was stressed for the purpose of the direct pressing of ASA crystals. There was no sign whatsoever of electrostatic charging during the pressing of ASA3. The crystal agglomerates can be compressed to tablets almost in a frictionless manner. This was confirmed by the slight variation of the pressing force, average weight and weight variation value. The extreme hardness of the tablets as well as their small standard deviation also proved the important role of the ASA3 sample.

## Conclusions

The typical spherical crystallization process can be used successfully to manufacture spherical crystals of ASA with good flowability, compactibility and cohesivity. The ASA3 crystallization process is optimized concerning the form of the crystal agglomerates and the reproducibility of the product. On the basis of the results this product can be recommended for direct tablet-making.

**Acknowledgement** This work was supported by OTKA T032707 and FKFP 04-89/1999 grants.

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# Mechanochemical Solid-State Polymerization (X): The Influence of Copolymer Structure in Copolymeric Prodrugs on the Nature of Drug Release

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From the standpoint of the mechanism of mechanochemical polymerization, two kinds of copolymeric prodrug, whose monomer sequence distribution (MSD) is different from each other, can be prepared by this polymerization under appropriate operational conditions: one is a random copolymer abundant in the longer block consisting of the same repeating units (multi-block copolymer), and the other is a block copolymer. To confirm the difference of MSD, the  $^{13}\text{C}$ -NMR spectra of poly(acrylamide-co-sodium acrylate) prepared by mechanochemical polymerization were measured and compared with the spectrum of that synthesized by a conventional radical-initiated solution polymerization, which produces the random copolymer normally. The results show that MSD in copolymers depends on the polymerization method (operational condition). We prepared three kinds of copolymeric prodrug consisting of acrylamide and vinyl monomer of 5-fluorouracil, whose MSD is different from one another. These copolymeric prodrugs had almost the same number average molecular weight, particle diameter and composition, and differed only in MSD. We compared the rate of drug release of these copolymeric prodrugs. The rate of drug release was the highest with the random copolymer, followed by the mechanochemically produced multi-block copolymer and the block copolymer. This result suggests that the rate of drug release depends on MSD of copolymeric prodrugs. These results are useful as they give a fundamental insight into the synthesis of copolymeric prodrugs having the desired rate of drug release.

**Key words** drug release; mechanochemical polymerization; copolymer structure; monomer sequence distribution; polymeric prodrug; 5-fluorouracil

The development of a sustained- and controlled-release system for drug delivery is one of the most active areas today in the entire field of drug research. Polymeric prodrugs, in which a drug is attached covalently to the polymer backbone and slowly released under appropriate conditions, possess unique properties distinct from those of the corresponding lower molecular weight prodrugs and can be utilized as a sustained-release delivery system. Thus, the chemotherapeutic utility of polymeric prodrugs has been the focus of intense research.<sup>1)</sup>

We have reported the synthesis and nature of drug release of novel polymeric prodrugs prepared by mechanochemical solid-state polymerization.<sup>2)</sup> Several important conclusions were reached from a series of such studies. The monomers prepared on the basis of the structural criteria derived from quantum chemical considerations underwent facile mechanochemical solid-state polymerizations to give corresponding polymeric prodrugs essentially quantitatively.<sup>2a,b,e)</sup> Thus, this method eliminates the need for any work-up of the reaction mixture. One of the most striking properties observed in such polymers is that the resulting polymeric prodrugs are of very low heterogeneity (narrow molecular weight distribution) represented by  $\bar{M}_w/\bar{M}_n$ , ( $\bar{M}_w$ , weight average molecular weight;  $\bar{M}_n$ , number average molecular weight), which is of great value in pharmaceuticals for highly functionalized polymeric prodrugs.<sup>2b)</sup> Therefore, the present reactions seem to be applicable to a wide variety of vinyl monomers of an important class of bioactive compounds with different physicochemical properties and provide a novel and simple methodology for syntheses of polymeric prodrugs through a totally dry process.

It is well known that the physicochemical property of copolymers largely depends on the composition, monomer

sequence distribution (MSD) and molecular weight. It is important, therefore, to establish the relationship between the nature of drug release and the physicochemical property of copolymers to synthesize a polymeric prodrug possessing a desired rate of drug release.

The copolymer of acrylamide (**1**) with sodium acrylate (**2**), poly(acrylamide-co-sodium acrylate) (**3**), prepared by radical-initiated solution polymerization is one of the copolymers whose MSD is known to be analyzed by the  $^{13}\text{C}$ -NMR.<sup>3)</sup> Accordingly, we first synthesized the same type of copolymers (**3**) by three kinds of polymerization method: conventional radical-initiated solution copolymerization of **1** with **2**, mechanochemical solid-state polymerization of **1** or polyacrylamide (**1p**) with **2**. The  $^{13}\text{C}$ -NMR spectra of these copolymers were measured and compared with one another to gain a fundamental insight into the characteristics of the resulting polymers.

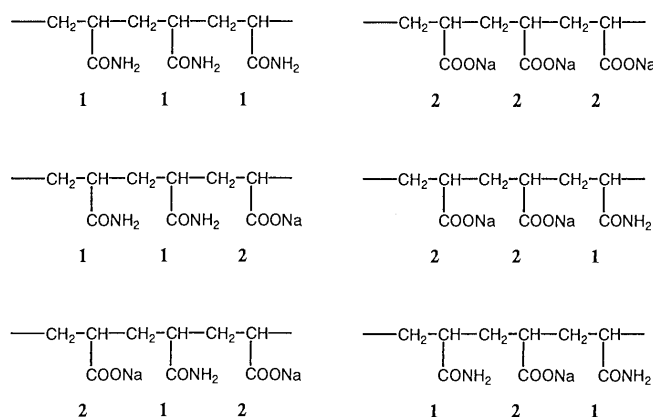


Chart 1

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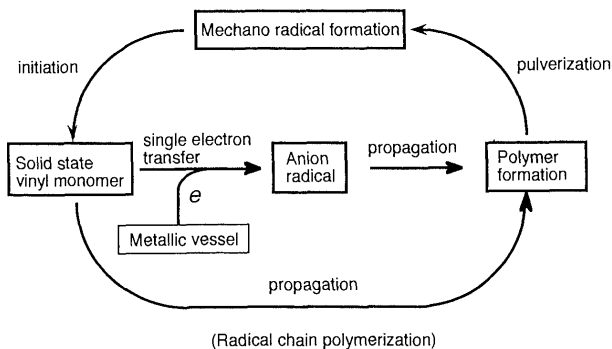


Fig. 1. Mechanism of Mechanochemical Polymerization

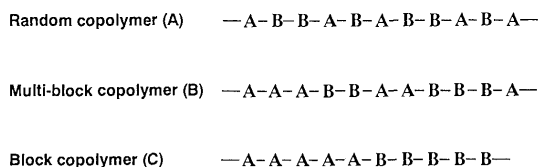


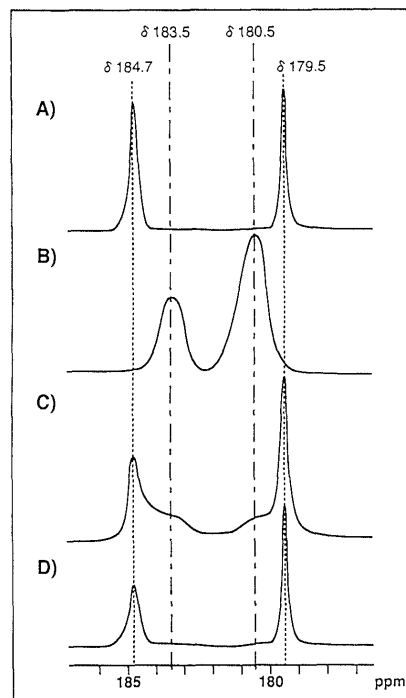
Fig. 2. Conceptional Illustration of Copolymer Structure

We have then prepared the copolymeric prodrugs of **1** with the methacryloyl derivative of 5-fluorouracil (5-Fu) (**4**), selected as a model drug, by the three kinds of method described above and examine the rate of drug release from the resulting copolymeric prodrugs. The influence of MSD on the nature of drug release of these copolymeric prodrugs is discussed.

## Results and Discussion

**Difference of the MSD of Poly(acrylamide-co-sodium acrylate) Depending on the Polymerization Methods** We have already clarified the mechanism of mechanochemical polymerization.<sup>2b,d)</sup> As shown in Fig. 1, the mechanism by which this polymerization is initiated involves solid-state single electron transfer (SSET) from the activated metal surface to the vinyl bond of the monomer to produce a corresponding anion radical, followed by the propagation through a radical chain polymerization. The polymerization initiated through the anion radical, however, is only a minor process in the mechanochemical polymerization. The main process is polymerization initiated by the mechanoradical produced in the course of polymer degradation, the so-called "mechanoradical-initiated polymerization".

In general, the radical-initiated solution copolymerization produces a random copolymer (Fig. 2A), since both monomers homogeneously dissolve in a solvent except for special cases such as copolymerization of maleic anhydride and styrene, which produces a perfectly alternating copolymer. On the other hand, the radical chain propagation reaction of mechanochemical solid-state copolymerization rapidly proceeds within each particle of powdered monomer, crystalline or amorphous. Therefore, it is assumed that the copolymer produced would be a random copolymer abundant in the longer block consisting of the same repeating units which may be denoted as multi-block copolymers (Fig. 2B). To our knowledge, however, there is no report on MSD of copolymers produced by mechanochemical solid-state copolymerization. Furthermore, if a mechanically-polymerizable monomer is milled in the presence of some other poly-

Fig. 3. Narrow Scan <sup>13</sup>C-NMR Spectra of Carbonyl Group of Blendmer of Polyacrylamide (**1p**) and Poly(sodium acrylate) (**2p**), and Poly(acrylamide-co-sodium acrylate) (**3**) Prepared by Three Kinds of Polymerization Method

A, Blendmer of polyacrylamide (**1p**) and poly(sodium acrylate) (**2p**); B, random copolymer prepared by radical-initiated solution copolymerization (**3rr**); C, multi-block copolymer prepared by mechanochemical copolymerization (**3rm**); D, block copolymer prepared by mechanochemical copolymerization (**3bm**).

mers under anaerobic conditions using a Teflon-made twin-shell blender in which the monomer itself does not polymerize,<sup>2b)</sup> block copolymers (Fig. 2C) would be produced by polymerization initiated by the polymer-derived mechanoradical generated by polymer main chain scission.

There are a number of NMR and IR studies on MSD of several kinds of copolymers. As described above, copolymer **3** prepared by radical-initiated solution copolymerization is one of the copolymers whose MSD can be analyzed by <sup>13</sup>C-NMR.<sup>3)</sup> To confirm the difference in MSD of copolymers dependent on the polymerization method, three kinds of copolymer **3** were prepared as follows. Copolymers **3rr** and **3rm** were prepared by radical-initiated solution and mechanochemical solid-state copolymerization of **1** and **2**, respectively. Copolymer **3bm** was produced by mechanochemical copolymerization of **1p** and **2** using a Teflon-made twin-shell blender for 2 h. Their <sup>13</sup>C-NMR spectra were measured and compared.

Figure 3 shows the narrow scan <sup>13</sup>C-NMR of the carbonyl group of three kinds of copolymer **3**, together with that of the blendmer produced by the mechanical mixing of **1p** and poly(sodium acrylate) (**2p**). The spectrum of the blendmer is shown in Fig. 3A, and two sharp peaks were observed at δ 179.5 and 184.7. Candau and Zekhnini reported chemical shifts for monomer triads, which denote MSD such as **111**, **112** and **221** in copolymer **3** (Chart 1).<sup>3)</sup> From the literature, two sharp peaks at δ 179.5 and 184.7 were assigned to **111** and **222** triads, respectively. This result shows that the mechanical blending causes no change in MSD within a detectable extent. The spectrum of copolymer **3rr** (radical-initiated solution copolymerization) is shown in Fig. 3B. This

spectrum is essentially identical to that of copolymers prepared by radical-initiated solution copolymerization reported by Candau and Zekhnini.<sup>3)</sup> From the literature, two broad peaks at  $\delta$  180.5 and 183.5 were assigned to **112** and **212** triads, and **221** and **121** triads, respectively. There is no peak assigned to **111** and **222** triads in Fig. 3B. These spectral features show that the copolymer prepared by radical-initiated solution copolymerization is a random copolymer.

Figure 3C shows the spectrum of copolymer **3rm** (mechanochemical solid-state polymerization of **1** and **2**). This spectrum has two relatively sharp and intense peaks at  $\delta$  179.5 and 184.7, and two broad and small peaks at  $\delta$  180.5 and 183.5. The former peaks, which are assigned to **111** and **222** triads (*vide supra*), denote the longer block consisting of the same repeating units (55%), and the latter peaks denote the features of the random copolymer (45%). Based on these MSD features, it is considered that copolymer **3rm** is a random copolymer abundant in the long block consisting of the same repeating units (multi-block copolymer). On the other hand, the spectrum of copolymer **3bm** (mechanochemical copolymerization of **1p** and **2** using a Teflon-made vessel) shown in Fig. 3D has two peaks of **111** and **222** triads (92%) with a broad and very small shoulder peak (8%). This spectral feature is very similar to that shown in Fig. 3A. Considering the mechanism and the spectral feature described above copolymer **3bm** should be a block copolymer. Thus, it was clearly shown that mechanochemical polymerization gave the copolymer whose MSD largely differed from that of radical-initiated solution polymerization.

**The Influence of MSD in Copolymeric Prodrugs on the Nature of Hydrolysis** It is well known that the rate of drug release from copolymeric prodrugs depends on the copolymer property. The copolymer structure (MSD) is one of the factors affecting the copolymer property. In order to synthesize copolymeric prodrugs possessing suitable drug release properties for chemotherapeutic use, it is important to gain a fundamental insight into the influence of MSD on the nature of drug release from copolymeric prodrugs.

We selected 5-Fu as a model compound and prepared its methacryloyl derivatives (**4**), 1-(2-methacryloyloxyethyl)carbamoyl-5-fluorouracil. Copolymeric prodrugs (**5**) consisting of **1** with **4** were synthesized by three kinds of polymerization method (Fig. 4): Copolymeric prodrug **5rr** was prepared by radical-initiated solution copolymerization of **1** and **4**. Mechanochemical copolymerization of **1** or **1p** with **4** produced copolymeric prodrug **5rm** or **5bm**, respectively. Random copolymeric prodrug **5rr** prepared was fractured by vibratory ball milling (60 Hz, 1 h) to obtain copolymeric prodrug with a limiting molecular weight. In this operation, the particle size of copolymeric prodrug **5rr** should have decreased more rapidly.<sup>4)</sup> We have confirmed that the particle size of copolymeric prodrug **5rr** became almost constant (3.31  $\mu\text{m}$ ) by the milling of about 5 min in this experimental condition.

The particle diameter (geometric mean diameter ( $\mu\text{m}$ )), number average molecular weight ( $\bar{M}_n$ ) and heterogeneity ( $\bar{M}_w/\bar{M}_n$ ) of the copolymeric prodrugs are shown in Table 1. These copolymeric prodrugs are insoluble in water, so that hydrolysis of copolymeric prodrugs was performed in a heterogeneous system.<sup>2e)</sup> The particle diameter of copolymeric prodrugs is an important factor affecting the rate of hydroly-

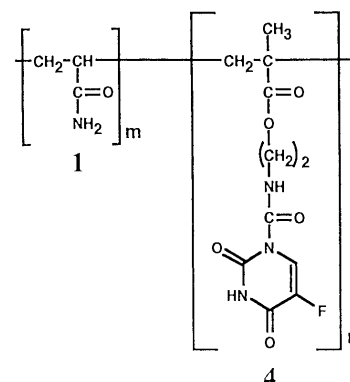


Fig. 4. Structure of Copolymeric Prodrug (**5**) of Acrylamide (**1**) with 1-(2-Methacryloyloxyethyl)carbamoyl-5-fluorouracil (**4**)

Table 1. Characterization of Copolymeric Prodrugs Consisting of Acrylamide (**1**) and 1-(2-Methacryloyloxyethyl)carbamoyl-5-fluorouracil (**4**)

|                                      | Geometric mean diameter ( $\mu\text{m}$ ) | Number average molecular weight ( $\bar{M}_n$ ) | Heterogeneity ( $\bar{M}_w/\bar{M}_n$ ) |
|--------------------------------------|-------------------------------------------|-------------------------------------------------|-----------------------------------------|
| Random copolymer ( <b>5rr</b> )      | 3.31                                      | 28,000                                          | 1.15                                    |
| Multi-block copolymer ( <b>5rm</b> ) | 3.00                                      | 28,000                                          | 1.15                                    |
| Block copolymer ( <b>5bm</b> )       | 2.96                                      | 29,000                                          | 1.14                                    |

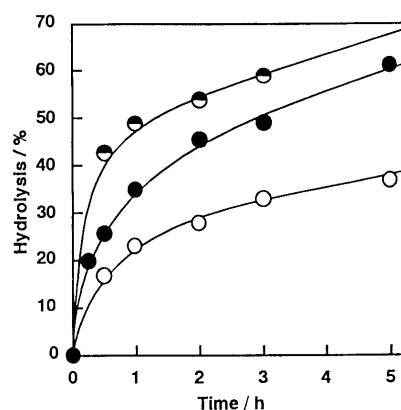


Fig. 5. Hydrolytic Profiles of Copolymeric Prodrugs (**5**) of Acrylamide (**1**) with 1-(2-Methacryloyloxyethyl)carbamoyl-5-fluorouracil (**4**) as a Function of Reaction Time

○, Random copolymer (**5rr**); ●, multi-block copolymer (**5rm**); ○, block copolymer (**5bm**).

sis in the heterogeneous system, because the rate of hydrolysis increases with a decrease of the particle diameter of copolymeric prodrugs. The geometric mean diameter of the copolymeric prodrugs shown in Table 1 is similar to one another. The number average molecular weight and heterogeneity of these copolymeric prodrugs were almost the same. We also note that the resulting copolymeric prodrugs are all amorphous based on the measurement of the X-ray diffraction of these copolymeric prodrugs exhibiting no peak for the crystallinity as shown in the monomers but only halo patterns. These results show that the characteristics of copolymeric prodrugs is similar to one another except for MSD.

Figure 5 shows progressive changes in hydrolysis of copolymeric prodrugs of **1** and **4** (1:1) as a function of the reaction time in a pH 7.4 phosphate buffer solution at 37 °C

in a heterogeneous system.

It is shown that the rate of drug release of these copolymeric prodrugs depends on MSD. Random copolymer **5rr** is hydrolyzed most easily and mechanochemically produced random copolymer **5rm** is the second. Block copolymer **5bm** released the drug most slowly. The hydrophilic group of copolymer **5rr** is homogeneously arranged in the polymer chain, but that of block copolymer **5bm** is arranged heterogeneously (Fig. 2). These results, therefore, suggest that the rate of drug release of copolymeric prodrugs depends on the arrangement of the hydrophilic group, *i.e.* MSD, in the polymer chain. Copolymeric prodrugs possessing a desired rate of drug release can be prepared, if the length of each block unit can be controlled by an operational condition of mechanochemical copolymerization. We have demonstrated herein that the rate of drug release can be controlled by MSD design of the copolymeric prodrug. The control of the length of each block unit will be the subject of a forthcoming paper.

## Experimental

Acrylamide (**1**) supplied by Tokyo Kasei Kogyo Co., Ltd. (Japan) is commercially available. It was purified by recrystallization from benzene and dried *in vacuo*. Sodium acrylate (**2**) was prepared by neutralization of acrylic acid with sodium hydroxide. Polyacrylamide (**1p**, 2 g) supplied by Kishida Chemicals Co., Ltd. (Japan) was purified by dissolving in water (50 ml) and after filtering, it was poured into a large amount of acetone (1.5 l). Precipitated polymers were collected and dried *in vacuo* at 60 °C for three days. Polysodium acrylate (**2p**) was prepared by a conventional radical-initiated solution polymerization. 1-(2-Methacryloyloxyethyl)carbonyl-5-fluorouracil (**4**) was prepared according to the literature.<sup>2f)</sup>

**Mechanochemical Copolymerization** A mixture of **1** (43 mg, 0.61 mmol) and **2** (57 mg, 0.61 mmol) was mechanically fractured under anaerobic conditions (*e.g.* in nitrogen) by ball milling (6.0 mm $\phi$ , 890 mg) in a stainless steel twin-shell blender (7.8 mm $\phi$ , 24 mm long) at room temperature for 2 h at 60 Hz according to the method previously reported.<sup>2b)</sup> Residual oxygen in this system was removed with high capacity gas purifier (Supelco, Inc.). The oxygen concentration was monitored with oxygen analyzer (LC 750/PC-120, Toray Engineering Co., Ltd.) and kept below 10 ppm. Mechanochemical copolymerization of **1** (20 mg, 0.28 mmol) and **4** (80 mg, 0.28 mmol) was performed in the same way as described above.

**Mechanochemical Block Copolymerization** A mixture of **1p** (43 mg) and **2** (57 mg, 0.61 mmol) was mechanically fractured in a Teflon-made twin-shell blender for 4 h under anaerobic conditions. Mechanochemical block copolymerization of **1p** (20 mg) and **4** (80 mg, 0.28 mmol) was performed in the same way as described above.

**Radical-Initiated Solution Copolymerization of 1 with 2** Radical initiated solution polymerization of **1** with **2** was carried out according to the literature.<sup>3)</sup> A mixture of **1** (0.6 g, 8.4 mmol), **2** (0.8 g, 7.4 mmol), and 2,2'-azobisisobutyronitrile (AIBN) (2 mg) in H<sub>2</sub>O (10 ml) was warmed at 60 °C in a sealed glass-made tube under nitrogen for 20 min. The content was poured into a large amount of methanol. The precipitated polymer was collected and dried *in vacuo* to yield 0.49 g (35%). The composition of this copolymer determined by elemental analysis was 1 : 1.

**Preparation of Blendmer of 1p and 2p** A mixture of **1p** (0.6 g, 8.4 mmol) and **2p** (0.8 g, 7.4 mmol) was mechanically fractured by ball milling in a stainless steel twin-shell blender for 2 h under anaerobic conditions.

**Radical Initiated Solution Copolymerization of 1 with 4** A mixture

of **1** (27.5 mg, 0.39 mmol), **4** (223 mg, 0.78 mmol), and AIBN (0.8 mg) in *N,N*-dimethylformamide (DMF) (3 ml) was warmed at 60 °C for 15 h in a manner similar to what was described above. After DMF (5 ml) was added, the content was poured into a large amount of acetone. The precipitated polymer was collected and dried *in vacuo* to yield 150 mg (60%). The composition of this polymer determined by elemental analysis was 1 : 1. In order to obtain copolymeric prodrugs of low molecular weight, the polymer formed (100 mg) was fractured for 1 h by ball milling in a metallic vessel.

**<sup>1</sup>H- and <sup>13</sup>C-NMR Spectral Measurement** <sup>1</sup>H- and <sup>13</sup>C-NMR spectra were recorded on a JEOL JNM-GX270 FT-NMR spectrometer in either dimethyl sulfoxide-*d*<sub>6</sub> (DMSO-*d*<sub>6</sub>) or D<sub>2</sub>O. Tetramethylsilane (TMS) was used as the internal or external standard.

**Molecular Weight Measurement** The molecular weight of copolymeric prodrugs was measured with a gel permeation chromatograph (GPC; Shimadzu LC-6A) equipped with a refractive index detector (Shimadzu, RID-6A), gel column (Shodex, KD-800M and KD-80M), and a data analyzer (Shimadzu, Chromatopac C-R4A), under the following conditions: elution solvent, DMF containing 10 mmol of LiBr; flow rate, 0.7 ml/min; column temperature, 40 °C. Calibration was carried out with a standard specimen of poly(ethylene oxide).

**Particle Diameter Measurement** The particle size of copolymeric prodrugs was measured with a light scattering apparatus (LS100Q, Coulter Co.), which uses diode laser light with a wavelength of 750 nm, equipped with a small volume module (SVM, Coulter Co.) in a methanol suspension. Data were collected and averaged over 60 s. Data analysis was performed using the Coulter LS control program (Coulter Co.) with a 32-bit microcomputer (NEC VALUESTAR NX).

**Method of Hydrolysis** Hydrolysis of powdered polymeric prodrugs (5.0–6.0 mg) was conducted in 10 ml of pH 7.4 phosphate buffer at 37 ± 0.2 °C in a heterogeneous system. Released 5-FU was periodically assayed by UV absorption spectrum (Recording Spectrophotometer UV-2200 (P/N 206-17000), Shimadzu Co.) at the wavelength of 266 nm. 5-FU was separately confirmed to be stable under the prescribed conditions of hydrolysis. The pH of the solution is unchanged before and after hydrolysis.

**Acknowledgments** This work was financially supported in part by a Grant-in-Aid of Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan (Grant No. 10771271), which is gratefully acknowledged.

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## New Potential Antimalarial Agents: Synthesis and Biological Activities of Original Diaza-analogs of Phenanthrene

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Several diaza-analogs of phenanthrene derived from 3-amino, 5-amino, 6-amino, 8-aminoquinolines, and 5-aminoisoquinoline were prepared to evaluate their antiplasmodial activities. All compounds showed mild to good activity *in vitro*, both on a Nigerian chloroquino-sensitive strain and on the chloroquino-resistant FcB1-Columbia and FcM29 strains. The position of the intracyclic nitrogen atom is shown to be crucial for the activities (best results are obtained with a 1,10-phenanthroline skeleton). In regard to the particular properties of this structure (metalloprotease inhibition activity by chelating divalent metal ions), the potential chelating site of the molecule was blocked. In this case, the biological activity of the compound was greatly enhanced, showing that the mechanism of action of such a compound is probably not correlated to metalloprotease inhibition activity.

**Key words** 1,10-phenanthroline; antiplasmodial; quinoline; Plasmodium falciparum; *in vitro*

Malaria is endemic throughout much of the tropics, and sub-tropics placing at risk some 40% of the world's population. More than 100 million clinical cases of the disease are thought to occur annually, resulting in at least 1—2 million deaths. In addition, since resistance to currently used antimalarials is spreading rapidly, there is a great need for new effective drugs. As part of our program concerning the synthesis and biological activities of aza-analogs of alkaloids,<sup>1)</sup> we were interested in the chemistry of diaza-analogs of phenanthrene (phenanthrene alkaloids are obtained from various families of the Angiospermae.<sup>2)</sup>) An example of particular interest of a non-natural antimalarial agent possessing a phenanthrolic skeleton is halofantrine, which possesses good therapeutic effects but some important side effects.<sup>3)</sup> In addition, heterocycles with two nitrogen atoms, such as pyronaridine<sup>4)</sup> (a benzo[*b*]-1,5-naphthyridine skeleton), are well-known for their antimalarial activities. In this context, we describe here our first results concerning the preparation and *in vitro* biological activities of original diaza-analogs of phenanthrene (compounds **A** and **B**), which can be further implicated in the preparation of new potent antimalarial agents (Chart 1).

**Chemistry** Our strategy for the synthesis of the tricyclic derivatives of quinoline or isoquinoline possessing an additional pyridinic ring is focused on the use of heterocyclic enamines as synthetic intermediates,<sup>5)</sup> which can then undergo regioselective heterocyclizations. Treatment of the different aminoquinolines **1—4** and aminoisoquinoline **5** by a 1.5 eq of 2-acetylbutyrolactone in refluxing toluene with a catalytic amount of *p*-toluenesulfonic acid gave the corresponding furanones **6—10** in moderate to good yield (Chart 2).

By treatment with phosphorus oxychloride (1 g of **6—10**, 10 ml of freshly distilled POCl<sub>3</sub>), these synthetic intermediates underwent regioselective cyclizations and subsequent ring and side chlorination to give **11—15** (Chart 3).<sup>6)</sup>

Structural determinations of these pyrido(iso)quinolines

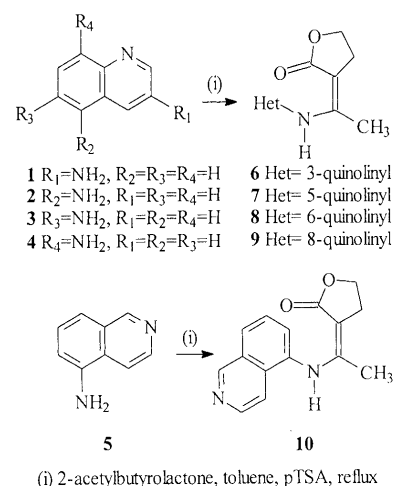
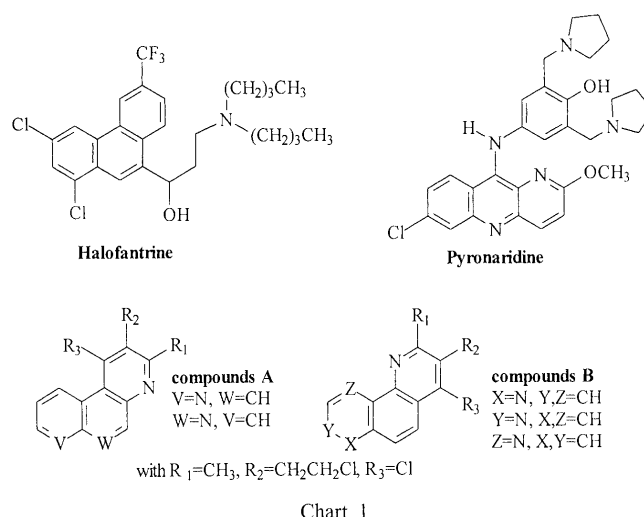


Chart 2

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**12–15** and benzonaphthyridines **11** were made on the basis of mass spectrometry and NMR studies. Regioselectivity of the cyclizations was easily determined by simple examination of  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data, except for **12**. In this case, examination of  $^1\text{H}$ - $^{13}\text{C}$  long-range correlations was necessary to confirm the position of cyclization on the C-6 position of the quinoline ring rather than on the C-4 position.

## Results and Discussion

Three strains of *P. falciparum* were used to evaluate the *in vitro* antiparasmodial activities of compounds **11–15**: the chloroquino-resistant FcB1-Columbia and FcM29-Cameron strains and a Nigerian chloroquino-sensitive strain.<sup>7)</sup> Para-

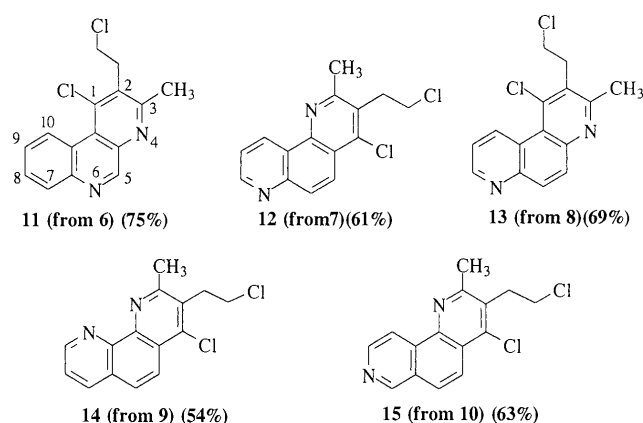
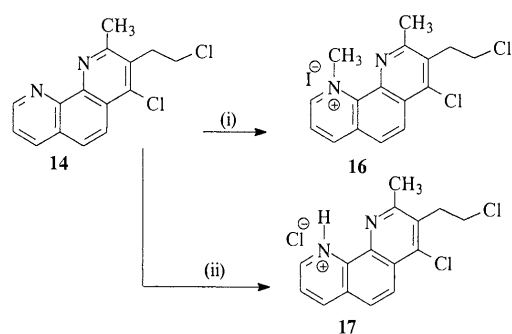


Chart 3



(i)  $\text{CH}_3\text{I}$ , acetone, reflux 12h  
(ii)  $\text{HCl}$  gas, acetone

Chart 4

sites were cultured according to the method originally described by Trager and Jensen.<sup>8)</sup> For each test, the parasite cultures were incubated with compounds at decreasing concentrations for 24 and 72 h. Results are summarized in Table 1. This first screening showed that all compounds were less active on the chloroquino-sensitive strain than chloroquine itself. Compound **14** displayed interesting antiplasmodial activity on the two chloroquino-resistant strains. Compound **11**, possessing a benzonaphthyridine skeleton, was the least active compound, especially after 24 h of contact between drug and parasite, with all the three strains. The three other compounds **12**, **13**, **15**, possessing as **14** a phenanthroline skeleton, exhibited mild activity. These results clearly indicate the primordial effect of the position of the intracyclic nitrogen atom: when the two pyridinic rings are joined together, only slight activity was observed (compound **11**). When the two pyridinic rings are joined toward a phenyl ring (phenanthrolines), the activities increased, especially in the case of the 1,10-phenanthroline ring system.

As the 1,10-phenanthroline ring system is well-known for its metalloprotease inhibition activities by chelating divalent metal ions,<sup>9)</sup> we could reasonably suppose that this phenomenon was implicated in the mechanism of action. In order to obtain such informations, we decided to block the potential chelating site by *N*-alkylation (Chart 4).

Surprisingly, compound **16** exhibited better activity than **14** on the three strains, both after 24 and 72 h incubation (Table 1). This result clearly demonstrated that the metalloprotease inhibition process was not correlated with the mechanism of action against *P. falciparum*. Furthermore, as **16** was more soluble in the aqueous biological phase, it was interesting to investigate the activities of the simple hydrosoluble phenanthroline hydrochloride salt **17**. In this case, antiparasmodial activity was not enhanced, showing that alkylation of the N-10 atom was essential to enhance the activities of such compounds.

Cytotoxic activities of these compounds (**11–17**) were also evaluated *in vitro* against the HeLa cell line. Results are summarized in Table 2. All compounds exhibited cytotoxic effects which were higher than chloroquine but lower than halofantrine. Moreover, these cytotoxic effects increased with antiparasmodial activities for **11–15**, while remaining close to compound **14** for derivatives **16** and **17**. A cytotoxic/antiparasmodial ratio (CAR.) was calculated at 72 h (Table 2), and showed an interesting value (higher than 100<sup>10)</sup> for com-

Table 1.  $\text{IC}_{50}$  ( $\mu\text{M}$ ) of Compounds **11–17** on the Three *P. falciparum* Strains Tested

|                   | Nigerian        |                 | FcB1            |                 | FcM29           |                 |
|-------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| IT <sup>a)</sup>  | 24              | 72              | 24              | 72              | 24              | 72              |
| <b>11</b>         | 72.5 $\pm$ 13.4 | 22.6 $\pm$ 7.5  | 60.6 $\pm$ 14.0 | 18.5 $\pm$ 17.8 | 68.1 $\pm$ 10.3 | 25.3 $\pm$ 3.8  |
| <b>12</b>         | 24.9 $\pm$ 7.2  | 8.9 $\pm$ 7.2   | 16.8 $\pm$ 3.7  | 7.5 $\pm$ 4.5   | 37.7 $\pm$ 20.9 | 6.9 $\pm$ 3.0   |
| <b>13</b>         | 17.4 $\pm$ 7.5  | 13.4 $\pm$ 5.1  | 27.4 $\pm$ 7.2  | 14.0 $\pm$ 4.5  | 18.5 $\pm$ 13.5 | 7.5 $\pm$ 11.6  |
| <b>14</b>         | 2.4 $\pm$ 0.4   | 1.5 $\pm$ 0.6   | 2.4 $\pm$ 0.8   | 1.3 $\pm$ 1.1   | 2.3 $\pm$ 0.4   | 1.8 $\pm$ 0.3   |
| <b>15</b>         | 15.7 $\pm$ 6.1  | 14.4 $\pm$ 3.8  | 18.8 $\pm$ 4.1  | 16.4 $\pm$ 6.8  | 10.6 $\pm$ 3.1  | 11.3 $\pm$ 6.5  |
| <b>16</b>         | 0.19 $\pm$ 0.05 | 0.16 $\pm$ 0.08 | 0.17 $\pm$ 0.07 | 0.02 $\pm$ 0.02 | 0.21 $\pm$ 0.02 | 0.10 $\pm$ 0.07 |
| <b>17</b>         | 2.33 $\pm$ 0.70 | 1.50 $\pm$ 0.50 | 2.27 $\pm$ 0.03 | 1.81 $\pm$ 0.06 | 22.1 $\pm$ 18.2 | 16.3 $\pm$ 13.4 |
| Hal <sup>b)</sup> | 0.003           | 0.0025          | 0.0034          | 0.0027          | 0.0031          | 0.0028          |
| CQ <sup>b)</sup>  | 0.076           | 0.076           | 0.145           | 0.145           | 0.210           | 0.210           |

a) Incubation time (h), b) Hal=halofantrine, CQ=chloroquine, values checked monthly.

Table 2. IC<sub>50</sub> and CAR Values on FcB1 of Compounds **11**–**17** on HeLa Cell Line

|                  | IC <sub>50</sub> (μM) |            | CAR <sup>a)</sup> |
|------------------|-----------------------|------------|-------------------|
| IT <sup>b)</sup> | 24                    | 72         | ///               |
| <b>11</b>        | 47.9±13.9             | 44.1±17.8  | 2.38              |
| <b>12</b>        | 26.7±7.2              | 33.9±11.3  | 4.52              |
| <b>13</b>        | 42.1±23.3             | 46.2±18.1  | 3.30              |
| <b>14</b>        | 17.4±3.8              | 14.0±6.2   | 10.77             |
| <b>15</b>        | 52.4±13.4             | 48.6±6.8   | 2.96              |
| <b>16</b>        | 19.6±8.0              | 15.1±1.8   | 755.00            |
| <b>17</b>        | 14.8±0.5              | 13.4±1.01  | 2.96              |
| Hal              | 4.28±0.75             | 7.26±1.17  | 2680.80           |
| CQ               | 68.61±7.85            | 75.15±9.13 | 518.27            |

a) Cytotoxic/antiplasmodial ratio on FcB1 calculated for 72 h of incubation time.

b) Incubation time (h).

pound **16** (CAR<sub>FcB1</sub>=755, CAR<sub>FcM29</sub>=151). These CAR values for **16** were obtained by an increasing antiplasmodial effect associated with stable cytotoxicity when compared to compound **14**.

## Conclusion

We have described the synthesis and evaluation of a five models of diaza-analogs of the phenanthrene skeleton. From this evaluation, the 1,10-phenanthroline ring system appeared as a new class of potential antimalarial compounds. From these results, and with respect to the particular properties of this skeleton, our first hypothesis about a possible mechanism of action led us to consider a new derivative (compound **16**) which exhibited higher antiplasmodial activity, with similar cytotoxic activity. Further studies concerning the pharmacomodulation, the mechanism of action, and the *in vivo* evaluation of such compounds are now underway.

## Experimental

Melting points were determined on a Büchi capillary melting point apparatus and are not corrected. Elemental analysis was performed by the Micro-analytical Center, ENSCM, Montpellier. Spectral measurements were taken using the following instruments: <sup>1</sup>H-NMR spectra were taken on Bruker AC 100 or WM 360 or EM 400WB; <sup>13</sup>C-NMR spectra were obtained at 26 °C, with proton noise decoupling at 25 MHz with a Bruker AC 100 instrument. Chemical shifts are expressed relative to residual chloroform. Mass spectra were recorded on an LKB 2091 spectrometer at 15 eV [θ(source)=180 °C]. Dichloromethane was dried over activated alumina and distilled from calcium hydride.

**3-[1-(3-Quinolin-3-ylamino)-ethylidene]-4,5-dihydrofuran-2-one (6)** A mixture of 2-acetyl-butylolactone (1 g, 8 mmol) and 3-aminoquinoline (**1**) (1.15 g, 8 mmol), and a catalytic amount of *p*-toluenesulfonic acid in toluene (25 ml), was refluxed under nitrogen with a Dean Stark apparatus for 6 h. After evaporation of the solvent, water was added and the mixture was basified (10% Na<sub>2</sub>CO<sub>3</sub>), saturated with NaCl, and extracted with dichloromethane. Organic layers were washed with brine (50 ml), dried over sodium sulfate and evaporated *in vacuo*. The remaining oil was treated with ether, cooled, and the precipitate was filtered. Recrystallization from ethanol gave (**6**) in 90% yield; mp 150–152 °C. MS (relative intensity) *m/z*: 254 (M<sup>+</sup>, 96), 195 (50), 128 (53). <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 2.07 (s, 3H), 2.91 (2H, t, *J*=7.0 Hz), 4.35 (2H, t, *J*=7.0 Hz), 7.61 (4H, m), 8.06 (1H, d, *J*=7.4 Hz), 8.65 (1H, d, *J*=2.5 Hz), 10.15 (1H, s). <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ: 17.4 (CH<sub>3</sub>), 25.9, 65.2, 91.7, 126.2, 126.9, 127.0, 127.6, 128.1, 128.7, 132.5, 144.8, 147.3, 151.9, 173.48. *Anal.* Calcd for C<sub>15</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub>: C, 70.85; H, 5.55; N, 11.02. Found: C, 70.69; H, 5.65; N, 11.12.

**3-[1-(5-Quinolin-3-ylamino)-ethylidene]-4,5-dihydrofuran-2-one (7)** This compound was obtained in 88% yield from 5-aminoquinoline (**2**) according to the general procedure used for the synthesis of **6**; mp 116–118 °C. MS (relative intensity) *m/z*: 254 (M<sup>+</sup>, 97), 195 (53), 128 (50). <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 1.79 (3H, s), 2.81 (2H, t, *J*=8 Hz), 4.27 (2H, t, *J*=8 Hz), 7.38 (4H, m), 7.80 (1H, d, *J*=9 Hz), 8.23 (1H, d, *J*=8 Hz), 8.78 (1H, d, *J*=5

Hz), 10.15 (1H, s). <sup>13</sup>C-NMR δ: 17.4, 25.1, 65.4, 90.24, 121.30, 122.7, 124.1, 127.1, 128.6, 130.9, 134.9, 148.6, 150.5, 154.0, 174.05. *Anal.* Calcd for C<sub>15</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub>: C, 70.85; H, 5.55; N, 11.02. Found: C, 70.95; H, 5.51; N, 11.32.

**3-[1-(6-Quinolin-3-ylamino)-ethylidene]-4,5-dihydrofuran-2-one (8)** This compound was obtained in 80% yield from 6-aminoquinoline (**3**) according to the general procedure used for the synthesis of **6**; mp 128–130 °C. MS (relative intensity) *m/z*: 254 (M<sup>+</sup>, 97), 209 (54), 169 (39). <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 2.10 (3H, s), 2.90 (2H, t, *J*=8.0 Hz), 4.35 (2H, t, *J*=8.0 Hz), 7.26 (3H, m), 7.92 (2H, d, *J*=11.0 Hz), 8.78 (1H, d, *J*=4.1 Hz), 10.18 (1H, s). <sup>13</sup>C-NMR δ: 17.3, 25.7, 64.81, 90.62, 118.1, 121.1, 125.9, 128.0, 129.8, 134.6, 136.7, 144.8, 148.8, 151.9, 173.2. *Anal.* Calcd for C<sub>15</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub>: C, 70.85; H, 5.55; N, 11.02. Found: C, 70.69; H, 5.69; N, 11.00.

**3-[1-(8-Quinolin-3-ylamino)-ethylidene]-4,5-dihydrofuran-2-one (9)** This compound was obtained in 85% yield from 8-aminoquinoline (**4**) according to the general procedure used for the synthesis of **6**; mp 130–132 °C. MS (relative intensity) *m/z*: 254 (M<sup>+</sup>, 53), 209 (84), 169 (96). <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 2.24 (3H, s), 2.98 (2H, t, *J*=8.0 Hz), 4.34 (2H, t, *J*=8.0 Hz), 7.33 (4H, m), 8.13 (1H, d, *J*=8.2 Hz), 8.91 (1H, d, *J*=3.7 Hz), 11.39 (1H, s). <sup>13</sup>C-NMR δ: 15.4, 26.8, 65.2, 92.81, 117.4, 121.6, 121.8, 121.9, 126.3, 128.8, 135.9, 136.8, 149.3, 151.3, 174.5. *Anal.* Calcd for C<sub>15</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub>: C, 70.85; H, 5.55; N, 11.02. Found: C, 70.89; H, 5.39; N, 10.89.

**3-[1-(5-Isoquinolin-3-ylamino)-ethylidene]-4,5-dihydrofuran-2-one (10)** This compound was obtained in 83% yield from 5-aminoisoquinoline (**5**) according to the general procedure used for the synthesis of **6**; mp 126–128 °C. MS (relative intensity) *m/z*: 254 (M<sup>+</sup>, 97), 226 (30), 195 (53). <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 1.79 (3H, s), 2.80 (2H, t, *J*=8.0 Hz), 4.25 (2H, t, *J*=8.0 Hz), 7.48 (4H, m), 8.37 (1H, d, *J*=6.0 Hz), 9.09 (1H, s), 10.13 (1H, s). <sup>13</sup>C-NMR δ: 17.6, 26.3, 65.6, 90.8, 115.4, 125.3, 126.2, 126.8, 129.4, 132.6, 134.5, 143.8, 152.8, 153.9, 174.3. *Anal.* Calcd for C<sub>15</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub>: C, 70.85; H, 5.55; N, 11.02. Found: C, 70.98; H, 5.49; N, 11.22.

**1-Chloro-2-(2-chloroethyl)-3-methylbenzo[*f*][1,7]naphthyridine (11)** Compound (**6**) (1 g, 4 mmol) was heated with phosphorus oxychloride (POCl<sub>3</sub>, 25 ml) until the end of the exothermic reaction (80–90 °C). The mixture was then refluxed for 4 h. Excess phosphorus oxychloride was removed, water was added and the mixture was neutralized with a saturated solution of sodium carbonate. After extraction with dichloromethane, the organic layers were washed with brine (50 ml), then dried (Na<sub>2</sub>SO<sub>4</sub>). Solvent evaporation gave a black oily product which was purified on a neutral alumina and eluted with dichloromethane to afford 0.87 g (75%) of **11**; mp 146–148 °C. (Recrystallization solvent ether–methanol 85/15). MS (relative intensity) *m/z*: 290 (M<sup>+</sup>, 55), 241 (97), 243 (35). <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 2.98 (3H, s), 3.56 (2H, t, *J*=7.0 Hz), 3.68 (2H, t, *J*=7.0 Hz), 7.79 (2H, m), 8.18 (1H, d, *J*=6.8 Hz), 9.37 (1H, s), 9.60 (1H, dd, *J*=9.2, 2.0 Hz). <sup>13</sup>C-NMR δ: 22.6, 34.1, 41.1, 122.3, 126.2, 126.7, 127.3, 129.3, 130.7, 133.7, 141.2, 142.3, 144.1, 154.4, 159.1. *Anal.* Calcd for C<sub>15</sub>H<sub>12</sub>N<sub>2</sub>Cl<sub>2</sub>: C, 61.87; H, 4.15; N, 9.62. Found: C, 61.76; H, 4.35; N, 9.51.

**3-(2-Chloroethyl)-4-chloro-2-methyl-1,7-phenanthroline (12)** This compound was obtained in 61% yield from **7** according to the general procedure used for the synthesis of **11**, mp 146–148 °C. (Recrystallization solvent ether–methanol 85/15). MS (relative intensity) *m/z*: 290 (M<sup>+</sup>, 46), 241 (96), 206 (32). <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 2.85 (3H, s), 3.41 (2H, t, *J*=7.0 Hz), 3.62 (2H, t, *J*=7.0 Hz), 7.52 (2H, dd, *J*=12.0, 4.7 Hz), 8.06 (1H, d, *J*=10.0 Hz), 8.20 (1H, d, *J*=10.0 Hz), 9.01 (1H, d, *J*=2.9 Hz), 9.42 (1H, d, *J*=8.5 Hz). <sup>13</sup>C-NMR δ: 24.5, 33.7, 41.6, 122.0, 122.7, 124.9, 128.7, 128.8, 129.2, 133.0, 142.2, 144.5, 149.22, 154.6, 158.0. *Anal.* Calcd for C<sub>15</sub>H<sub>12</sub>N<sub>2</sub>Cl<sub>2</sub>: C, 61.87; H, 4.15; N, 9.62. Found: C, 61.99; H, 4.22; N, 9.56.

**1-Chloro-2-(2-chloroethyl)-3-methyl-4,7-phenanthroline (13)** This compound was obtained in 69% yield from **8** according to the general procedure used for the synthesis of **11**; mp 120–122 °C. (Recrystallization solvent ether–methanol 85/15). MS (relative intensity) *m/z*: 290 (M<sup>+</sup>, 37), 241 (94), 206 (23). <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 2.66 (3H, s), 3.37 (2H, t, *J*=7.0 Hz), 3.63 (2H, t, *J*=7.0 Hz), 7.35 (2H, dd, *J*=4.5, 3.9 Hz), 7.88 (1H, d, *J*=9.0 Hz), 7.93 (1H, d, *J*=9.0 Hz), 8.70 (1H, d, *J*=8.0 Hz), 9.75 (1H, d, *J*=9.0 Hz). <sup>13</sup>C-NMR δ: 24.1, 33.8, 41.2, 120.4, 124.2, 124.5, 124.5, 131.7, 132.9, 134.4, 141.5, 147.7, 148.2, 149.4, 158.0. *Anal.* Calcd for C<sub>15</sub>H<sub>12</sub>N<sub>2</sub>Cl<sub>2</sub>: C, 61.87; H, 4.15; N, 9.62. Found: C, 62.01; H, 4.08; N, 9.69.

**4-Chloro-3-(2-chloroethyl)-2-methyl-1,10-phenanthroline (14)** This compound was obtained in 54% yield from **9** according to the general procedure used for the synthesis of **11**, mp 204–206 °C. (Recrystallization solvent hexane–dichloromethane 85/15). MS (relative intensity) *m/z*: 291 (M<sup>+</sup>, 38), 290 (50), 241 (97). <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 3.05 (3H, s), 3.55 (2H, t, *J*=7.0 Hz), 3.92 (2H, t, *J*=7.0 Hz), 7.74 (1H, dd, *J*=12.3, 4.3 Hz), 7.84 (1H, d, *J*=9.3 Hz), 8.19 (1H, d, *J*=9.3 Hz), 8.22 (1H, m), 9.19 (1H, d, *J*=2.6 Hz).

$^{13}\text{C}$ -NMR  $\delta$ : 24.5, 33.5, 41.1, 122.1, 123.4, 125.0, 126.5, 128.1, 130.3, 135.7, 142.1, 144.7, 145.1, 150.4, 160.2. *Anal.* Calcd for  $\text{C}_{15}\text{H}_{12}\text{N}_2\text{Cl}_2$ : C, 61.87; H, 4.15; N, 9.62. Found: C, 61.99; H, 4.23; N, 9.76.

**4-Chloro-3-(2-chloroethyl)-2-methyl-1,8-phenanthroline (15)** This compound was obtained in 63% yield from **10** according to the general procedure used for the synthesis of **11**; mp 148–150 °C. (Recrystallization solvent ether–ethyl acetate 70/30). MS (relative intensity)  $m/z$ : 290 ( $\text{M}^+$ , 48), 241 (94), 206 (29).  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ )  $\delta$ : 2.86 (3H, s), 3.52 (2H, t,  $J=7.0$  Hz), 3.76 (2H, t,  $J=7.0$  Hz), 7.77 (1H, d,  $J=9.0$  Hz), 8.03 (1H, d,  $J=9.0$  Hz), 8.74 (1H, d,  $J=5.5$  Hz), 8.92 (1H, d,  $J=5.5$  Hz), 9.24 (1H, s).  $^{13}\text{C}$ -NMR  $\delta$ : 24.4, 33.7, 41.4, 117.4, 122.7, 124.8, 125.9, 127.9, 130.0, 135.0, 142.1, 143.5, 145.7, 151.0, 158.1. *Anal.* Calcd for  $\text{C}_{15}\text{H}_{12}\text{N}_2\text{Cl}_2$ : C, 61.87; H, 4.15; N, 9.62. Found: C, 61.69; H, 4.31; N, 9.69.

**4-Chloro-3-(2-chloroethyl)-2,10-dimethyl-1,10-phenanthroline Iodide (16)** A solution of 2-methyl-3-(1'-chloroethyl)-4-chloropyrido[2,3-*i*]quinoline (**14**) (0.29 g, 1 mmol) and methyl iodide (5 mmol) in acetone (15 ml) was refluxed for 12 h. The resulting mixture was then cooled. The precipitate which formed was filtered, and washed with acetone to give 0.35 g (80%) of **16**; mp 186–188 °C. (Recrystallization solvent dichloromethane–ether 1/1).  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ )  $\delta$ : 2.86 (3H, s), 3.48 (2H, t,  $J=7.0$  Hz), 3.70 (2H, t,  $J=7.0$  Hz), 5.31 (3H, s), 8.14 (1H, d,  $J=9.0$  Hz), 8.16 (1H, dd,  $J=10.0, 3.0$  Hz), 8.45 (1H, d,  $J=9.0$  Hz), 9.14 (1H, d,  $J=5.0$  Hz), 9.66 (1H, d,  $J=4.5$  Hz).  $^{13}\text{C}$ -NMR  $\delta$ : 24.5, 33.2, 41.1, 55.1, 124.8, 126.7, 126.9, 128.7, 132.1, 135.7, 136.8, 139.2, 143.0, 146.8, 151.7, 159.1. *Anal.* Calcd for  $\text{C}_{16}\text{H}_{15}\text{N}_2\text{Cl}_2\text{I}$ : C, 44.45; H, 3.50; N, 6.48. Found: C, 44.23; H, 3.37; N, 6.29.

**4-Chloro-3-(2-chloroethyl)-2-methyl-1,10-phenanthroline Chloride (17)** This product was obtained after bubbling HCl gas through a solution of **14** in ethanol until the hydrochloride salt of **14** was precipitated out of the solution; yield 95%, mp >260 °C.  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ )  $\delta$ : 2.75 (3H, s), 3.37 (2H, t,  $J=7.0$  Hz), 3.81 (2H, t,  $J=7.0$  Hz), 7.71–8.07 (3H, m), 8.73 (1H, d,  $J=8.6$  Hz), 8.88 (1H, d,  $J=5.2$  Hz).

**Plasmodium falciparum Growth Inhibition Assay** Three strains of *P. falciparum* were used: the chloroquine resistant FcB1-Columbia and FcM29 strains, and a Nigerian chloroquine-sensitive strain.<sup>11)</sup> Parasites were cultured according to the method originally described by Trager and Jensen.<sup>8)</sup> Culture medium was replaced daily and the cultures were synchronized every 48 h by 5% D-sorbitol lysis (Merck, Darmstadt, Germany).<sup>12)</sup> The method used for *in vitro* antimalarial activity testing was adapted from the radioactive micromethod of Desjardins *et al.*<sup>13)</sup> The molecules were tested 3 times in triplicate in 96-well plates (TPP, Switzerland) with cultures at ring stage at 0.5–1% parasitaemia (hematocrit: 1%). For each test, the parasite cultures were incubated with the chemicals at decreasing concentrations for 24 and 72 h. The first dilution of the product (10 mg/ml) was performed with dimethylsulfoxide (DMSO, Merck), and the following with RPMI 1640. Parasite growth was estimated by [ $^3\text{H}$ ]-hypoxanthine (Amersham-France, Les Ulis, France) incorporation.<sup>11)</sup> The parasite [ $^3\text{H}$ ]-hypoxanthine incorporation in the presence of chemicals was compared with that of control cultures without chemicals (mean of the corresponding wells was referred to as

100%) or with DMSO. Concentrations inhibiting 50% of the parasite [ $^3\text{H}$ ]-hypoxanthine incorporation ( $\text{IC}_{50}$ ) were determined graphically in concentration *versus* percent inhibition curves. Chloroquine (diphosphate, Sigma) sensitivity was checked every month: the  $\text{IC}_{50}$  value for the Nigerian chloroquine-sensitive strain was 41 (<70 nM) and for the chloroquine-resistant FcB1-Columbia and FcM29 strains, the  $\text{IC}_{50}$  were 145 nM and 210 nM respectively (>100 nM).<sup>14)</sup> Toxicity of the chemicals was estimated on human fibroblasts (HeLa). The cell line was cultured under the same conditions as *P. falciparum*, except for 5% human serum which was replaced by 5% fetal calf serum (Boehringer). Subcultures were obtained after treatment with Trypsine (0.125%) EDTA (0.02%) in PBS (Gibco). For determination of the cytotoxicity, cells were distributed in 96-well plates at  $2 \times 10^3$  cells/well in 100  $\mu\text{l}$ , then 100  $\mu\text{l}$  of culture medium containing chemicals at various concentrations were added. Cell growth was estimated by [ $^3\text{H}$ ]-hypoxanthine (Amersham) incorporation after 24 and 72 h incubations. The [ $^3\text{H}$ ]-hypoxanthine incorporation in the presence of drugs was compared with that of control cultures without chemicals.<sup>11)</sup>

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of analytical reagent grade, purchased from Nakalai Tesque, Kyoto, Japan.

**Thermal Analysis** The crystals of lisinopril dihydrate with or without preheating were examined by DSC (DSC-910, TA Instru. Inc., New Castle, DE, U.S.A.) at a heating rate of 3 °C/min with an open pan system in a stream of N<sub>2</sub> gas. TGA (TGA-951, TA Instru. Inc., New Castle, DE, U.S.A.) was also performed at the same heating rate to determine the weight loss of sample.

**Thermal FT-IR Microspectroscopic Study**<sup>10–15</sup> A small amount of lisinopril dihydrate was sealed within two pieces of KBr pellets by hydraulic press. This compressed KBr disc was directly put on a DSC microscopy cell (FP 84, Mettler, Greifensee, Switzerland) and the cell was then placed on the stage of the microscope within the FT-IR microscopic spectrometer (Micro FTIR-200, Jasco, Tokyo, Japan) by a mercury-cadmium-telluride (MCT) detector. The system was operated in the transmission mode. The position and focus of sample were adjusted by the microscope. Temperature of DSC microscopy cell was monitored with a central processor (FT80HT, Mettler). The heating rate of the DSC assembly was controlled at 3 °C/min in ambient condition. During the experiment, the sample disc was first equilibrated to the starting temperature (25 °C) for about 3 min and then heated from 25 to 200 °C. The thermal-responsive IR spectra were recorded while the sample disc was heated on the DSC microscope stage.

## Results and Discussion

The DSC thermograms and TGA curve of lisinopril dihydrate are depicted in Fig. 1. Three main endothermic peaks at 76, 99 and 167 °C were observed from the DSC thermograms of the solid-state sample of lisinopril dihydrate (Fig. 1, a). In the same temperature range, two obvious steps of weight loss before 120 °C were seen from the TGA curve. The first loss in the curve corresponding to the endothermic peak at 76 °C was about 4.0%, and the second loss corresponding to the endothermic peak at 99 °C was also about 4.0%. About 1% of weight loss found at the initial stage was due to evaporation of the adsorbed water. The 4.0% weight loss was equal to almost 4.07% of water loss from lisinopril dihydrate and to 4.25% of water loss from lisinopril monohydrate (molecular weight of lisinopril dihydrate: 441.5). This suggests that the endothermic peaks at 76 and 99 °C might be the result of a two-step dehydration process from the solid sample. The endothermic peak at 167 °C might have to do with the melting of the sample, which will be discussed in the following section. To confirm the two-step dehydration, the solid-state sample of lisinopril dihydrate was pre-heated to 80 or 100 °C, respectively, and then again determined by DSC. It is evident that the endothermic peak at 76 °C disappeared from the DSC curve of the 80 °C-preheated sample, but the peaks at 95 and 167 °C remained (Fig. 1, b). Both endothermic peaks at 76 and 99 °C disappeared from the DSC curve of the 100 °C-preheated sample, but the peak at 167 °C remained (Fig. 1, c). This strongly indicates that dehydration from the solid-state lisinopril dihydrate occurred in a two-step process from dihydrate to monohydrate and then from monohydrate to anhydrate.

Figure 2 shows the three-dimensional plots of FT-IR spectra of lisinopril dihydrate as a function of temperature between 3700–2500 and 1800–1200 cm<sup>-1</sup> wavenumbers. The peak at 3554 cm<sup>-1</sup> is assigned to the stretching vibrations of an O–H band of water. The peaks at 3340 and 3296 cm<sup>-1</sup> are due to the asymmetric and symmetric N–H band of primary amine with hydrogen bonding. The peak at 3099 cm<sup>-1</sup> is due to the aromatic C–H stretching, and the peaks at 2962 and 2925 cm<sup>-1</sup> are assigned to the asymmetric C–H stretching vibrations, respectively. The peak at 1655 cm<sup>-1</sup> is attributed to the carbonyl stretching of tertiary amide and/or scissoring

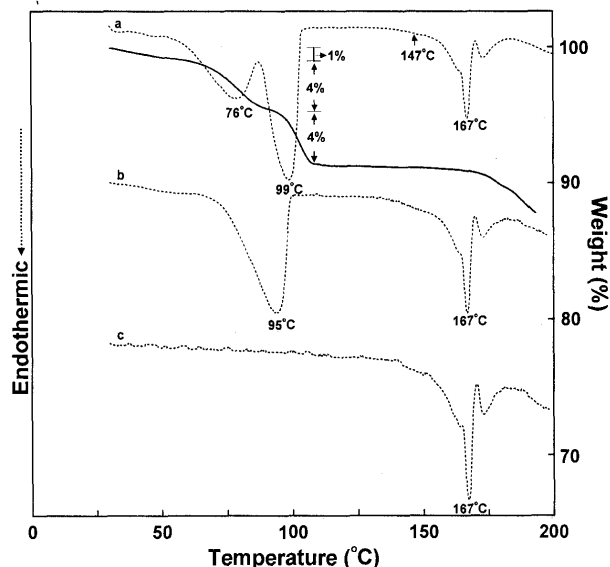


Fig. 1. DSC Thermograms (Dotted Line) and TGA Curve (Solid Line) of Lisinopril Dihydrate

(a) Sample without preheating, (b) 80 °C-preheated sample, (c) 100 °C-preheated sample.

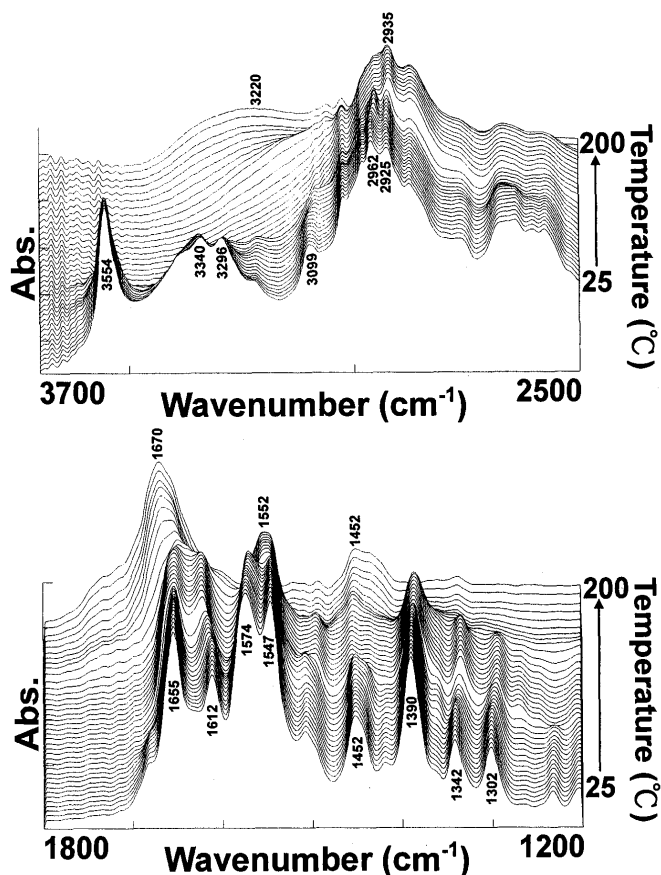


Fig. 2. Three-Dimensional Plots of FT-IR Spectra of Lisinopril Dihydrate as a Function of Temperature

NH<sub>2</sub>; that at 1612 cm<sup>-1</sup> is due to the aromatic ring mode, and those at 1574 and 1547 cm<sup>-1</sup> correspond to the asymmetric carboxylate and/or ring mode of the aromatic group; the peak at 1452 cm<sup>-1</sup> indicates the CH<sub>2</sub> scissoring mode and the peak at 1390 cm<sup>-1</sup> is due to the symmetric carboxylate.<sup>16</sup> With the increase of temperature, the IR spectral contour and several frequencies corresponding to the water (3554 cm<sup>-1</sup>), hydro-

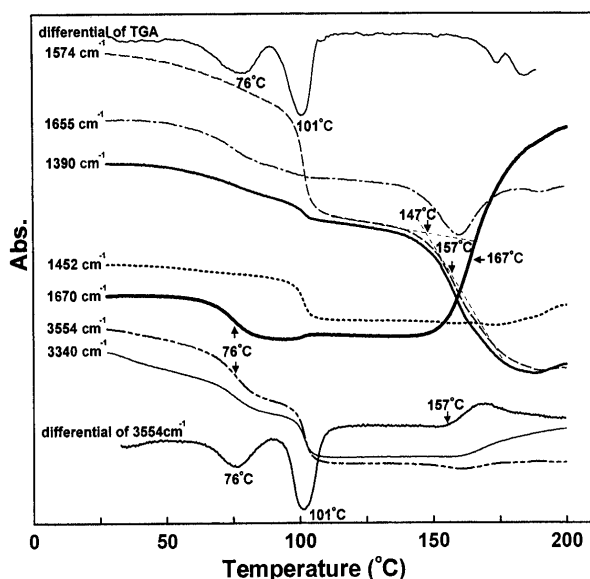


Fig. 3. Temperature-Dependent Changes in Peak Intensity of the Selected IR Bands of Lisinopril Dihydrate

gen bonding of primary amine ( $3340$ ,  $3296\text{ cm}^{-1}$ ) and carboxylate ( $1574$ ,  $1390\text{ cm}^{-1}$ ) significantly changed. Moreover, a new peak at  $1670\text{ cm}^{-1}$  assigned to the carbonyl band of DKP formation due to intramolecular cyclization was clearly evidenced.<sup>10,16,17</sup> The conformational changes result in the disappearance of hydrogen-bonding bands and the formation of DKP in solid-state lisinopril dihydrate. The water of the reaction by-product was liberated after the temperature reached  $157^\circ\text{C}$  and appeared on the broad IR spectra near about  $3200\text{--}3400\text{ cm}^{-1}$ . The persistence of the IR spectra of water ranging from  $3200\text{--}3400\text{ cm}^{-1}$  was still observed even at higher temperature and might due to the by-product "water" being tightly sealed within KBr discs. In addition, the interaction of water with KBr might induce the peak at  $3500\text{ cm}^{-1}$  (assigned to free water) to shift to a lower wavenumber ( $3200\text{--}3400\text{ cm}^{-1}$ ). Due to the DKP formation the peak at  $1574\text{ cm}^{-1}$  assigned to carboxylate shifted to  $1552\text{ cm}^{-1}$ .

To verify the dehydration process and DKP formation from lisinopril dihydrate, the changes in IR peak intensity of several specific bands with temperature are displayed in Fig. 3. Obviously, the peak intensity of these specific bands of lisinopril dihydrate decreased gradually with temperature and were decreased apparently, especially near  $76\text{--}101^\circ\text{C}$ . The bands at  $3554$  ( $\nu\text{OH}$ ) and  $3340$  ( $\nu\text{NH}$ )  $\text{cm}^{-1}$  were observed with two-step change, as confirmed by the differential curves of TGA and the  $3340\text{ cm}^{-1}$  band. Factors such as crystal packing, nucleation and hydrogen bonding are assumed to influence solid-state desolvation reaction.<sup>18,19</sup> The overall dehydration process might consist of two simple processes: the breaking of hydrogen bonding and the rapid escape of water from hydrate. The former is more related with IR spectral change, while the latter is easily obtained by TGA. In the present study, dehydration was found to undergo a two-step process at the same temperature, one with a differential curve of the  $3340\text{ cm}^{-1}$  band due to the breaking of hydrogen bonding, and the other with the differential TGA curve due to the escape of water from hydrate. The breaking of hydrogen bonding was a rate-determining step. Other peaks at  $1574$ ,

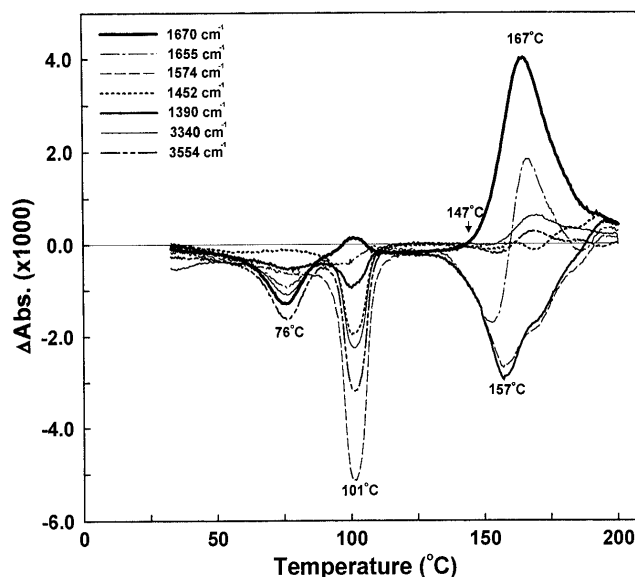


Fig. 4. Differential Curves of Peak Intensity of the Selected IR Bands of Lisinopril Dihydrate

$1390$  and  $1542\text{ cm}^{-1}$  changed in peak intensity at  $101^\circ\text{C}$ , and the peak at  $1655\text{ cm}^{-1}$  altered more pronouncedly from  $76^\circ\text{C}$ . Although the peak at  $1670\text{ cm}^{-1}$  also showed a slight change within  $76\text{--}101^\circ\text{C}$ , the overlapping of  $1655\text{ cm}^{-1}$  might be responsible for this.

The dehydration process seemed to induce the structural transformation of lisinopril dihydrate to change the IR peak intensity of different functional bands with temperature. The peaks at  $1655$ ,  $1574$  and  $1390\text{ cm}^{-1}$  assigned to the carboxylate groups reduced in intensity gradually from above  $147^\circ\text{C}$  and markedly at  $157^\circ\text{C}$ . In the meantime, the appearance of  $1670\text{ cm}^{-1}$  also slightly changed in peak intensity from  $157^\circ\text{C}$  but was significantly altered near  $167^\circ\text{C}$ . Moreover, the peak intensity at  $3340\text{ cm}^{-1}$  (N-H band of primary amine), also assigned to the stretching OH vibration of water increased again from  $157^\circ\text{C}$ . This strongly implies that DKP and water can be produced by the thermal-dependent intramolecular cyclization in anhydrous lisinopril between  $147\text{--}157^\circ\text{C}$ . The changes of FT-IR spectra give more evidence of the DKP formation in lisinopril than do those from DSC thermogram. Three main processes corresponding to dehydration were also observed in the differential IR bands: from dihydrate to monohydrate at  $76^\circ\text{C}$ , from monohydrate to anhydrate at  $101^\circ\text{C}$ , and DKP formation *via* intramolecular cyclization at  $>147^\circ\text{C}$  (Fig. 4). The process of dehydration and DKP formation of solid-state lisinopril dihydrate is postulated in Chart 1.

To verify that the DKP formation *via* intramolecular cyclization was a solid state reaction, an isothermal study of solid-state lisinopril dihydrate was made at  $135^\circ\text{C}$  before the fusion point of  $167^\circ\text{C}$ . Figure 5 shows the three-dimensional plots of IR spectra of lisinopril dihydrate between  $1800$  and  $1200\text{ cm}^{-1}$ , with respect to isothermal time. Only a slight shift in IR spectra for the sample was found due to the difference in temperature ( $135$ ,  $25^\circ\text{C}$ ). With the increase of isothermal time at  $135^\circ\text{C}$ , however, the peaks at  $1612$  and  $1651\text{ cm}^{-1}$  gradually disappeared and were replaced by a broad spectrum near  $1651\text{ cm}^{-1}$ . If the sample was directly changed to a non-isothermal study from  $135^\circ\text{C}$  (solid state)

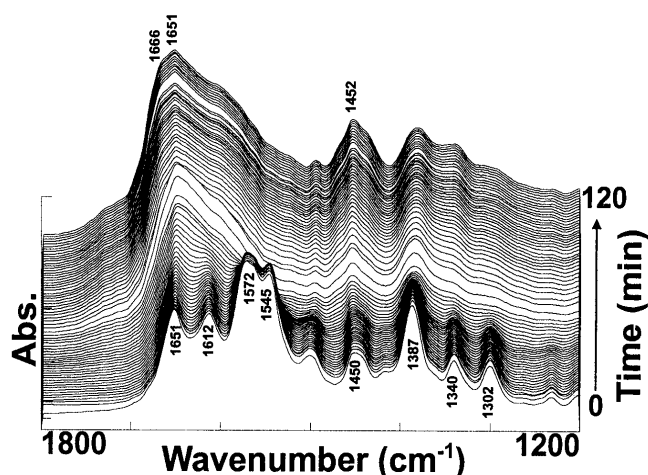


Fig. 5. Three-Dimensional Plots of FT-IR Spectra of Lisinopril Dihydrate between 1800 and 1200  $\text{cm}^{-1}$  with Respect to the Isothermal Time at 135 °C

to 200 °C (liquid state), the IR peak at 1651  $\text{cm}^{-1}$  shifted to 1670  $\text{cm}^{-1}$ . This broad spectrum found at 1651  $\text{cm}^{-1}$  might be assigned to the solid-state DKP formation through the isothermal study at 135 °C, implying that the DKP formation occurred in the solid-state lisinopril. Thus, it should be noted that the fusion peak at 167 °C in the DSC curve might be attributed to the melting of DKP rather than to lisinopril.

In conclusion, the thermal-dependent dehydration process and intramolecular cyclization of lisinopril dihydrate in the solid state were effectively studied using a novel FT-IR microspectroscope equipped with differential scanning

calorimetry. Two-step dehydration was clearly evidenced from the changes in FT-IR spectra. Moreover, the solid-state DKP formation in lisinopril *via* intramolecular cyclization was also quickly and directly investigated by this thermal FT-IR microscopic system.

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## Determination of Residual Solvents in Bulk Pharmaceuticals by Thermal Desorption/Gas Chromatography/Mass Spectrometry

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Received June 2, 2000; accepted August 3, 2000

Thermal desorption (TD) techniques followed by capillary GC/MS were applied for the analysis of residual solvents in bulk pharmaceuticals. Solvents desorbed from samples by heating were cryofocused at the head of a capillary column prior to GC/MS analysis. This method requires a very small amount of sample and no sample pretreatment. Desorption temperature was set at the point about 20 °C higher than the melting point of each sample individually. The relative standard deviations of this method tested by performing six consecutive analyses of 8 different samples were 1.1 to 3.1%, and analytical results of residual solvents were in agreement with those obtained by direct injection of *N,N*-dimethylformamide solution of the samples into the GC. This novel TD/GC/MS method was demonstrated to be very useful for the identification and quantification of residual solvents in bulk pharmaceuticals.

**Key words** thermal desorption; GC/MS; residual solvent; bulk pharmaceutical

The analysis of residual solvents in pharmaceuticals is very important because of the potential risk to human health from the toxicity of many of these solvents<sup>1)</sup>; the amounts of such solvents are therefore restricted by International Conference on Harmonization (ICH) guidelines.<sup>2)</sup> Gas chromatography (GC) is generally used to determine residual solvents because of its excellent separation ability and low detection limit. Samples are dissolved in a suitable solvent followed by direct injection (DI)<sup>3,4)</sup> or headspace sampling,<sup>5,6)</sup> or are extracted with a suitable solvent.<sup>7,8)</sup> Although the DI method is both rapid and convenient, less volatile sample components or dissolving solvents could interfere with the subsequent analysis, and interactions between dissolving solvents and other sample components in the injection port can cause various problems.<sup>9)</sup> Headspace sampling has the advantage of introducing only a limited number of solvents onto the GC column, however, it requires specialized apparatus and is an imprecise analysis. Solvent extraction may introduce experimental problems such as selectivity of extraction, inconvenient sample treatment, and variable recoveries.

Thermal desorption (TD) followed by GC/MS has been successfully employed in the analysis of volatile compounds in many samples<sup>10–14)</sup>; several kinds of pyrolyzers have been used in these studies. However, few studies applying this technique to the determination of residual solvents in pharmaceuticals have been performed<sup>14)</sup>; moreover, they are not based on quantitative determination.

The aim of the work described here was to develop and evaluate a new method for the identification and quantification of residual solvents in bulk pharmaceuticals on capillary GC/MS without sample pretreatment, such as dissolution in a solvent or solvent extraction. This method was based on TD of residual solvents from samples using a double shot pyrolyzer. Desorbed solvents are cryofocused at the head of a separation column prior to capillary GC/MS analysis. To investigate the optimum conditions for the determination method, 8 different bulk pharmaceuticals containing various residual solvents were used.

### Experimental

**Materials and Reagents** Ethyl aminobenzoate and phenacetin were commercial products of JP XIII grade and obtained from Maruishi Pharmaceutical Co., Ltd. (Osaka, Japan). Isoniazid was a commercial product of JP XIII grade from Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan). Other samples were of biochemical grade and obtained from Wako Pure Chemical Industries, Ltd. (Osaka). Solvents were of reagent grade and also obtained from Wako Pure Chemical Industries, Ltd.

**TD of Residual Solvents in Pharmaceutical Samples** A vertical furnace-type double shot pyrolyzer (Frontier Lab Co., Ltd., Koriyama, Japan) mounted on top of a Shimadzu Model QP5050A GC/MS with an electron impact ion source was used. Figure 1 shows the schematic diagram of this pyrolyzer.

About 1 mg of a sample accurately weighed was placed inside the sample cup (5.0 mm×3.8 mm i.d.) and TD was performed. TD was achieved at 100 °C, or by raising the temperature of the furnace from 100 °C to the desired temperature (120 to 220 °C range) at a rate of 50 °C/min. The TD period was set for 3 min. The volatile substances were desorbed from the sample in a stream of carrier gas and collected at the head of a capillary column, where

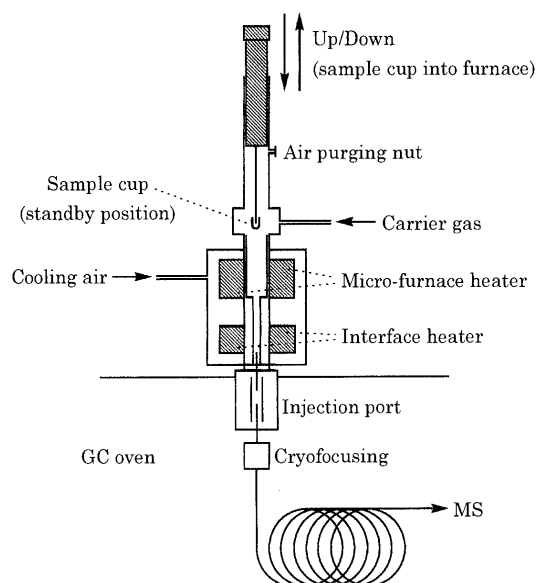


Fig. 1. Schematic Diagram of Vertical Furnace-type Double Shot Pyrolyzer

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cryogenic cooling was done with liquid nitrogen during the period of TD. By discontinuing cryofocusing, the temperature of the column head was raised to 40 °C (initial column oven temperature), and the trapped compounds were transferred to an analytical column.

**Standard Preparation for TD Based Analysis** Stock standard solvents were prepared by weighing approximately 1.0 g of methanol, ethanol, acetone, toluene, and heptane into a 20-ml volumetric flask, diluting to volume with *N,N*-dimethylformamide (DMF), and mixing. Further, standard solvents for calibration were prepared by suitably diluting the stock standard solvents with DMF to give solutions over the concentration range of 25 to 1000 µg/ml, which corresponds to a solvent level of 25 to 1000 ppm by weight in the sample. One microliter of these standard solvents was transferred into a sample cup for calibration, and heated up to 160 °C with cryofocusing in the above manner.

**GC and MS Conditions for TD Based Analysis** The column used was a 30 m×0.25 mm i.d. fused silica column coated with a 0.5 µm film of polyethyleneglycol (DB-Wax, J & W Scientific Co., Ltd., Folsom, CA, U.S.A.). The split injection mode was used with an approximate splitting ratio of 1:50. The carrier gas was helium, the average column linear velocity was about 35 cm/s, and injector temperature was 270 °C. The column temperature was programmed at 40 °C for 5 min, increased to 175 °C at 8 °C/min, then to 250 °C at 35 °C/min, and finally maintained at 250 °C for at least 16 min. Mass spectra were recorded under electron-impact ionization at 70 eV from *m/z* 35 to 300 and the ion source was maintained at 280 °C.

**Sample and Standard Preparation for DI Based Analysis** Approximately 0.1 g of a sample was weighed, put into a 5-ml volumetric flask, dissolved and diluted to the volume with DMF, and mixed. Standard solvents were prepared by diluting with the stock standard solvents, described in the previous section, 10000 times with DMF, which corresponds to a solvent level of 250 ppm by weight in the sample. Equal volumes (about 1 µl) of the sample and standard were separately injected into the GC.

**GC Conditions for DI Based Analysis** Quantitative analyses were performed on a Shimadzu Model GC-15A gas chromatograph equipped with a flame ionization detector and a Shimadzu Model AOC-17 auto injector. Sample and standard solutions were injected in the direct mode. The analytical column used was a 30 m×0.53 mm i.d. fused silica column coated with a 5.0 µm film of 5% diphenyl, 95% dimethylsiloxane (SPB-5, Supelco Co., Ltd., Bellefonte, PA, U.S.A.). A 5 m×0.53 mm i.d. fused silica column coated with 0.5 µm film of SPB-5 was used as a guard column to retain the injected compounds and prevent contamination of the analytical column.

Table 1. List of Sample Names and Residual Solvents Contained

| No. | Sample              | Residual solvent  | Class |
|-----|---------------------|-------------------|-------|
| 1   | Lidocaine           | Heptane           | 3     |
| 2   | Ethyl aminobenzoate | Toluene           | 2     |
| 3   | Antipyrine          | Methanol          | 2     |
| 4   | Phenacetin          | Methanol, toluene | 2, 2  |
| 5   | Cimetidine          | Ethanol           | 3     |
| 6   | Famotidine          | Methanol          | 2     |
| 7   | Isoniazid           | Methanol          | 2     |
| 8   | Carbamazepine       | Acetone           | 3     |

The carrier gas was helium, and the average column linear velocity determined by injections of methane was about 35 cm/s. Nitrogen was used as the make-up gas at a flow rate of 50 ml/min. The injector and detector temperatures were 140 and 260 °C, respectively. The column temperature was programmed at 35 °C for 10 min, raised to 175 °C at 8 °C/min, then to 260 °C at 35 °C/min, and finally maintained at 260 °C for at least 16 min. The injection volume was 1.0 µl.

## Results and Discussion

**Effect of Desorption Temperature** The effect of temperature on the TD was examined using 8 different samples in a 100 to 220 °C range. Table 1 lists the sample names together with the residual solvents contained. These solvents are classified as Class 2 (solvents to be limited) or Class 3 (solvents with low toxic potential) based on the ICH guidelines.<sup>2)</sup>

Desorption at high temperature may be accompanied by the pyrolysis of samples, while low temperature may result in the incomplete desorption of solvents. Therefore, TD was performed at a temperature range set for each individual sample. A desorption period of 3 min seemed advisable, since a longer period increased overall analysis time and consumed a large quantity of the liquid nitrogen for the cryofocusing. Quantitative data were calculated from total ion current (TIC) peak areas. The results are summarized in Table 2 and are expressed as the relative value (%) to the maximum amount of the solvent found in the sample.

When desorption temperature was at least 20 °C higher than the melting point of each sample, desorption of solvents reached a plateau. This desorption characteristic was observed regardless of the kinds of sample and residual solvent. Although famotidine started to pyrolyze above its melting (decomposition) point, it did not interfere with the analysis. Taking into account the damage of the analytical column caused by pyrolytic compounds, TD temperature was set at a point about 20 °C higher than the melting point of each sample.

**Standard Linearity** The linearity of the relationship between peak area and concentration (ppm) for the 5 solvents contained in the samples (Table 1) used in the present study was investigated by linear regression analysis by the method of least squares. The linear range investigated for each solvent was from 25 to 1000 ng, which corresponds to a solvent level of 25 to 1000 ppm based on a sample amount of 1 mg. The equation of the best-fit line was found for each solvent. The detection limits of these solvents were defined as the

Table 2. Effect of Temperatures on the Desorption of Residual Solvents for Different Samples

| Sample <sup>a)</sup> | Melting point (°C) | Residual solvent | Relative concentration (%) |              |              |              |              |              |             |
|----------------------|--------------------|------------------|----------------------------|--------------|--------------|--------------|--------------|--------------|-------------|
|                      |                    |                  | 100 °C                     | 120 °C       | 140 °C       | 160 °C       | 180 °C       | 200 °C       | 220 °C      |
| 1                    | 68                 | Heptane          | <b>99.3</b>                | <b>99.6</b>  | <b>99.6</b>  | <b>100.0</b> | —            | —            | —           |
| 2                    | 89                 | Toluene          | <b>99.7</b>                | <b>100.0</b> | <b>99.9</b>  | <b>99.4</b>  | —            | —            | —           |
| 3                    | 112                | Methanol         | 83.4                       | <b>99.5</b>  | <b>100.0</b> | <b>98.7</b>  | —            | —            | —           |
| 4                    | 134                | Methanol         | —                          | 84.1         | <b>99.2</b>  | <b>100.0</b> | <b>99.6</b>  | —            | —           |
|                      |                    | Toluene          | —                          | 90.5         | <b>99.8</b>  | <b>98.8</b>  | <b>100.0</b> | —            | —           |
| 5                    | 142                | Ethanol          | —                          | 0            | 84.2         | <b>98.3</b>  | <b>99.6</b>  | <b>100.0</b> | <b>99.9</b> |
| 6                    | 163                | Methanol         | —                          | 2.5          | 4.8          | 96.9         | <b>100.0</b> | <b>98.9</b>  | <b>99.4</b> |
| 7                    | 171                | Methanol         | —                          | 0            | 93.1         | <b>99.8</b>  | <b>100.0</b> | <b>98.8</b>  | <b>99.8</b> |
| 8                    | 191                | Acetone          | —                          | 7.4          | 24.8         | 87.9         | <b>100.0</b> | <b>98.9</b>  | <b>99.6</b> |

a) Numbers refer to sample listed in Table 1. Data are expressed as the relative value (%) to the maximum amount of solvent found in the sample. Data in bold are within the plateau range. Data indicated by — are not determined.

concentration which would give a peak height equal to three times the baseline noise. The slopes and intercepts of the best-fit lines, the correlation coefficients and detection limits for each solvent are presented in Table 3. Figure 2B shows a typical chromatogram of standard containing 5 solvents at concentrations of 500 ppm by weight in the sample. No appreciable difference was observed in the retention time between sample and standard analysis.

**Method Precision and Accuracy (Recovery)** The precision of the proposed method was tested by performing six consecutive TD/GC/MS analyses using all samples. Temperature of TD was set at a point about 20 °C higher than the melting point of each sample, as described above. The mean contents of residual solvents and relative standard deviations (RSDs) were calculated and compared with those obtained by conventional DI/GC method; the results are given in Table 4.

In spite of the very small sample amount used (1 mg), each sample has an RSD of less than 3.5%, indicating almost the same precision as the DI/GC method. To evaluate the accuracy, the percentage recoveries were calculated as the relative value of the amount obtained by the proposed method to those obtained by the DI/GC method (Table 4). The results show virtually complete accuracy (95.7–102.1%) of the solvents in every sample, and solvents detected by DI/GC method could also be detected by TD/GC/MS method. These satisfactory data of method precision and accuracy at the concentration level from 50 to 800 ppm suggest that Class 1 solvents (the lowest concentration limit is 2 ppm) in the ICH guidelines could not be determined, however, Class 2 solvents (the lowest concentration limit is 50 ppm) could be by the proposed method. Figures 2C–J shows typical chromatograms from analyses of the samples.

Table 3. Linearity Data for the Solvents Analyzed by the Proposed TD/GC/MS Method

| Solvent  | Slope               | Intercept           | Correlation coefficient ( <i>r</i> ) | Detection limit (ppm) |
|----------|---------------------|---------------------|--------------------------------------|-----------------------|
| Heptane  | $2.432 \times 10^4$ | $6.309 \times 10^3$ | 1.0000                               | 2                     |
| Acetone  | $1.310 \times 10^4$ | $2.317 \times 10^2$ | 0.9995                               | 5                     |
| Methanol | $1.305 \times 10^4$ | $1.210 \times 10^3$ | 0.9996                               | 25                    |
| Ethanol  | $1.612 \times 10^4$ | $7.686 \times 10^3$ | 1.0000                               | 25                    |
| Toluene  | $2.697 \times 10^4$ | $7.447 \times 10^3$ | 1.0000                               | 10                    |

## Conclusion

We have demonstrated that the proposed novel TD/GC/MS method is very useful for the identification and quantification of residual solvents in bulk pharmaceuticals. The conventional method of GC analysis of residual solvents in these pharmaceuticals requires sample pretreatment such as disso-

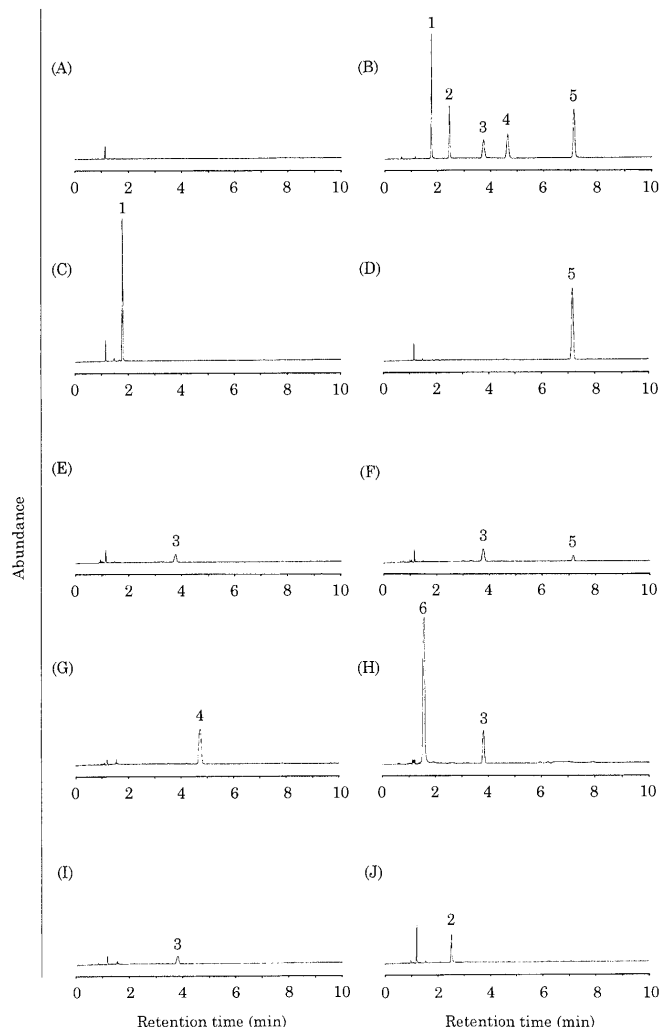


Fig. 2. TD/GC/MS Chromatograms Obtained from Blank, Standard and Samples

(A) Blank, (B) standard (500 ppm of each solvent), (C) lidocaine, (D) ethyl aminobenzoate, (E) antipyrine, (F) phenacetin, (G) cimetidine, (H) famotidine, (I) isoniazid, (J) carbamazepine. Peaks: 1=heptane, 2=acetone, 3=methanol, 4=ethanol, 5=toluene, 6=thermal degradation compound.

Table 4. Analytical Results of 8 Different Samples by TD/GC/MS Method

| Sample              | Residual solvent | Desorption temperature (°C) | TD/GC/MS <sup>a)</sup> |         | DI/GC <sup>a)</sup> |         | Accuracy <sup>b)</sup> (%) |
|---------------------|------------------|-----------------------------|------------------------|---------|---------------------|---------|----------------------------|
|                     |                  |                             | Mean value (ppm)       | RSD (%) | Mean value (ppm)    | RSD (%) |                            |
| Lidocaine           | Heptane          | 100                         | 565                    | 2.7     | 590                 | 2.5     | 95.8                       |
| Ethyl aminobenzoate | Toluene          | 110                         | 736                    | 2.5     | 756                 | 3.1     | 97.4                       |
| Antipyrine          | Methanol         | 130                         | 232                    | 2.2     | 239                 | 4.7     | 97.1                       |
| Phenacetin          | Methanol         | 155                         | 340                    | 2.8     | 345                 | 5.2     | 98.6                       |
|                     | Toluene          |                             | 56                     | 2.9     | 56                  | 3.6     | 100.0                      |
| Cimetidine          | Ethanol          | 160                         | 877                    | 2.4     | 888                 | 2.9     | 98.8                       |
| Famotidine          | Methanol         | 185                         | 697                    | 3.1     | 715                 | 1.4     | 97.5                       |
| Isoniazid           | Methanol         | 190                         | 230                    | 2.4     | 225                 | 4.2     | 102.2                      |
| Carbamazepine       | Acetone          | 210                         | 289                    | 1.1     | 290                 | 1.6     | 99.7                       |

a) Results of 6 analyses. b) Determined as the percent recovery by [(concentration obtained from TD/GC/MS)/(concentration from DI/GC) × 100].

lution of the sample in a solvent or solvent extraction using more than 100 mg of sample. The proposed method, based on direct TD followed by GC/MS analysis, eliminates the need for such pretreatment as well as the possibility of interaction between dissolving solvent and other sample components in the injection port; moreover, it reduces the sample amount required to 1 mg. To achieve sufficient precision and accuracy, this technique requires strict control of desorption temperatures set for individual samples.

**Acknowledgments** The authors thank Mr. Shunji Terada for constructive advice and suggestions.

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# A New Synthesis of Indoloquinolizines by Pictet–Spengler Reaction of Tryptamine Type 1,2-Dihydropyridines Utilizing *sec*-Nitrodienamine

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Received June 13, 2000; accepted August 23, 2000

**The Pictet–Spengler reaction of tryptamine type 1,2-dihydropyridine **5c** derived from the cycloaddition of the *sec*-nitrodienamine **3c** with acetaldehyde afforded the indoloquinolizine derivatives **6** and **7**.**

**Key words** nitrodienamine; tryptamine; 1,2-dihydropyridine derivative; indoloquinolizine; Pictet–Spengler reaction

The reactivities of *tert*-nitrodienamines [ex. 1-(*N,N*-dimethylamino)-4-nitro-1,3-butadiene (**1**)] and *sec*-nitrodienamines [ex. 4-nitro-1-phenethylamino-1,3-butadiene (**3a**)] have been studied because of their potentially useful synthons in organic synthesis. The chemistry of nitrodienamines exploits the enaminic, dienic, and electronic “push pull” character of these molecules, and leads to interesting cycloaddition reactions as well as aminodienyl esters and aminoacrylate synthons.<sup>1–4</sup> Dihydropyridine chemistry is of interest from the point of view of pure research on heterocyclic compounds and also from a biological standpoint.<sup>5</sup>

Here, we studied the reactivities of aryl and alkylethyl-dienamine derivatives, especially indolyethyldienamine derivative and the Pictet–Spengler reaction of tryptamine type 1,2-dihydropyridine derivative **5c**. We earlier found that the reaction of *sec*-nitrodienamine **3a** with acetaldehyde (**4**) provided 2-methyl-3-nitro-1-phenethyl-1,2-dihydropyridine (**5a**) in 92% yield.<sup>1d</sup> Similarly, the *sec*-nitrodienamines **3a–c** were prepared by the reaction of the *tert*-nitrodienamine **1** with 2-phenylethylamine (**2a**), propylamine (**2b**), and tryptamine (**2c**), respectively (Chart 1, Table 1). The 2-methyl-3-nitro-1,2-dihydropyridine derivatives **5a–c** were prepared from the corresponding *sec*-dienamines **3a–c**.

We investigated the Pictet–Spengler reaction of tryptamine type 1,2-dihydropyridine derivative **5c** and expected to get information on indole alkaloid synthesis.<sup>6a</sup> The Pictet–Spengler reaction is the most important and powerful method of alkaloid chemistry. It has been applied in numerous cases for the construction of  $\beta$ -carboline and indoloquinolizine of natural products.<sup>6b</sup> Refluxing treatment of tryptamine type 1,2-dihydropyridine derivative **5c** in 10% H<sub>2</sub>SO<sub>4</sub>/MeOH gave ( $\pm$ )-*rel*-(2*S*,3*S*,4*S*,12*bS*)-2-methoxy-4-methyl-3-nitro-1,2,3,4,6,7,12,12*b*-octahydroindolo[2,3-*a*]quinolizine (**6**) in 52% yield. The structure of product **6** was proposed on the basis of the following spectroscopic analyses. The molecular

formula of **6** was found to be C<sub>17</sub>H<sub>21</sub>N<sub>3</sub>O<sub>3</sub>. The <sup>1</sup>H-NMR spectrum of **6** showed the presence of two methyl protons at  $\delta$  1.29 (3H, d, *J*=6.1 Hz), 3.40 (3H, s), three methylene protons at  $\delta$  1.64 (1H, q, *J*=12.1 Hz), 2.54 (1H, ddd, *J*=12.1, 5.0, 2.4 Hz), 2.59 (1H, ddd, *J*=11.5, 8.9, 5.0 Hz), 2.77 (1H, dtd, *J*=15.3, 5.0, 1.5 Hz), 2.82–2.88 (1H, m), 3.08 (1H, dt, *J*=11.5, 5.0 Hz), four methine protons at  $\delta$  3.06 (1H, dq, *J*=10.1, 6.1 Hz), 3.70 (1H, dd, *J*=12.1, 2.4 Hz), 3.98 (1H, ddd, *J*=12.1, 10.1, 5.0 Hz), 4.38 (1H, t, *J*=10.1 Hz), four aromatic protons at  $\delta$  7.11 (1H, t, *J*=7.6 Hz), 7.17 (1H, t, *J*=7.6 Hz), 7.32 (1H, d, *J*=7.6 Hz), 7.48 (1H, dd, *J*=7.6, 1.5 Hz), and a nitrogen proton of the indole ring at  $\delta$  7.73 (1H, br s). The IR spectrum of **6** revealed absorption bands at 3400 cm<sup>−1</sup> (NH group), 1550 cm<sup>−1</sup> (NO<sub>2</sub> group), and 1480, 1470, 1460, 1395 cm<sup>−1</sup> (indoloquinolizine ring). The nuclear Overhauser effect correlation spectroscopy (NOESY) of **6** exhibited the presence of cross-peaks between the methine proton of H $\alpha$ -12*b* at  $\delta$  3.70 and the methine protons of H $\alpha$ -2 and H $\alpha$ -4 at  $\delta$  3.98 and 3.06, and cross-peaks between the methine proton of H $\beta$ -3 at  $\delta$  4.38 and the methyl protons of OMe $\beta$ -2 and Me $\beta$ -4 at  $\delta$  3.40 and 1.29. It can therefore be deduced that indoloquinolizine **6** has an energetically stable equatorial methoxy group, an equatorial nitro group, and an equatorial methyl group in *trans*-decaline type configuration as shown in Chart 2. On the other hand, refluxing treatment of **5c** in 10% H<sub>2</sub>SO<sub>4</sub>/tetrahydrofuran (THF) provided ( $\pm$ )-*rel*-(4*R*,12*bS*)-4-methyl-3-nitro-1,4,6,7,12,12*b*-hexahydroindolo[2,3-*a*]quinolizine (**7**) in 18% yield. Compound (**7**) has an axial methyl group at C-4, which may provide less steric interference to a nitro group than an equatorial methyl group.

On the basis of our earlier report on the formation of 2-methyl-3-nitro-1,2-dihydropyridines **5** by the reaction of *sec*-nitrodienamines **3** with acetaldehyde (**4**),<sup>1d,f</sup> we attempted to prepare the product 3-nitro-1,2-dihydropyridines **10a, b** by cycloaddition reaction of the *sec*-nitrodienamine **3a** with ace-

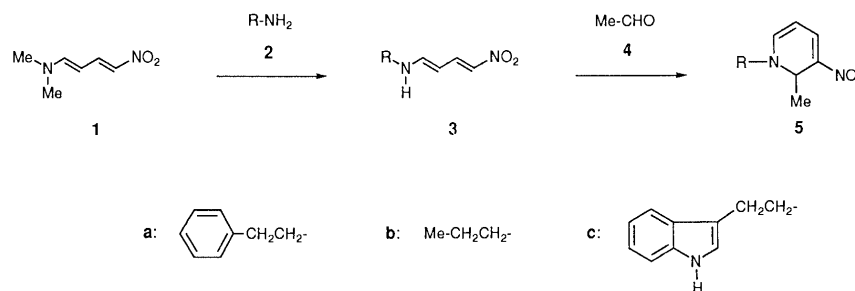


Chart 1

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Table 1. Physical Data of Compounds

| Compound <sup>a)</sup> | Yield (%) | Appearance [solvent, mp (°C)]               | <sup>1</sup> H-NMR, $\delta$ (ppm)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | IR (cm <sup>-1</sup> )                     | Formula, HR-MS <i>m/z</i> or Analysis (%)<br>Calcd (Found)                                                                     |
|------------------------|-----------|---------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|
| <b>3b</b>              | 71        | Brown oil                                   | 0.99 (3H, t, <i>J</i> =7.3 Hz, Me), 1.52—1.80 (2H, m, methylene H), 3.04—3.26 (2H, m, methylene H), 5.17 (1H, br s, NH), 5.33 (1H, t, <i>J</i> =12.3 Hz, olefinic H), 7.00 (1H, d, <i>J</i> =12.3 Hz, olefinic H), 7.00 (1H, d, <i>J</i> =12.3 Hz, olefinic H), 7.80 (1H, t, <i>J</i> =12.3 Hz, olefinic H), [CDCl <sub>3</sub> ]                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 3219, 1609, 1582, 1543, 1468, 1433, (neat) | C <sub>7</sub> H <sub>12</sub> N <sub>2</sub> O <sub>2</sub><br>156.0901<br>(156.0899)                                         |
| <b>5b</b>              | 85        | Dark red oil                                | 0.94 (3H, t, <i>J</i> =7.1 Hz, Me), 1.20 (1H, d, <i>J</i> =6.2 Hz, Me), 1.71 (2H, sextet, <i>J</i> =7.1 Hz, methylene H), 3.02—3.61 (2H, m, methylene H), 4.94 (1H, d, <i>J</i> =7.3 Hz, olefinic H), 5.16 (1H, dd, <i>J</i> =7.3, 6.2 Hz, olefinic H), 5.16 (1H, q, <i>J</i> =6.2 Hz, methine H), 6.71 (1H, d, <i>J</i> =6.2 Hz, olefinic H), [CDCl <sub>3</sub> ]                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 1616, 1516, 1481, 1435, 1358, 1290, (neat) | C <sub>9</sub> H <sub>14</sub> N <sub>2</sub> O <sub>2</sub><br>182.1054<br>(182.1074)                                         |
| <b>3c</b>              | 95        | Dark yellow prisms [AcOEt–MeOH, 125—126]    | 3.04 (2H, t, <i>J</i> =6.6 Hz, methylene H), 3.45—3.62 (2H, m, methylene H), 5.45 (1H, t, <i>J</i> =12.2 Hz, olefinic H), 6.91 (1H, d, <i>J</i> =12.2 Hz, olefinic H), 6.97—7.62 (6H, m, aromatic and olefinic H), 7.76 (1H, t, <i>J</i> =12.2 Hz, olefinic H), 10.11 (1H, br s, NH), [CD <sub>3</sub> COCD <sub>3</sub> ]                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | 3239, 1605, 1570, 1530, 1439, 1416, (KBr)  | C <sub>14</sub> H <sub>13</sub> N <sub>3</sub> O <sub>2</sub><br>C, 65.35; H, 5.88; N, 16.33.<br>(C, 65.23; H, 5.90; N, 16.03) |
| <b>5c</b>              | 85        | Dark red oil                                | 1.16 (3H, d, <i>J</i> =6.2 Hz, Me), 3.17 (2H, t, <i>J</i> =7.0 Hz, methylene H), 3.74 (1H, dt, <i>J</i> =13.8, 7.0 Hz, methylene H), 3.90 (1H, dt, <i>J</i> =13.8, 7.0 Hz, methylene H), 4.84 (1H, dd, <i>J</i> =7.4, 6.2 Hz, olefinic H), 5.22 (1H, q, <i>J</i> =6.2 Hz, methine H), 6.89 (1H, d, <i>J</i> =7.4 Hz, olefinic H), 7.02 (1H, t, <i>J</i> =7.5 Hz, aromatic H), 7.10 (1H, t, <i>J</i> =7.5 Hz, aromatic H), 7.14 (1H, s, aromatic H), 7.38 (1H, d, <i>J</i> =7.5 Hz, aromatic H), 7.48 (1H, d, <i>J</i> =6.2 Hz, olefinic H), 7.61 (1H, d, <i>J</i> =7.5 Hz, aromatic H), 10.09 (1H, br s, NH), [CD <sub>3</sub> COCD <sub>3</sub> , <sup>1</sup> H-NMR]. 15.52, 26.03, 53.17, 55.75, 93.23, 111.65, 112.25, 119.02, 119.58, 122.23, 124.07, 127.02, 128.15, 133.21, 137.67, 148.50, [CD <sub>3</sub> COCD <sub>3</sub> , <sup>13</sup> C-NMR]                                                                                                                                                                                                                                                     | 3330, 1610, 1550, 1510, 1490, 1430, (neat) | C <sub>16</sub> H <sub>17</sub> N <sub>3</sub> O <sub>2</sub><br>283.1319<br>(283.1347)                                        |
| <b>6</b>               | 52        | Colorless prisms [ether–hexane, 148—150]    | 1.29 (3H, d, <i>J</i> =6.1 Hz, Me), 1.64 (1H, q, <i>J</i> =12.1 Hz, methylene H), 2.54 (1H, ddd, <i>J</i> =12.1, 5.0, 2.4 Hz, methylene H), 2.59 (1H, ddd, <i>J</i> =11.5, 8.9, 5.0 Hz, methylene H), 2.77 (1H, ddt, <i>J</i> =15.3, 5.0, 1.5 Hz, methylene H), 2.82—2.88 (1H, m, methylene H), 3.06 (1H, dq, <i>J</i> =10.1, 6.1 Hz, methine H), 3.08 (1H, dt, <i>J</i> =11.5, 5.0 Hz, methylene H), 3.40 (3H, s, OMe), 3.70 (1H, dd, <i>J</i> =12.1, 2.4 Hz, methine H), 3.98 (1H, ddd, <i>J</i> =12.1, 10.1, 5.0 Hz, methine H), 4.38 (1H, t, <i>J</i> =10.1 Hz, methine H), 7.11 (1H, t, <i>J</i> =7.6 Hz, aromatic H), 7.17 (1H, t, <i>J</i> =7.6 Hz, aromatic H), 7.32 (1H, d, <i>J</i> =7.6 Hz, aromatic H), 7.48 (1H, dd, <i>J</i> =7.6, 1.5 Hz, aromatic H), 7.73 (1H, br s, NH), [CDCl <sub>3</sub> , <sup>1</sup> H-NMR]. 16.44 (C4-Me), 22.06 (C7), 32.62 (C1), 44.16 (C6), 56.37 (C12b), 56.82 (OMe), 59.01 (C4), 78.88 (C2), 93.24 (C3), 108.93 (C7a), 110.87 (C11), 118.32 (C8), 119.73 (C9), 121.92 (C10), 126.97 (C7b), 133.05 (C12a), 136.20 (C11a), [CDCl <sub>3</sub> , <sup>13</sup> C-NMR] | 3400, 1550, 1480, 1470, 1460, 1395, (KBr)  | C <sub>17</sub> H <sub>21</sub> N <sub>3</sub> O <sub>3</sub><br>C, 64.74; H, 6.71; N, 13.33.<br>(C, 64.61; H, 6.72; N, 13.38) |
| <b>7</b>               | 18        | Colorless prisms [ether–hexane, 196 (dec.)] | 1.37 (3H, d, <i>J</i> =4.1 Hz, Me), 2.52 (1H, ddt, <i>J</i> =22.1, 10.1, 2.8 Hz, methylene H), 2.75 (1H, dt, <i>J</i> =22.1, 4.6 Hz, methylene H), 2.83—2.94 (2H, m, methylene H), 3.06 (1H, ddd, <i>J</i> =15.0, 8.6, 4.9 Hz, methylene H), 3.34 (1H, dt, <i>J</i> =15.0, 4.9 Hz, methylene H), 4.16 (1H, dd, <i>J</i> =10.1, 4.6 Hz, methine H), 4.35 (1H, q, <i>J</i> =4.1 Hz, methine H), 7.12 (1H, t, <i>J</i> =7.6 Hz, aromatic H), 7.18 (1H, t, <i>J</i> =7.6 Hz, aromatic H), 7.33 (1H, d, <i>J</i> =7.6 Hz, aromatic H), 7.34 (1H, dd, <i>J</i> =4.6, 2.8 Hz, olefinic H), 7.51 (1H, d, <i>J</i> =7.6 Hz, aromatic H), 7.73 (1H, br s, NH), [CDCl <sub>3</sub> , <sup>1</sup> H-NMR]. 13.31 (C4-Me), 21.91 (C7), 31.10 (C1), 46.01 (12b), 48.12 (C6), 54.08 (C4), 108.88 (C7a), 110.82 (C11), 118.38 (C8), 119.73 (C9), 121.96 (C10), 126.98 (C7b), 131.02 (C2), 133.78 (C12a), 136.44 (C11a), 153.00 (C3), [CDCl <sub>3</sub> , <sup>13</sup> C-NMR]                                                                                                                                                   | 3390, 1680, 1620, 1540, 1510, 1470, (KBr)  | C <sub>16</sub> H <sub>17</sub> N <sub>3</sub> O <sub>2</sub><br>283.1319<br>(283.1299)                                        |
| <b>11</b>              | 10        | Yellow oil                                  | 2.16 (3H, s, Me), 2.60 (3H, s, Me), 2.80 (2H, t, <i>J</i> =7.5 Hz, methylene H), 2.96 (2H, t, <i>J</i> =7.5 Hz, methylene H), 7.38 (1H, t, <i>J</i> =7.8 Hz, aromatic H), 7.41 (1H, d, <i>J</i> =7.8 Hz, aromatic H), 7.79 (1H, s, aromatic H), 7.79 (1H, d, <i>J</i> =7.8 Hz, aromatic H), [CDCl <sub>3</sub> ]                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 1715, 1682, 1610, 1585, 1520, 1485, (neat) | C <sub>12</sub> H <sub>14</sub> O <sub>2</sub><br>190.0992<br>(190.0965)                                                       |

<sup>a)</sup> Compounds **3a** and **5a** were reported in reference **1d**.

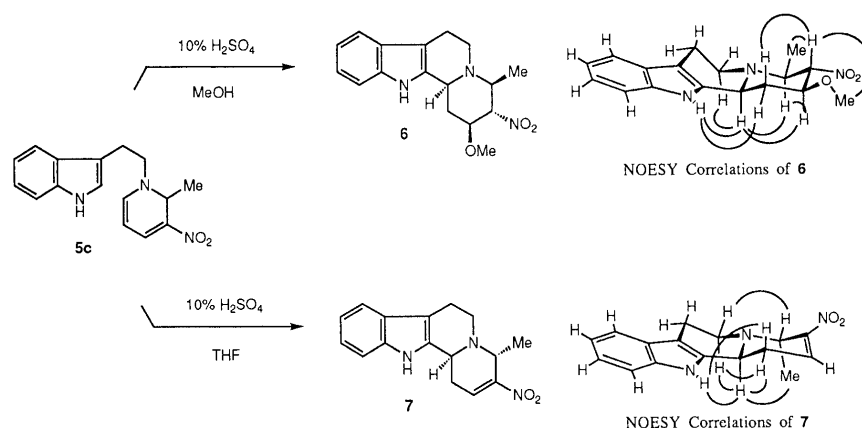


Chart 2

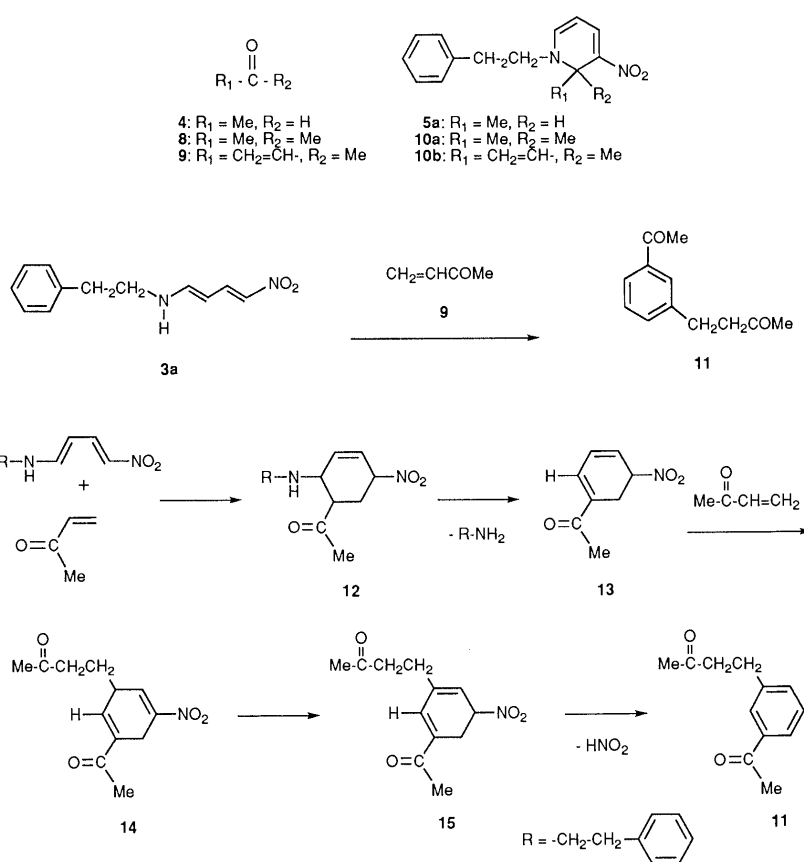


Chart 3

cular Diels-Alder reaction of 1-[2-(3-indolyl)ethyl]amino]-2-nitro-1,3-butadiene (**3c**).<sup>7)</sup> Unexpectedly, refluxing treatment of **3c** in xylene afforded 1-[2-(3-indolyl)ethyl]-2-methyl-3-

1,2-dihydropyridine (**5c**), 1,3-dinitrobenzene (**17**) and obenzaldehyde (**18**) in 11%, 37% and 14% yields, respectively, as shown in Chart 4. The self-cycloaddition reaction of **3c** can be explained as follows. Initially, the self-conjugation reaction of **3c** might generate the intermediates **19**, and following aromatization with deamination it affords the 3-nitrobenzaldehyde (**18**), 1,3-dinitrobenzene (**17**) and acetaldehyde (**4**), respectively. Then, the condensation reaction of **3c** with acetaldehyde (**4**) might generate the intermediate **22**, followed by an intramolecular ring closure with aromatization, which could lead to 1,2-dihydropyridine **5c**, as shown in Chart 5.

These results indicate a new synthetic method of indoloquinolizines **6** and **7** by the Pictet-Spengler reaction of tryptamine type 1,2-dihydropyridine derivative **5c** utilizing *sec*-ni-

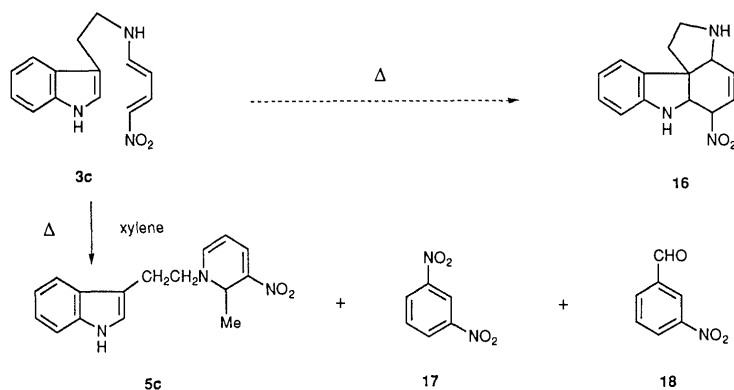


Chart 4

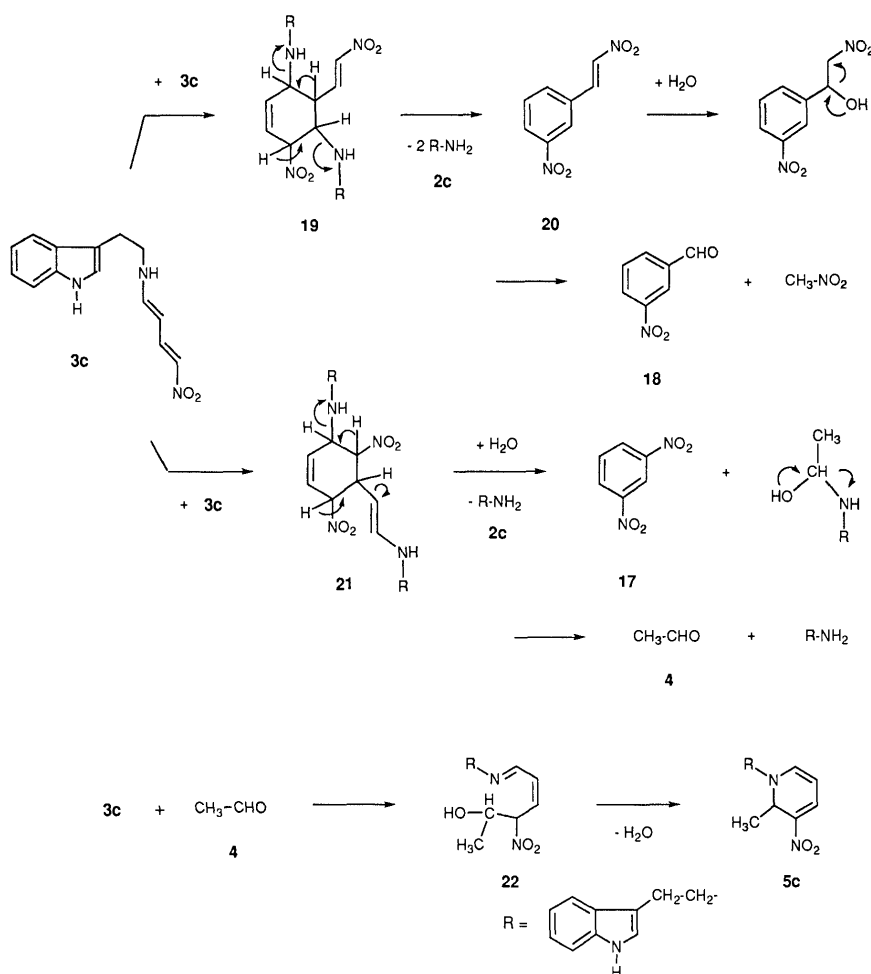


Chart 5

trodienamine **3c**.

### Experimental

All melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. IR spectra were recorded on either a JASCO FT/IR-200 or JASCO FT/IR-8000 spectrometer, and  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR spectra on either a JEOL EX-90 or JEOL JNM- $\alpha$ 500 spectrometer with tetramethylsilane as an internal standard. NOESY spectra were obtained with the usual pulse sequence and data processing was performed with the standard JEOL software. MS spectra were recorded on a JEOL JMS-D 300 spectrometer. Elemental analyses were recorded on a Yanaco CHN-corder MT-3. Wakogel C-200 (silica gel) and Merck Kieselgel G nach Stahl (silica gel) and NH-DM 1020 (basic 100 Å type silica gel, Fuji Silysia Chemical, Ltd.) were used for column chromatography and thin layer chromatography

(TLC), respectively. All runs were carried out under an argon atmosphere.

**General Procedure for Reactions of *tert*-Nitrodienamine **1** with Primary Amines **2**** A solution of the *tert*-nitrodienamine **1** (40 mg, 0.28 mmol) and an amine **2** (0.98–14.5 mmol) in benzene (8 ml) or THF (4 ml) was stirred at room temperature for an appropriate period until the disappearance of **1** (checked by TLC). The reaction mixture was concentrated in a vacuum, then the residue was subjected to silica gel column chromatography with appropriate solvents. The properties of the prepared compounds **3** are shown in Table 1.

4-Nitro-1-phenethylamino-1,3-butadiene (**3a**) and 2-methyl-3-nitro-1-phenethyl-1,2-dihydropyridine (**5a**) were synthesized by the previously reported method.<sup>1d)</sup>

4-Nitro-1-propylamino-1,3-butadiene (**3b**): Substrate: propylamine (**2b**) (856 mg, 14.5 mmol). Reaction solvent: benzene. Reaction time: 1.2 h. Sol-

vent for chromatography: 30% ethyl acetate in hexane. Product **3b**: 31 mg.

1-[2-(3-Indolyl)ethylamino]-4-nitro-1,3-butadiene (**3c**): Substrate: tryptamine (**2c**) (450 mg, 2.81 mmol). Reaction solvent: THF. Reaction time: 4.5 h. Solvent for chromatography: 20% hexane in ethyl acetate. Product **3c**: 69 mg.

**General Procedure for Reactions of *sec*-Nitrodienamine **3** with Acetaldehyde (**4**)** A solution of a *sec*-nitrodienamine **3** (0.214 mmol) and acetaldehyde (**4**) (0.4 ml, 7.16 mmol) in THF (3 ml) in a sealed tube was stirred at room temperature for an appropriate period until **3** disappeared (checked by TLC). The reaction mixture was concentrated in a vacuum, then the residue was subjected to silica gel column chromatography. The properties of the prepared compounds **5** are shown in Table 1.

2-Methyl-3-nitro-1-propyl-1,2-dihydropyridine (**5b**): Substrate: 4-nitro-1-propylamino-1,3-butadiene (**3b**) (33 mg, 0.214 mmol). Reaction time: 1.2 h. Solvent for chromatography: 20% ethyl acetate in hexane. Product **5b**: 33 mg.

1-[2-(3-Indolyl)ethyl]-2-methyl-3-nitro-1,2-dihydropyridine (**5c**): Substrate: 1-[2-(3-indolyl)ethylamino]-4-nitro-1,3-butadiene (**3c**) (55 mg, 0.214 mmol). Reaction time: 1.5 h. Solvent for chromatography: 50% ethyl acetate in hexane. Product **5c**: 52 mg.

**The Pictet-Spengler Reactions of 1-[2-(3-Indolyl)ethyl]-2-methyl-3-nitro-1,2-dihydropyridine (**5c**)** A solution of **5c** (40 mg, 0.141 mmol) and 10% H<sub>2</sub>SO<sub>4</sub> (0.5 ml) in MeOH (2 ml) was refluxed for 3 h. The reaction mixture was concentrated in a vacuum, and then the residue was made alkaline with a K<sub>2</sub>CO<sub>3</sub> solution. The whole was extracted with AcOEt and the organic phase was washed with water, dried and evaporated. The residue was subjected to silica gel column chromatography [solvent: 20% acetone in hexane]. Product: 23 mg of (±)-*rel*-(2*S*,3*S*,4*S*,12*bS*)-2-methoxy-4-methyl-3-nitro-1,2,3,4,6,7,12,12*b*-octahydroindolo[2,3-*a*]quinolizine (**6**).

A solution of **5c** (40 mg, 0.141 mmol) and 10% H<sub>2</sub>SO<sub>4</sub> (0.5 ml) in THF (2 ml) was refluxed for 3 h. The reaction mixture was concentrated in a vacuum, and then the residue was made alkaline with a K<sub>2</sub>CO<sub>3</sub> solution. The whole was extracted with AcOEt and the organic phase was washed with water, dried and evaporated. The residue was subjected to silica gel column chromatography [solvent: 50% ether in hexane]. Product: 7 mg of (±)-*rel*-(4*R*,12*bS*)-4-methyl-3-nitro-1,4,6,7,12,12*b*-hexahydroindolo[2,3-*a*]quinolizine (**7**).

**The Reaction of 4-Nitro-1-phenethylamino-1,3-butadiene (**3a**) with Methyl Vinyl Ketone (**9**)** A solution of **3a** (40 mg, 0.183 mmol) with methyl vinyl ketone (**9**) (1.5 ml, 18.3 mmol) and phenethylamine (2 drops) in liquid reaction mixture (no solvent) in a sealed tube was stirred at room temperature for 16 h. The reaction mixture was concentrated in a vacuum, then the residue was subjected to silica gel column chromatography [solvent: 30% ethyl acetate in hexane]. Product: 4 mg of 4-(3-acetylphenyl)butan-2-one (**11**).

**The Self-Cycloaddition Reaction of 1-[2-(3-Indolyl)ethylamino]-4-nitro-1,3-butadiene (**3c**)** A solution of **3c** (50 mg, 0.195 mmol) in xylene (10 ml) was refluxed for 12 h in a sealed tube. The reaction mixture was concentrated in a vacuum, then the residue was subjected to silica gel column chromatography [solvent: 50% ethyl acetate in hexane]. Product: 6 mg (37%) of 1,3-dinitrobenzene (**17**), light yellow plates (AcOEt-hexane), mp 89 °C (lit.<sup>8</sup>) mp 88–90 °C and 2 mg (14%) of 3-nitrobenzaldehyde (**18**),<sup>17</sup> yellow needles (AcOEt-hexane), mp 58 °C (lit.<sup>8</sup>) mp 57–59 °C and 6 mg (11%) of **5c**, red oil. These products were identical with authentic samples on the basis of IR, MS, and NMR spectral comparisons.

## References and Notes

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- 8) This product was identified by comparison with an authentic sample obtained from a commercial supplier.

# Asymmetric Synthesis and Determination of the Absolute Configuration of FK584, an Agent for the Treatment of Overactive Detrusor

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**FK584[(-)-*N*-*tert*-butyl-4,4-diphenyl-2-cyclopentenylamine hydrochloride, (-)-4·HCl], a potential candidate for the treatment of overactive detrusor, was synthesized in a 4-step approach starting with Sharpless oxidation of cyclopentenol **6** (kinetic resolution). This epoxidation is a rare case in that the empirical rule does not work. Regio- and stereoselective introduction of *tert*-butylamine to the obtained epoxycyclopentanol **5** and subsequent conversion of the resulting diol to an olefin completed the synthesis. The absolute configuration of FK584 was determined to be *S* by X-ray crystallographic analysis of the salt of *S*-(+)-mandelic acid.**

**Key words** FK584; *N*-*tert*-butyl-4,4-diphenyl-2-cyclopentenylamine; overactive detrusor; Sharpless oxidation

We earlier reported<sup>1)</sup> that FK584 has good pharmacological properties for the treatment of overactive detrusor and its clinical development is in progress. In the course of developing a new clinical agent, pharmacokinetic studies are necessary which require radiolabelled compound. The synthesis of FK584<sup>1)</sup> was carried out by optical resolution of the synthetic racemate but this method is not applicable to the synthesis of radiolabelled compound. Consequently, we pursued an asymmetric synthesis of FK584 by two different methods, A and B, using 2-methyl-[2-<sup>14</sup>C]-propylamine as a labelled precursor which is available from the Amersham Company (Fig. 1). Method A consists of the introduction of *tert*-butylamine to  $\pi$ -allylpalladium **3** generated from cyclopentenyl acetate **1** or **2**, while method B involves the introduction of *tert*-butylamine to 5,5-diphenyl-2,3-epoxycyclopentan-1-ol (**5**) and subsequent conversion to the required final product. Herein, we report the details of the asymmetric synthesis of FK584 and its absolute configuration.

Our initial attempt at the synthesis of FK584 involved a kinetic resolution of **6**<sup>1)</sup> or **7**<sup>2)</sup> with lipase PS (Amano) and iso-

propenyl acetate to afford optically active cyclopentenol (+)-**7** and cyclopentenyl acetate (-)-**2**, respectively, and the results are shown in Table 1 (Chart 1).<sup>3)</sup> In the case of **6**, no reaction occurred, probably due to too large steric hindrance of the diphenyl group in the  $\alpha$ -position of the hydroxyl group. With chiral cyclopentenyl acetate (-)-**2** in hand, the regio- and stereoselective introduction of *tert*-butylamine to it by a double inversion reaction using palladium catalyst<sup>4)</sup> was investigated (Chart 2). For convenience, we first attempted the reaction with racemic cyclopentenyl acetate **1** which was prepared by acetylation of **6**: a mixture of compound **1** (1 mol), tetrakis triphenylphosphine palladium (0.05 mol), *tert*-butylamine (1 mol) and triethylamine (1 mol) in tetrahydrofuran (THF) was stirred at room temperature overnight under nitrogen atmosphere to afford cyclopentadiene **8**<sup>5)</sup> in 68% yield and no desired product was detected by <sup>1</sup>H-NMR measurement. Elimination probably occurred *via*  $\sigma$ -allylpalladium equilibrated with  $\pi$ -allylpalladium through the action of the amine as base. This idea was partly supported by the result that in the case of a neutral nucleophile (azide),<sup>6)</sup> regioselective

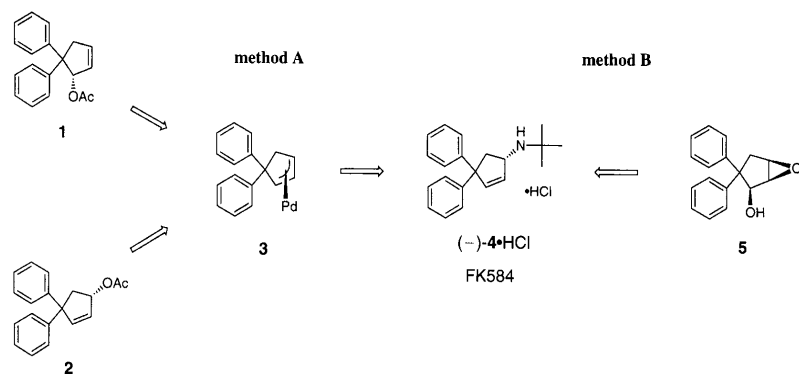


Fig. 1

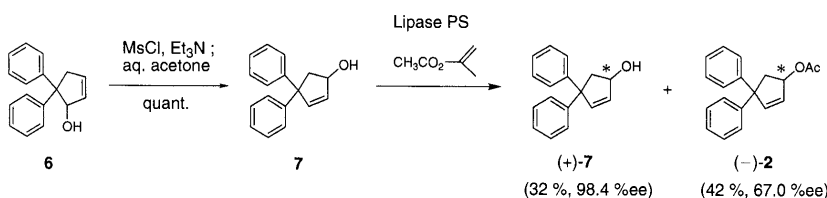


Chart 1

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Table 1. Kinetic Resolution of Cyclopentenol **7**

| Run | Reaction condition | <b>7</b>  |                                   |                   | <b>2</b>  |                                  |                   |
|-----|--------------------|-----------|-----------------------------------|-------------------|-----------|----------------------------------|-------------------|
|     |                    | Yield (%) | $[\alpha]_D$                      | +/- <sup>a)</sup> | Yield (%) | $[\alpha]_D$                     | +/- <sup>b)</sup> |
| 1   | 40 °C, 41 h        | 60        | +57.8<br>( <i>c</i> =2.18, MeOH)  | 75.1/24.9         | 37        | -86.3<br>( <i>c</i> =1.50, MeOH) | 8.8/91.2          |
| 2   | 40 °C, 110 h       | 32        | +113.4<br>( <i>c</i> =0.63, MeOH) | 99.2/0.8          | 42        | -72.9<br>( <i>c</i> =0.98, MeOH) | 16.5/83.5         |

a, b) The enantiomer ratio of compounds **7** and **2** was determined by HPLC analysis as described in the Experimental section.

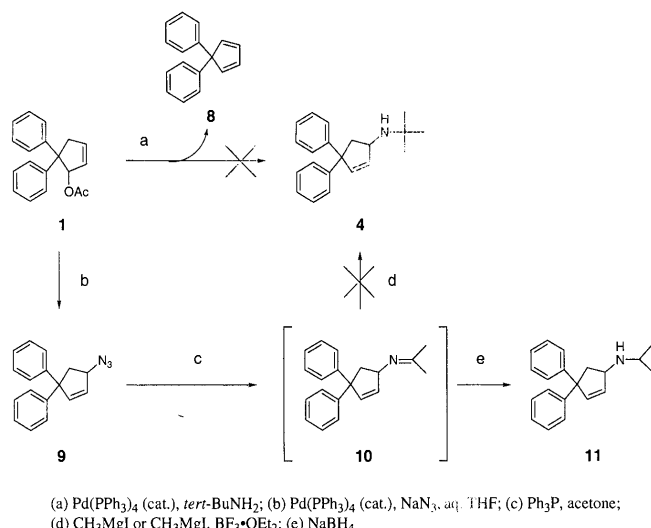


Chart 2

tive substitution occurred to afford an azide derivative **9** in 79% yield. The hindered nature of the diphenyl group is probably responsible for this selectivity. Since <sup>14</sup>C-methyl magnesium iodide is also available, we pursued an alternate route to **4** from **9**: reaction of methyl magnesium iodide and an imine **10** which was prepared by Staudinger reaction of the azide **9** with triphenylphosphine and acetone. The imine **10** was identified as amine **11** by reducing it with sodium borohydride. Contrary to our expectations, *in situ* treatment of the imine **10** with methyl magnesium iodide in the absence or presence of Lewis acid (boron trifluoride etherate)<sup>7)</sup> afforded a complex mixture and no desired product was obtained.

This result led us to pursue method B starting with an asymmetric epoxidation of cyclopentenol **6** by Sharpless oxidation<sup>8)</sup> (Chart 3). A mixture of cyclopentenol **6** (1 mol), dimethyl D-(−)-tartrate (0.2 mol), *tert*-butylhydroperoxide (0.50 mol), titanium(IV) isopropoxide (0.15 mol) and 4-Å molecular sieves (MS4A) was stirred at −10 °C for 2 d to afford (−)-**5**<sup>9)</sup> and (+)-**6** in 39.2% and 56.4% yield, respectively.<sup>10)</sup> The enantiomeric excess of these compounds was determined to be 50.2% for (−)-**5** and 30.4% for (+)-**6** by HPLC analysis; fortunately, purification of (−)-**5** was accomplished by removing the more crystallizable racemate from ethyl acetate (EtOAc). According to the empirical rule,<sup>11)</sup> the absolute configuration of (−)-5,5-diphenyl-2,3-epoxycyclopentan-1-ol ((−)-**5**) was believed to be 1*R*, 2*R*, 3*S*. However, applying the proposed structure of the ‘loaded’ catalyst at the time of oxygen transfer in the Sharpless oxidation of an allylic alcohol, the absolute configuration of (−)-**5** was

thought to be 1*S*, 2*S*, 3*R* (the antipode). The reason was that the restricted conformation of the substrate and contact between the large diphenyl group adjacent to the hydroxyl of the substrate and the ligand ester group of the active epoxidation catalyst seriously impede the necessary approach of alkene of (1*S*)-**6** to the oxidant as shown in Fig. 2 (in the path which would yield 1*R*, 2*R*, 3*S* according to the empirical rule). The absolute configuration of (−)-**5** was not determined at this point and conversion of (−)-**5** to the objective compound **4** proceeded. Reaction of (−)-**5** (99.5% ee) with *tert*-butylamine in the presence of titanium(IV) isopropoxide,<sup>12)</sup> followed by treatment with 3*N* hydrochloric acid afforded cyclopentenylamine hydrochloride (−)-**12**·HCl (>99.4% ee) in 99% yield. The C-3 opened product was the only isomer detected by <sup>1</sup>H-NMR analysis and a stereoelectronic effect through an intramolecular coordination of a cyclopentanoyltitanium to the epoxide oxygen atom was probably responsible for the regio- and stereoselectivity. Conversion of (−)-**12**·HCl to **4** was carried out by the following procedure: 1) treatment of (−)-**12**·HCl with methyl orthoformate in the presence of catalytic amount of *p*-toluenesulfonic acid (TsOH) to afford orthoester **13** as a 10 : 1 diastereomer mixture<sup>13)</sup> (97% yield); 2) refluxing of a mixture of **13**, acetic acid and a catalytic amount of acetic anhydride in xylene,<sup>14)</sup> followed by salt formation with hydrogen chloride to afford (−)-**4**·HCl (>99.8% ee) in 44% isolated yield. Synthetic **4**·HCl showed all spectroscopic data to be in accord with the reported values,<sup>1)</sup> including the sign of the optical rotation; this means that asymmetric synthesis of FK584 ((−)-**4**·HCl) was completed in a 3-step approach starting with (−)-**5** which was obtained by Sharpless oxidation of cyclopentenol **6** with dimethyl D-(−)-tartrate, *tert*-butylhydroperoxide, titanium(IV) isopropoxide and MS4A.

The absolute configuration of FK584 was determined to be *S* by X-ray crystallographic analysis of the salt of (*S*)-(+)-mandelic acid as shown in Fig. 3. Based on this result and the fact that regio- and stereoselective conversion of (−)-**5** to the (*S*)-(+)-**4**·HCl was attained, the absolute configuration of (−)-**5** was confirmed to be 1*S*, 2*S*, 3*R*. The proposed structure of ‘loaded’ catalyst at the time of oxygen transfer state of allylic alcohol was confirmed to work well in this conformationally restricted cyclopentenol case and to afford moderate optical purity. However, the reaction time of the epoxidation was much longer than that of the usual allylic alcohol, probably due to the poor fit to the requirements of the active epoxidation catalyst as in the case of allylic alcohols having *cis*-3-substituents. According to method B, (*S*)-*N*-{2-methyl-[2-<sup>14</sup>C]-propyl}-4,4-diphenyl-2-cyclopentenylamine hydrochloride (<sup>14</sup>C-FK584, 303 MBq/mmol) was synthesized from (−)-**5** and 2-methyl-[2-<sup>14</sup>C]-propylamine, and pharma-

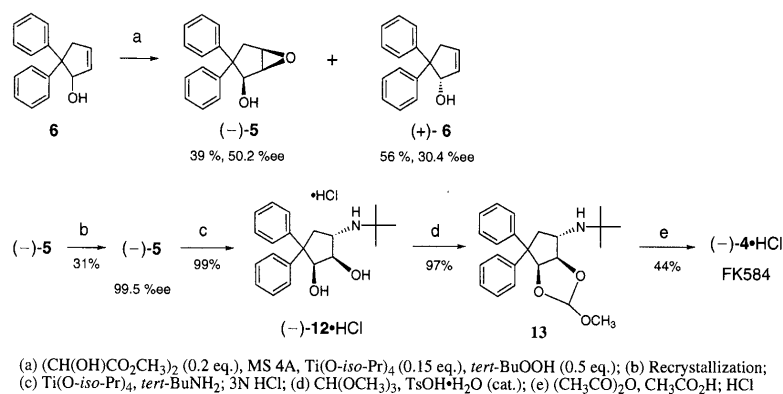


Chart 3

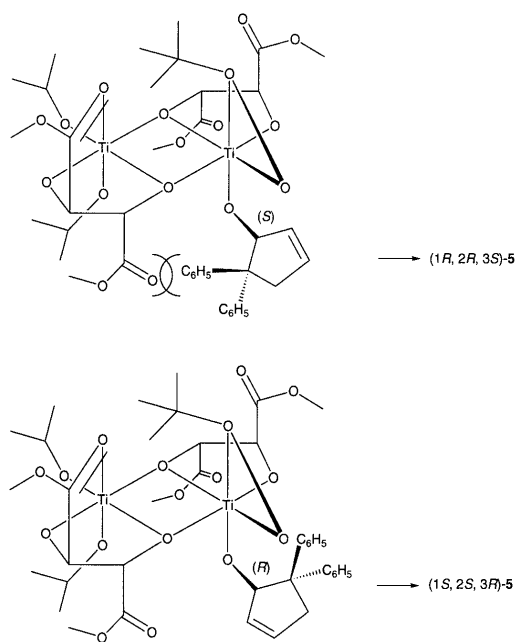


Fig. 2

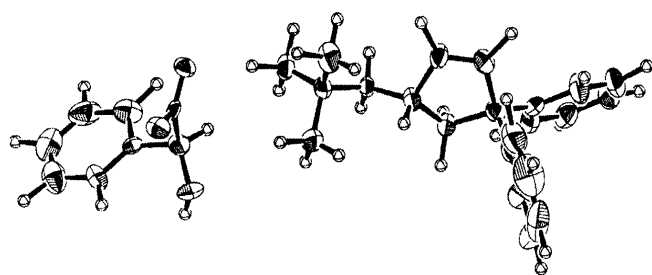


Fig. 3. ORTEP Drawing of the Crystal Structure of (-)-4·(S)-(+)-Mandelic acid Determined by X-Ray Crystallographic Analysis

cokinetic studies are currently ongoing.

In conclusion, (1*S*,2*S*,3*R*)-(-)-5,5-diphenyl-2,3-epoxycyclopentan-1-ol ((-)-5) was synthesized by Sharpless oxidation of cyclopentenol **6** (kinetic resolution) in a rare case in which the empirical rule did not work. Regio- and stereoselective introduction of *tert*-butylamine to the obtained (-)-5 and subsequent conversion of the resulting diol to an olefin afforded FK584. The absolute configuration of FK584 was determined to be *S* by X-ray crystallographic analysis of the salt of (*S*)-(+)-mandelic acid.

### Experimental

All melting points were determined in open glass capillaries on a Thomas-Hoover apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Hitachi 260-10 IR spectrophotometer. <sup>1</sup>H-NMR spectra were recorded on a Hitachi R-90H or a Bruker AC-200P NMR spectrometer with tetramethylsilane as an internal standard ( $\delta$  value, ppm). Mass (MS) spectra were recorded on a JEOL JMS D-300 MS spectrometer. Elemental analyses were carried out on a Perkin-Elmer 2400CHN elemental analyzer.

**(Method A) 4,4-Diphenyl-2-cyclopenten-1-ol (7)** Methanesulfonyl chloride (MsCl) (0.79 ml, 10.2 mmol) and triethylamine (1.42 ml, 10.2 mmol) were successively added to a solution of **6** (2.00 g, 8.46 mmol) in acetone (20 ml) below  $-5^\circ\text{C}$  and the whole was stirred for 0.5 h. The mixture was poured into ice water (20 ml) and the mixture was stirred for 15 min. Brine and EtOAc were added to the mixture and the organic layer was separated, washed with brine, dried over sodium sulfate ( $\text{Na}_2\text{SO}_4$ ), and evaporated *in vacuo*. The residue was purified by column chromatography on silica gel with a mixture of *n*-hexane and EtOAc (5:1) to afford **7** (2.00 g, 100%) as an oil. The physical data of this compound was identical to those of **7** reported in the preceding paper.<sup>1)</sup>

**(-)-4,4-Diphenyl-2-cyclopentenyl Acetate [(-)-2]** A mixture of **7** (0.30 g, 1.27 mmol) and lipase PS (Amano, 0.70 g) in isopropenyl acetate (2.20 ml) was stirred at  $40^\circ\text{C}$  for 41 h; then the lipase was removed by filtration and the filtrate was evaporated *in vacuo*. The residue was purified by column chromatography on silica gel with a mixture of *n*-hexane and EtOAc (20:1) to afford (+)-**7** (0.18 g, 60%) and (-)-**2** (0.13 g, 37%) as oils, respectively. (+)-**7**:  $[\alpha]_D^{24} +57.9^\circ$  ( $c=2.18$ , MeOH). IR (neat):  $3350\text{ cm}^{-1}$ . NMR ( $\text{CDCl}_3$ , 200 MHz):  $\delta$  1.61 (1H, s), 2.40 (1H, dd,  $J=4.7, 13.8\text{ Hz}$ ), 3.00 (1H, dd,  $J=6.9, 13.8\text{ Hz}$ ), 4.90–5.10 (1H, brs), 5.97 (1H, dd,  $J=2.0, 5.6\text{ Hz}$ ), 6.38 (1H, dd,  $J=1.1, 5.6\text{ Hz}$ ), 7.10–7.42 (10H, m). MS  $m/z$ : 236 ( $\text{M}^+$ ). The enantiomeric excess of this compound was determined to be 50.2% by HPLC analysis (column, CHIRALCEL-OD (Daicel)  $4.6\times 250\text{ mm}$ ; eluent, 20:1 *n*-hexane–EtOH mixture; flow rate, 1.0 ml/min; detector, 220 nm;  $t_R$  of (+)-**7**, 10.6 min;  $t_R$  of (-)-**7**, 16.0 min). (-)-**2**:  $[\alpha]_D^{24} -86.3^\circ$  ( $c=1.50$ , MeOH). IR (neat):  $1730\text{ cm}^{-1}$ . NMR ( $\text{CDCl}_3$ , 200 MHz):  $\delta$  2.03 (3H, s), 2.47 (1H, dd,  $J=5.0, 14.2\text{ Hz}$ ), 3.12 (1H, dd,  $J=7.2, 14.2\text{ Hz}$ ), 5.80–5.92 (1H, m), 5.95 (1H, dd,  $J=2.0, 5.5\text{ Hz}$ ), 6.44 (1H, dd,  $J=1.2, 5.5\text{ Hz}$ ), 7.10–7.38 (10H, m). MS  $m/z$ : 278 ( $\text{M}^+$ ). The enantiomeric excess of this compound was determined to be 82.4% by HPLC analysis (column, CHIRALCEL-OD (Daicel)  $4.6\times 250\text{ mm}$ ; eluent, 100:1 *n*-hexane–EtOH mixture; flow rate, 1.0 ml/min; detector, 220 nm;  $t_R$  of (-)-**2**, 6.9 min;  $t_R$  of (+)-**2**, 8.1 min).

**5,5-Diphenyl-2-cyclopentenyl Acetate (1)** Acetic anhydride (0.84 ml, 8.90 mmol) and a catalytic amount of 4-dimethylaminopyridine were added to a solution of **6** (0.70 g, 2.96 mmol) in pyridine (5 ml) at room temperature. Stirring was continued for 30 min, then the pH of the reaction mixture was adjusted to 1.0 with dilute HCl and extracted with EtOAc. The extract was washed with brine, dried over magnesium sulfate ( $\text{MgSO}_4$ ), and evaporated *in vacuo* to afford **1** (0.82 g, 100%) as an oil. IR (neat):  $1720\text{ cm}^{-1}$ . NMR ( $\text{CDCl}_3$ , 200 MHz):  $\delta$  1.68 (3H, s), 2.80–3.00 (1H, m), 3.48–3.70 (1H, m), 6.00–6.10 (1H, m), 6.14–6.26 (1H, m), 6.49 (1H, s), 7.10–7.35 (10H, m). MS  $m/z$ : 278 ( $\text{M}^+$ ).

**4,4-Diphenyl-2-cyclopentenyl Azide (9)** A mixture of **1** (216 mg, 0.78 mmol), sodium azide (84 mg, 1.3 mmol) and tetrakis(triphenylphosphine)palladium(0) (62 mg, 0.05 mmol) in a mixture of THF (3 ml) and water (1.5 ml) was stirred at room temperature for 14.5 h under an argon at-

mosphere. EtOAc was added to the solution and the organic layer was separated, washed with brine, dried over  $\text{MgSO}_4$ , and evaporated *in vacuo*. The residue was purified by column chromatography on silica gel with a mixture of *n*-hexane and EtOAc (100:1) to afford **9** (0.16 g, 78%) as an oil. IR (neat): 2100  $\text{cm}^{-1}$ . NMR ( $\text{CDCl}_3$ , 90 MHz):  $\delta$  2.45 (1H, dd,  $J=6$ , 14 Hz), 3.05 (1H, dd,  $J=14$ , 8 Hz), 4.42–4.70 (1H, m), 5.90 (1H, dd,  $J=2$ , 6 Hz), 6.42 (1H, dd,  $J=1$ , 6 Hz), 6.95–7.30 (10H, m). MS  $m/z$ : 261 ( $\text{M}^+$ ), 232, 219.

***N*-Isopropyl-4,4-diphenyl-2-cyclopentenylamine Methanesulfonate (11·MsOH)** Triphenylphosphine (330 mg, 1.26 mmol) was added to a solution of **9** (300 mg, 1.15 mmol) in acetone (3 ml) at room temperature and the whole was refluxed for 21 h. After cooling, the solvent was removed by evaporation and the residue was dissolved in MeOH (3 ml). Sodium borohydride (220 mg, 5.81 mmol) was added to the solution and the whole was stirred for 20 min. Brine and EtOAc were then added to the mixture and the organic layer was separated, washed with brine, dried over  $\text{MgSO}_4$ , and evaporated *in vacuo*. The residue was purified by flash column chromatography on silica gel with a mixture of chloroform ( $\text{CHCl}_3$ ) and methanol (MeOH) (50:1) to afford **11** (0.26 g) as an oil. MsOH (92 mg, 0.95 mmol) in MeOH was added to a solution of the free amine **11** obtained in the above manner (0.26 g) in  $\text{CHCl}_3$  and the solution was evaporated *in vacuo*. The residue was triturated with a mixture of diethyl ether and EtOAc to afford **11·MsOH** (260 mg, 61%), mp 148–150 °C. Anal. Calcd for  $\text{C}_{21}\text{H}_{27}\text{NSO}_3 \cdot 0.4\text{H}_2\text{O}$ : C, 66.25; H, 7.35; N, 3.67. Found: C, 66.35; H, 7.26; N, 3.57. IR (nujol): 2700, 2510, 2470, 1610, 1490  $\text{cm}^{-1}$ . NMR ( $\text{CDCl}_3$ , 200 MHz):  $\delta$  1.38 (3H, d,  $J=6.2$  Hz), 1.39 (3H, d,  $J=6.2$  Hz), 2.52 (3H, s), 2.64 (1H, dd,  $J=8.7$ , 13.0 Hz), 3.19 (1H, dd,  $J=6.7$ , 13.0 Hz), 3.20–3.50 (1H, m), 4.35 (1H, brs), 6.12 (1H, d,  $J=5.7$  Hz), 6.42 (1H, d,  $J=5.7$  Hz), 7.10–7.40 (10H, m), 8.75 (2H, brs).

**(Method B) (1S,2S,3R)-(–)-5,5-Diphenyl-2,3-epoxycyclopentan-1-ol ((–)-5)** Titanium(IV) isopropoxide (22.70 ml, 76.3 mmol) was added to a suspension of **6** (120.09 g, 508 mmol), dimethyl D-(–)-tartrate (18.10 g, 102 mmol) and molecular sieves 4A (activated powder, 24.00 g) in  $\text{CH}_2\text{Cl}_2$  (1200 ml) at –10––9 °C under nitrogen atmosphere. Stirring was continued for 2 h, then *tert*-butyl hydroperoxide (3.0 M solution in 2,2,4-trimethylpentane, 85.00 ml) was added to the suspension and the whole was stirred at –10 °C for 2 d. The mixture was poured into 0.4 N HCl (1550 ml) and the resulting emulsion was filtered through a celite pad. The organic layer was separated and the aqueous layer was extracted with dichloromethane ( $\text{CH}_2\text{Cl}_2$ ). The combined organic layers were washed with brine, dried over  $\text{MgSO}_4$ , and evaporated *in vacuo*. The residue was purified by column chromatography on silica gel with a mixture of  $\text{CH}_2\text{Cl}_2$  and EtOAc (1:0 to 0:1) to afford (+)-**6** (67.73 g),  $[\alpha]_{\text{D}}^{21.2} +100.8^\circ$  ( $c=1.19$ , MeOH) and (–)-**5** (50.20 g),  $[\alpha]_{\text{D}}^{22.4} -150.5^\circ$  ( $c=0.63$ ,  $\text{CH}_2\text{Cl}_2$ ).

The crude product (–)-**5** was purified as follows: The crude product (50.03 g) was recrystallized from EtOAc (150 ml) to afford its racemate (16.46 g, mp 140–141 °C,  $[\alpha]_{\text{D}}^{18} -4.2^\circ$  ( $c=0.61$ ,  $\text{CH}_2\text{Cl}_2$ ). Anal. Calcd for  $\text{C}_{17}\text{H}_{16}\text{O}_2$ : C, 80.93; H, 6.39. Found: C, 80.74; H, 6.36. IR (nujol): 3400, 1100  $\text{cm}^{-1}$ . NMR ( $\text{CDCl}_3$ , 200 MHz):  $\delta$  1.62 (1H, d,  $J=12.2$  Hz), 2.41 (1H, d,  $J=15.2$  Hz), 3.46 (1H, d,  $J=15.2$  Hz), 3.65 (2H, s), 4.96 (1H, d,  $J=12.2$  Hz), 7.04–7.36 (10H, m). The filtrate was evaporated *in vacuo* and the residue was recrystallized from EtOAc (50 ml) to afford racemate (1.42 g,  $[\alpha]_{\text{D}}^{16} -5.7^\circ$  ( $c=0.53$ ,  $\text{CH}_2\text{Cl}_2$ )). The filtrate was evaporated *in vacuo* and the residue was stirred in a mixture of *n*-hexane (100 ml) and petroleum ether (20 ml). The resulting precipitates were collected by filtration to afford (–)-**5** (25.67 g,  $[\alpha]_{\text{D}}^{16} -298.6^\circ$  ( $c=0.52$ ,  $\text{CH}_2\text{Cl}_2$ )). This compound was recrystallized from EtOAc (25 ml) to afford pure (–)-**5** (15.51 g, 12%), mp 97–98 °C.  $[\alpha]_{\text{D}}^{18} -300.2^\circ$  ( $c=0.48$ ,  $\text{CH}_2\text{Cl}_2$ ). Anal. Calcd for  $\text{C}_{17}\text{H}_{16}\text{O}_2$ : C, 80.93; H, 6.39. Found: C, 80.94; H, 6.48. IR (nujol): 3420  $\text{cm}^{-1}$ . NMR ( $\text{CDCl}_3$ , 200 MHz):  $\delta$  1.61 (1H, d,  $J=12.3$  Hz), 2.42 (1H, dd,  $J=15.2$ , 1.3 Hz), 3.47 (1H, d,  $J=15.2$  Hz), 3.61–3.70 (2H, m), 4.97 (1H, dd,  $J=12.3$ , 1.3 Hz), 7.05–7.40 (10H, m). The enantiomeric excess of this compound was determined to be 99.5% by HPLC analysis (column, SUMIPAX OA-4500 (Sumitomo) 4.6×250 mm; eluent, 25:1 *n*-hexane–EtOH mixture; flow rate, 1.0 ml/min; detector, 220 nm;  $t_{\text{R}}$  of (–) isomer, 8.9 min;  $t_{\text{R}}$  of (+) isomer, 9.9 min). The enantiomeric excess of the compound (+)-**6** was determined to be 30.4% by HPLC analysis (column, CHIRALCEL-OD (Daicel) 4.6×250 mm; eluent, 50:1 *n*-hexane–EtOH mixture; flow rate, 1.0 ml/min; detector, 220 nm;  $t_{\text{R}}$  of (–) isomer, 10.1 min;  $t_{\text{R}}$  of (+) isomer, 12.5 min).

**(1S,2R,3S)-(–)-3-*tert*-Butylamino-5,5-diphenylcyclopentane-1,2-diol Hydrochloride [(–)-12·HCl]** Titanium(IV) isopropoxide (2.15 ml, 7.22 mmol) was added to a solution of (–)-**5** (1.40 g, 5.55 mmol) in a mixture of  $\text{CH}_2\text{Cl}_2$  (14 ml) and isopropyl alcohol (5.6 ml) with ice bath cool-

ing. Stirring was continued for 30 min, then *tert*-butylamine (0.70 ml, 6.66 mmol) was added to the solution and the whole was stirred at room temperature overnight. The solution was evaporated *in vacuo* and the residue was dissolved in diethyl ether. 3 N HCl was added to the solution in an ice bath and the whole was stirred for 3 h. The resulting precipitates were collected by filtration, washed with diethyl ether and dried to afford (–)-**12·HCl** (1.98 g, 99%), mp 279–281 °C (dec.). Anal. Calcd for  $\text{C}_{21}\text{H}_{27}\text{NO}_2 \cdot \text{HCl} \cdot \text{H}_2\text{O}$ : C, 66.39; H, 7.96; N, 3.69. Found: C, 66.27; H, 8.21; N, 3.60.  $[\alpha]_{\text{D}}^{22} -70.5^\circ$  ( $c=0.37$ , MeOH). IR (nujol): 3520, 3320  $\text{cm}^{-1}$ . NMR ( $\text{DMSO}-d_6$ , 200 MHz):  $\delta$  1.36 (9H, s), 2.63 (1H, dd,  $J=14.0$ , 7.7 Hz), 3.12 (1H, dd,  $J=14.0$ , 9.9 Hz), 3.48–3.72 (1H, brs), 4.09–4.26 (1H, m), 4.68–4.84 (1H, m), 5.15 (1H, d,  $J=5.8$  Hz), 5.23 (1H, d,  $J=4.9$  Hz), 7.00–7.50 (10H, m), 8.60–8.78 (1H, m), 9.00–9.18 (1H, m). The enantiomeric excess of the compound (–)-**12·HCl** was determined to be >99.4% by HPLC analysis (column, CHIRALCEL-OD (Daicel) 4.6×250 mm; eluent, 9:1 *n*-hexane–EtOH mixture; flow rate, 1.0 ml/min; detector, 220 nm;  $t_{\text{R}}$  of (+) isomer, 6.5 min;  $t_{\text{R}}$  of (–) isomer, 8.0 min).

**(S)-(–)-*N*-*tert*-Butyl-4,4-diphenyl-2-cyclopentenylamine Hydrochloride ((–)-4·HCl, FK584)** Trimethyl orthoformate (2.80 ml, 25.6 mmol) was added to a suspension of (–)-**12** (1.85 g, 5.11 mmol) and TsOH monohydrate (0.10 g, 0.51 mmol) in  $\text{CH}_2\text{Cl}_2$  (18.5 ml) and the whole was stirred at room temperature for 2 h. Then, the mixture was evaporated *in vacuo* and 1 N NaOH solution (18.5 ml) and EtOAc were added to the residue. The organic layer was separated, washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , and evaporated *in vacuo*. The residue was purified by column chromatography on silica gel with a mixture of  $\text{CHCl}_3$  and MeOH (50:1) to afford **13** (1.83 g, 97%) as an oil. IR (neat): 3350, 1735, 1600, 1580, 1490, 1445, 1365  $\text{cm}^{-1}$ . NMR ( $\text{CDCl}_3$ , 200 MHz) for a major diastereomer A:  $\delta$  1.01 (9H, s), 2.12 (1H, dd,  $J=5.1$ , 13.2 Hz), 3.13 (1H, dd,  $J=7.5$ , 13.2 Hz), 3.25 (3H, s), 3.35–3.51 (1H, m), 4.67 (1H, d,  $J=6.0$  Hz), 5.41 (1H, s), 5.58 (1H, d,  $J=6.0$  Hz), 7.06–7.36 (10H, m). NMR ( $\text{CDCl}_3$ , 200 MHz) for a minor diastereomer B:  $\delta$  0.92 (9H, s), 2.12 (1H, dd,  $J=5.1$ , 13.2 Hz), 3.13 (1H, dd,  $J=7.5$ , 13.2 Hz), 3.15 (3H, s), 3.35–3.51 (1H, m), 4.76 (1H, d,  $J=5.5$  Hz), 5.46 (1H, d,  $J=5.5$  Hz), 5.70 (1H, s), 7.06–7.36 (10H, m). The ratio of the diastereomer A/B was 10/1. MS  $m/z$ : 367 ( $\text{M}^+$ ), 352. To a solution of **13** (0.80 g, 2.18 mmol) in xylene (16 ml) was added acetic acid (0.69 ml, 12.05 mmol) and acetic anhydride (0.04 ml, 0.42 mmol) and the whole was refluxed for 3.5 h. After cooling, 1.5 N of NaOH solution (20 ml) and EtOAc were added to the solution. The organic layer was separated, washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , and evaporated *in vacuo*. The residue was purified by column chromatography on silica gel with a mixture of  $\text{CHCl}_3$  and MeOH (30:1) to afford (–)-**4** (0.58 g) as an oil. A 4 N solution of HCl in EtOAc (0.55 ml) was added to a solution of (–)-**4** (0.58 g) in a mixture of isopropyl alcohol (1.2 ml) and EtOAc (5.8 ml) and the whole was stirred at 0 °C for 3.5 h. The resulting precipitates were collected by filtration and dried to afford (–)-**4·HCl** (0.32 g, 44%, >99.8% ee). The physical data of this compound was identical with those of FK584 reported in the preceding paper.<sup>1)</sup>

**X-Ray Crystallography of (–)-4·(S)-(+)-Manderate** A mixture of (–)-**4** (1.00 g, 3.43 mmol) and (S)-(+)-mandelic acid (522 mg, 3.43 mmol) in isopropyl alcohol (20 ml) was refluxed and the obtained solution was cooled to room temperature overnight. The resulting precipitates were collected by filtration and dried to give (–)-**4·(S)-(+)-manderate** (1.40 g, 92%) as crystals, mp 151–155 °C (recrystallized from ethanol).  $[\alpha]_{\text{D}}^{20} -102.0^\circ$  ( $c=1.03$ , MeOH). Anal. Calcd for  $\text{C}_{29}\text{H}_{33}\text{O}_3\text{N}$ : C, 78.52; H, 7.50; N, 3.16. Found: C, 78.56; H, 7.69; N, 3.12. IR (nujol): 3090, 2800–2300, 1615, 1575  $\text{cm}^{-1}$ . NMR ( $\text{DMSO}-d_6$ , 200 MHz):  $\delta$  1.24 (9H, s), 2.31 (1H, dd,  $J=7.9$ , 13.4 Hz), 3.22 (1H, dd,  $J=7.1$ , 13.4 Hz), 4.27 (1H, br t,  $J=7.4$  Hz), 4.60 (1H, s), 5.94 (1H, br d,  $J=5.7$  Hz), 6.58 (1H, dd,  $J=5.7$ , 1.9 Hz), 7.15–7.40 (15H, m). Crystal data of (–)-**4·(S)-(+)-manderate** were as follows:  $\text{C}_{29}\text{H}_{33}\text{O}_3\text{N}$ , monoclinic,  $P2_1$  (#4),  $a=8.328$  (2) Å,  $b=10.306$  (1) Å,  $c=14.568$  (1) Å,  $\beta=98.91$  (1)°,  $V=1235.2$  (3) Å<sup>3</sup>,  $Z=2$ ,  $D_{\text{calcd}}=1.193$  g/cm<sup>3</sup>,  $F_{(000)}=476.00$ ,  $\mu$  (MoK $\alpha$ )=5.68  $\text{cm}^{-1}$ . Intensities were collected on a Rigaku AFC5R diffractometer with graphite monochromated CuK $\alpha$  radiation ( $\lambda=1.54178$  Å), and 2185 unique reflections with  $I_0 \geq 3\sigma$  were obtained using the  $\omega-2\theta$  scanning method within  $6^\circ \leq 2\theta \leq 130^\circ$ . The structure was solved using MULTAN 84 based on direct methods, and refined. The final R value was 0.058. An ORTEP drawing of (–)-**4·(S)-(+)-manderate** is shown in Fig. 3.

**Acknowledgements** We wish to thank Dr. T. Tada and the staff of the Analytical Research Laboratories, Fujisawa Pharmaceutical Co., Ltd. for X-ray crystallographic analyses and measurement of the two dimensional (2D)-NMR (nuclear Overhauser enhancement and exchange spectroscopy NOESY) spectrum.

## References and Notes

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- 2) This compound was obtained by reducing 4,4-diphenyl-2-cyclopenten-1-one with diisopropyl aluminum hydride as reported<sup>1)</sup> or, more conveniently, from **6** by treatment with MsCl and triethylamine, followed by quenching with water in quantitative yield.
- 3) Lipase PS shows a preference for the (*R*)-configuration, so the absolute configuration of (+)-**7** and (–)-**2** was believed to be *S* and *R*, respectively, however, we have not determined these configurations. *a*) Kazlauskas R. J., Weissfloch A. N. E., Rappaport A. T., Cuccia L. A., *J. Org. Chem.*, **56**, 2656—2665 (1991); *b*) Cygler M., Grochulski P., Kazlauskas R. J., Schrag J. D., Bouthillier F., Rubin B., Serreque A. N., Gupta A. K., *J. Am. Chem. Soc.*, **116**, 3180—3186 (1994).
- 4) The oxidative addition forming  $\pi$ -allylpalladium usually proceeds with inversion of the configuration, while the stereochemistry at the nucleophilic substitution of the  $\pi$ -allylpalladium is dependent upon the nature of the nucleophile. When dimethylamine was used as a nucleophile, it attacked the  $\pi$ -allyl ligand from the side opposite the palladium. *a*) For reviews, see: Frost C. G., Howarth J., Williams J. M. J., *Tetrahedron Asymmetry*, **3**, 1089—1122 (1992); Godleski S. A., "Comprehensive Organic Synthesis," ed. by Trost B. M., Fleming I., Pergamon Press, Oxford, 1991, Vol. 4, Chapter 3.3, pp. 585—661; Consiglio G., Waymouth R. M., *Chem. Rev.*, **89**, 257—276 (1989); *b*) Hayashi T., Konishi M., Kumada M., *J. Chem. Soc., Chem. Commun.*, **1984**, 107—108 and references cited therein.
- 5) IR (neat): 3060, 3025, 2950  $\text{cm}^{-1}$ . NMR ( $\text{CDCl}_3$ , 90 MHz):  $\delta$  6.36 (2H, br d,  $J=6$  Hz), 6.80 (2H, br d,  $J=6$  Hz), 7.20 (10H, s). MS  $m/z$ : 218 ( $\text{M}^+$ ).
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- 9) Nuclear Overhauser effects were observed between hydrogens at the C-1, C-2, C-3, and C-4 positions by a NOESY experiment of (–)-**5**, so the relative configuration of the hydroxyl group and the epoxide is *cis*.
- 10) Opposite results ((+)-**5**, (–)-**6**) were obtained with dimethyl L-(+)-tartrate. (+)-**5**: 39%, 58.4% ee,  $[\alpha]_D^{22} +175.8^\circ$  ( $c=0.50$ ,  $\text{CH}_2\text{Cl}_2$ ). (–)-**6**: 59%, 38.0% ee,  $[\alpha]_D^{22} +127.4^\circ$  ( $c=0.52$ , MeOH).
- 11) The empirical rule in Sharpless oxidation is that the allylic alcohol with a C-1 substituent is drawn so that the olefinic carbons and the hydroxyl lie in the plane of the paper, and the carbinol carbon is at the lower right, D-(–)-tartrates mediate delivery of the oxygen atom from the top face, so one enantiomer having the C-1 substituent oriented away from the direction of oxygen delivery, *i.e.*, (1*S*)-**6** was thought to be oxidized much faster than the other enantiomer. The kinetic resolutions of 2-cyclohexen-1-ol and 2-cyclohepten-1-ol with 1 eq of titanium(IV) isopropoxide, 1.2 eq of diisopropyl L-(+)-tartrate and 0.6 eq of *tert*-butylhydroperoxide at  $-20^\circ\text{C}$  afforded (1*R*)-2-cyclohexen-1-ol and (1*R*)-2-cyclohepten-1-ol, respectively, in accord with the empirical rule. Martin V. S., Woodard S. S., Katsuki T., Yamada Y., Ikeda M., Sharpless K. B., *J. Am. Chem. Soc.*, **103**, 6237—6240 (1981).
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## 5,11-Epoxymegastigmanes from the Leaves of *Asclepias fruticosa*<sup>1)</sup>

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Received June 14, 2000; accepted July 21, 2000

Five new megastigmane glucosides and one of their aglycones were isolated, along with (6*S*,9*R*)-roseoside, from the polar fraction of the leaves of *Asclepias fruticosa*, and the structures were determined by spectroscopic methods. Most of them have an epoxy-linkage between C-5 and C-11. The configurations at C-3 and C-9 for each compound were confirmed to be *S* and *R*, respectively, by application of the modified Mosher's method.

**Key words** 5,11-epoxymegastigmane; ascleposide; *Asclepias fruticosa*; Asclepiadaceae

During investigations of the polar constituents of Asclepiadaceous plants, we isolated an 11-glucosyloxymegastigmane, along with conduritol F and its 3-*O*- and 4-*O*-glucosides from *Cynanchum liukuense* WARB.<sup>1)</sup> Since the occurrence of 11-hydroxylated megastigmane is rare in Asclepiadaceous plants, we examined other species in this family. This paper deals with the isolation and structure determination of five new glucosides of 5,11-epoxymegastigmanes, named ascleposides A—E (**2**, **4**—**7**), and the aglycone of **2** (**3**), along with (6*S*,9*R*)-roseoside (**1**),<sup>2)</sup> from the leaves of *Asclepias fruticosa* L.

Fresh leaves were soaked in 50% aqueous acetone and the extract was partitioned between *n*-BuOH/H<sub>2</sub>O. The H<sub>2</sub>O layer was chromatographed on a charcoal column and the column was eluted with 5—50% EtOH in H<sub>2</sub>O. Compounds **2** and **3** were obtained from 10% EtOH, **4** from 20% EtOH, and **1**, **6** and **7** from 50% EtOH effluent. Compound **5** was isolated from the *n*-BuOH soluble fraction, along with **6**, after Diaion HP-20 and silica gel column chromatography. Compound **1** was identified as (6*S*,9*R*)-roseoside, based on a comparison of its optical rotation and the <sup>1</sup>H- and <sup>13</sup>C-NMR signals reported in the literature.<sup>2)</sup>

Based on high resolution (HR)-FAB-MS, the molecular formula of ascleposide A (**2**) was assigned as C<sub>19</sub>H<sub>32</sub>O<sub>9</sub> (*m/z*: 427.1943, [M+Na]<sup>+</sup>). The presence of an anomeric proton signal at  $\delta$  4.35 (d, *J*=8 Hz) and the molecular formula suggested **2** to be a glucoside of megastigmane. In the <sup>1</sup>H-NMR

spectrum of **2**, only two tertiary methyl signals ( $\delta$  0.91, 1.12, each s) and hydroxymethyl signals ( $\delta$  3.72 (d, *J*=8 Hz) and  $\delta$  3.77 (dd, *J*=8, 2 Hz)) were observed instead of three tertiary methyl signals in **1**, and the <sup>1</sup>H—<sup>1</sup>H shift correlation spectroscopy (COSY) spectrum suggested a connection from the terminal methyl signal (H-10) to H-7 in the side chain as observed in **1**. A signal at  $\delta$  4.23 (m) seemed to be due to an axial methine proton at C-3. In the <sup>13</sup>C-NMR spectrum, three quaternary carbon signals were assigned to C-1 ( $\delta$  48.8), C-6 ( $\delta$  82.6) and C-5 ( $\delta$  87.3) and the carbon signals due to glucose were also ascribable. Since three bond correlations were observed between protons and carbons (H-12/C-2,6,11; H-13/C-4,6; H-11/C-5,6) as shown in Chart 2 in the heteronuclear multiple bond correlation (HMBC) spectrum, **2** was considered to be a glucoside of 5,11-epoxy-7,8-dehydro-3,6,9-trihydroxymegastigmane.

Upon hydrolysis with cellulase, **2** afforded an aglycone (**2a**, C<sub>13</sub>H<sub>22</sub>O<sub>4</sub>) along with glucose which was confirmed to be in the D-form from its optical rotation ([ $\alpha$ ]<sub>D</sub> +66.0°). The location of D-glucopyranose was assigned to be the 3-hydroxy group based on the glucosylation shifts of C-3 (+7.6 ppm) along with C-2 (−1.8 ppm, pro-*R*) and C-4 (−3.2 ppm, pro-*S*) as well as cross peaks (H-3/C-1', H-1'/C-3) in the HMBC spectrum. The configuration of C-3 was also suggested to be *S*.<sup>3)</sup>

Since nuclear Overhauser effect (NOE) responses were observed between H-7/H-2 $\beta$ ,4 $\beta$  and H-3/H-2 $\alpha$ ,4 $\alpha$  in **2a**, a

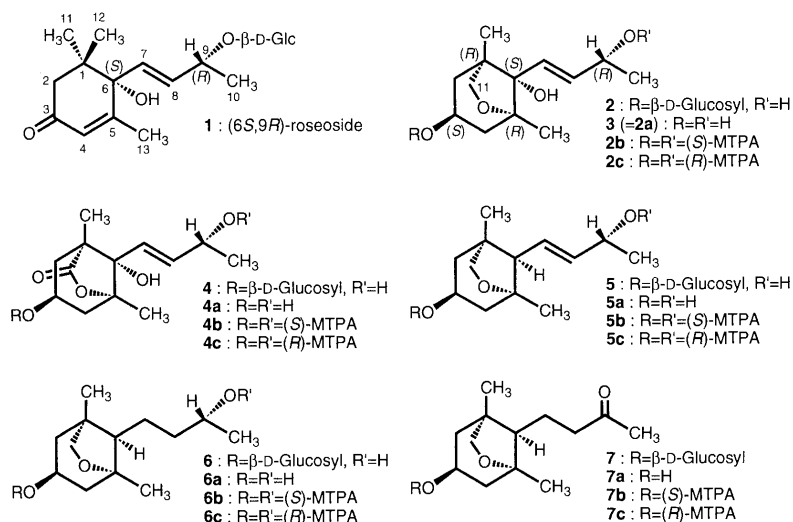


Chart 1

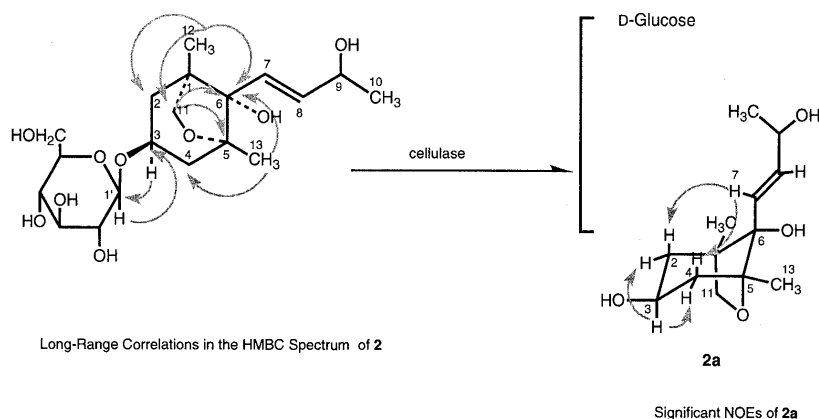
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Table 1.  $^{13}\text{C}$  Spectral Data for **2**—**7** and **4a**—**7a** in  $\text{CD}_3\text{OD}$  ( $\delta$  ppm from tetramethylsilane (TMS))

| C  | <b>2</b> | <b>3 (=2a)</b> | <b>4</b> | <b>4a</b> | <b>5</b> | <b>5a</b> | <b>6</b> | <b>6a</b> | <b>7</b> | <b>7a</b> |
|----|----------|----------------|----------|-----------|----------|-----------|----------|-----------|----------|-----------|
| 1  | 48.8     | 48.8           | 53.1     | 53.1      | 44.7     | 44.7      | 44.0     | 44.0      | 44.0     | 44.0      |
| 2  | 42.7     | 44.5           | 39.4     | 40.9      | 39.1     | 40.8      | 38.8     | 40.6      | 38.7     | 40.5      |
| 3  | 73.6     | 66.0           | 72.8     | 65.2      | 74.5     | 66.9      | 74.4     | 66.7      | 74.3     | 66.6      |
| 4  | 42.7     | 45.9           | 39.3     | 42.2      | 39.5     | 42.7      | 39.1     | 42.3      | 39.0     | 42.3      |
| 5  | 87.3     | 87.4           | 89.7     | 89.9      | 84.7     | 84.9      | 85.3     | 85.4      | 85.1     | 85.3      |
| 6  | 82.6     | 82.6           | 82.2     | 82.3      | 60.0     | 60.0      | 55.6     | 55.5      | 54.8     | 54.7      |
| 7  | 126.9    | 126.9          | 124.5    | 124.6     | 124.4    | 124.3     | 22.1     | 22.1      | 19.6     | 19.6      |
| 8  | 139.5    | 139.6          | 141.3    | 141.4     | 141.8    | 141.9     | 40.0     | 40.0      | 43.6     | 43.6      |
| 9  | 69.1     | 69.1           | 68.8     | 68.8      | 69.1     | 69.0      | 68.8     | 68.8      | 211.0    | 211.0     |
| 10 | 24.0     | 24.0           | 23.9     | 23.8      | 24.0     | 23.9      | 23.5     | 23.5      | 29.8     | 29.8      |
| 11 | 77.0     | 77.2           | 181.2    | 181.4     | 78.7     | 78.8      | 78.1     | 78.2      | 78.0     | 78.2      |
| 12 | 16.2     | 16.2           | 14.3     | 14.4      | 21.4     | 21.4      | 21.8     | 21.8      | 21.7     | 21.7      |
| 13 | 19.5     | 19.4           | 18.3     | 18.3      | 25.0     | 25.0      | 25.8     | 25.7      | 25.7     | 25.6      |
| 1' | 102.8    |                | 103.1    |           | 102.7    |           | 102.7    |           | 102.7    |           |
| 2' | 75.2     |                | 75.1     |           | 75.2     |           | 75.1     |           | 75.1     |           |
| 3' | 78.1     |                | 78.0     |           | 78.1     |           | 78.1     |           | 78.1     |           |
| 4' | 71.7     |                | 71.6     |           | 71.7     |           | 71.7     |           | 71.7     |           |
| 5' | 77.9     |                | 77.9     |           | 77.9     |           | 77.9     |           | 77.9     |           |
| 6' | 62.8     |                | 62.6     |           | 62.8     |           | 62.8     |           | 62.8     |           |

Table 2.  $^1\text{H}$  Spectral Data for **2**—**6** and **7** in  $\text{CD}_3\text{OD}$  ( $\delta$  ppm from TMS,  $J$  in Hz)

| H          | <b>2</b>              | <b>3</b>              | <b>4</b>             | <b>5</b>          | <b>6</b>          | <b>7</b>          |
|------------|-----------------------|-----------------------|----------------------|-------------------|-------------------|-------------------|
| 2 $\alpha$ | 1.93 (ddd, 14, 7, 1)  | 1.80 (ddd, 13, 7, 2)  | 2.03 (ddd, 14, 7, 1) | 1.82 (dd, 13, 7)  | 1.77 (dd, 13, 7)  | 1.77 (dd, 13, 7)  |
| 2 $\beta$  | 1.80 (ddd, 14, 10, 2) | 1.67 (ddd, 13, 11, 2) | 1.90 (dd, 14, 11)    | 1.64 (br t, 13)   | 1.59 (br t, 13)   | 1.57 (br t, 13)   |
| 3          | 4.23 (m)              | 4.09 (m)              | 3.99 (m)             | 4.17 (m)          | 4.11 (m)          | 4.10 (m)          |
| 4 $\alpha$ | 2.14 (ddd, 14, 7, 1)  | 1.99 (ddd, 13, 7, 2)  | 2.41 (ddd, 14, 7, 1) | 1.98 (dd, 13, 7)  | 1.94 (dd, 13, 7)  | 1.95 (dd, 13, 7)  |
| 4 $\beta$  | 1.79 (dd, 14, 10)     | 1.72 (dd, 13, 10)     | 1.94 (dd, 14, 10)    | 1.56 (dd, 13, 10) | 1.55 (dd, 13, 10) | 1.52 (dd, 13, 10) |
| 6          |                       |                       |                      | 2.04 (br d, 9)    | 1.30—1.37 (m)     | 1.35 (t, 6)       |
| 7          | 6.08 (d, 16)          | 6.07 (d, 16)          | 6.11 (d, 16)         | 5.74 (m)          | 1.30—1.37 (m)     | 1.60 (m)          |
|            |                       |                       |                      |                   | 1.67 (m)          | 1.79 (m)          |
| 8          | 6.04 (dd, 16, 5)      | 6.03 (dd, 16, 5)      | 6.07 (dd, 16, 5)     | 5.74 (m)          | 1.52—1.58 (m)     | 2.62 (2H, t, 8)   |
| 9          | 4.36 (qd, 7, 5)       | 4.35 (qd, 7, 5)       | 4.37 (qd, 7, 5)      | 4.28 (m)          | 3.72 (m)          |                   |
| 10         | 1.27 (d, 7)           | 1.27 (d, 7)           | 1.28 (d, 7)          | 1.25 (d, 6)       | 1.18 (d, 6)       | 2.15 (s)          |
| 11         | 3.72 (d, 8)           | 3.68 (d, 7)           |                      | 3.46 (dd, 8, 2)   | 3.41 (dd, 8, 2)   | 3.41 (dd, 8, 2)   |
|            | 3.77 (dd, 8, 2)       | 3.78 (dd, 7, 2)       |                      | 3.76 (d, 8)       | 3.66 (d, 8)       | 3.66 (d, 8)       |
| 12         | 0.91 (s)              | 0.91 (s)              | 1.05 (s)             | 0.94 (s)          | 1.00 (s)          | 1.00 (s)          |
| 13         | 1.12 (s)              | 1.10 (s)              | 1.31 (s)             | 1.16 (s)          | 1.24 (s)          | 1.24 (s)          |
| 1'         | 4.35 (d, 8)           |                       | 4.33 (d, 8)          | 4.37 (d, 8)       | 4.35 (d, 8)       | 4.34 (d, 8)       |
| 2'         | 3.13 (dd, 8, 9)       |                       | 3.13 (dd, 8, 9)      | 3.14 (dd, 8, 9)   | 3.12 (dd, 8, 9)   | 3.12 (dd, 8, 9)   |
| 3'         | 3.35 (t, 9)           |                       | 3.34 (t, 9)          | 3.35 (t, 9)       | 3.34 (t, 9)       | 3.34 (t, 9)       |
| 4'         | 3.28 (t, 9)           |                       | 3.28 (t, 9)          | 3.28 (t, 9)       | 3.28 (t, 9)       | 3.27 (t, 9)       |
| 5'         | 3.27 (m)              |                       | 3.27 (m)             | 3.27 (m)          | 3.27 (m)          | 3.26 (m)          |
| 6'         | 3.66 (dd, 11, 5)      |                       | 3.66 (dd, 12, 5)     | 3.66 (dd, 12, 5)  | 3.66 (dd, 12, 5)  | 3.66 (dd, 11, 4)  |
|            | 3.86 (dd, 11, 1)      |                       | 3.84 (dd, 12, 2)     | 3.86 (dd, 12, 2)  | 3.86 (dd, 12, 2)  | 3.85 (dd, 11, 1)  |



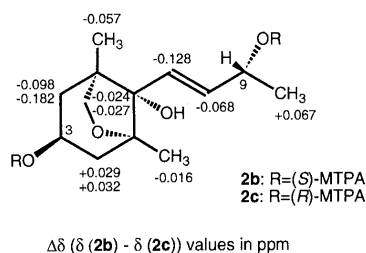


Fig. 1

*trans* diaxial relationship between H-3 and the side chain at C-6 was assigned. Comparison of the chemical shifts of (*S*)- and (*R*)- $\alpha$ -methoxy- $\alpha$ -trifluoromethylphenyl acetate (MTPA) derivatives of **2a** (**2b** and **2c**) by the modified Mosher's procedure<sup>4)</sup> resulted in the configurations at C-3 and C-9 being assigned as *S* and *R*, respectively (Fig. 1). Therefore, **2** was confirmed to be (1*R*,3*S*,5*R*,6*S*,9*R*)-5,11-epoxy-3,6,9-trihydroxy-7-megastigmen-3-*O*- $\beta$ -D-glucopyranoside. Compound **3** was identified as **2a**, based on a comparison of the <sup>1</sup>H- and <sup>13</sup>C-NMR spectra and optical rotations of the two compounds.

Ascleposide B (**4**) afforded a  $[M+Na]^+$  peak at  $m/z$  441.1737, suggesting the molecular formula, C<sub>19</sub>H<sub>30</sub>O<sub>10</sub>. In the <sup>1</sup>H-NMR spectrum of **4**, signals due to the side chain and the NOE responses were similar to those of **2**. A carbonyl carbon signal, which has a correlation from H-12 in the HMBC spectrum, was observed at  $\delta$  181.2 instead of the hydroxymethylene carbon due to C-11 in **2**, suggesting **4** to be an 11-oxo-derivative of **2**, forming a lactone linkage between C-5 and C-11. The aglycone of **4** (**4a**), obtained by enzymatic hydrolysis of **4**, was subjected to the modified Mosher's procedure<sup>4)</sup> as in **2a**, to yield (*S*)- and (*R*)-MTPA derivatives (**4b**, **4c**), in which the signals of H-2, 7, 8 were observed at a higher field in **4b** than in **4c**, while H-4 and H-10 were at a lower field. Thus, the configurations at C-3 and C-9 in **4** were confirmed to be *S* and *R*, respectively, as in **2**. The  $\beta$ -glucopyranose at C-3-OH was assigned as being in the D-form since C-2 (pro-*R*) and C-4 (pro-*S*) showed glucosylation shifts of -1.5 ppm and -2.9 ppm, respectively, along with the deshielding of C-3 (+7.6 ppm).<sup>3)</sup>

The molecular formula of ascleposide C (**5**) was considered to be C<sub>19</sub>H<sub>32</sub>O<sub>8</sub>, one oxygen less than **2**, based on HR-FAB-MS. A methine proton signal at  $\delta$  2.04 (br d,  $J=9$  Hz) showed a correlation to the olefinic proton signal at  $\delta$  5.74 (H-7) in the <sup>1</sup>H-<sup>1</sup>H COSY spectrum and a 3-bond correlation to C-8 ( $\delta$  141.8) in the HMBC spectrum, suggesting it to be due to H-6. Since the corresponding carbon signal (C-6) was observed at  $\delta$  60.0, showing cross peaks from H-11, 12, 13 in the HMBC spectrum, **5** was assigned to be the 6-deoxy derivative of **2**. The presence of an NOE between H-6 and H-11a strongly suggested the same orientation of the C-6 side chain as that of **2**—**4**. The configurations at C-3 and C-9, and the presence of a  $\beta$ -D-glucosyl residue at C-3-OH were determined as described above.

The molecular formula of ascleposide D (**6**) was shown to be C<sub>19</sub>H<sub>34</sub>O<sub>8</sub>, by HR-FAB-MS, 2H more than **5** and no olefinic proton signals were observed in the <sup>1</sup>H-NMR spectrum. The presence of two additional methylene carbon signals at  $\delta$  22.1 and 40.0, instead of two olefinic carbons observed in **1**—**5**, suggested that **6** is a 7,8-saturated derivative

of **5**. NOE correlations were observed at H-6/H-11a ( $\delta$  3.41) and H-3/H-11b ( $\delta$  3.66). Therefore, **6** was considered to have the same 5,11-epoxymegastigmane structure as **5**, and the configurations at C-3 and C-9 were finally determined based on the modified Mosher's procedure for the aglycone (**6a**)<sup>4)</sup> after enzymatic hydrolysis of **6**. Glucose was also confirmed to be in D-form by glucosylation shifts of C-2 and C-4.<sup>3)</sup>

HR-FAB-MS of ascleposide E (**7**) showed the same molecular formula as **5**, 2H less than **6**. In the NMR spectra, one carbonyl carbon signal was observed at  $\delta$  211.0, and one additional 3H singlet signal was observed at  $\delta$  2.15, instead of a 3H doublet signal in **2**—**6**, suggesting **7** to be a 9-oxo-derivative of **6**. The stereochemistry of the megastigmane nucleus in **7** was shown to be the same as **2**—**6** by the NOE correlation and the  $\Delta\delta$  value ( $\delta$  (**7b**)— $\delta$  (**7c**)).

Previously, we reported that the 11-*O*- $\beta$ -D-glucoside of 6,9,11-trihydroxy-4,7-megastigmadien-3-one from *Cynanchum liukiuense* was transformed into an 5,11-epoxy-structure by splitting the glucosyl linkage.<sup>1)</sup> The possibility that 5,11-epoxymegastigmanes are biosynthesized in the plant through an 11-glucosyloxy-4-megastigmen-3-one intermediate cannot be excluded.

## Experimental

<sup>1</sup>H- and <sup>13</sup>C-NMR spectra were recorded on a JEOL JNM-A500 spectrometer in CD<sub>3</sub>OD. Chemical shifts are given as  $\delta$  values referred to the internal standard, TMS, and the following abbreviations are used: s=singlet, d=doublet, t=triplet, m=multiplet, br=broad. The  $J$  value in HMBC experiment was 8 Hz. HR-FAB-MS was recorded on a JEOL HX-110 spectrometer. Optical rotations were measured on a JASCO DIP 360 polarimeter. For silica gel column chromatography and TLC, the following solvent systems were used: CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O (7:3:1.6—7:3:1.0, bottom layer, solvent 1), EtOAc-MeOH-H<sub>2</sub>O (8:1:1.2—6:1:1.2, top layer, solvent 2). For HPLC (Capcell Pak NH<sub>2</sub> column, UG 80 type, 10 mm i.d.×250 mm), CH<sub>3</sub>CN-H<sub>2</sub>O was used. Megastigmanes were visualized on TLC plates by spraying with 10% H<sub>2</sub>SO<sub>4</sub> and heating.

**Plant Materials** *Asclepias fruticosa* L. was cultivated in the medicinal plant garden of Fukuoka University in 1998, and harvested in September.

**Extraction and Isolation of Megastigmanes** Fresh leaves (2.4 kg) were soaked in 50% aqueous acetone for 2 months and filtered. The filtrate was concentrated *in vacuo* and partitioned between *n*-BuOH-H<sub>2</sub>O. The H<sub>2</sub>O layer was concentrated *in vacuo* to dryness (extract 265 g) and chromatographed on a charcoal column. The column was eluted with H<sub>2</sub>O (2 l), and then with 5, 10, 20, 50% EtOH in H<sub>2</sub>O. Each eluate with 10—50% EtOH was further chromatographed on a silica gel column with solvents 1 and 2, and HPLC (80—90% CH<sub>3</sub>CN). **1**: 44 mg (50% EtOH elution), **2**: 151 mg (10% EtOH), **3**: 17 mg (10% EtOH), **4**: 24 mg (20% EtOH), **6**: 55 mg (50% EtOH), **7**: 9 mg (50% EtOH). The BuOH layer (extract 18.7 g) was chromatographed on a Diaion HP-20 column (Mitsubishi Chem. Ind. Ltd.) with H<sub>2</sub>O-MeOH and a silica gel column with solvents 1 and 2 to afford **5** (11 mg) and **6** (31 mg).

Ascleposide A (**2**): A solid,  $[\alpha]_D^{20} -23.2^\circ$  ( $c=1.37$ , MeOH), HR-FAB-MS  $m/z$ : 427.1943 ( $[M+Na]^+$ ) (Calcd for C<sub>19</sub>H<sub>32</sub>O<sub>9</sub>+Na: 427.1944). Compound **2** (50 mg) was subjected to hydrolysis with cellulase (Sigma, grade II) (100 mg) in H<sub>2</sub>O (2 ml) for 8 h at 38 °C, and the mixture was extracted with *n*-BuOH. The BuOH layer was purified on a silica gel column with solvent 1 to give **2a** as a solid,  $[\alpha]_D^{20} +1.0^\circ$  ( $c=1.05$ , MeOH), HR-FAB-MS  $m/z$ : 265.1415 ( $[M+Na]^+$ ) (Calcd for C<sub>13</sub>H<sub>22</sub>O<sub>4</sub>+Na: 265.1416). From H<sub>2</sub>O layer D-glucose was obtained,  $[\alpha]_D^{20} +66.0^\circ$  ( $c=0.80$ , H<sub>2</sub>O, 24 h).

Compound **3** (**2a**): A solid,  $[\alpha]_D^{28} +3.2^\circ$  ( $c=0.65$ , MeOH), HR-FAB-MS  $m/z$ : 265.1414 (Calcd for C<sub>13</sub>H<sub>22</sub>O<sub>4</sub>+Na: 265.1416).

Ascleposide B (**4**): A solid,  $[\alpha]_D^{24} -32.4^\circ$  ( $c=1.30$ , MeOH), HR-FAB-MS  $m/z$ : 441.1737 ( $[M+Na]^+$ ) (Calcd for C<sub>19</sub>H<sub>30</sub>O<sub>10</sub>+Na: 441.1737). Upon hydrolysis with cellulase under the same conditions as for **2**, an aglycone (**4a**) was obtained as a solid,  $[\alpha]_D^{22} -22.6^\circ$  ( $c=0.35$ , MeOH), HR-FAB-MS (negative)  $m/z$ : 255.1234 ( $[M-H]^-$ ) (Calcd for C<sub>13</sub>H<sub>19</sub>O<sub>5</sub>: 255.1232). Glucose in the H<sub>2</sub>O layer was confirmed by TLC (solvents 1 and 2).

Ascleposide C (**5**): A solid,  $[\alpha]_D^{20} -19.3^\circ$  ( $c=0.55$ , MeOH), HR-FAB-MS  $m/z$ : 411.1992 (Calcd for C<sub>19</sub>H<sub>32</sub>O<sub>8</sub>+Na: 411.1994). Upon hydrolysis in the

same manner as **2** and **4**, an aglycone (**5a**) was obtained as a solid,  $[\alpha]_D^{22} + 11.8^\circ$  ( $c=0.28$ , MeOH), HR-FAB-MS (negative)  $m/z$ : 225.1499 (Calcd for  $C_{13}H_{22}O_3-H$ : 225.1491). Glucose was confirmed by TLC (solvents 1, 2).

Ascleposide D (**6**): A solid,  $[\alpha]_D^{26} - 12.7^\circ$  ( $c=1.01$ , MeOH), HR-FAB-MS  $m/z$ : 413.2161 (Calcd for  $C_{19}H_{34}O_8+Na$ : 413.2151). Upon hydrolysis, an aglycone (**6a**) was obtained as a solid,  $[\alpha]_D^{21} + 20.4^\circ$  ( $c=0.28$ , MeOH), HR-FAB-MS  $m/z$ : 251.1629 (Calcd for  $C_{13}H_{24}O_3+Na$ : 251.1623). Glucose was confirmed by TLC (solvents 1 and 2).

Ascleposide E (**7**): Colorless fine prisms, mp 138–139 °C,  $[\alpha]_D^{22} - 7.1^\circ$  ( $c=0.47$ , MeOH), HR-FAB-MS  $m/z$ : 411.1992 (Calcd for  $C_{19}H_{32}O_8+Na$ : 411.1994). Upon hydrolysis, an aglycone (**7a**) was obtained as a solid,  $[\alpha]_D^{22} + 22.5^\circ$  ( $c=0.29$ , MeOH), HR-FAB-MS (negative)  $m/z$ : 225.1487 (Calcd for  $C_{13}H_{22}O_3-H$ : 225.1491). Glucose was confirmed by TLC (solvents 1, 2).

MTPA Derivatives of **2a** and **4a–7a**: Each solution of **2a**, **4a–7a** (1–3 mg) in  $CH_2Cl_2$  (0.2–0.3 ml) was treated with (*S*)-MTPA (8–18 mg) in  $CH_2Cl_2$  (0.2 ml) in the presence of dicyclohexylcarbodiimide (DCC) (10–15 mg) and 4-dimethylaminopyridine (DMAP) (8–12 mg) and the mixture was allowed to stand for 2 d. The reaction mixture was subjected to a silica gel column chromatography directly and eluted with benzene and benzene–acetone (20:1–10:1) to give **2b**, **4b–7b**. In a similar procedure, **2c**, **4c–7c** were obtained with (*R*)-MTPA.

$\Delta\delta$  (**2b–2c**) ppm: See Fig. 1.

$\Delta\delta$  (**4b–4c**) ppm: H-2 (–0.110, –0.194), H-4 (+0.031, +0.014), H-7

(–0.165), H-8 (–0.056), H-10 (+0.060), H-12 (–0.059), H-13 (–0.019).

$\Delta\delta$  (**5b–5c**) ppm: H-2 (–0.092, –0.178), H-4 (+0.030, +0.054), H-6 (–0.058), H-7 (–0.120), H-8 (–0.095), H-10 (+0.063), H-11 (–0.025, –0.005), H-12 (–0.052), H-13 (–0.018).

$\Delta\delta$  (**6b–6c**) ppm: H-2 (–0.164, –0.245), H-4 (+0.011, +0.090), H-6 (–0.103), H-8 (–0.19, –0.07), H-10 (+0.074), H-11 (–0.048, –0.052), H-12 (–0.147), H-13 (–0.016).

$\Delta\delta$  (**7b–7c**) ppm: H-2 (–0.066, –0.135), H-4 (+0.065, +0.140), H-11 (–0.011, –0.032), H-12 (–0.023), H-13 (+0.022).

**Acknowledgements** We thank Ms. Y. Iwase for obtaining the NMR spectra and Mr. H. Hanazono for operating the MS.

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## New Access to 7,17-*Seco* Norditerpenoid Alkaloids *via* Reduction of the Corresponding 8-Chloro Derivatives

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**New access to the 7, 17-*seco* norditerpenoid alkaloids **9** (60%) from yunnaconitine (**5**), as well as **14** (46%) and **15** (22%) from isotalatizidine (**10**), *via* selective hydrolysis, chlorination and reduction with NaBH<sub>4</sub> is described.**

**Key words** norditerpenoid alkaloid; 7,17-*seco* norditerpenoid alkaloid; yunnaconitine; isotalatizidine

The norditerpenoid alkaloids are a group of highly oxygenated and complex natural products. Apart from analgesic, local anesthetic, anti-inflammatory and antiarrhythmic activities,<sup>1)</sup> these alkaloids afford novel derivatives.<sup>2)</sup> In order to search for more active, less toxic compounds, we have carried out structural modifications of the norditerpenoid alkaloids since 1986.<sup>3)</sup> One of the key reactions of the aconitine-type norditerpenoid alkaloid is cleavage of the C(7)–C(17) bond. The reactions of these type of alkaloids *via*: pyrolysis of *N*-oxides,<sup>3b,4)</sup> pyrolysis-reduction,<sup>5a)</sup> photolysis-reduction,<sup>6)</sup> rearrangement-reduction,<sup>7)</sup> oxidation-reduction<sup>5b,8)</sup> and neighboring group participation<sup>9)</sup> have been summarized. However, most of them led to complicated products with low yields<sup>7)</sup> or difficult purification.<sup>3b)</sup> Recently, we reported a new method for the preparation of the 7,17-*seco* norditerpenoid alkaloids *via* rearrangement of chloramine.<sup>3k)</sup> In this paper, we report in detail the cleavage of the C(7)–C(17) bond in the aconitine-type norditerpenoid alkaloids yunnaconitine (**5**) and isotalatizidine (**10**) by selective hydrolysis, chlorination and reduction with NaBH<sub>4</sub>.

### Results and Discussion

In 1987, Kulanthaivel and Pelletier<sup>10)</sup> reported a method for the cleavage of the C(11)–C(17) bond in the lycoctonine-type norditerpenoid alkaloid deltaline (**1**) (Fig. 1). Srivastava, *et al.* reported that an 11,17-*seco* compound **4** is obtained by the treatment of **2**, which has an anti-periplanar relationship between the lone pair of the nitrogen atom and the C(10)–Cl bond (MeOH/H<sub>2</sub>O).<sup>11)</sup> Observation of the Dreiding model of the norditerpenoid alkaloids showed that both C(8)–Cl and C(10)–Cl bonds are located similarly to the lone electron pair of the nitrogen atom. We surmised that fragmentation of (**2**) gave (**3**) *via* pathway a (A<sub>1</sub>). We have designed a method for the preparation of 7,17-*seco* compounds starting from yunna-

conitine (**5**), probably proceeding by pathway b (B<sub>1</sub>) (Fig. 2).

Heating yunnaconitine (**5**) with Ac<sub>2</sub>O/TsOH afforded **6** (99% yield), and refluxing **6** in diglyme–H<sub>2</sub>O (4:1) gave **7** (90% yield). The <sup>1</sup>H (<sup>13</sup>C)-NMR spectra of **7** showed the non signal of the 8-OAc group. Reaction of **7** in dry benzene with freshly distilled SOCl<sub>2</sub> at room temperature afforded a major product (*Rf*, 0.15), which is probably an intermediate **8** (Fig. 3), followed by treatment with NaBH<sub>4</sub>/MeOH, which gave a white powder in 60% yield in addition to other minor products. Its MS spectrum displayed a molecular ion peak at *m/z* 685, and the formula C<sub>37</sub>H<sub>51</sub>NO<sub>11</sub> was established by the HRFAB-MS spectrum. The <sup>1</sup>H- and <sup>13</sup>C-NMR spectra of the compound showed a characteristic trisubstituted double bond ( $\delta_H$  5.67, 1H, br s;  $\delta_C$  126.5 d, 132.4 s). Its structure was deduced to be **9** by careful analysis of its 2D-NMR data (<sup>1</sup>H–<sup>1</sup>H COSY, HMQC, HMBC) (Table 1). In addition, in the NOE experiments of **9**, enhancement of the signal at  $\delta$  2.64 ppm (H<sub>2</sub>-15) was observed when the H-7 signal at  $\delta$  5.67 ppm was irradiated.

Similarly, acetylation of **10** with Ac<sub>2</sub>O–TsOH gave the compound **11**, and selective hydrolysis of **11** afforded compound **12**, which was treated with SOCl<sub>2</sub>; then the possible intermediate **13** formed (Fig. 4) was subsequently reduced with NaBH<sub>4</sub> to give a residue. Chromatographic separation of the reaction products on a Chromatotron gave compounds **14** and **15** (Fig. 4). Compound **14** was obtained as an amorphous powder, C<sub>27</sub>H<sub>41</sub>NO<sub>6</sub> (HRFAB-MS). The <sup>1</sup>H- and <sup>13</sup>C-NMR spectra of **14** showed a trisubstituted double bond ( $\delta_H$  5.37, 1H, d, *J*=6.8 Hz, H-7;  $\delta_C$  123.7 d, C-7; 135.2 s, C-8), and its structure was confirmed on the basis of 2D-NMR data (Table 2). Compound **15** was also obtained as a white amorphous powder, C<sub>26</sub>H<sub>37</sub>NO<sub>5</sub> (HRFAB-MS). Compared with compound **14**, the <sup>1</sup>H- and <sup>13</sup>C-NMR spectra of compound **15** are similar, except for a conjugated diene moiety ( $\delta_H$  5.54,

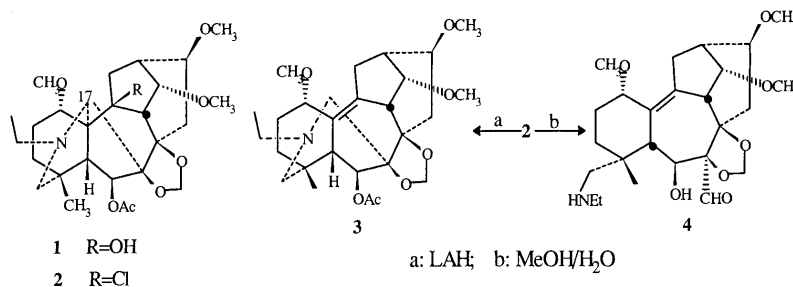


Fig. 1

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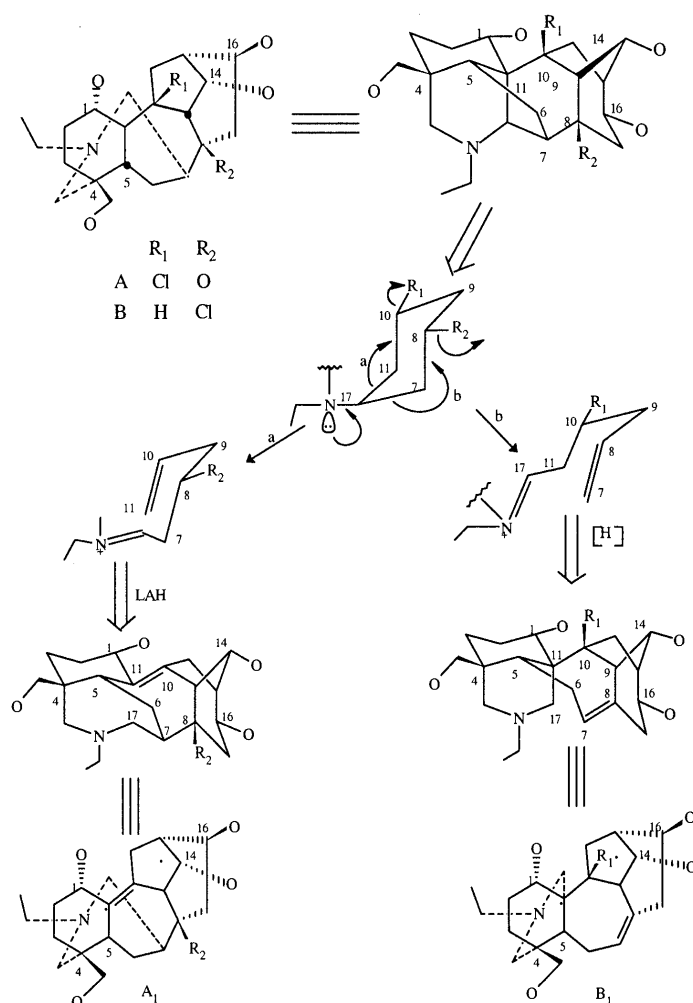


Fig. 2

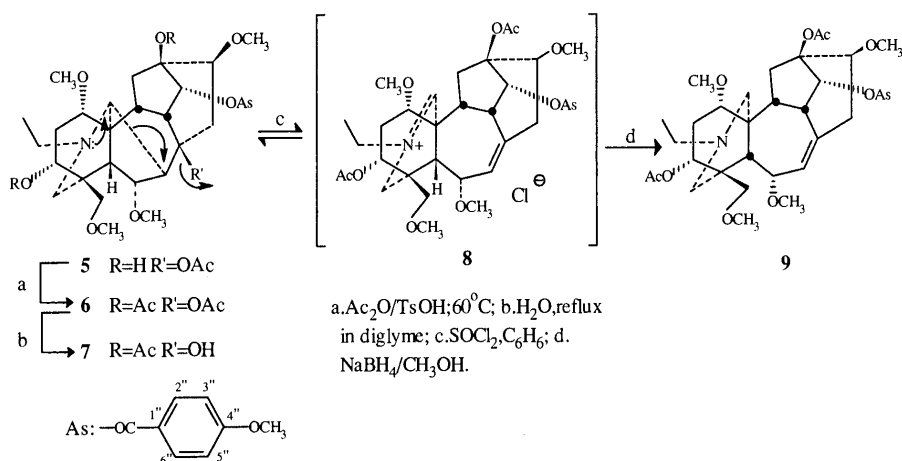


Fig. 3

1H, t,  $J=7.2$  Hz, H-7; 6.24, 1H, d,  $J=7.2$  Hz, H-15; 5.84, 1H, t,  $J=7.2$  Hz, H-16;  $\delta_{\text{C}}$  125.5 d, C-7; 137.3 s, C-8; 129.2 d, C-15; 129.5 d, C-16) and only one methoxyl group ( $\delta_{\text{H}}$  3.29, 3H, s;  $\delta_{\text{C}}$  59.0 q), as well as some C-13 signals, such as C-12, C-14 and C-17. Finally, its structure **15** was confirmed based on its 2D-NMR data (Table 3) and NOE experiments.

Treatment of **12** with  $\text{SOCl}_2$  ( $\text{MeOH}/\text{H}_2\text{O}$ ,  $40^\circ\text{C}$ , 5 h)<sup>12)</sup> or refluxing in 10% HCl solution gave the compound **16** (20% yield) and the starting material **10**, instead of the expected

7,17-*seco* products, respectively.

In summary, preparation of the 7,17-*seco* norditerpenoid alkaloids *via* reducing the corresponding probable 8-chloro derivatives with  $\text{NaBH}_4$  in moderate yield is a novel method.

#### Experimental

**General. Experimental Procedures**  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra were acquired on a Bruker AC-E200 or a Varian INOVA-400/54 spectrometer. IR spectra were recorded on a Nicolet FT-IR 20BXB spectrometer. EIMS and FABMS were obtained with a VG Auto-spec 3000 mass spectrometer, and

Table 1.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR Data of Compound 9

| No.                       | $\delta_{\text{H}}$ | Mult ( $J=\text{Hz}$ )      | $\delta_{\text{C}}$ | HMBC (H $\rightarrow$ C) |
|---------------------------|---------------------|-----------------------------|---------------------|--------------------------|
| 1                         | 2.91                | dd (7.2, 6.4)               | 84.3 d              | 17, 1'                   |
| 2                         | 2.50 (a)/2.80 (e)   | m/m                         | 31.4 t              | 1, 3, 11/1, 3            |
| 3                         | 4.97                | dd (11.6, 6.8)              | 72.0 d              | 4, 19                    |
| 4                         | —                   | —                           | 42.3 s              | —                        |
| 5                         | 1.60                | br s                        | 40.8 d              | 1, 3, 4, 11              |
| 6                         | 4.20                | br s                        | 79.3 d              | —                        |
| 7                         | 5.67                | br s                        | 126.5 d             | 5, 9                     |
| 8                         | —                   | —                           | 132.4 s             | —                        |
| 9                         | 3.11                | m                           | 40.7 d              | 10, 11, 13               |
| 10                        | 2.70                | m                           | 41.4 d              | 9, 11, 12                |
| 11                        | —                   | —                           | 42.5 s              | —                        |
| 12                        | 2.10 (a)/2.35 (e)   | m/m                         | 38.8 t              | 11, 16/11, 14, 16        |
| 13                        | —                   | —                           | 83.7 s              | —                        |
| 14                        | 5.20                | br s                        | 80.9 d              | 13                       |
| 15                        | 2.64 (a)/3.00 (e)   | dd (12.0, 8.4)/<br>(hidden) | 39.5 t              | 9, 13/9, 16              |
| 16                        | 4.03                | t (8.0)                     | 82.6 d              | 12, 1, 14, 15, 16'       |
| 17                        | 2.50                | (hidden)                    | 50.9 d              | 10, 11                   |
| 18                        | 3.10                | (hidden)                    | 71.9 t              | 3, 4                     |
| 19                        | 2.27/2.65           | (hidden)                    | 50.0 t              | 4', 21/4, 5, 17, 18      |
| $\text{NCH}_2\text{CH}_3$ | 2.20/2.30           | m/m                         | 51.6 t              | 17, 19/22                |
| $\text{NCH}_2\text{CH}_3$ | 0.98                | t (6.8)                     | 11.8 q              | 17, 19, 21               |
| 1'                        | 3.40                | s                           | 57.8 q              | —                        |
| 6'                        | 3.39                | s                           | 55.8 q              | 16                       |
| 16'                       | 3.35                | s                           | 57.5 q              | —                        |
| 18'                       | 3.20                | s                           | 58.8 q              | 18                       |
| 3-OAc                     | 2.05                | s                           | 170.1 s/21.3 q      | —                        |
| 13-OAc                    | 2.06                | s                           | 170.5 s/21.1 q      | —                        |
| COO                       | —                   | —                           | 165.9 s             | —                        |
| 1''                       | —                   | —                           | 122.6 s             | —                        |
| 2'', 6''                  | 8.05                | AA'BB' (9.0)                | 131.8 d             | 3'', 4'', 5'', COO       |
| 3'', 5''                  | 6.93                | AA'BB' (9.0)                | 113.5 d             | 1'', 4''                 |
| 4''                       | —                   | —                           | 163.3 s             | —                        |
| 4''-OCH <sub>3</sub>      | 3.85                | s                           | 55.3 q              | —                        |

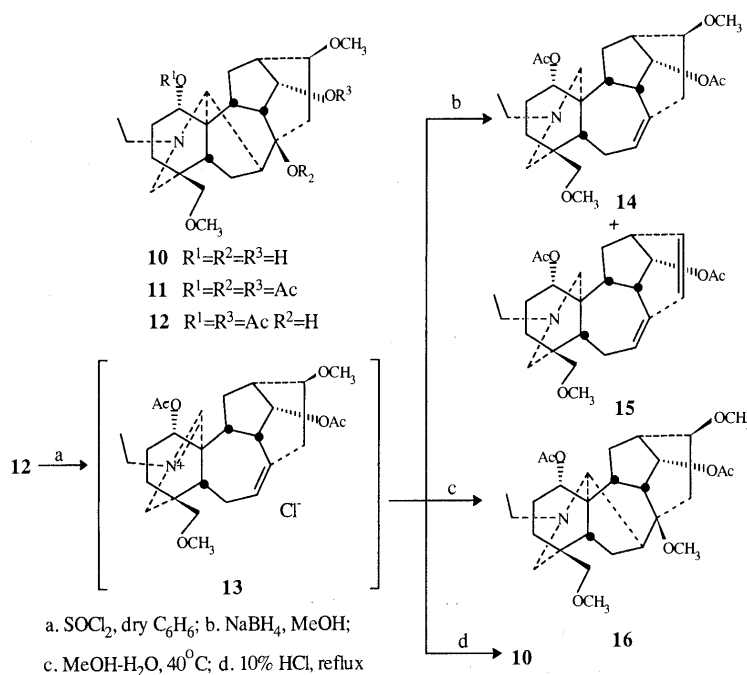


Fig. 4

TLC was performed on silica gel GF<sub>254</sub> precoated plates sprayed with a modified Dragendorff's reagent for detection. Column chromatography was performed using silica gel H. Chromatographic separations on a Chromatotron were carried out on rotors coated with 1mm thick layers of silica gel

H and 0.5% CMC. All of the silica gel GF<sub>254</sub> and silica gel H in the experimental were purchased from Qingdao Sea Chemical Factor, People's Republic of China.

**Compound 6** To a solution of yunnaconitine (**5**) (200 mg, 0.3 mmol) in

Table 2.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR Data of Compound **14**

| No.                        | $\delta_{\text{H}}$   | Mult ( $J=\text{Hz}$ ) | $\delta_{\text{C}}$ | HMBC ( $\text{H} \rightarrow \text{C}$ ) |
|----------------------------|-----------------------|------------------------|---------------------|------------------------------------------|
| 1                          | 4.53                  | dd (11.2, 5.6)         | 85.3 d              | 17, 1-O $\text{COCH}_3$                  |
| 2                          | 1.72 (a)/2.69 (e)     | m/m                    | 27.1 t              | 1, 3                                     |
| 3                          | 1.60                  | m                      | 33.3 t              | 1, 2, 4, 5, 18                           |
| 4                          | —                     | —                      | 39.1 s              | —                                        |
| 5                          | 1.88                  | dd (10.4, 5.6)         | 38.6 d              | 1, 3, 4, 6, 7, 17                        |
| 6                          | 2.11 (a)/2.39 (e)     | d (10.8)               | 24.1 t              | 5, 7, 8                                  |
| 7                          | 5.37                  | d (6.8)                | 123.7 d             | 5, 9                                     |
| 8                          | —                     | —                      | 135.2 s             | —                                        |
| 9                          | 3.03                  | m                      | 42.2 d              | 14                                       |
| 10                         | 1.64                  | m                      | 43.4 d              | 5, 8, 9, 11, 12                          |
| 11                         | —                     | —                      | 40.6 s              | —                                        |
| 12                         | 1.13                  | m                      | 29.4 t              | 10, 11, 13, 14, 16                       |
| 13                         | 2.57                  | m                      | 35.7 d              | 9, 10, 12, 14, 15, 16                    |
| 14                         | 4.69                  | t (4.0)                | 76.2 d              | 8, 9, 13, 16, 14-O $\text{COCH}_3$       |
| 15                         | ca. 1.80 (a)/2.47 (e) | m/m                    | 34.0 t              | 8, 9, 13, 16/7, 8, 9, 16                 |
| 16                         | 3.25                  | m                      | 83.9 d              | 12, 14, 15, 16, 16'                      |
| 17                         | 2.1/2.79              | ABq (11.2)             | 52.4 t              | 1, 11, 20/1, 5, 11, 20                   |
| 18                         | 2.95/3.15             | ABq (9.2)              | 78.35 t             | 3, 4, 5, 18                              |
| 19                         | 2.16                  | (hidden)               | 52.2 t              | 17                                       |
| N $\text{CH}_2\text{CH}_3$ | 2.29                  | q (7.2)                | 54.2 t              | 17, 19, 22                               |
| N $\text{CH}_2\text{CH}_3$ | 1.00                  | t (6.8)                | 12.0 q              | 17, 19 ( $^4J$ )                         |
| 16'                        | 3.23                  | s                      | 55.6 q              | 16                                       |
| 18'                        | 3.26                  | s                      | 59.0 q              | 18                                       |
| 1-OAc                      | 1.99                  | s                      | 170.3 s, 21.3 q     |                                          |
| 14-OAc                     | 2.03                  | s                      | 170.9 s, 21.0 q     |                                          |

Table 3.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR Data of Compound **15**

| No.                        | $\delta_{\text{H}}$ | Mult ( $J=\text{Hz}$ ) | $\delta_{\text{C}}$ | HMBC ( $\text{H} \rightarrow \text{C}$ ) |
|----------------------------|---------------------|------------------------|---------------------|------------------------------------------|
| 1                          | 4.60                | dd (11.6, 6.0)         | 84.9 d              | 1'                                       |
| 2                          | 1.70 (a)/2.60 (e)   | m/m                    | 27.0 t              | 1, 3, 4, /1, 3                           |
| 3                          | 1.63                | m                      | 33.6 t              | 1, 4, 5                                  |
| 4                          | —                   | —                      | 39.6 s              | —                                        |
| 5                          | 1.95                | m                      | 41.6 d              | 4, 10, 17                                |
| 6                          | 2.75                | m                      | 23.3 t              | 7, 8                                     |
| 7                          | 5.54                | t (7.2)                | 125.5 d             | 15                                       |
| 8                          | —                   | —                      | 137.3 s             | —                                        |
| 9                          | 3.25                | m                      | 42.8 d              | 8, 12                                    |
| 10                         | 2.05                | m                      | 41.9 d              | 8, 9, 12                                 |
| 11                         | —                   | —                      | 41.8 s              | —                                        |
| 12                         | 1.60                | m                      | 32.7 t              | 10, 13, 14, 16                           |
| 13                         | 2.65                | m                      | 36.2 d              | 15, 16                                   |
| 14                         | 4.77                | t (4.4)                | 73.7 d              | 8, 16, 14'                               |
| 15                         | 6.24                | d (7.2)                | 129.2 d             | 7, 8                                     |
| 16                         | 5.84                | d (7.2)                | 129.5 d             | 8                                        |
| 17                         | 2.16/2.78           | ABq (11.6)             | 55.0 t              | 1, 10                                    |
| 18                         | 2.96/3.25           | ABq (9.2)              | 79.1 t              | 4, 19                                    |
| 19                         | 2.18/2.48           | ABq (10.4)             | 51.2 t              | 4, 5                                     |
| N $\text{CH}_2\text{CH}_3$ | 2.29                | q (6.8)                | 52.0 t              | 19                                       |
| N $\text{CH}_2\text{CH}_3$ | 0.99                | t (6.8)                | 11.9 q              | 21                                       |
| 18'                        | 3.29                | s                      | 59.0 q              | 18                                       |
| 1-OAc                      | 2.03                | s                      | 170.2 s, 21.5 q     |                                          |
| 14-OAc                     | 2.01                | s                      | 170.9 s, 21.0 q     |                                          |

EtOAc (6 ml), TsOH (200 mg, 1.15 mmol) was added, and the solution was maintained at 60 °C for 3 h. After the work-up, compound **6** (210 mg, 99% yield) was obtained as an amorphous powder:  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ , 200 MHz)  $\delta$ : 1.09 (3H, t,  $J=7.0$  Hz, N- $\text{CH}_2\text{CH}_3$ ), 1.29 (3H, s, 8-OAc), 2.02, 2.04 (each 3H, s, 2 $\times$ OAc), 3.16, 3.17, 3.21, 3.36, 3.84 (each 3H, s, 5 $\times$ OCH $_3$ ), 4.86 (1H, dd,  $J=10.0$ , 8.0 Hz, H-3 $\beta$ ), 5.05 (1H, d,  $J=5.0$  Hz, H-14 $\beta$ ), 6.90, 8.02 (each 2H, AA'BB' system,  $J=9.0$  Hz, Ar-H);  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ , 50 MHz)  $\delta$ : 81.4 (d, C-1), 31.7 (t, C-2), 71.3 (d, C-3), 42.2 (s, C-4), 46.0 (d, C-5), 79.8 (d, C-6), 43.5 (d, C-7), 85.1 (s, C-8), 48.9 (d, C-9), 40.9 (d, C-10), 49.8 (s, C-11), 35.1 (t, C-12), 81.8 (s, C-13), 76.6 (d, C-14), 39.4 (t, C-15), 83.2 (d, C-16), 61.0 (d, C-17), 71.5 (t, C-18), 48.8 (t, C-19), 55.9 (q, C-1'), 57.9 (q, C-

6'), 57.9 (q, C-16'), 58.6 (q, C-18'), 47.4 (t, N $\text{CH}_2$ ), 13.2 (q, N- $\text{CH}_2\text{CH}_3$ ), 170.1 (s), 170.1 (s), 169.6 (s); 21.4 (q), 21.3 (q), 21.2 (q) (3 $\times$ OAc); 165.8 (s, Ar-COO), 122.3 (s, C-1''), 131.7 (d, C-2'', 6''), 113.6 (d, C-3'', 5''), 163.3 (s, C-4''), 55.2 (q, 4'-OCH $_3$ ); EI-MS  $m/z$ : 743 [ $\text{M}^+$ ] (5), 712 [ $\text{M}-\text{OCH}_3$ ] (100), 684 (73), 652 (52), 135 (98); HREI-MS  $m/z$ : 743.3489 (Calcd for  $\text{C}_{39}\text{H}_{53}\text{NO}_{13}$ , 743.3514).

**Compound 7** Compound **6** (380 mg, 0.51 mmol) was dissolved in diglyme-H $_2\text{O}$  (4 : 1, 10 ml). The solution was refluxed for 6 h, then evaporated to dryness under reduced pressure to give a residue, which was chromatographed on a  $\text{SiO}_2$  column (26 g) and eluted with  $\text{CHCl}_3$ -CH $_3\text{OH}$  (95 : 5) to afford amorphous **7** (322 mg, 90% yield).  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ , 200

MHz)  $\delta$ : 1.11 (3H, t,  $J=7.0$  Hz,  $\text{N-CH}_2\text{CH}_3$ ), 1.99, 2.05 (each 3H, s,  $2\times\text{OAc}$ ), 3.19, 3.21, 3.26, 3.30, 3.84 (each 3H, s,  $5\times\text{OCH}_3$ ), 4.88 (1H, t,  $J=7.0$  Hz, H-3 $\beta$ ), 5.44 (1H, d,  $J=5.0$  Hz, H-14 $\beta$ ), 6.92, 8.03 (each 2H, AA'BB' system,  $J=9.0$  Hz, Ar-H);  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 50 MHz)  $\delta$ : 81.6 (d, C-1), 31.4 (t, C-2), 71.6 (d, C-3), 42.3 (s, C-4), 46.1 (d, C-5), 79.1 (d, C-6), 46.0 (d, C-7), 73.3 (s, C-8), 53.6 (d, C-9), 42.0 (d, C-10), 49.8 (s, C-11), 35.2 (t, C-12), 83.0 (s, C-13), 76.7 (d, C-14), 42.2 (t, C-15), 82.6 (d, C-16), 61.4 (d, C-17), 71.7 (t, C-18), 48.9 (t, C-19), 55.9 (q, C-1'), 57.5 (q, C-6'), 57.6 (q, C-16'), 58.7 (q, C-18'), 47.8 (q,  $\text{N-CH}_2$ ), 14.0 (q,  $\text{N-CH}_2\text{CH}_3$ ), 170.2 (s), 170.3 (s); 21.0 (q), 21.3 (q) ( $2\times\text{OAc}$ ); 165.5 (s, Ar-COO), 122.2 (s, C-1''), 131.8 (d, C-2'', 6''), 113.5 (d, C-3'', 5''), 163.3 (s, C-4''), 55.2 (q, 4''-OCH<sub>3</sub>). EI-MS  $m/z$ : 701 [ $\text{M}]^+$  (2), 670 [ $\text{M-OCH}_3$ ] $^+$  (97), 610 (85), 135 (100); HREI-MS  $m/z$ : 701.3378 (Calcd for  $\text{C}_{37}\text{H}_{51}\text{NO}_{12}$ , 701.3411).

**Compound 9** Compound 7 (200 mg, 0.28 mmol) was dissolved in benzene (10 ml). To the solution,  $\text{SOCl}_2$  (2 ml, 2.8 mmol) was added, maintained for 10 h, and evaporated to dryness under reduced pressure to give a residue (210 mg) which was dissolved in methanol (5 ml). To the solution,  $\text{NaBH}_4$  (200 mg) was added on an ice-bath, and the mixture was allowed to sit for 30 min. Evaporation under reduced pressure gave a residue. It was chromatographed on a  $\text{SiO}_2$  (25 g) column and eluted with  $\text{CHCl}_3$ -MeOH (98:2) to afford amorphous 9 (112 mg, 60% yield). IR (KBr)  $\nu_{\text{max}}$  3000, 1740, 1730, 1620, 1260, 780  $\text{cm}^{-1}$ ;  $^1\text{H}$  ( $\text{CDCl}_3$ , 400 MHz) and  $^{13}\text{C}$  ( $\text{CDCl}_3$ , 100 MHz) NMR spectra, see Table 1; The NOE experiments: the enhancement of the H<sub>(a)</sub>-15 at  $\delta$  2.64 ppm was observed when irradiating the H-7 at  $\delta$  5.67 ppm; EI-MS  $m/z$ : 685 [ $\text{M}]^+$  (29), 626 [ $\text{M-59}]^+$  (23), 534 (45), 135 (100); HRFAB-MS  $m/z$ : 686.3590 (Calcd for  $\text{C}_{37}\text{H}_{52}\text{NO}_{11}$ , 686.3590).

**Compound 11** Isotalatizidine (10) (1.2 g, 2.5 mmol) was dissolved in the mixture of  $\text{Ac}_2\text{O}$  (15 ml) and TsOH (1.2 g). The solution was kept at 60 °C for 6 h, and usual work-up gave 11 (1.28 g, 96%) as an amorphous powder;  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 200 MHz)  $\delta$ : 1.02, (3H, t,  $J=7.1$  Hz,  $\text{N-CH}_2\text{CH}_3$ ), 1.90, 1.95, 1.98 (each 3H, s,  $3\times\text{OAc}$ ), 3.21, 3.24 (each 3H, s,  $2\times\text{OCH}_3$ ), 4.67 (1H, t,  $J=4.8$  Hz, H-14 $\beta$ ), 4.83 (1H, dd,  $J=10.2$ , 6.8 Hz, H-1 $\beta$ );  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 50 MHz)  $\delta$ : 74.6 (d, C-1), 27.4 (t, C-2), 32.2 (t, C-3), 38.0 (s, C-4), 45.9 (d, C-5), 24.6 (t, C-6), 42.1 (d, C-7), 85.4 (s, C-8), 41.6 (d, C-9), 43.8 (d, C-10), 47.6 (s, C-11), 28.4 (t, C-12), 37.9 (d, C-13), 77.7 (d, C-14), 37.3 (t, C-15), 82.8 (d, C-16), 60.7 (d, C-17), 79.0 (t, C-18), 53.1 (t, C-19), 56.3 (q, C-16'), 59.2 (q, C-18'), 48.6 (t,  $\text{NCH}_2$ ), 13.2 (q,  $\text{NCH}_2\text{CH}_3$ ), 169.5 (s), 170.2 (s), 170.7 (s); 21.0 (q), 21.8 (q), 22.2 (q) ( $3\times\text{OAc}$ ); EI-MS  $m/z$ : 533 [ $\text{M}]^+$  (5), 474 [ $\text{M-59}]$  (100), 432 (50), 414 (28), 400 (28); HREI-MS  $m/z$ : 533.2953 (Calcd for  $\text{C}_{29}\text{H}_{43}\text{NO}_8$ , 533.2988).

**Compound 12** Compound 11 (535 mg, 1.0 mmol) was dissolved in diglyme- $\text{H}_2\text{O}$  (4:1, 20 ml). The solution was refluxed for 6 h and evaporated to dryness under reduced pressure to give a residue which was chromatographed on a  $\text{SiO}_2$  (20 g) column and eluted with  $\text{CHCl}_3$ -MeOH (98:2) to afford compound 12 (419 mg, 85% yield). Compound 12: IR (KBr)  $\nu_{\text{max}}$  1742  $\text{cm}^{-1}$ ;  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 200 MHz)  $\delta$ : 1.07 (3H, t,  $J=7.2$  Hz,  $\text{NCH}_2\text{CH}_3$ ), 2.02, 2.03 (each 3H, s,  $2\times\text{OAc}$ ), 3.21, 3.27 (each 3H, s,  $2\times\text{OCH}_3$ ), 4.75 (1H, t,  $J=4.6$  Hz, H-14 $\beta$ ), 4.89 (1H, dd,  $J=10.1$ , 6.7 Hz, H-1 $\beta$ );  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 50 MHz)  $\delta$ : 76.2 (d, C-1), 27.2 (t, C-2), 32.2 (t, C-3), 38.2 (s, C-4), 45.0 (d, C-5), 24.8 (t, C-6), 46.0 (d, C-7), 73.3 (s, C-8), 45.9 (d, C-9), 43.8 (d, C-10), 47.4 (s, C-11), 27.9 (t, C-12), 34.9 (d, C-13), 78.0 (d, C-14), 40.6 (t, C-15), 81.5 (d, C-16), 61.1 (d, C-17), 79.1 (t, C-18), 53.2 (t, C-19), 56.0 (q, C-16'), 59.3 (q, C-18'), 48.7 (t,  $\text{N-CH}_2\text{CH}_3$ ), 13.2 (q,  $\text{N-CH}_2\text{CH}_3$ ), 170.3 (s), 170.4 (s); 21.2 (q), 21.8 (q) ( $2\times\text{OAc}$ ); EI-MS:  $m/z$ : 491 [ $\text{M}]^+$  (5), 432 [ $\text{M-59}]^+$  (100), 400 (45); HREI-MS  $m/z$ : 491.2832 (Calcd for  $\text{C}_{27}\text{H}_{41}\text{NO}_7$ , 491.2883).

**Compounds 14 and 15** Compound 12 (400 mg, 0.81 mmol) was dissolved in benzene (10 ml). To the solution,  $\text{SOCl}_2$  (2 ml, 8.1 mmol) was added; the mixture sat for 12 h, and was evaporated to dryness under reduced pressure to give a residue (408 mg) which was dissolved in MeOH (10 ml). To the solution,  $\text{NaBH}_4$  (300 mg) was added in an ice bath, and it sat for 30 min. Work-up gave a residue, which was chromatographed on a Chromatotron ( $\text{SiO}_2$  G) and eluted with petroleum ether-acetone (91:9) to give compounds 14 (212 mg, 46% yield) and 15 (95 mg, 22% yield). Compound 14: IR (KBr)  $\nu_{\text{max}}$  3480, 3000, 1740, 1260  $\text{cm}^{-1}$ ;  $^1\text{H}$  ( $\text{CDCl}_3$ , 400 MHz) and  $^{13}\text{C}$  ( $\text{CDCl}_3$ , 100 MHz) NMR spectra, see Table 2; EIMS:  $m/z$ : 475 [ $\text{M}]^+$  (15), 432 (11), 416 (100), 400 (10); HRFAB-MS  $m/z$ : 476.3012 (Calcd for

$\text{C}_{27}\text{H}_{42}\text{NO}_6$ , 476.3012). Compound 15: IR (KBr)  $\nu_{\text{max}}$  3460, 3000, 1740, 1650, 1250  $\text{cm}^{-1}$ ;  $^1\text{H}$  ( $\text{CDCl}_3$ , 400 MHz) and  $^{13}\text{C}$  ( $\text{CDCl}_3$ , 100 MHz) NMR spectra, see in Table 3; The NOES (400 MHz) experiments: enhancement of H-15 ( $\delta$  6.24 ppm) and H-7 ( $\delta$  2.16 ppm) were observed in terms of irradiating the H-7 and H-15, respectively; EI-MS  $m/z$ : 443 [ $\text{M}]^+$  (37), 400 (100); HRFAB-MS  $m/z$ : 444.2749 (Calcd for  $\text{C}_{26}\text{H}_{38}\text{NO}_5$ , 444.2749).

**Compound 16** Treatment of 13 (200 mg, 0.4 mmol) with  $\text{SOCl}_2$  for 30 min as the above mentioned method gave a residue (200 mg) which was treated with MeOH- $\text{H}_2\text{O}$  (5:1, 12 ml) at 50 °C for 6 h to afford the complex mixture. This was chromatographed on a Chromatotron ( $\text{SiO}_2$  G) and eluted with ether-acetone (2:1) to give 16 (40 mg, 20% yield). IR (KBr)  $\nu_{\text{max}}$  1734  $\text{cm}^{-1}$ ;  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 200 MHz)  $\delta$ : 1.07, (3H, t,  $J=7.1$  Hz,  $\text{N-CH}_2\text{CH}_3$ ), 1.99, 2.02 (each 3H, s,  $2\times\text{OAc}$ ), 3.10, 3.26, 3.31 (each 3H, s,  $3\times\text{OAc}$ ), 4.66 (1H, t,  $J=4.6$  Hz, H-14 $\beta$ ), 4.88 (1H, dd,  $J=10.0$ , 8.0 Hz, H-1 $\beta$ );  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 50 MHz)  $\delta$ : 75.2 (d, C-1), 27.6 (t, C-2), 31.3 (t, C-3), 38.2 (s, C-4), 42.9 (d, C-5), 23.9 (t, C-6), 46.1 (d, C-7), 77.5 (s, C-8), 44.2 (d, C-9), 40.0 (d, C-10), 47.9 (s, C-11), 29.2 (t, C-12), 37.7 (d, C-13), 78.0 (d, C-14), 37.3 (t, C-15), 83.4 (d, C-16), 60.6 (d, C-17), 79.5 (t, C-18), 53.4 (t, C-19), 48.1 (q, C-8'), 56.2 (q, C-16'), 59.4 (q, C-18'), 48.6 (t,  $\text{N-CH}_2$ ), 13.4 (q,  $\text{N-CH}_2\text{CH}_3$ ), 170.3 (s), 171.4 (s); 21.2 (q), 21.9 (q,  $2\times\text{OAc}$ ); EI-MS  $m/z$ : 505 [ $\text{M}]^+$  (19), 490 [ $\text{M-15}]$  (10), 474 [ $\text{M-31}]$  (30), 447 (100), 446 [ $\text{M-59}]$  (85), 430 (40), 414 (35), 402 (29), 372 (24); HREI-MS  $m/z$ : 505.2962 (Calcd for  $\text{C}_{28}\text{H}_{43}\text{NO}_7$ , 505.3039).

**Acknowledgements** We thank the National Natural Science Foundation of China (Grant No. 3937080) and the Chengdu Diao Pharmaceutical Company for supporting this work. We are also grateful to Prof. Xiao Tian Liang for helpful discussion on the subject and advice regarding of this manuscript.

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## Novel A-Seco-Rearranged Lanostane Triterpenoids from *Abies sachalinensis*

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From the needles of *Abies sachalinensis*, novel rearranged lanostane type triterpenes, 1–4, were isolated along with a known triterpene (5). The structures of the new compounds, 1–4, were elucidated to be 3,4-*seco*-8-(14→13*R*)*abeo*-17,13-*friedo*-9β-*lanosta*-4(28),7,14(30),22*Z*,24-pentaene-26,23-olide-3-*oic* acid, methyl 3,4-*seco*-8-(14→13*R*)*abeo*-17,13-*friedo*-9β-*lanosta*-4(28),7,14(30),22*Z*,24-penten-26,23-olide-3-*oate*, 3,4-*seco*-8-(14→13*R*)*abeo*-17,13-*friedo*-9β-*lanosta*-4(28),7,14,22*Z*,24-pentaene-26,23-olide-3-*oic* acid and methyl 3,4-*seco*-8-(14→13*R*)*abeo*-17,13-*friedo*-9β-*lanosta*-4(28),7,14,22*Z*,24-pentaene-26,23-olide-3-*oate*, respectively, by means of spectral experiments, especially two dimensional NMR spectroscopy, such as <sup>1</sup>H-detected multiple quantum coherence (HMQC), <sup>1</sup>H-detected heteronuclear multiple bond connectivity (HMBC) and <sup>1</sup>H–<sup>1</sup>H-correlation spectroscopy (COSY) experiments. These new compounds have novel structures containing A-*seco*, rearranged spiro structure and a γ-lactone conjugated with a diene. Some of these compounds showed potent antibacterial activity against gram positive bacteria.

**Key words** *Abies sachalinensis*; Pinaceae; rearranged lanostane; A-*seco*-lanostane; antibacterial activity

Gymnosperm trees are very important in forestry and paper manufacturing, but large amounts of the trees are discarded in the manufacturing process for wooden products and papers. There have been fewer reports on bioactive constituents from gymnosperm plants compared with other plants. In the course of our program on researching biological active constituents from the abandoned parts of forestry trees, we found that several coniferous plants including *Abies sachalinensis* (Pinaceae, Japanese name “Todomatsu”) showed potent anti-bacterial activity against gram positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis*. *A. sachalinensis* trees are distributed in Hokkaido, Japan and Sakhalin, Russia. From *Abies* spp. plants, many kinds of lanostane triterpenes, including A-*seco* and rearranged derivatives, were isolated.<sup>1–4</sup> From *A. sibirica*, an abiesonic acid having an A-*seco* and rearranged structure, was isolated<sup>5</sup> and from *A. mariesii*, a mariesiic acid C having a rearranged structure, was isolated.<sup>6</sup> From *A. sachalinensis*, epijuvabione type sesquiterpenes were isolated,<sup>7</sup> but there have been few reports about constituents from *A. sachalinensis*.<sup>8</sup> From the antibacterially active AcOEt soluble fraction of needles of *A. sachalinensis*, four new A-*seco*-rearranged lanostane derivatives (1–4) were isolated along with a known triterpene (5).<sup>9</sup> This paper deals with the isolation and structural determination of these novel lanostane derivatives and their antibacterial activity against gram positive bacteria.

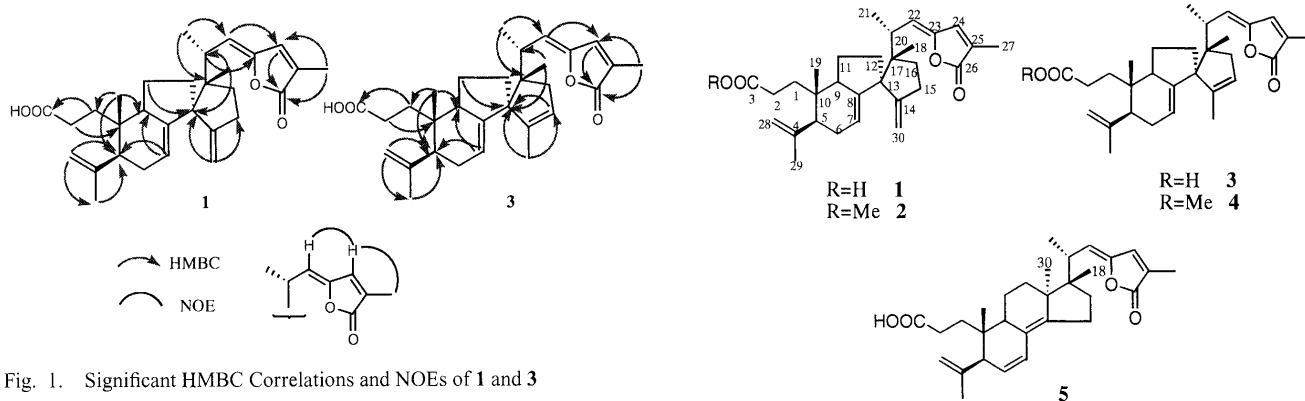
### Results and Discussion

A methanol extract of the needles of *A. sachalinensis* showed potent antibacterial activity against gram positive bacteria. The activity partitioned into the AcOEt soluble fraction of the methanol extract, so that fraction was chromatographed on silica gel. The resultant fractions were further separated by HPLC using an octadecyl silica (ODS) column as described in the Experimental to give four new rearranged lanostane triterpenes, named abiesanolide A (1), B (2), C (3) and D (4) along with a known rearranged lanostane

(5).

Abiesanolide A (1) gave pseudo molecular ion peak, *m/z* 465.2983 [MH]<sup>+</sup>, consistent with the formula, C<sub>30</sub>H<sub>40</sub>O<sub>4</sub> from high resolution (HR)-FAB-MS. The UV spectrum of 1 showed an absorption band at 281 nm (ε 18537). The IR spectrum of 1 showed an absorption band at 1754 cm<sup>−1</sup> (strained lactone carbonyl) and 1715 cm<sup>−1</sup> (carboxyl carbonyl). The <sup>1</sup>H-NMR spectrum of 1 showed the characteristic signals for two exomethylene [δ 4.82 (1H, s), 4.86 (1H, s), 4.73 (1H, s), 4.69 (1H, s)], three olefinic protons [δ 5.39 (1H, dd, *J*=6.8, 3.6 Hz), 5.15 (1H, d, *J*=10.4 Hz), 6.93 (1H, q, *J*=1.6 Hz)] two singlet methyl groups [δ 0.86 (3H, s), 0.94 (3H, s)], two vinylmethyl groups [δ 2.02 (3H, s), 1.78 (3H, s)] and a doublet methyl group [δ 1.02 (3H, d, *J*=7.6 Hz)]. The <sup>13</sup>C-NMR spectrum of 1 showed thirty carbon signals and showed the presence of a carboxyl group (δ 180.8), a lactone group (δ 171.2), two exomethylene carbons (δ 112.3, 105.7), three trisubstituted olefine groups (δ 121.1, 144.8, 118.8, 147.0, 128.4, 138.2) and a quaternary carbon (δ 63.8). The <sup>1</sup>H-NMR and <sup>13</sup>C-NMR data in the A–D ring part of 1 showed a similar signal pattern and chemical shifts with those of dimethyl abiesonate having a 3,4-*seco*-8-(14→13*R*)*abeo*-17,13-*friedo*-9β-*lanosta*-4(28),7,14(30)-trien structure, whose stereo structure was determined by X-ray analysis.<sup>5</sup> These data indicated that 1 was an A-*seco*-rearranged lanostane triterpene having a γ-lactone conjugated with a diene moiety. The H–H-correlation spectroscopy (COSY) spectrum of 1 was utilized for an alignment of the side chain and <sup>1</sup>H-detected multiple quantum coherence (HMQC) spectrum of 1 was utilized for the assignment of <sup>1</sup>H-NMR and <sup>13</sup>C-NMR signals. The <sup>1</sup>H-detected heteronuclear multiple bond correlation (HMBC) experiment of 1 showed many C–H long range correlations as shown in Fig. 1. The Me-29 protons at δ 1.78 showed a correlation with an exomethylene carbon (δ 112.3) and C-5 (δ 30.8). The exomethylene protons at C-30 showed a correlation with a spiro carbon (δ 63.8) at C-13 and C-15 (δ 28.9). The doublet

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Fig. 1. Significant HMBC Correlations and NOEs of **1** and **3**

methyl protons ( $\delta$  1.02) showed a correlation with a methine carbon ( $\delta$  118.8) at C-22 and a quaternary carbon ( $\delta$  50.5) at C-17. The Me-18 protons showed a correlation with a methine ( $\delta$  37.9) at C-20 and C-13. The Me-19 protons showed a correlation with C-1 ( $\delta$  30.2), C-5 ( $\delta$  44.2) and C-9 ( $\delta$  48.8). The C-1 methylene protons ( $\delta$  1.5–1.7) showed a correlation with a carboxyl carbon ( $\delta$  180.0). The olefinic proton ( $\delta$  5.39) at C-7 showed a correlation with C-5 ( $\delta$  44.2) and C-9 ( $\delta$  48.8). The olefinic proton ( $\delta$  5.15) showed a correlation with C-17 ( $\delta$  50.5) and C-24 ( $\delta$  138.2). The vinylmethyl protons ( $\delta$  2.02) showed a correlation with C-24 ( $\delta$  138.2) and a  $\gamma$ -lactone carbon ( $\delta$  171.2) at C-26. These HMBC data showed the presence of an isopentenyl group, a carboxyethyl group, a spiro structure and a  $\gamma$ -lactone conjugated with a diene moiety, and confirmed the positions of these functional groups. From these data, the planar structure of **1** was determined. The difference nuclear Overhauser effect (NOE) spectrum of **1** showed a correlation between Me-18 and H-7. This fact indicated that **1** has the same stereochemistry at C-13 and C-17 as that of dimethyl abiesonate. The geometrical isomerism of the double bond at C-22,23 was determined to be *Z* by a difference NOE experiment as shown in Fig. 1. Thus the structure of **1** was determined to be 3,4-*seco*-8(14 $\rightarrow$ 13*R*)*abeo*-17,13-*friedo*-9 $\beta$ -lanosta-4(28),7,14(30),22*Z*,24-pentaen-26,23-olide-3-oic acid.

Abiesanolide B (**2**) gave the molecular formula,  $C_{31}H_{42}O_4$ , from a pseudo molecular ion  $[MH]^+$  at  $m/z$  479.3170 in HR-FAB-MS. IR spectrum of **2** showed an absorption band at  $1767\text{ cm}^{-1}$  (strained lactone carbonyl),  $1740\text{ cm}^{-1}$  (ester carbonyl) and  $1198\text{ cm}^{-1}$  (ester C–O). The  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR spectra of **2** showed almost the same signal patterns with those of **1** (See Table 1 and Experimental section) except for the presence of the carbomethoxy group [ $\delta$  3.66 (s) in  $^1\text{H}$ -NMR and  $\delta$  51.6, 174.8 in  $^{13}\text{C}$ -NMR]. These indicated that **2** was the methyl ester of **1**. This was confirmed by HMBC experiment. Thus the structure of **2** was determined to be methyl 3,4-*seco*-8(14 $\rightarrow$ 13*R*)*abeo*-17,13-*friedo*-9 $\beta$ -lanosta-4(28),7,14(30),22*Z*,24-pentaen-26,23-olide-3-oate.

Abiesanolide C (**3**) gave the molecular formula,  $C_{40}H_{40}O_4$  from HR-FAB-MS,  $m/z$  465.2983. The UV spectrum of **3** showed a similar absorption band ( $\epsilon$  24615 at 279 nm) to that of **1**. The IR spectrum of **3** showed an absorption band at  $1771\text{ cm}^{-1}$  (strained lactone) and  $1711\text{ cm}^{-1}$  (carboxyl). The  $^1\text{H}$ -NMR spectrum of **3** showed almost the same signal pattern with that of **1** except for the absence of the exomethylene at C-30 and a newly arisen trisubstituted olefin group [ $\delta$  5.25 (1H, br s)] and a vinylmethyl group [ $\delta$  1.53 (3H, br s)].

Table 1.  $^{13}\text{C}$ -NMR Data for Compounds **1**–**5** ( $\text{CDCl}_3$ , 125 MHz)

| C-No               | <b>1</b> | <b>2</b> | <b>3</b> | <b>4</b> | <b>5</b> |
|--------------------|----------|----------|----------|----------|----------|
| 1                  | 30.2     | 3.05     | 29.7     | 29.9     | 28.4     |
| 2                  | 29.2     | 29.3     | 29.2     | 29.2     | 29.5     |
| 3                  | 180.0    | 174.8    | 180.2    | 174.9    | 180.1    |
| 4                  | 149.2    | 149.4    | 148.8    | 148.9    | 145.8    |
| 5                  | 44.2     | 44.2     | 44.6     | 44.7     | 50.8     |
| 6                  | 30.8     | 30.8     | 30.0     | 30.0     | 126.8    |
| 7                  | 121.1    | 121.1    | 121.9    | 121.9    | 125.1    |
| 8                  | 144.8    | 145.0    | 144.7    | 144.7    | 125.2    |
| 9                  | 48.8     | 48.9     | 47.2     | 47.3     | 39.9     |
| 10                 | 36.6     | 36.6     | 36.3     | 36.2     | 37.2     |
| 11                 | 23.5     | 23.5     | 23.9     | 24.6     | 23.8     |
| 12                 | 31.7     | 31.7     | 28.2     | 28.3     | 30.5     |
| 13                 | 63.8     | 63.8     | 55.0     | 66.0     | 47.5     |
| 14                 | 161.6    | 161.7    | 148.4    | 148.4    | 146.2    |
| 15                 | 28.9     | 28.9     | 119.5    | 119.5    | 19.6     |
| 16                 | 35.0     | 35.0     | 39.0     | 39.0     | 35.8     |
| 17                 | 50.5     | 50.5     | 51.7     | 51.7     | 49.0     |
| 18                 | 19.1     | 19.1     | 19.0     | 19.0     | 16.2     |
| 19                 | 24.6     | 24.6     | 24.7     | 24.7     | 21.9     |
| 20                 | 37.9     | 37.9     | 38.0     | 38.0     | 36.5     |
| 21                 | 17.6     | 17.6     | 14.4     | 14.4     | 17.2     |
| 22                 | 118.8    | 118.8    | 119.5    | 119.5    | 118.3    |
| 23                 | 147.0    | 147.0    | 147.5    | 147.5    | 146.5    |
| 24                 | 138.2    | 138.2    | 138.0    | 138.0    | 137.9    |
| 25                 | 128.4    | 128.4    | 128.7    | 128.8    | 129.0    |
| 26                 | 171.2    | 171.2    | 171.4    | 171.4    | 171.1    |
| 27                 | 10.5     | 10.5     | 10.5     | 10.5     | 10.5     |
| 28                 | 112.3    | 112.2    | 112.4    | 112.4    | 115.5    |
| 29                 | 26.4     | 26.3     | 25.5     | 25.5     | 24.7     |
| 30                 | 105.7    | 105.7    | 22.6     | 22.4     | 21.7     |
| CO <sub>2</sub> Me |          | 51.6     |          | 51.6     |          |

All assignments were confirmed by HMQC and HMBC experiments.

This was supported from the  $^{13}\text{C}$ -NMR spectrum data as shown in Table 1. These facts indicated that **3** was transformed from **1** by double bond migration from C-14,30 to C-14,15. This was confirmed by the HMBC experiments as shown in Fig. 1. Thus the structure of **3** was determined to be 3,4-*seco*-8(14 $\rightarrow$ 3*R*)*abeo*-17,13-*friedo*-9 $\beta$ -lanosta-4(28),7,14,22*Z*,24-pentaen-26,23-olide-3-oic acid.

Abiesanolide D (**4**) gave the molecular formula,  $C_{31}H_{42}O_4$ , from pseudo molecular ion  $[MH]^+$  at  $m/z$  479.3177 in HR-FAB-MS. The UV spectrum of **4** showed an absorption band at 280 nm ( $\epsilon$  15512). The IR spectrum of **4** showed absorption bands at  $1768\text{ cm}^{-1}$  (strained lactone carbonyl), at  $1740\text{ cm}^{-1}$  (ester carbonyl) and at  $1193\text{ cm}^{-1}$  (ester C–O). The  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR spectra showed almost the same signal patterns with those of **3** except for the presence of car-

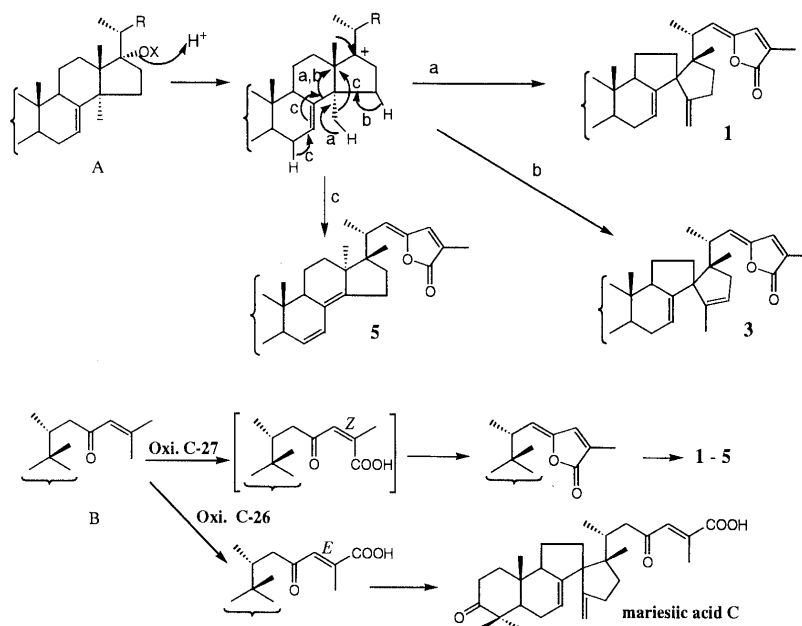


Fig. 2. Hypothetical Reaction Mechanisms for Rearranged Lanostanes Having a  $\gamma$ -Lactone

bomethoxy group [ $\delta$  3.66 (3H, s) in  $^1\text{H-NMR}$  and  $\delta$  51.6, 174.9 in  $^{13}\text{C-NMR}$ ] instead of a free carboxyl group in **3**. Thus the structure of **4** was determined to be methyl 3,4-*seco*-8(14 $\rightarrow$ 13*R*)*abeo*-17,13-*friedo*-9 $\beta$ -lanosta-4(28),7,14,22*Z*,24-pentaen-26,23-olide-3-oate.

Compound **5** was identified as 3,4-*seco*-4(28),6,8(14),22*Z*,24-mariesapentaen-26,23-olide-3-oic acid by means of spectral data, which was isolated from *A. sibirica*.<sup>9)</sup> The  $^{13}\text{C-NMR}$  data for **5** is reported for the first time.

The five *A-seco*-rearranged lanostane derivatives, **1–5**, were tested for their antibacterial activity against gram positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis*, in the same way as described in the previous paper.<sup>10)</sup> Of these, **1** and **5** showed a minimum inhibitory concentration (MIC) at 12.5  $\mu\text{g/ml}$ , but **2**, **3** and **4** showed no activity up to a concentration of 200  $\mu\text{g/ml}$ .

Compounds **1–5** have novel structures transformed from lanostane triterpenes. A leaving group at C-17 of the hypothetical intermediate A migrates to give a carbocation at C-17, to which the Me-18 group moved, followed next by three different pathways (a, b, c) to give **1**, **2** and **5**, respectively, as shown in Fig. 2. The Me-27 group of the hypothetical intermediate B was oxidized to give the *Z* form followed by enolization and lactonization to give the  $\gamma$ -lactone derivatives such as **1–5**. Oxidation of Me-26 of B gave *E* form, which gave enone carboxylic acid, such as mariesiic acid as shown in Fig. 2.

#### Experimental

Optical rotations were determined with JASCO P-1010 polarimeter in MeOH at 25  $^\circ\text{C}$ . UV spectra were obtained with a Hitachi U3410 spectrometer. IR spectra were obtained with a Perkin Elmer GX FT-IR spectrometer.  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  spectra were recorded on a JEOL  $\alpha$ -500 spectrometer in  $\text{CDCl}_3$  using tetramethylsilane (TMS) as an internal standard ( $^1\text{H-NMR}$  at 500 MHz,  $^{13}\text{C-NMR}$  at 125 MHz). H-H COSY, HMQC and HMBC and difference NOE experiments were carried out on the same spectrometer. HR-FAB-MS were obtained with a JEOL HX110 mass spectrometer. Analytical TLC were performed by using precoated Silica gel 60 F<sub>254</sub> (Merck) plates, and detection for the spots was accomplished by UV absorption and spraying with 50%  $\text{H}_2\text{SO}_4$  followed by heating. HPLC was carried out using

an ODS column (YMC R-ODS7 packed column).

**Extraction and Isolation** Air dried needles of *A. sachalinensis* (1.5 kg), collected in Assabu, Hokkaido, Japan, in September 1997, were extracted with MeOH under reflux to give the MeOH extract, which was suspended in water and extracted with AcOEt to give the AcOEt soluble fraction (100 g). The residual water layer was extracted with *n*-BuOH to give the *n*-BuOH soluble fraction (60 g) and the aqueous fraction. Of these three fraction, the AcOEt soluble fraction showed most potent antibacterial activity against gram positive bacteria, *S. aureus* and *B. subtilis*. The active AcOEt fraction (50 g) was separated by silica gel ( $\text{SiO}_2$ ) column chromatography using gradient  $\text{CHCl}_3$ –MeOH (50:1–3:1) solvent system to give 10 fractions. Fraction 1 (30 g) was chromatographed on a  $\text{SiO}_2$  column using a hexane–AcOEt gradient solvent system to give eight fractions (1'–8'), of which fraction 2' (5 g) was further chromatographed on a  $\text{SiO}_2$  column using a hexane–AcOEt solvent system to give eleven fractions (1''–11''). Of these, fraction 11'' (200 mg) was repeatedly purified by HPLC using an ODS column and 85%  $\text{CH}_3\text{CN}$  to give **2** (10 mg) and **4** (12 mg). Fraction 3 (8 g) was chromatographed on a  $\text{SiO}_2$  column using a gradient hexane–AcOEt solvent system to give seven fractions (1'''–7'''), of which fraction 2''' (500 mg) was repeatedly purified by HPLC using an ODS column and 85%  $\text{CH}_3\text{CN}$  to give **1** (40 mg), **3** (25 mg) and **5** (70 mg).

**Abiesanolide A (1):** White amorphous solid,  $[\alpha]_D^{25} -310^\circ$  ( $c=0.016$ , MeOH), UV  $\lambda_{\text{max}}$  nm ( $\epsilon$ ) (MeOH); 203.5 (11252), 281 (18537), IR  $\nu_{\text{max}}$   $\text{cm}^{-1}$  (KBr); 3080, 1754, 1713, 1640, HR-FAB-MS;  $m/z$  465.2983  $[\text{MH}]^+$  (Calcd for  $\text{C}_{30}\text{H}_{41}\text{O}_4$ ; 465.2304),  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : 0.86 (3H, s, Me-19), 0.94 (3H, Me-18), 1.02 (3H, d,  $J=7.6$  Hz, Me-21), 1.4 (2H, overlap, H-11, 12), 1.5–1.7 (2H, overlap, H-1), 1.6 (2H, overlap, H-16), 1.7 (1H, overlap, H-11), 1.78 (3H, s, Me-29), 1.82 (1H, m, H-12), 2.02 (3H, s, Me-27), 2.03 (1H, overlap, H-5), 2.11 (1H, br d,  $J=18.8$  Hz, H-6), 2.30 (2H, t,  $J=8.4$  Hz, H-2), 2.33 (1H, br d,  $J=18.8$  Hz, H-6), 2.46 (2H, m, H-15), 3.07 (1H, dq,  $J=10.4$ , 7.6 Hz, H-20), 4.73 (1H, br s, H-30), 4.69 (1H, br s, H-30), 4.82 (1H, br s, H-28), 4.86 (1H, br s, H-28), 5.15 (1H, d,  $J=10.4$  Hz, H-22), 5.39 (1H, dd,  $J=6.8$ , 3.6 Hz, H-7), 6.93 (1H, q,  $J=1.6$  Hz, H-24),  $^{13}\text{C-NMR}$  data is shown in Table 1.

**Abiesanolide B (2):** White amorphous powder,  $[\alpha]_D^{25} -297^\circ$  ( $c=0.5$ , MeOH), IR  $\nu_{\text{max}}$   $\text{cm}^{-1}$  (KBr); 3080, 1768, 1740, 1193, HR-FAB-MS;  $m/z$  479.3170  $[\text{MH}]^+$  (Calcd for  $\text{C}_{31}\text{H}_{43}\text{O}_4$ ; 479.3161),  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : 0.85 (3H, s, Me-19), 0.94 (3H, s, Me-18), 1.02 (3H, d,  $J=7.2$  Hz, Me-21), 1.58 (2H, overlap, H-1), 1.6 (2H, overlap, H-16), 1.78 (3H, s, Me-29), 1.99 (3H, s, Me-27), 2.27 (2H, t,  $J=8.4$  Hz, H-2), 2.47 (2H, m, H-15), 3.07 (1H, dq,  $J=10.4$ , 7.2 Hz, H-20), 3.66 (3H, s, COOMe), 4.70 (1H, br s, H-30), 4.73 (1H, br s, H-30), 4.82 (1H, br s, H-28), 4.85 (1H, br s, H-28), 5.14 (1H, d,  $J=10.4$  Hz, H-22), 5.39 (1H, dd,  $J=6.4$ , 3.6 Hz, H-7), 6.93 (1H, q,  $J=1.6$  Hz, H-24),  $^{13}\text{C-NMR}$  data is shown in Table 1.

**Abiesanolide C (3):** A white amorphous powder,  $[\alpha]_D^{25} -370^\circ$  ( $c=0.034$ , MeOH), UV  $\lambda_{\text{max}}$  nm ( $\epsilon$ ) (MeOH); 278 (24615), IR  $\nu_{\text{max}}$   $\text{cm}^{-1}$  (KBr); 3090,

1771, 1711, HR-FAB-MS:  $m/z$  465.2983  $[MH]^+$  (Calcd for  $C_{30}H_{41}O_4$ ; 465.2304),  $^1H$ -NMR ( $CDCl_3$ )  $\delta$ : 0.88 (3H, d,  $J=6.8$  Hz, Me-21), 0.91 (3H, s, Me-18), 0.92 (3H, s, Me-19), 1.53 (3H, br s, Me-30), 1.76 (3H, s, Me-29), 1.99 (3H, s, Me-27), 3.02 (1H, dq,  $J=10.8, 6.8$  Hz, H-20), 4.79 (1H, br s, H-28), 4.82 (1H, br s, H-28), 5.14 (1H, d,  $J=10.8$  Hz, H-22), 5.25 (1H, br s, H-15), 5.30 (1H, dd,  $J=6.4, 4.0$ , H-7), 6.96 (1H, q,  $J=1.2$  Hz, H-24),  $^{13}C$ -NMR data is shown in Table 1.

Abiesanolide D (4): White amorphous powder,  $[\alpha]_D -314^\circ$  ( $c=1.2$ , MeOH), UV  $\lambda_{max}$  nm ( $\epsilon$ ) (MeOH); 280 (15512), IR  $\nu_{max}$   $cm^{-1}$  (KBr); 3080, 1768, 1740, 1193. HR-FAB-MS:  $m/z$  479.3177  $[MH]^+$  (Calcd for  $C_{31}H_{43}O_4$ ; 479.3161),  $^1H$ -NMR ( $CDCl_3$ )  $\delta$ : 0.88 (3H, d,  $J=6.8$  Hz, Me-21), 0.90 (3H, s, Me-18), 0.92 (3H, s, Me-19), 1.54 (3H, br s, Me-30), 1.76 (3H, s, Me-29), 1.99 (3H, s, Me-27), 3.03 (1H, dq,  $J=10.8, 6.8$  Hz, H-20), 3.66 (3H, s, COOMe), 4.79 (1H, br s, H-28), 4.81 (1H, br s, H-28), 5.14 (1H, d,  $J=10.8$  Hz, H-22), 5.24 (1H, br s, H-15), 5.29 (1H, dd,  $J=6.4, 4.3$  Hz, H-7), 6.96 (1H, q,  $J=1.6$  Hz, H-24),  $^{13}C$ -NMR data is shown in Table 1.

3,4-Seco-4(28),6,8(14),22Z,24-mariesapentaen-26,23-olide-3-oic Acid (5): White amorphous powder,  $[\alpha]_D -343^\circ$  ( $c=0.036$ , MeOH), UV  $\lambda_{max}$  nm ( $\epsilon$ ) (MeOH); 254 (29249), IR  $\nu_{max}$   $cm^{-1}$  (KBr); 3400(br), 1754, 1715, HR-FAB-MS:  $m/z$  464.2925  $[M]^+$  (Calcd for  $C_{30}H_{40}O_4$ ; 464.2926),  $^1H$ -NMR ( $CDCl_3$ )  $\delta$ : 0.78 (3H, s, Me-30), 0.83 (3H, s, Me-18), 0.99 (3H, s, Me-19), 1.01 (3H, d,  $J=7.6$  Hz, Me-21), 1.77 (3H, s, Me-29), 2.00 (3H, s, Me-27), 2.61 (1H, d,  $J=5.6$  Hz, H-5), 3.15 (1H, dq,  $J=10.4, 7.6$  Hz, H-20), 4.75 (1H, br s, H-28), 4.95 (1H, br s, H-28), 5.18 (1H, d,  $J=10.4$  Hz, H-22), 5.36 (1H, dd,  $J=10.0, 5.6$  Hz, H-6), 6.20 (1H, d,  $J=10.0$  Hz, H-7), 6.97 (1H, q,  $J=1.6$  Hz, H-24),  $^{13}C$ -NMR data is shown in Table 1.

**Antibacterial Activity** The antibacterial activity test was carried out by the agar plate dilution method against gram positive bacteria, *Staphyrococ-*

*cus aureus* and *Bacillus subtilis* as previously reported.<sup>10)</sup>

**Acknowledgements** The authors thank Dr. K. Masuda and Mr. Y. Takase (Showa College of Pharmaceutical Science) for HR-FAB-MS measurements. This work was supported by the Japan Health Sciences Foundation.

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## Pharmacokinetic Disposition of Polyethylene Glycol-Modified Salmon Calcitonins in Rats

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Received June 19, 2000; accepted September 4, 2000

This study first reports the pharmacokinetic disposition of polyethylene glycol (PEG)-modified salmon calcitonin (sCT) based on the number of attached PEG molecules. PEG-modified sCT was prepared by covalent linkage with succinimidyl carbonate monomethoxy polyethylene glycol. Mono- and di-PEG-sCTs were separated by size exclusion and reverse phase HPLC, and radiiodinated by the chloramine-T method with Na<sup>125</sup>I. <sup>125</sup>I-mono-PEG sCT, <sup>125</sup>I-di-PEG-sCT and unmodified <sup>125</sup>I-sCT were administered to rats by i.v. injection. Serial blood samples, urine and various tissue samples were taken for the determination of radioactivity. Di-PEG-sCT exhibited significantly reduced systemic clearance (2.3 vs. 11.1 ml/min/kg) and steady-state volume of distribution (229.9 vs. 603.1 ml/kg), while mono-PEG-sCT showed a prolonged elimination half-life (189.1 min vs. 59.8 min) compared with unmodified sCT. The extent of urinary excretion of the PEG-modified sCTs was higher than for the unmodified sCT, but all these chemicals were excreted in urine in small quantities ( $\leq 0.6\%$ ). There was a tendency toward reduced accumulation of PEGylated sCTs in tissues, with its reduction being inversely proportional to the molecular size. Accumulation of the total radioactivity of the unmodified and PEG-modified sCTs was highest in the liver, followed by kidneys, lungs, spleen, heart and thyroid. When expressed per tissue gram weight, however, the highest radioactivity was found in the kidneys. PEGylated sCTs may have greater therapeutic potential *via* reduced systemic clearance and prolonged elimination half-life over unmodified sCT.

**Key words** salmon calcitonin; PEGylation; pharmacokinetics; tissue distribution

Salmon calcitonin (sCT) is a therapeutic polypeptide hormone consisting of 32 amino acids (3432 Da), with an N-terminal disulfide bond between the 1 and 7 positions and a C-terminal proline amide residue. It is currently marketed either as a solution for intramuscular or subcutaneous injection, or as a nasal spray in the treatment of postmenopausal osteoporosis, symptomatic Paget's disease of the bone, and hypercalcemia due to a malignancy. As with other peptide therapeutics, sCT is subject to enzymatic degradation and has a short elimination half-life (approximately 1 h) in humans.<sup>1,2)</sup> The mean absolute bioavailability of sCT ranges from 11.2–23.1% after subcutaneous injection to rats.<sup>3)</sup> Animal studies suggest that sCT is rapidly metabolized to smaller inactive fragments primarily in the kidneys, but also in blood and other tissues.<sup>4–6)</sup> Only small amounts of unchanged sCT and inactive metabolites are excreted in the urine.<sup>7)</sup>

Chemical modification of therapeutic polypeptides with polyethylene glycol (PEG) is a technique widely used to provide functional bioconjugates with increased resistance to proteolytic degradation, increased solubility, and reduced immunogenicity.<sup>8–11)</sup> Clinically available PEGylated polypeptides are, however, limited, mainly due to restricted distribution to target tissues and reduced receptor binding affinity.<sup>12)</sup> In addition, difficulty in physicochemical characterization due to heterogeneity with respect to the distribution in the number and position of the attached PEG molecules and inherent polydispersity of PEG itself is a barrier to the development of polypeptide therapeutics.

We previously reported a chemical modification of sCT by covalent linkage with PEG, and further isolated positional isomers of PEGylated sCTs.<sup>13,14)</sup> PEG may bind to sCT at ly-

sine 11, lysine 18 and N-terminus (cysteine 1) positions, yielding mono-, di-, and tri-PEGylated sCTs depending on the number of attached PEG molecules per molecule of sCT.<sup>14)</sup> The formation of mono-PEG-sCT appears to be favored over that of di-PEG-sCT, while the formation of tri-PEG-sCT is minimal. PEGylated sCTs exhibit substantially improved stability in rat liver and kidney homogenates over unmodified sCT, yet they retain biological activity similar to that of unmodified sCT, as examined by the adenosine cyclic 3',5'-phosphate (cAMP) assay.<sup>13,14)</sup>

The purpose of this study was to characterize the pharmacokinetic disposition of mono- and di-PEG-sCTs and their tissue distribution after i.v. injection to rats. Our results showed that the systemic clearance and the steady-state volume of distribution were significantly reduced while the AUC was increased for di-PEG-sCT, and the elimination half-life was significantly prolonged for mono-PEG-sCT compared with unmodified sCT.

### Materials and Methods

**Chemicals** Salmon calcitonin (synthetic cyclic sCT) and Na<sup>125</sup>I were purchased from BACHEM (Torrance, CA, U.S.A.) and Dupont NEN (Boston, MA, U.S.A.), respectively. Succinimidyl carbonate monomethoxy polyethylene glycol (SC-mPEG, M.W. 5000) was purchased from Shearwater Polymers, Inc., (Huntsville, AL, U.S.A.). Chloramine T was purchased from Shinyo Pure Chemicals Co. (Osaka, Japan), and ketamine and xylazine from Sigma Chem. Co. (St. Louis, MO, U.S.A.). HPLC grade acetonitrile and trifluoroacetic acid were purchased from J.T. Baker (Phillipsburg, NJ, U.S.A.) and Acros (Springfield, NJ, U.S.A.), respectively. Other chemicals used in the study were of analytical grade.

**Preparation and Purification of SC-mPEG-Modified sCT** SC-mPEG (40  $\mu$ g in 0.2 ml of 0.1 M phosphate buffer, pH 8.0) was added to 0.2 ml of sCT solution (2 mg/ml in 0.1 M phosphate buffer, pH 8.0) (SC-mPEG:sCT molar ratio, 1:3). The mixture was shaken gently at room temperature for 20 min and the reaction was stopped by the addition of an excess amount of

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1.0 M glycine solution. The reaction mixture was then subjected to size-exclusion chromatography on a Superose HR 12/30 column (Pharmacia LKB, Uppsala, Sweden) eluted with 10 mM phosphate buffer (pH 7.4) at a flow rate of 0.4 ml/min. In fluorescence measurements, the excitation and emission wavelengths were set at 280 and 315 nm, respectively (Hitachi F4010 spectrofluorometer, Tokyo, Japan). Fractions corresponding to mono- and di-PEGylated sCT were collected and concentrated to 100 µg/ml using Centricon-10 concentrators (Amicon, Beverly, MA) and were kept at 4°C until use.

**HPLC Conditions for sCT and PEGylated sCT** The HPLC system consisted of 2 Gilson pumps (Model 307), a UV-visible detector (Model 118) and an auto-injector (Model 234) (Gilson, Villiers-le-Bel, France). The analytical column used was a LiChrosphere 100 RP-18 cartridge (4.0×125 mm, 5 µm) with a guard column (4.0×4 mm, 5 µm) (Merck, Darmstadt, Germany). A linear gradient elution was carried out at a flow rate of 1.2 ml/min at a solvent A (0.1% trifluoroacetic acid in distilled water):solvent B (0.1% trifluoroacetic acid in acetonitrile) ratio of 70:30% to 100% B over 20 min. Additional elution was allowed for 10 min with 30% B between injections. UV responses were measured at 215 nm after the injection of a portion (40 µl) of sCT and PEGylated sCT samples by using an auto-injector.

**Radioiodination of sCT and PEGylated sCT** Radioiodinated sCT and PEGylated sCT were prepared as described previously.<sup>15)</sup> A portion (15 µl) of a chloramine T solution (50 µmole/ml in 0.1 M phosphate buffer, pH 7.4) and 50 µl of sCT (200 µg/ml) in 0.1 M phosphate buffer (pH 7.4) were added to 0.2 mCi Na<sup>125</sup>I (1 mCi/100 µl 0.1 M phosphate buffer, pH 7.4). After reaction for 30 s, the reaction mixture was quickly transferred to a Sephadex G-25 desalting column. The loaded mixture was eluted with 0.1 M phosphate buffer (pH 7.4), and fractions were collected in test tubes every 1 min over a 60 min period. The radioactivity of these collected samples was measured by gamma counting (Cobra™ Series Auto-Gamma Counting System, Packard Instrument Co., Groningen, the Netherlands).

**HPLC Conditions for Radioiodinated sCT and PEGylated sCT** The HPLC system consisted of two Waters pumps (Model 510), a 6-port switching valve, a UV visible detector (Model 440) (Milford, MA, U.S.A.) and a Ramona 2000 flow-through radioisotope detector (Raytest, Straubenhardt, Germany). The columns and elution conditions were identical to those described above. A portion (100 µl) of the radioisotope samples was injected onto the switching valve. The UV detection was carried out at 215 nm for the eluted samples to determine the concentration of PEG-modified and unmodified sCTs. The radioactivity of the effluents was determined using a flow-through radioactive detector. Data handling was performed by means of an integration computer program Winnie 2.01 (Raytest, Straubenhardt, Germany).

**Animals** Male Sprague Dawley rats (7–9 weeks of age, 200–250 g) were obtained from Hanlim, Inc. (Suwon, Korea). The rats were kept in plastic rat cages and were housed in an animal facility (temperature 23±2°C) with a light/dark cycle of 12/12 h and a relative humidity of 50±10%. The animals were fed a standard rat diet (DaeJong Co., Seoul, Korea) and had free access to water. At least one week of acclimatization was allowed prior to experimentation.

**I.v. Injection Study** Rats were anesthetized with i.m. injection of ketamine and xylazine (90/10 mg/kg), and were cannulated with PE tubing (0.58 mm i.d. and 0.96 mm o.d., Natsume Co., Tokyo, Japan) in the right jugular and left femoral veins. After at least two days of recovery, the rats were administered <sup>125</sup>I-sCT, <sup>125</sup>I-mono-PEG-sCT or <sup>125</sup>I-di-PEG-sCT by i.v. bolus injection (5 µg) into the femoral venous catheter (injection volume 0.5 ml) in three groups of rats (*n*=4 each). The animals were unrestrained and conscious and kept in metabolic cages during the dosing and sampling period. The average specific radioactivity of administered <sup>125</sup>I-sCT, <sup>125</sup>I-mono-PEG-sCT and <sup>125</sup>I-di-PEG-sCT was in the range of approximately 6–8×10<sup>6</sup> cpm. Serial blood samples (approximately 0.3 ml each) were withdrawn from the jugular vein at 0, 2, 5, 10, 15, 30 and 45 min, and at 1, 1.5, 2, 4 and 6 h into Eppendorf tubes which were then placed in an ice bath. Equal volumes of isotonic saline were replaced after each sampling. Serum samples were harvested immediately by centrifugation at 10000 rpm for 10 min. A portion (200 µl) of 20% TCA solution was added to a 100 µl of serum samples. The mixture was mixed on a vortex mixer for 10 s and centrifuged at 10000 rpm for 10 min. The radioactivity of the resulting supernatants and precipitates was measured by gamma counting. In addition, the rats were sacrificed at 6 h after i.v. injection, and the liver, kidneys, thyroid, spleen, heart, lungs and muscle were excised, blot dried and weighed. The radioactivity was determined for these tissue samples by gamma counting. Urine samples were also collected for 6 h in metabolic cages. A portion (200 µl) of the 20% TCA solution was added to the urine samples, mixed on a vortex

mixer and centrifuged at 10000 rpm for 10 min. The radioactivity of the resulting supernatants and precipitates was then measured by gamma counting.

**Data Analysis** Serum concentration vs. time data obtained after i.v. administration were analyzed by fitting a bi-exponential equation to the time profiles using the nonlinear least squares regression program WinNonlin (Scientific Consulting, Inc., Cary, NC, U.S.A.). Area under the concentration vs. time curve (*AUC*) and the area under the first moment of the concentration vs. time curve (*AUMC*) were determined from the coefficients and exponents of these fitted relationships.<sup>16)</sup> Pharmacokinetic parameters were calculated from the following equations: systemic clearance (*Cl*)=dose/*AUC*, volume of distribution at steady-state (*V<sub>ss</sub>*)=dose·*AUMC*/*AUC*<sup>2</sup>, distribution half-life (*t*<sub>1/2,λ<sub>1</sub></sub>)=0.693/λ<sub>1</sub>, and elimination half-life (*t*<sub>1/2,λ<sub>2</sub></sub>)=0.693/λ<sub>2</sub>. Pharmacokinetic parameter values were expressed as the mean±S.D.

## Results

Conjugation of sCT with SC-mPEG (M.W. 5000) produced a heterogeneous mixture of PEG-modified and unmodified sCT species. The mixture was subjected to size-exclusion chromatography, and unmodified sCT, mono-PEG-sCT and di-PEG-sCT were separated from each other. Their retention times were 44.5, 36.8 and 33.4 min, respectively. Tri-PEG-sCT was eluted as a minor shoulder peak. The percentage of the peak area counts was 11.9% for sCT, 42.9% for mono-PEG-sCT and 33.4% for di-PEG-sCT. HPLC analysis of the radioiodinated sCT, mono-sCT and di-sCT prepared by the chloramine-T method showed that the free to labeled <sup>125</sup>I ratio was 21.5±11.5% for sCT, 85.5±2.5% for mono-PEG-sCT and 66.4±8.6% for di-PEG-sCT.

Figure 1 shows average decay curves of the total radioactivity and the radioactivities corresponding to intact and degradation species after i.v. injection of sCT, mono-PEG-sCT and di-PEG-sCT to rats (*n*=4 each). The average serum concentration vs. time curves of the unmodified sCT, mono-PEG-sCT and di-PEG-sCT are shown in Fig. 2. Serum levels of the unmodified and PEGylated sCTs declined bi-exponentially, with the mean initial distribution half-lives ranging from 3.6–6.2 min and the terminal elimination half-lives ranging from 59.8–189.1 min (Table 1). The systemic clearance of mono-PEG-sCT was not significantly altered over unmodified sCT, although it was reduced (5.1 vs. 11.1 ml/min/kg). Also, the steady-state volume of distribution (821.8 vs. 603.1 ml/kg) and *AUC* (5670 vs. 3288 ng·min/ml) of mono-PEG-sCT were not significantly altered over unmodified sCT. In contrast, di-PEG-sCT showed significantly reduced systemic clearance (2.3 ml/min/kg) and steady-state volume of distribution (229.9 ml/kg), and increased *AUC* (11008 ng·min/ml) over unmodified and mono-PEGylated sCT. The extent of urinary excretion of intact and degradation species after i.v. injection of unmodified and PEG-modified sCTs is shown in Fig. 3. PEG-modified and unmodified sCTs were excreted intact in urine in small quantities (0.1–0.6%). The urinary excretion of degradation products was also low (range 1.5–2.5%). The total radioactivity found in various tissues is shown in Table 2. The highest radioactivity was found in the liver, followed by the kidneys, lungs, spleen, heart and thyroid for unmodified and PEG-modified sCTs. Figure 4 shows the total radioactivity in various tissues normalized by gram tissue weight. The weight-normalized total radioactivity was highest in the kidneys for both unmodified and PEG-modified sCTs. Nevertheless, the accumulation of mono- and di-PEG-sCTs in the kidneys was significantly reduced over unmodified sCT. The accumulation of di-PEG-sCT was also significantly reduced in the liver and

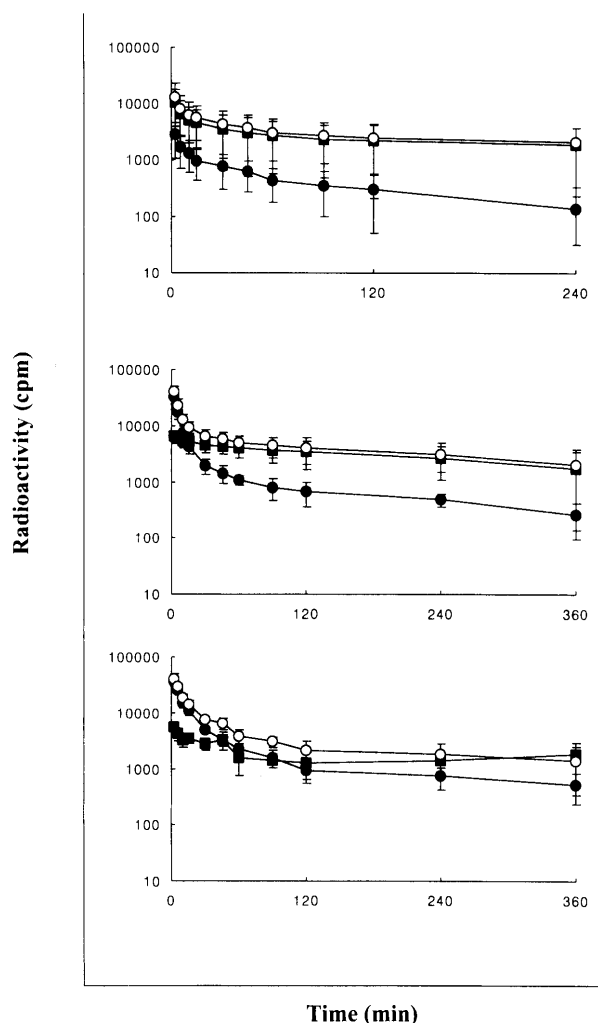


Fig. 1. Average Decay Curves of the Total Radioactivity (○) and the Radioactivities Corresponding to the Intact (●) and Degradation (■) Species Found in the Serum after I.v. Injection of sCT (Top Panel), Mono-PEG-sCT (Middle Panel) and Di-PEG-sCT (Bottom Panel) to Rats

$n=4$  each.

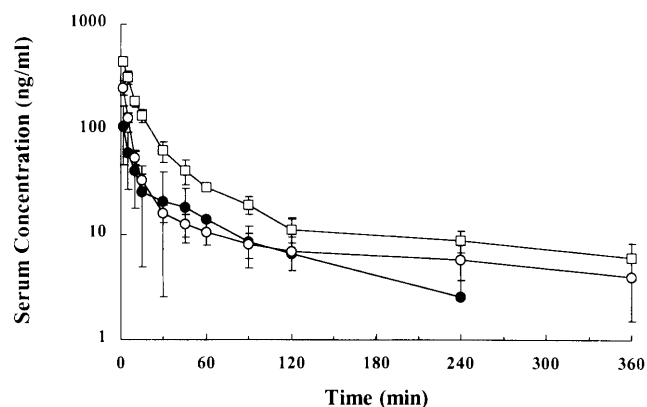


Fig. 2. Average Serum Concentration vs. Time Curves of sCT (●), Mono-PEG-sCT (○) and Di-PEG-sCT (□) in Rats after Their Respective I.v. Injections

$n=4$  each.

spleen.

## Discussion

To our knowledge, this study first examined the pharmaco-

Table 1. Pharmacokinetic Parameters (Mean $\pm$ S.D.) of sCT, Mono-PEG-sCT and Di-PEG-sCT Obtained after Their Respective I.v. Injections (5  $\mu$ g) in the Rat ( $n=4$  Each)

| Parameter                 | sCT               | Mono-PEG-sCT                    | Di-PEG-sCT                     |
|---------------------------|-------------------|---------------------------------|--------------------------------|
| Weight (g)                | 190 $\pm$ 6       | 193 $\pm$ 5                     | 190 $\pm$ 8                    |
| $t_{1/2,\lambda_1}$ (min) | 3.6 $\pm$ 3.0     | 3.3 $\pm$ 0.7                   | 6.2 $\pm$ 0.9                  |
| $t_{1/2,\lambda_2}$ (min) | 59.8 $\pm$ 45.2   | 189.1 $\pm$ 119.6 <sup>a)</sup> | 107.2 $\pm$ 32.8               |
| AUC (ng $\cdot$ min/ml)   | 3288 $\pm$ 1553   | 5670 $\pm$ 2183                 | 11008 $\pm$ 224 <sup>b)</sup>  |
| Cl (ml/min/kg)            | 11.1 $\pm$ 9.0    | 5.1 $\pm$ 1.8                   | 2.3 $\pm$ 0.2 <sup>b)</sup>    |
| $V_{ss}$ (ml/kg)          | 603.1 $\pm$ 332.3 | 821.8 $\pm$ 324.3               | 229.9 $\pm$ 68.3 <sup>b)</sup> |

a)  $p<0.05$  over unmodified sCT. b)  $p<0.05$  over unmodified and mono-PEG-ylated sCT.

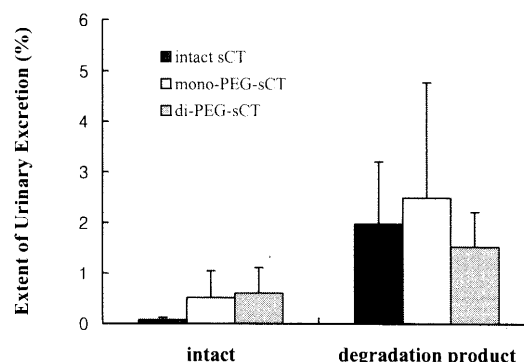


Fig. 3. Extent of Urinary Excretion (%) of the Unmodified sCT, Mono-PEG-sCT and Di-PEG-sCT, as well as Their Degradation Products Determined after I.v. Injection to Rats

$n=4$  each.

Table 2. The Total Radioactivity (Mean $\pm$ S.D.) Found in Various Organs Determined at 6 h after I.v. Injection of sCT, Mono-PEG-sCT and Di-PEG-sCT in the Rat ( $n=4$  Each)

| Tissue  | Radioactivity (cpm) found in whole organ |                                 |                                 |
|---------|------------------------------------------|---------------------------------|---------------------------------|
|         | sCT                                      | Mono-PEG-sCT                    | Di-PEG-sCT                      |
| Liver   | 99776 $\pm$ 34685                        | 73271 $\pm$ 57572               | 46342 $\pm$ 13377 <sup>b)</sup> |
| Kidney  | 67657 $\pm$ 31261                        | 44376 $\pm$ 22806 <sup>a)</sup> | 21704 $\pm$ 3441 <sup>a)</sup>  |
| Lung    | 12503 $\pm$ 10764                        | 10745 $\pm$ 6357                | 9071 $\pm$ 2728                 |
| Spleen  | 6985 $\pm$ 4507                          | 7846 $\pm$ 3566                 | 4875 $\pm$ 2418                 |
| Heart   | 6829 $\pm$ 6281                          | 6935 $\pm$ 2472                 | 4265 $\pm$ 808                  |
| Thyroid | 1284 $\pm$ 1303                          | 3115 $\pm$ 3195                 | 3145 $\pm$ 458                  |

a)  $p<0.05$  over unmodified sCT. b)  $p<0.05$  over unmodified and mono-PEG-ylated sCT.

kinetics of PEGylated sCT based on the number of attached PEG molecules. sCT contains three potential sites for PEGylation at the primary amine moiety of the N-terminus (cysteine 1) and two lysine residues (lysine 11 and 18). Mono-PEG-sCT is, therefore, a mixture consisting of the N-terminus-, lysine 11- and lysine 18-modified sCTs, whereas di-PEG-sCT is a mixture consisting of the N-terminus- and lysine 11-, N-terminus- and lysine 18-, and lysine 11- and lysine 18-modified sCTs. Tri-PEG-sCT is sCT PEGylated at all three available sites, and is formed as a minor product. The administered dose of sCT was within a linear i.v. dose range (1–10  $\mu$ g) reported previously in rats.<sup>3)</sup> Consistent with previous findings,<sup>3,17)</sup> serum levels of unmodified sCT declined bi-exponentially. Also, serum levels of mono- and di-PEGylated sCTs declined bi-exponentially. The pharmacokinetic parameters of unmodified sCT determined in this study (ter-

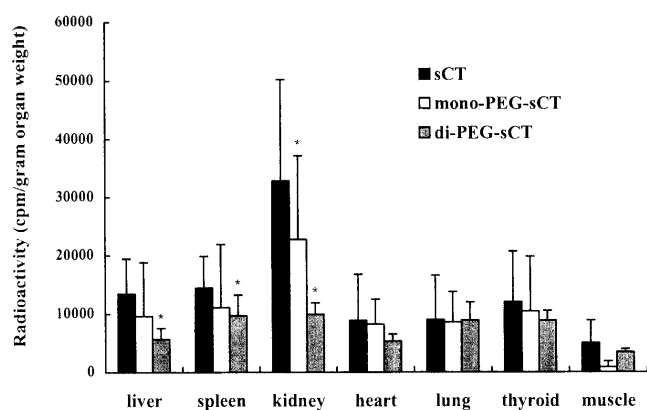


Fig. 4. Total Radioactivity in Tissues (cpm per Gram Organ Weight) Determined at 6 h after I.v. Injection of sCT, Mono-PEG-sCT and Di-PEG-sCT to Rats

$n=4$  each.

minimal elimination half-life of 59.8 min, systemic clearance of 11.1 ml/min/kg, and steady-state volume of distribution of 603.1 ml/kg) are comparable to those previously reported in rats.<sup>3)</sup> Attachment of one PEG molecule to sCT did not significantly alter the systemic clearance or steady-state volume of distribution. On the other hand, attachment of two PEG molecules to sCT resulted in significantly reduced systemic clearance and steady-state volume of distribution. The reduced systemic clearance of di-PEG-sCT is consistent with its improved stability in rat kidney homogenates over that of unmodified sCT.<sup>13)</sup> The elimination half-life of mono-PEG-sCT was prolonged as the systemic clearance was lowered and the volume of distribution was greater compared to those of unmodified sCT. However, the elimination half-life of di-PEG-sCT was unaltered as its steady-state volume of distribution and systemic clearance were simultaneously reduced. These observations are somewhat different from those of other PEGylated peptides, e.g., recombinant methioninase,<sup>18)</sup> tumor necrosis factor- $\alpha$ ,<sup>19)</sup> recombinant human granulocyte colony-stimulating factor,<sup>20)</sup> ribonuclease,<sup>21)</sup> recombinant human interferon gamma,<sup>22)</sup> and recombinant human interleukin-2,<sup>23)</sup> in that their systemic clearances were reduced and elimination half-lives were increased simultaneously. Hence, the distribution volume of these peptides appears to be minimally affected by PEG modification.<sup>23)</sup> The molecular masses of these latter peptides are relatively large (>14 kDa) compared to that of sCT (3432 Da). The molecular masses of mono- and di-PEG-sCT were increased to approximately 8410 and 13230 Da, respectively, as measured by MALDI-TOF mass spectrometry. Whether a molecular size threshold exists beyond which the tissue distribution of PEG-modified peptides is minimally affected deserves further examination.

The kidney is known to be a major biotransformation site of sCT, accounting for two-thirds of its metabolism.<sup>24,25)</sup> The removal of calcitonin from renal circulation appears to occur primarily by a glomerular filtration-independent pathway, with the urinary excretion being minimal.<sup>25)</sup> In our study, the urinary excretion of unmodified and PEG-modified sCTs was also low. Nevertheless, their total radioactivities were highest in the kidney when expressed per gram weight. When compared with unmodified sCT, mono- and di-PEGylated sCTs were accumulated in the kidney to a significantly lesser ex-

tent (Table 2, Fig. 4). Similarly, the accumulation of di-PEGylated sCT into the liver and spleen was significantly reduced. These reductions occurred despite the serum levels of PEGylated sCTs being higher than those of unmodified sCT at the time the rats were sacrificed (Fig 1). Therefore, PEG modification appears to have altered the tissue distribution characteristics of sCT depending on the degree of increase in the molecular mass.

In conclusion, PEGylated sCT showed altered pharmacokinetics depending on the number of attached PEG molecules. Given the similar biological activity of mono- and di-PEG-sCTs to that of unmodified sCT,<sup>13)</sup> PEGylated sCTs may have greater therapeutic potential via prolonged elimination half-life and reduced systemic clearance.

**Acknowledgements** This work was supported by the Korean Science and Engineering Foundation Grant KOSEF 97-04-12-01-3 and partially by the Brain Korea 21 by the Ministry of Education.

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## Structure–Affinity Relationships of C-terminal Cyclic Analogue of Neuropeptide Y for the Y<sub>1</sub>-Receptor

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Received June 22, 2000; accepted September 5, 2000

We previously reported that a cyclic octapeptide amide, c[D-Cys<sup>29</sup>, Cys<sup>34</sup>]NPY Ac-29–36 (YM-42454) showed a high affinity for Y<sub>1</sub>-receptors in SK-N-MC cells ( $K_i=0.047\ \mu\text{M}$ ) but not for Y<sub>2</sub>-receptors in the porcine hippocampus membranes ( $K_i>10\ \mu\text{M}$ ). To explore the critical residues of this unique cyclic peptide for Y<sub>1</sub>-binding activity, the structure–affinity relationships were investigated by means of amino acid replacement. The results indicated that the hydrophobic side-chains of Leu<sup>30</sup> and Ile<sup>31</sup>, the guanidinium groups of Arg<sup>33</sup> and Arg<sup>35</sup>, and the C-terminal amide are critical for the binding affinity of YM-42454 to the Y<sub>1</sub>-receptor. On the other hand, Thr<sup>32</sup> in YM-42454 might not be critical for the Y<sub>1</sub>-binding affinity. <sup>1</sup>H-NMR studies for YM-42454 and its derivatives have suggested that the critical residues are involved in the direct interaction with a Y<sub>1</sub>-receptor rather than in maintaining the bioactive conformation.

**Key words** neuropeptide Y; Y<sub>1</sub>-receptor; cyclic peptide; structure–affinity relationship; binding affinity

Neuropeptide Y (NPY) is a 36-amino-acid C-terminally amidated peptide initially isolated from porcine brain by Tatemoto and co-workers.<sup>2)</sup> It is a member of the pancreatic polypeptide family (PP-family) and is one of the most abundant neuropeptides in the mammalian central and peripheral nervous systems. It has various biological functions such as vasoconstriction,<sup>3,4)</sup> stimulation of food intake<sup>5,6)</sup> and anxiolysis.<sup>7)</sup>

The three-dimensional structure of one member of the PP-family, avian PP (aPP), has been determined by X-ray crystallography.<sup>8)</sup> It consists of a polyproline type II-like helix (residues 1–8), a  $\beta$ -turn (residues 9–13), an  $\alpha$ -helix (residues 14–32), and a flexible tail (residues 33–36). The polyproline helix and  $\alpha$ -helix pack with hydrophobic interactions and form the hairpin-like structure. A modeling study based on the aPP structure predicted that NPY would also have the hairpin-like structure.<sup>9)</sup> This has been supported by NMR investigations,<sup>10–12)</sup> although both N- and C-terminals are not well defined.

Several receptor subtypes were proposed based on the various biological responses by NPY and its analogues.<sup>13–16)</sup> At present, five receptor subtypes (Y<sub>1</sub>, Y<sub>2</sub>, Y<sub>4</sub>, Y<sub>5</sub>, Y<sub>6</sub>) have been cloned.<sup>17–21)</sup> The Y<sub>1</sub>-receptor mainly mediates vasoconstriction, anxiolysis, sedation, and food intake. The Y<sub>2</sub>-receptor is related to the suppression of transmitter release.<sup>22)</sup> It was previously thought that the entire NPY molecule was required to activate the Y<sub>1</sub>-receptor whereas C-terminal fragments such as NPY13–36 can activate the Y<sub>2</sub>-receptor.<sup>13,23)</sup> Structure–activity relationship studies of NPY including L-Ala and D-amino-acid scans have revealed that Arg<sup>33</sup>, Arg<sup>35</sup>, and Tyr<sup>36</sup>-amide are the most crucial residues for binding to the both Y<sub>1</sub> and Y<sub>2</sub>-receptor subtypes.<sup>24–27)</sup> Several NPY analogues—such as truncated, branched, and cyclic peptide derivatives—have been reported to have various biological activities and specificities.<sup>28–33)</sup>

We previously reported that a C-terminal cyclic peptide of

NPY, c[D-Cys<sup>29</sup>, Cys<sup>34</sup>]NPY Ac-29–36 (YM-42454), showed a high affinity and selectivity for Y<sub>1</sub>-receptors in SK-N-MC cells ( $K_i=0.047\ \mu\text{M}$ ) despite the lack of the N-terminal sequence of NPY.<sup>34)</sup> The affinity was about 200-fold higher than the corresponding C-terminal linear peptide, NPY Ac-28–36 ( $K_i=8.7\ \mu\text{M}$ ), and about 1/500 of porcine NPY (pNPY,  $K_i=0.092\ \text{nM}$ ). YM-42454 was designed to stabilize the hypothetical bioactive conformation of the C-terminal octapeptide of human NPY (hNPY) based on the homology modeling with the crystal structure of aPP.

In this report, the structure–affinity relationships of YM-42454 for the Y<sub>1</sub>-receptor were investigated by means of amino acid replacement and <sup>1</sup>H-NMR experiments.

### Results and Discussion

YM-42454 is an interesting Y<sub>1</sub>-receptor ligand that has only a region corresponding to the C-terminal residues of NPY and does not show Y<sub>2</sub>-binding activity.<sup>34)</sup> To investigate which residues of YM-42454 were critical for Y<sub>1</sub>-binding activity, structure–activity relationships were studied with a total of eighteen derivatives (Table 1). In general, the crucial residues are considered to play an important role in the direct interaction with the Y<sub>1</sub>-receptor and/or in maintaining the bioactive conformation. To explore the role of the crucial residues, <sup>1</sup>H-NMR studies were carried out on YM-42454 and some of its derivatives in aqueous solution. In following discussions, the numbering of the residue positions in YM-42454 is according to that of NPY.

**Structure–Affinity Relationships of YM-42454** The first set of experiments involved replacing Arg<sup>33</sup> or Arg<sup>35</sup> of YM-42454 with Nle (norleucine), Asn, His and Lys (1–7). The corresponding Arg residues in NPY are essential for its Y<sub>1</sub>-binding activity.<sup>24–27)</sup> All the substitutions drastically decreased the receptor binding ability although peptide **2** partially retained Y<sub>1</sub>-binding affinity ( $K_i=4.2\ \mu\text{M}$ ). These results suggest that both Arg residues are also important for the

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Table 1. Binding Affinity to SK-N-MC Cells ( $Y_1$ ) and Analytical Data of YM-42454 Derivatives

| Peptide      | Amino acid sequence <sup>a)</sup>                      | SK-N-MC (Y <sub>1</sub> ) <sup>b)</sup> |                                    | RP-HPLC <sup>c)</sup> | (M+H) <sup>+</sup>  |                    |
|--------------|--------------------------------------------------------|-----------------------------------------|------------------------------------|-----------------------|---------------------|--------------------|
|              |                                                        | K <sub>i</sub> (μM)                     | t <sub>R</sub> <sup>d)</sup> (min) | (purity, %)           | Calcd <sup>e)</sup> | Obsd <sup>f)</sup> |
| YM-42454     | Ac-cys-Leu-Ile-Thr-Arg-Cys-Arg-Tyr-NH <sub>2</sub>     | 0.047                                   |                                    | 7.2 (97)              | 1066.5              | 1066.2             |
| NPY Ac-28—36 | Ac-Ile-Asn-Leu-Ile-Thr-Arg-Gln-Arg-Tyr-NH <sub>2</sub> | 8.7                                     |                                    | 7.2 (99)              | 1217.7              | 1217.2             |
| <b>1</b>     | Ac-cys-Leu-Ile-Thr-Nle-Cys-Arg-Tyr-NH <sub>2</sub>     | >10                                     |                                    | 26.8 (99)             | 1023.5              | 1023.5             |
| <b>2</b>     | Ac-cys-Leu-Ile-Thr-Lys-Cys-Arg-Tyr-NH <sub>2</sub>     | 4.2                                     |                                    | 6.7 (99)              | 1038.5              | 1038.5             |
| <b>3</b>     | Ac-cys-Leu-Ile-Thr-Asn-Cys-Arg-Tyr-NH <sub>2</sub>     | >10                                     |                                    | 8.1 (99)              | 1024.5              | 1024.6             |
| <b>4</b>     | Ac-cys-Leu-Ile-Thr-His-Cys-Arg-Tyr-NH <sub>2</sub>     | >10                                     |                                    | 6.7 (97)              | 1047.5              | 1047.6             |
| <b>5</b>     | Ac-cys-Leu-Ile-Thr-Arg-Cys-Nle-Tyr-NH <sub>2</sub>     | >10                                     |                                    | 16.8 (99)             | 1023.5              | 1023.7             |
| <b>6</b>     | Ac-cys-Leu-Ile-Thr-Arg-Cys-Lys-Tyr-NH <sub>2</sub>     | >10                                     |                                    | 6.9 (97)              | 1038.5              | 1038.7             |
| <b>7</b>     | Ac-cys-Leu-Ile-Thr-Arg-Cys-His-Tyr-NH <sub>2</sub>     | >10                                     |                                    | 7.1 (99)              | 1047.5              | 1047.7             |
| <b>8</b>     | Ac-cys-Leu-Ile-Thr-Arg-Cys-Arg-Nal-NH <sub>2</sub>     | 1.5                                     |                                    | 26.3 (100)            | 1100.6              | 1100.7             |
| <b>9</b>     | Ac-cys-Leu-Ile-Thr-Arg-Cys-Arg-Tyr-NHCH <sub>3</sub>   | >10                                     |                                    | 8.1 (96)              | 1080.5              | 1080.5             |
| <b>10</b>    | Ac-cys-Ala-Ile-Thr-Arg-Cys-Arg-Tyr-NH <sub>2</sub>     | 3.2                                     |                                    | 4.2 (99)              | 1024.5              | 1024.5             |
| <b>11</b>    | Ac-cys-Leu-Ala-Thr-Arg-Cys-Arg-Tyr-NH <sub>2</sub>     | 5.0                                     |                                    | 4.1 (99)              | 1024.5              | 1024.6             |
| <b>12</b>    | Ac-cys-Leu-Pro-Thr-Arg-Cys-Arg-Tyr-NH <sub>2</sub>     | >10                                     |                                    | 4.2 (99)              | 1050.5              | 1050.6             |
| <b>13</b>    | Ac-cys-Leu-pro-Thr-Arg-Cys-Arg-Tyr-NH <sub>2</sub>     | >10                                     |                                    | 4.4 (100)             | 1050.5              | 1050.7             |
| <b>14</b>    | Ac-cys-Leu-Ile-Ala-Arg-Cys-Arg-Tyr-NH <sub>2</sub>     | 0.68                                    |                                    | 6.4 (97)              | 1036.5              | 1036.7             |
| <b>15</b>    | Ac-cys-Leu-Ile-Val-Arg-Cys-Arg-Tyr-NH <sub>2</sub>     | 0.23                                    |                                    | 12.2 (99)             | 1064.6              | 1064.7             |
| <b>16</b>    | Ac-cys-Leu-Ile-Trp-Arg-Cys-Arg-Tyr-NH <sub>2</sub>     | 0.49                                    |                                    | 16.6 (98)             | 1151.6              | 1151.6             |
| <b>17</b>    | Ac-cys-Leu-Ile-thr-Arg-Cys-Arg-Tyr-NH <sub>2</sub>     | 1.9                                     |                                    | 6.6 (99)              | 1066.5              | 1066.7             |
| <b>18</b>    | Ac-cys-Leu-Ile-trp-Arg-Cys-Arg-Tyr-NH <sub>2</sub>     | 5.3                                     |                                    | 17.3 (100)            | 1151.6              | 1151.6             |

a) Amino acids are expressed by 3 letter codes. Codes starting with capital letters refer to L-isomers, whereas the others refer to D-isomers. Cysteines are cross-linked by an intramolecular disulfide bond. b)  $K_i$  value of porcine NPY in SK-N-MC was 0.092 nM. c) CAPCELL PAK C<sub>18</sub> SG300 S5 (250 mm×4.6 mm), 0.1% aqueous Trifluoroacetic acid (TFA)/CH<sub>3</sub>CN 75/25, flow rate 1 ml/min, detection at 220 nm. d) Retention time. e) Theoretical values calculated with monoisotopic mass. f) Data obtained by MALDI-TOF-MS.

binding of YM-42454 to the  $Y_1$ -receptor. However, it is interesting that the replacement of Arg<sup>33</sup> with Lys resulted in weak affinity to the  $Y_1$ -receptor, while only Arg was permissible at position 35. The guanidinium group of Arg is not only positively charged but also has the ability to form plural hydrogen bonds. In addition, it has a pseudo-aromatic character that is a consequence of its planar nature and  $\pi$ -electron system. These versatile properties of Arg in YM-42454 may play an important role for receptor recognition, especially at position 35.

The replacement of Tyr<sup>36</sup> with 2-naphthyl-Ala (Nal) decreased the binding affinity about 30-fold. In the case of NPY, the replacement of Tyr<sup>36</sup> with Phe showed a small reduction of  $Y_1$ -binding affinity while the replacement of Tyr<sup>36</sup> with Ala significantly reduced its binding ability.<sup>27)</sup> The moderate reduction of peptide **8** might be due to the lack of a hydroxyl group and/or a somewhat larger aromatic ring than that of Tyr residue.

When the C-terminal amide of YM-42454 was converted to the N-methyl-amide, this derivative (**9**) showed a drastically reduced affinity to the  $Y_1$ -receptor. This result indicated that the C-terminal carbamoyl group is essential for  $Y_1$ -binding affinity in YM-42454. The deamidated derivative of NPY showed complete loss of affinity to the  $Y_1$ -receptor, indicating that the C-terminal amide of NPY is also essential for receptor binding.<sup>14,35)</sup>

The derivatives in which Leu<sup>30</sup> or Ile<sup>31</sup> were replaced with Ala (**10**, **11**) exhibited a distinct reduction of  $Y_1$ -binding affinity ( $K_i$  values of 3.3 and 5.0  $\mu$ M, respectively). These results suggest that the hydrophobic side-chains of Leu<sup>30</sup> and Ile<sup>31</sup> are important for  $Y_1$ -binding. In the case of NPY, the replacement of Ile<sup>31</sup> with Ala resulted in a significantly reduced affinity for the  $Y_1$ -receptor, while the replacement of Leu<sup>30</sup> with Ala resulted in a less reduced affinity for the  $Y_1$ -receptor.<sup>27)</sup> The modeling and NMR studies of NPY suggest that

these hydrophobic residues interact with the N-terminal polyproline-like helix and that this interaction stabilizes the hairpin-like structure.<sup>9,10)</sup> Since YM-42454 does not have the N-terminal portion of NPY, these side-chains are probably exposed and could be involved in the direct binding interaction rather than in maintaining the binding conformation. The derivatives with L-Pro and D-Pro at position 31 (**12**, **13**) showed drastically reduced binding affinities. As D- and L-Pro are structurally restricted amino acids, their introduction might have an adverse effect on the conformation responsible for the  $Y_1$ -receptor binding. Conformational aspects of peptides **10** and **13** are described below in the <sup>1</sup>H-NMR study section.

The replacements of Thr<sup>32</sup> with Ala, Val and Trp (**14**–**16**) retained the  $Y_1$ -binding ability better than other substitutions ( $K_i$  values of 0.68, 0.23 and 0.49  $\mu$ M, respectively). These results suggest that position 32 does not appear to be crucial for the binding of YM-42454 to the  $Y_1$ -receptor. They are interesting results because the substitution of Thr<sup>32</sup> in NPY with Ala significantly reduced the  $Y_1$ -binding affinity.<sup>27)</sup> On the other hand, the conversion of the residues at position 32 to D-amino acids (**17**, **18**) reduced the  $Y_1$ -binding affinity compared to that of the original L-amino acid derivatives (YM-42454, **16**, respectively). A D-amino acid has the same side-chain as the corresponding L-amino acid but the orientation of its side-chain is different. To investigate the effect of the replacement with D-amino acid at position 32 on the overall conformation, a <sup>1</sup>H-NMR study was performed for the peptide **17** (see next section).

**<sup>1</sup>H-NMR Study of YM-42454 and Its Derivatives** A <sup>1</sup>H-NMR study was carried out on YM-42454, NPY Ac-28—36, and six of the derivatives (**2**, **6**, **9**, **10**, **13**, **17**) in aqueous solution. All the investigated peptide analogues gave only one set of signals in each <sup>1</sup>H-NMR spectrum. Chemical shifts, temperature coefficients and <sup>3</sup>J<sub>NH $\alpha$</sub>  coupling constants

Table 2. Amide Proton Chemical Shifts of YM-42454 and Its Related Peptides<sup>a)</sup> (ppm)

| Peptide      | Position <sup>b)</sup> |      |      |      |      |      |      |      |      |
|--------------|------------------------|------|------|------|------|------|------|------|------|
|              | 28                     | 29   | 30   | 31   | 32   | 33   | 34   | 35   | 36   |
| YM-42454     |                        | 8.48 | 8.76 | 8.18 | 7.83 | 8.38 | 8.63 | 8.35 | 8.29 |
| NPY Ac-28—36 | 8.32                   | 8.65 | 8.29 | 8.21 | 8.24 | 8.38 | 8.45 | 8.46 | 8.32 |
| <b>2</b>     |                        | 8.49 | 8.76 | 8.21 | 7.81 | 8.32 | 8.63 | 8.35 | 8.30 |
| <b>6</b>     |                        | 8.48 | 8.75 | 8.19 | 7.82 | 8.38 | 8.64 | 8.47 | 8.27 |
| <b>9</b>     |                        | 8.48 | 8.76 | 8.18 | 7.82 | 8.37 | 8.63 | 8.37 | 8.33 |
| <b>10</b>    |                        | 8.53 | 8.88 | 8.11 | 7.86 | 8.36 | 8.54 | 8.29 | 8.30 |
| <b>13</b>    |                        | 8.42 | 8.07 | —    | 8.48 | 8.44 | 7.96 | 8.33 | 8.23 |
| <b>17</b>    |                        | 8.61 | 8.68 | 8.08 | 8.34 | 8.68 | 8.33 | 8.42 | 8.30 |

a) Measured in aqueous solution (90% H<sub>2</sub>O/10% D<sub>2</sub>O) at 283 K, pH 4.5. b) The numbering of the positions is according to that of NPY.

Table 3. <sup>3</sup>J<sub>NHHA</sub> Coupling Constants of YM-42454 and Its Related Peptides<sup>a)</sup> (Hz)

| Peptide      | Position <sup>b)</sup> |               |     |     |               |               |               |               |               |
|--------------|------------------------|---------------|-----|-----|---------------|---------------|---------------|---------------|---------------|
|              | 28                     | 29            | 30  | 31  | 32            | 33            | 34            | 35            | 36            |
| YM-42454     |                        | 6.9           | 6.3 | 5.8 | 6.2           | 5.9           | 6.6           | 6.9           | 7.8           |
| NPY Ac-28-36 | <sup>c)</sup>          | 7.4           | 6.5 | 7.5 | 7.4           | 6.8           | <sup>c)</sup> | <sup>c)</sup> | <sup>c)</sup> |
| <b>2</b>     |                        | 6.9           | 6.2 | 5.7 | 6.6           | 6.0           | 6.8           | 6.9           | 7.8           |
| <b>6</b>     |                        | 6.9           | 6.3 | 5.6 | 6.9           | 6.3           | 6.6           | 7.1           | 7.9           |
| <b>9</b>     |                        | 6.6           | 5.9 | 6.0 | 6.0           | 5.9           | 6.6           | 6.9           | 7.4           |
| <b>10</b>    |                        | 7.1           | 4.6 | 5.8 | 7.5           | 6.4           | 6.9           | <sup>c)</sup> | <sup>c)</sup> |
| <b>13</b>    |                        | <sup>c)</sup> | 8.8 | —   | 7.0           | <sup>c)</sup> | 6.3           | 6.8           | 6.8           |
| <b>17</b>    |                        | 6.3           | 7.8 | 8.7 | <sup>c)</sup> | 6.9           | <sup>c)</sup> | 6.9           | 7.8           |

a) Measured in aqueous solution (90% H<sub>2</sub>O/10% D<sub>2</sub>O) at 283 K, pH 4.5. b) The numbering of the positions is according to that of NPY. c) Coupling constant could not be measured due to overlapping.

Table 4. Amide Temperature Coefficients of YM-42454 and Its Related Peptides<sup>a)</sup> (−Δδ/ΔT, ppb/K)

| Peptide      | Position <sup>b)</sup> |               |      |      |      |               |      |     |      |
|--------------|------------------------|---------------|------|------|------|---------------|------|-----|------|
|              | 28                     | 29            | 30   | 31   | 32   | 33            | 34   | 35  | 36   |
| YM-42454     |                        | 7.4           | 8.0  | 9.7  | 3.3  | 7.6           | 9.8  | 8.6 | 10.3 |
| NPY Ac-28-36 | 8.9                    | 7.7           | 8.4  | 7.5  | 8.7  | 9.1           | 8.8  | 9.5 | 10.0 |
| <b>2</b>     |                        | 7.8           | 8.3  | 10.0 | 3.0  | 7.5           | 10.3 | 8.9 | 10.6 |
| <b>6</b>     |                        | 7.8           | 8.3  | 10.3 | 3.1  | 7.6           | 10.3 | 9.3 | 10.3 |
| <b>10</b>    |                        | 7.8           | 9.4  | 8.8  | 3.0  | 7.8           | 7.8  | 6.9 | 10.7 |
| <b>13</b>    |                        | <sup>c)</sup> | 7.2  | —    | 6.0  | <sup>c)</sup> | 0.7  | 8.2 | 8.3  |
| <b>17</b>    |                        | 5.1           | 11.0 | 12.2 | 10.4 | 11.0          | 7.9  | 8.8 | 9.8  |

a) Temperature coefficients were measured in aqueous solution (90% H<sub>2</sub>O/10% D<sub>2</sub>O) from 277 K to 303 K at pH 4.5. b) The numbering of the positions is according to that of NPY. c) Temperature coefficient could not be measured due to overlapping.

for the amide protons of these peptide analogues are shown in Tables 2–4.

In the linear peptide, NPY Ac-28—36, all of the <sup>1</sup>H-NMR parameters were in the motionally averaged range, suggesting that this peptide is very flexible in aqueous solution. In contrast, some spectral features of YM-42454 indicated that the conformation of this peptide, at least its cyclic portion, should be restricted. The amide proton signals for the cyclic portion of YM-42454 were somewhat broad compared to those of exocyclic moiety. The amide proton of Thr<sup>32</sup> showed a relatively small temperature coefficient (3.3 ppb/K). In the case of a small peptide, a small temperature coefficient is generally considered to indicate that this amide proton is somewhat shielded from the solvent.<sup>36)</sup> The relatively small temperature coefficient of Thr<sup>32</sup> amide proton in YM-42454 suggests that this amide proton could be involved in the hy-

drogen bond. This amide proton also had a somewhat higher field chemical shift (7.83 ppm) than that of other residues (8.18–8.76 ppm), which is probably due to the conformational feature of YM-42454. Moreover, the reasonable number and intensity of inter-residue nuclear Overhauser effects (NOEs) were observed for YM-42454 in the two-dimensional (2D) NOE spectroscopy (NOESY) spectrum, while few and weak inter-residue NOEs were observed for NPY Ac-28—36 (data not shown). This restricted conformation of YM-42454 should be suitable for the Y<sub>1</sub>-binding since the affinity of YM-42454 for the Y<sub>1</sub>-receptor was 185-fold higher than that of its corresponding linear peptide, NPY Ac-28—36, which might be flexible in solution. The solution structure of YM-42454 based on the NOEs will be reported in detail elsewhere.

The NMR parameters of peptides **2**, **6**, **9** and **10** are similar

to those of YM-42454, indicating that these substitutions did not cause a notable conformational change. The results suggest that the reduction of  $Y_1$ -binding affinity for these derivatives is due to the loss of the crucial interactions in the  $Y_1$ -receptor binding rather than to the overall conformational change. Therefore, the hydrophobic side-chain of Leu<sup>30</sup>, the guanidinium groups of Arg<sup>33</sup> and Arg<sup>35</sup>, and C-terminal amide of YM-42454 are involved in the direct interaction with the  $Y_1$ -receptor rather than in maintaining the bioactive conformation.

On the contrary, some NMR parameters of peptide **13** and **17** differed from those of YM-42454. The temperature coefficient of the Thr<sup>32</sup> amide proton was larger in peptide **13** (6.0 ppb/K) than in YM-42454 (3.3 ppb/K), and this amide proton showed a lower field chemical shift (8.48 ppm) than the corresponding proton in YM-42454 (7.83 ppm). In addition, the amide proton of Cys<sup>34</sup> in peptide **13** showed a small temperature coefficient (0.7 ppb/K) and showed a higher field chemical shift (7.96 ppm) than the corresponding proton in YM-42454 (8.63 ppm). And the  $^3J_{\text{NH}\alpha}$  coupling constant for the Leu<sup>30</sup> in peptide **13** was larger (8.8 Hz) than that for the corresponding residue in YM-42454 (6.3 Hz). As for the peptide **17**, the D-Thr<sup>32</sup> amide proton showed a large temperature dependence (10.4 ppb/K) and showed a lower field chemical shift (8.34 ppm) than the corresponding proton in YM-42454 (7.83 ppm). And the  $^3J_{\text{NH}\alpha}$  coupling constants for the Leu<sup>30</sup> and Ile<sup>31</sup> in peptide **17** (7.8, 8.7 Hz, respectively), were larger than those of the corresponding amino acids in YM-42454 (6.3, 5.8 Hz, respectively). These results suggest that the solution conformations of peptides **13** and **17** differ from that of YM-42454 and that the reduced binding affinities of these peptides are probably caused by the overall conformational changes. But, since peptide **17** retained a certain degree of  $Y_1$ -binding affinity, its conformational changes might be not so drastic.

## Conclusion

The structure-affinity relationships of YM-42454 were investigated by means of amino acid replacement. The guanidinium groups of Arg<sup>33</sup> and Arg<sup>35</sup>, the hydrophobic side-chains of Leu<sup>30</sup> and Ile<sup>31</sup>, and the C-terminal amide in YM-42454 were considered to be crucial for  $Y_1$ -receptor binding. These critical residues nearly agreed with those of NPY although the substitutions at position 32 in YM-42454 influenced  $Y_1$ -binding affinity less than they did in NPY. These results suggest that the binding mode of YM-42454 to the  $Y_1$ -receptor is similar to that of the C-terminal of NPY. <sup>1</sup>H-NMR studies of YM-42454 and some of its derivatives indicated that the crucial residues play an important role in the direct interaction with the  $Y_1$ -receptor rather than in maintaining the conformation. Further investigations of these cyclic peptides, including structural and pharmacological studies, would provide insight into the bioactive conformation of NPY and would lead to the discovery of more potent ligands that are more selective for the  $Y_1$ -receptor.

## Experimental

**Materials** Porcine NPY was purchased from Peptide Institute, Inc. (Osaka, Japan). PYY, [Leu<sup>31</sup>, Pro<sup>34</sup>]NPY, bacitracin, phenylmethylsulfonyl-fluoride (PMSF) and fraction V bovine serum albumin (BSA) were purchased from Sigma (St. Louis, MO, U.S.A.). Minimum essential medium (MEM) was obtained from GIBCO Laboratories (Grand Island, NY, U.S.A.)

and fetal bovine serum was obtained from Bioserum (Australia). [<sup>125</sup>I]PYY was obtained from DuPont/NEN Research Products (Wilmington, DE, U.S.A.). All other chemicals were of analytical grade.

Production of the NPY peptide analogues was commissioned to Peptide Institute, Inc. (Osaka, Japan). All peptide analogues were synthesized by solid-phase peptide synthesis using the Boc-strategy on an Applied Biosystems 430A peptide synthesizer (Foster City, CA, U.S.A.).<sup>37)</sup> After the protected peptide resin was treated with hydrogen fluoride to remove the protecting groups and resin, the intramolecular disulfide bond was formed by a ferricyanide procedure.<sup>38)</sup> The products were purified by preparative reversed-phase high-performance liquid chromatography (RP-HPLC) and purity was established (>95%) by analytical RP-HPLC in two diverse systems and by amino acid analysis (AAA). All peptides were characterized by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and AAA. Analytical data are shown in Table 1. The structure of YM-42454 was confirmed by 1D and 2D <sup>1</sup>H-NMR. (Data are not shown.)

**Cell Cultures** Human neuroblastoma cell line SK-N-MC was kindly provided by Dr. June L. Biedler (Memorial Sloan-Kettering Cancer Center, New York, NY, U.S.A.). SK-N-MC cells were cultured in MEM supplemented with 10% fetal bovine serum. Penicillin-G (100 IU/ml) and streptomycin (100 µg/ml) were added to the culture media. Cells were grown in a 150-mm tissue culture dish at 37°C in a humidified atmosphere of 95% air-5% CO<sub>2</sub>.

**Binding Experiments** Porcine brains were obtained from local slaughterhouses. The hippocampal tissues<sup>39)</sup> were homogenized in 5 vol. ice-cold 234 mM sucrose with a motor-driven Teflon-glass homogenizer. The homogenate was then centrifuged at 700×g for 10 min to remove cellular and nuclear debris. The tumor cells<sup>40)</sup> were washed twice with 25 mM Tris buffer (pH 7.4) containing 1 mM PMSF and 1 mg/ml bacitracin, sonicated, and centrifuged at 700×g for 10 min. The supernatant was centrifuged at 100000×g for 20 min at 4°C. The pellets were resuspended in 25 mM Tris buffer and centrifuged again. The resulting membrane fractions were resuspended by homogenization in the same buffer and used for the study. Membrane protein content was determined by Lowry's method,<sup>41)</sup> using BSA as a standard.

**Binding Assay for the  $Y_1$ -Receptor:** All assays were performed at 25°C for 180 min in 0.5 ml of 25 mM Tris buffer (pH 7.4) containing SK-N-MC cell membranes, 50 pM [<sup>125</sup>I]PYY, various concentrations of peptide analogue, 10 mM MgCl<sub>2</sub>, 1 mM PMSF, 1 mg/ml bacitracin, and 5 mg/ml BSA. Bound and free peptides were separated by centrifugation at 10000×g for 3 min in a microcentrifuge. Specific binding was calculated as the difference in radioactivity bound in the presence and absence of 1 µM unlabeled NPY. IC<sub>50</sub> values were computed by logit-log analysis (Hill plot) and K<sub>i</sub> (inhibition constant) values were determined using the Cheng-Prusoff relationship.<sup>42)</sup>

**Binding Assay for the  $Y_2$ -Receptor:** All assays were performed at 25°C for 120 min in 0.5 ml of 25 mM Tris buffer (pH 7.4) containing porcine hippocampal membranes, 10 pM [<sup>125</sup>I]PYY, various concentrations of peptide analogue, 10 mM MgCl<sub>2</sub>, 1 mM PMSF, 1 mg/ml bacitracin, and 5 mg/ml BSA. Bound and free peptides were separated by rapid filtration under vacuum through a GF/B filter treated with 0.1% polyethylenimine. The filter was immediately washed three times with 4 ml of 25 mM Tris buffer containing 10 mM MgCl<sub>2</sub>. Nonspecific binding was determined in the presence of 0.2 µM unlabeled NPY. IC<sub>50</sub> and K<sub>i</sub> values were determined in the same way as in  $Y_1$ -receptor binding assay.

**<sup>1</sup>H-NMR Experiments** All NMR samples were dissolved to a final concentration of 4 mM in 90% H<sub>2</sub>O/10% D<sub>2</sub>O and the pH was adjusted to 4.5 with dilute NaOH and dilute HCl. <sup>1</sup>H-NMR spectra were acquired at 600 MHz on a Bruker AMX 600 spectrometer immediately after sample preparation. Chemical shift values were given relative to the water signal whose chemical shift was previously determined on the basis of sodium 3-(trimethylsilyl)propionate-2,2,3,3-*d*<sub>4</sub> (TSP) as an internal standard reference at the corresponding temperature. Resonance assignments for the <sup>1</sup>H-NMR spectrum were carried out using double quantum filtered 2D correlation spectroscopy (DQF-COSY), total correlation spectroscopy (TOCSY) and NOESY experiments. Water suppression was achieved by a weak presaturation of the water signal during the relaxation delay and, in the case of NOESY experiments, during the mixing time. Coupling constants were measured directly from the 1D spectra using a Gaussian window function. Temperature coefficients of the amide protons were calculated from 1D spectra obtained at six temperatures (277, 283, 288, 293, 298, 303 K).

**Acknowledgements** We thank Mr. M. Shimizu and Mr. M. Funatsu for analytical support. We also thank Dr. K. Nakajima and Ms. K. Kumagai of Peptide Institute, Inc. for synthesis of peptide analogues and thank Dr. M.

Wälchli of Bruker Japan Co., Ltd. for advice about the <sup>1</sup>H-NMR measurements.

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## Constituents of a Fern, *Diplazium subsinuatum*. III.<sup>1)</sup> Four New Hopane-Triterpene Lactone Glycosides

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Received June 30, 2000; accepted September 2, 2000

From a whole plant of a fern, *Diplazium subsinuatum*, three new hopane-triterpene lactone glycosides, diplaziosides V—VII (1—3), were isolated, together with a new monoacetyl derivative (4) of diplazioside VII (3). Compounds 1—3 were defined as the respective 3-*O*-[ $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 2)]- $\beta$ -D-glucopyranosides of 3 $\beta$ ,24-dihydroxyhopan-28,22-olide (1), of 3 $\beta$ ,17,24-trihydroxyhopan-28,22-olide (2), and of (22*R*)-3 $\beta$ ,24,30-trihydroxyhopan-28,22-olide (3), and 4 as the 6'-*O*-acetate of 3, respectively, on the basis of spectral evidence. 1—3 are new in their glycoside structures but also in their triterpene structures. Furthermore, in compounds 1—4, the coupling between the 24-hydroxy proton and one of the 24-methylene protons showed a very large *J*-value (11.4 Hz); based on this <sup>1</sup>H-NMR evidence, *etc.*, preferred conformations of the 24-hydroxymethylene groups in 1—4 are also inferred. In a similar manner, <sup>1</sup>H-NMR coupling patterns of the 24-hydroxymethylene in the monoglucoside (2a) and aglycone (2b), derived from 2, are also reported and discussed here.

**Key words** *Diplazium subsinuatum*; fern; hopane-triterpene lactone glycoside; diplazioside V; diplazioside VI; diplazioside VII

The whole plant of the fern *Diplazium subsinuatum* (WALL. ex HOOK. et GREV.) Tagawa (Herashida in Japanese; Woodsiaceae) has been used as a folk medicine (diuretic, hydragogue, *etc.*) in China.<sup>2)</sup> From the fronds of the fern, Tanaka *et al.* characterized a hopane-triterpene lactone, together with three glycosides,<sup>3)</sup> and from the whole plant, we further identified four hopane-triterpene glycosides, diplaziosides I—IV, together with three acetyl derivatives.<sup>1,4)</sup> All triterpenes already identified in free and bonded forms from this fern species belonged to hopane-type triterpenes having no hydroxy group at C-3. Upon continued chemical investigation of the 1-butanol fraction from the hot-water extract of *D. subsinuatum*, we isolated three new glycosides of hopane-triterpene lactone with a 3 $\beta$ -hydroxy group, that is, diplaziosides V—VII (1—3), together with an acetate (4) of 3. This report describes the isolation and the structural elucidation of these four new compounds. In addition, based on the <sup>1</sup>H-NMR data and Dreiding model inspections, preferable conformations of the 24-hydroxymethylene moieties on 1—4 in pyridine-*d*<sub>5</sub> solutions are also dealt with in this paper.

The 1-butanol fraction was separated on silica gel and octadecyl silica gel (ODS) column chromatography, successively, followed by HPLC separation to each isolate of 1—4.

In the structural elucidation of each compound, the <sup>1</sup>H- and <sup>13</sup>C-NMR assignments were performed with the aid of two-dimensional (2D)-NMR [<sup>1</sup>H—<sup>1</sup>H and <sup>13</sup>C—<sup>1</sup>H shift-correlation spectroscopy (COSY), nuclear Overhauser enhancement spectroscopy (NOESY), and heteronuclear multiple bond correlation spectroscopy (HMBC)] experiments.

Diplazioside V (1), colorless needles of mp 297—298 °C, [ $\alpha$ ]<sub>D</sub> -1.7° (*c*=0.69, pyridine) showed strong bands at 3420 and 1732 cm<sup>-1</sup> due to hydroxy and lactone carbonyl residues, respectively, in the IR spectrum. In the FAB-MS (negative mode) spectrum, 1 gave the [M-H]<sup>-</sup> ion at *m/z* 795, and based on the high resolution (HR) spectrum in the same mode, 1 was formulated as C<sub>42</sub>H<sub>68</sub>O<sub>14</sub>. In addition, the FAB-MS (negative mode) afforded significant fragments at *m/z* 633 and 471, arising from the loss of one and two hexosyl units from the [M-H]<sup>-</sup> ion, respectively. In the <sup>1</sup>H-NMR spectrum (Table 1), 1 showed the presence of six *tert*

methyls, a hydroxymethylene, and an oxygenated methine in the aglycone part and two anomeric protons [ $\delta$  4.89 (d, *J*=7.8 Hz, Glc 1'-H), 5.54 (d, *J*=7.8 Hz, Glc 1''-H)] in the sugar part. These data by MS and NMR suggested that 1 is a triterpene diglycoside carrying two hexoses as the sugar part.

The chemical shifts of carbons (Table 2) attributed to the aglycone part of 1 were compared with those of diplazioside I (=24-*O*-glycoside of 24-hydroxyhopan-28,22-olide).<sup>4)</sup> The chemical shifts of the D/E ring carbons (C<sub>13</sub>—C<sub>22</sub> and C<sub>27</sub>—C<sub>30</sub>) of 1 were superimposed on those of diplazioside I, suggesting that a hopane-triterpene with a 28,22-lactone moiety is reasonably assigned as the partial structure for the agly-

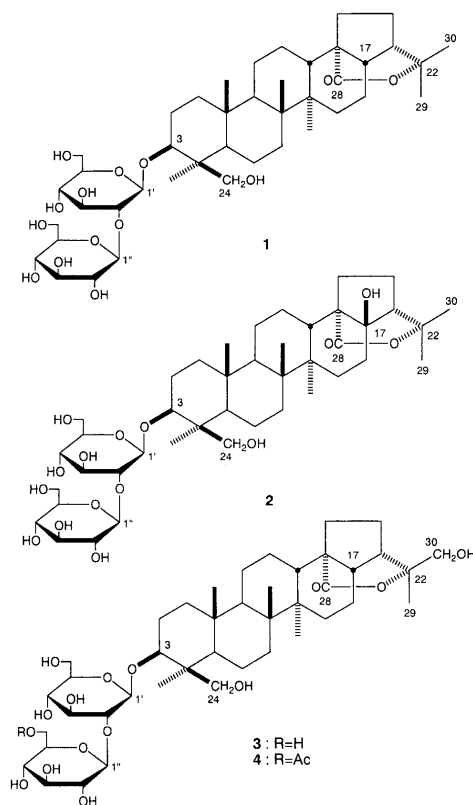


Chart 1

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Table 1.  $^1\text{H}$ -NMR Data of **1**, **2**, **3** and **4** in  $\text{C}_5\text{D}_5\text{N}$  (600 MHz)<sup>a)</sup>

| Proton No.       | 1                                      | 2                                      | 3                                      | 4                                      |
|------------------|----------------------------------------|----------------------------------------|----------------------------------------|----------------------------------------|
| 1 $\alpha$ (ax)  | 0.73 (1H, ddd, 13.2, 13.2, 3.6)        | 0.74 (1H, ddd, 13.2, 13.2, 3.6)        | 0.74 (1H, ddd, 13.2, 13.2, 3.6)        | 0.77 <sup>b)</sup>                     |
| $\beta$ (eq)     | 1.45 <sup>b)</sup>                     | 1.46 <sup>b)</sup>                     | 1.46 <sup>b)</sup>                     | 1.50 <sup>b)</sup>                     |
| 2 $\alpha$ (eq)  | 2.33 (1H, m)                           | 2.32 <sup>b)</sup>                     | 2.33 (1H, m)                           | 2.26 <sup>b)</sup>                     |
| $\beta$ (ax)     | 1.90 (1H, m)                           | 1.89 <sup>b)</sup>                     | 1.90 <sup>b)</sup>                     | 1.95 <sup>b)</sup>                     |
| 3 $\alpha$ (ax)  | 3.46 (1H, dd, 11.7, 4.2)               | 3.45 (1H, dd, 11.7, 4.2)               | 3.45 (1H, dd, 11.7, 4.2)               | 3.45 (1H, dd, 11.7, 4.2)               |
| 5 $\alpha$ (ax)  | 0.77 (1H, d, 12.0)                     | 0.79 (1H, d, 12.0)                     | 0.78 (1H, br d, 12.0)                  | 0.79 <sup>b)</sup>                     |
| 6 $\alpha$ (eq)  | 1.54 <sup>b)</sup>                     | 1.51 <sup>b)</sup>                     | 1.53 (1H, br d, 13.2)                  | 1.61 <sup>b)</sup>                     |
| $\beta$ (ax)     | 1.23 <sup>b)</sup>                     | 1.25 <sup>b)</sup>                     | 1.24 <sup>b)</sup>                     | 1.38 <sup>b)</sup>                     |
| 7 $\alpha$ (ax)  | 1.25 <sup>b)</sup>                     | 1.25 <sup>b)</sup>                     | 1.24 <sup>b)</sup>                     | 1.25 <sup>b)</sup>                     |
| $\beta$ (eq)     | 1.38 <sup>b)</sup>                     | 1.44 <sup>b)</sup>                     | 1.37 <sup>b)</sup>                     | 1.38 <sup>b)</sup>                     |
| 9 $\alpha$ (ax)  | 1.28 <sup>b)</sup>                     | 1.30 <sup>b)</sup>                     | 1.30 <sup>b)</sup>                     | 1.30 <sup>b)</sup>                     |
| 11 $\alpha$ (eq) | 1.45 <sup>b)</sup>                     | 1.51 <sup>b)</sup>                     | 1.46 <sup>b)</sup>                     | 1.48 <sup>b)</sup>                     |
| $\beta$ (ax)     | 1.13 <sup>b)</sup>                     | 1.25 <sup>b)</sup>                     | 1.15 <sup>b)</sup>                     | 1.16 <sup>b)</sup>                     |
| 12 $\alpha$ (ax) | 2.75 (1H, dddd, 13.2, 13.2, 13.2, 4.2) | 2.84 (1H, dddd, 13.2, 13.2, 13.2, 4.2) | 2.77 (1H, dddd, 13.2, 13.2, 13.2, 4.2) | 2.77 (1H, dddd, 13.2, 13.2, 13.2, 4.2) |
| $\beta$ (eq)     | 1.74 (1H, br d, 13.2)                  | 1.77 (1H, br d, 13.2)                  | 1.77 (1H, br d, 13.2)                  | 1.78 (1H, br d, 13.2)                  |
| 13 $\beta$ (ax)  | 1.56 <sup>b)</sup>                     | 2.64 (1H, dd, 13.2, 3.2)               | 1.61 <sup>b)</sup>                     | 1.61 <sup>b)</sup>                     |
| 15 $\alpha$ (eq) | 1.25 <sup>b)</sup>                     | 1.11 <sup>b)</sup>                     | 1.28 <sup>b)</sup>                     | 1.28 <sup>b)</sup>                     |
| $\beta$ (ax)     | 1.32 <sup>b)</sup>                     | 2.42 (1H, ddd, 13.2, 13.2, 4.2)        | 1.37 <sup>b)</sup>                     | 1.36 <sup>b)</sup>                     |
| 16 $\alpha$ (ax) | 1.76 <sup>b)</sup>                     | 2.27 (1H, ddd, 13.2, 13.2, 4.2)        | 1.83 (1H, dddd, 13.2, 13.2, 13.2, 3.6) | 1.85 (1H, dddd, 13.2, 13.2, 13.2, 3.6) |
| $\beta$ (eq)     | 1.67 (1H, br d, 13.2)                  | 2.04 (1H, br d, 13.2)                  | 1.74 (1H, br d, 13.2)                  | 1.73 (1H, br d, 13.2)                  |
| 17 $\beta$ (ax)  | 1.28 <sup>b)</sup>                     |                                        | 1.36 <sup>b)</sup>                     | 1.33 <sup>b)</sup>                     |
| (ax)-OH          |                                        | 5.89 (1H, s)                           |                                        |                                        |
| 19               | 1.57 <sup>b)</sup>                     | 1.92 <sup>b)</sup>                     | 1.63 <sup>b)</sup>                     | 1.62 <sup>b)</sup>                     |
|                  | 1.86 (1H, m)                           | 2.11 (1H, ddd, 12.8, 12.8, 3.6)        | 2.00 (1H, m)                           | 2.00 (1H, m)                           |
| 20               | 1.57 <sup>b)</sup>                     | 1.91 <sup>b)</sup>                     | 1.63 <sup>b)</sup>                     | 1.62 <sup>b)</sup>                     |
|                  | 1.79 <sup>b)</sup>                     | 2.39 <sup>b)</sup>                     | 2.24 (1H, m)                           | 2.25 (1H, m)                           |
| 21 $\beta$       | 1.93 (1H, br dd, 4.8, 4.8)             | 2.23 (1H, d, 6.6)                      | 2.30 (1H, br dd, 4.8, 4.8)             | 2.31 (1H, br dd, 4.8, 4.8)             |
| 23               | 1.36 (3H, s)                           | 1.36 (3H, s)                           | 1.36 (3H, s)                           | 1.39 (3H, s)                           |
| 24a              | 4.32 (1H, d, 10.8)                     | 4.31 (1H, d, 10.8)                     | 4.32 (1H, d, 10.8)                     | 4.34 (1H, d, 10.8)                     |
| b                | 3.38 (1H, dd, 10.8, 11.4)              | 3.36 (1H, dd, 10.8, 11.4)              | 3.38 (1H, dd, 10.8, 11.4)              | 3.50 (1H, dd, 10.8, 11.4)              |
| 24-OH            | 4.36 (1H, d, 11.4)                     | 4.34 (1H, d, 11.4)                     | 4.35 (1H, d, 11.4)                     | 3.69 (1H, d, 11.4)                     |
| 25               | 0.66 (3H, s)                           | 0.65 (3H, s)                           | 0.67 (3H, s)                           | 0.75 (3H, s)                           |
| 26               | 0.92 (3H, s)                           | 1.12 (3H, s)                           | 0.94 (3H, s)                           | 0.94 (3H, s)                           |
| 27               | 1.10 (3H, s)                           | 1.18 (3H, s)                           | 1.13 (3H, s)                           | 1.14 (3H, s)                           |
| 29               | 1.39 (3H, s)                           | 1.51 (3H, s)                           | 1.62 (3H, s)                           | 1.62 (3H, s)                           |
| 30               | 1.19 (3H, s)                           | 1.27 (3H, s)                           | 3.76 (2H, s)                           | 3.76 (2H, s)                           |
| 3-O-Glc 1'       | 4.89 (1H, d, 7.8)                      | 4.88 (1H, d, 7.8)                      | 4.88 (1H, d, 7.8)                      | 4.89 (1H, d, 7.8)                      |
| 2'               | 4.09 (1H, dd, 7.8, 9.0)                | 4.08 (1H, dd, 7.8, 9.0)                | 4.08 (1H, dd, 7.8, 9.0)                | 4.20 (1H, dd, 7.8, 9.0)                |
| 3'               | 4.29 (1H, dd, 9.0, 9.0)                | 4.28 (1H, dd, 9.0, 9.0)                | 4.28 (1H, dd, 9.0, 9.0)                | 4.28 (1H, dd, 9.0, 9.0)                |
| 4'               | 4.17 (1H, dd, 9.0, 9.0)                | 4.17 (1H, dd, 9.0, 9.0)                | 4.16 (1H, dd, 9.0, 9.0)                | 4.14 (1H, dd, 9.0, 9.0)                |
| 5'               | 3.94 (1H, ddd, 9.0, 5.4, 2.4)          | 3.94 (1H, ddd, 9.0, 5.4, 2.4)          | 3.94 (1H, ddd, 9.0, 5.4, 2.4)          | 3.92 (1H, ddd, 9.0, 5.4, 2.4)          |
| 6'               | 4.36 <sup>b)</sup>                     | 4.36 <sup>b)</sup>                     | 4.36 <sup>b)</sup>                     | 4.37 (1H, dd, 11.7, 5.4)               |
|                  | 4.59 (1H, dd, 11.7, 2.4)               | 4.58 (1H, dd, 11.7, 2.4)               | 4.59 (1H, dd, 11.7, 2.4)               | 4.58 (1H, dd, 11.7, 2.4)               |
| 2'-O-Glc 1''     | 5.54 (1H, d, 7.8)                      | 5.53 (1H, d, 7.8)                      | 5.53 (1H, d, 7.8)                      | 5.55 (1H, d, 7.8)                      |
| 2''              | 4.10 (1H, dd, 7.8, 9.0)                | 4.09 (1H, dd, 7.8, 9.0)                | 4.10 (1H, dd, 7.8, 9.0)                | 4.15 (1H, dd, 7.8, 9.0)                |
| 3''              | 4.20 (1H, dd, 9.0, 9.0)                | 4.19 (1H, dd, 9.0, 9.0)                | 4.19 (1H, dd, 9.0, 9.0)                | 4.19 (1H, dd, 9.0, 9.0)                |
| 4''              | 4.38 (1H, dd, 9.0, 9.0)                | 4.37 (1H, dd, 9.0, 9.0)                | 4.38 (1H, dd, 9.0, 9.0)                | 4.12 (1H, dd, 9.0, 9.0)                |
| 5''              | 3.74 (1H, ddd, 9.0, 2.4, 2.4)          | 3.73 (1H, ddd, 9.0, 2.4, 2.4)          | 3.73 (1H, ddd, 9.0, 2.4, 2.4)          | 4.01 (1H, ddd, 9.0, 6.6, 1.8)          |
| 6''              | 4.34 <sup>b)</sup>                     | 4.34 <sup>b)</sup>                     | 4.34 <sup>b)</sup>                     | 4.87 (1H, dd, 11.4, 6.6)               |
|                  | 4.39 <sup>b)</sup>                     | 4.39 <sup>b)</sup>                     | 4.38 <sup>b)</sup>                     | 5.08 (1H, dd, 11.4, 1.8)               |
| 6'-O-Ac          |                                        |                                        |                                        | 2.07 (3H, s)                           |

a) Chemical shifts are in  $\delta$ -values from internal TMS and are followed by multiplicities and  $J$ -values (in Hz). b) Overlapping with other signal(s), hence, its multiplicity and  $J$ -value are both obscure.

cone of **1**. This comparison also suggested the presence of additional hydroxyl (or oxygenated) groups at  $\text{C}_3$  and  $\text{C}_{24}$  on the aglycone of **1**. Furthermore, the location and configuration of these hydroxy (or oxygenated) groups were substantiated based on the following  $^1\text{H}$ -NMR evidence. A doublet signal at  $\delta$  3.46 ( $J=11.7, 4.2$  Hz) is assigned to an axial  $3\alpha$ -proton, indicative of the presence of a  $3\beta$ -OH or  $3\beta$ -O-sugar moiety in **1**. The position of a hydroxymethylene [ $\delta$  4.32 (H-24a; d,  $J=10.8$  Hz), 3.38 (H-24b; dd,  $J=10.8, 11.4$  Hz), 4.36 (OH-24; d,  $J=11.4$  Hz)]<sup>5)</sup> was decided to be at

$\text{C}_{24}$  based on the presence of cross peaks between both protons of 24-H<sub>2</sub> and both methyls of 23-H<sub>3</sub> and 25-H<sub>3</sub> in the NOESY spectrum. In this hydroxymethylene [ $\text{C}_{(24)}\text{H}_2\text{OH}$ ], the hydroxy proton coupled with one of the methylene protons, demonstrating that the 24-hydroxy group exists in a free alcohol form in **1**. These spectral findings, in conjunction with the presence of correlation in the NOESY (between  $3\alpha$ -H and Glc 1'-H) and HMBC (between Glc 1'-H and 3-C) spectra, revealed the disaccharide moiety to be connected at the  $3\beta$ -OH via a glycosidic linkage. Assignments of a  $\beta$ -D-

Table 2.  $^{13}\text{C}$ -NMR Data for **1**—**4**, **2a** and **2b** in  $\text{C}_5\text{D}_5\text{N}^{(a)}$ 

| Carbon No.   | <b>1</b>            | <b>2</b>            | <b>2a</b>           | <b>2b</b>           | <b>3</b>            | <b>4</b>            |
|--------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| 1            | 38.8                | 38.8                | 38.8                | 39.0                | 38.8                | 39.1                |
| 2            | 26.8                | 26.8                | 27.0                | 28.6                | 26.8                | 26.8                |
| 3            | 90.6                | 90.6                | 88.9                | 80.2                | 90.6                | 90.0                |
| 4            | 43.8                | 43.8                | 44.5                | 43.3                | 43.8                | 44.4                |
| 5            | 56.1                | 56.2                | 56.1                | 56.4                | 56.1                | 56.3                |
| 6            | 18.6                | 18.6                | 18.9                | 19.2                | 18.6                | 18.8                |
| 7            | 34.3                | 34.4                | 34.6                | 34.7                | 34.3                | 34.5                |
| 8            | 42.9 <sup>(b)</sup> | 42.0 <sup>(b)</sup> | 42.2 <sup>(b)</sup> | 42.2 <sup>(b)</sup> | 43.0 <sup>(b)</sup> | 43.0 <sup>(b)</sup> |
| 9            | 51.0                | 50.9                | 51.0                | 51.3                | 51.0                | 51.1                |
| 10           | 36.7                | 36.7                | 36.9                | 37.4                | 36.7                | 36.9                |
| 11           | 22.4                | 22.6                | 22.7                | 22.7                | 22.4                | 22.4                |
| 12           | 26.0                | 25.3                | 25.4                | 25.4                | 26.0                | 26.1                |
| 13           | 49.9                | 41.1                | 41.1                | 41.1                | 50.1                | 50.1                |
| 14           | 41.5 <sup>(b)</sup> | 42.3 <sup>(b)</sup> | 42.3 <sup>(b)</sup> | 42.3 <sup>(b)</sup> | 41.6 <sup>(b)</sup> | 41.6 <sup>(b)</sup> |
| 15           | 32.8                | 27.6                | 27.7                | 27.7                | 32.8                | 32.8                |
| 16           | 25.0                | 31.5                | 31.5                | 31.5                | 24.9                | 24.9                |
| 17           | 50.1                | 77.9                | 77.9                | 77.9                | 50.1                | 50.1                |
| 18           | 50.7                | 55.7                | 55.8                | 55.8                | 51.5                | 51.5                |
| 19           | 35.5                | 33.8                | 33.9                | 33.8                | 36.2                | 36.2                |
| 20           | 25.2                | 23.5                | 23.5                | 23.5                | 25.1                | 25.1                |
| 21           | 45.8                | 53.3                | 53.4                | 53.4                | 43.8                | 43.8                |
| 22           | 81.7                | 80.2                | 80.2                | 80.2                | 83.4                | 83.4                |
| 23           | 22.6                | 22.6                | 23.3                | 23.6                | 22.6                | 22.8                |
| 24           | 63.4                | 63.4                | 63.3                | 64.6                | 63.4                | 63.0                |
| 25           | 16.5                | 16.3                | 16.2                | 16.9                | 16.4                | 16.4                |
| 26           | 16.3                | 16.6                | 16.7                | 16.7                | 16.3                | 16.3                |
| 27           | 16.1                | 15.9                | 15.9                | 15.9                | 16.1                | 16.1                |
| 28           | 175.7               | 176.7               | 176.7               | 176.7               | 176.3               | 176.3               |
| 29           | 29.4                | 29.3                | 29.3                | 29.3                | 25.0                | 25.0                |
| 30           | 29.8                | 30.5                | 30.5                | 30.5                | 69.0                | 69.0                |
| 3-O-Glc 1'   | 104.6               | 104.5               | 106.2               |                     | 104.5               | 104.5               |
| 2'           | 82.6                | 82.6                | 75.7                |                     | 82.6                | 81.6                |
| 3'           | 78.9                | 78.9                | 78.8                |                     | 78.9                | 78.8                |
| 4'           | 71.2                | 71.2                | 72.0                |                     | 71.2                | 71.6                |
| 5'           | 78.6                | 78.5                | 78.7                |                     | 78.6                | 78.4                |
| 6'           | 62.8                | 62.8                | 63.1                |                     | 62.8                | 62.8                |
| 2'-O-Glc-1'' | 105.3               | 105.2               |                     |                     | 105.2               | 104.9               |
| 2''          | 75.8                | 75.8                |                     |                     | 75.8                | 75.8                |
| 3''          | 78.3                | 78.3                |                     |                     | 78.3                | 78.3                |
| 4''          | 70.0                | 69.9                |                     |                     | 70.0                | 71.4                |
| 5''          | 78.5                | 78.4                |                     |                     | 78.5                | 75.4                |
| 6''          | 61.6                | 61.6                |                     |                     | 61.6                | 64.9                |
| Ac at C-6''  |                     |                     |                     |                     |                     | 171.2               |
|              |                     |                     |                     |                     |                     | 21.0                |

a) Assignments were determined based on  $^1\text{H}$ - $^{13}\text{C}$  COSY and HMBC experiments.  
 b) Signal assignments may be interchanged in each column.

glucopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -D-glucopyranosyl residue for the disaccharide part was performed based on detailed analyses of the  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data (Tables 1 and 2, respectively) and of NOESY (between Glc 1''-H and Glc 2'-H) and HMBC (between Glc 1''-H and Glc 2'-C) correlations.<sup>(6)</sup> In conclusion, diplazioside V is defined as 3 $\beta$ -O- $[\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 2)]- $\beta$ -D-glucopyranosyl-24-hydroxy-hopane-28,22-olide, as shown in formula **1**.

Diplazioside VI (**2**), colorless needles of mp 293–295 °C,  $[\alpha]_{\text{D}}^{20} +2.9^\circ$  ( $c=1.0$ , pyridine) gave a carbonyl band due to  $\delta$ -lactone at  $1732\text{ cm}^{-1}$ , along with a hydroxy absorption at  $3420\text{ cm}^{-1}$  in the IR spectrum. Negative ion (=neg.) FAB-MS afforded an  $[\text{M}-\text{H}]^-$  peak at  $m/z$  811 and the HR-MS of neg. FAB revealed the molecular formula to be  $\text{C}_{42}\text{H}_{68}\text{O}_{15}$ , which corresponds to a substance larger by one oxygen-atom unit than  $\text{C}_{42}\text{H}_{68}\text{O}_{14}$  for **1**. In the  $^1\text{H}$ -NMR spectrum, **2** gave signals due to six *tert* methyls, a hydroxymethylene [ $\delta$  4.31 (H-24a; d,  $J=10.8\text{ Hz}$ ), 3.36 (H-24b; dd,  $J=10.8, 11.4\text{ Hz}$ ),

4.34 (OH-24; d,  $J=11.4\text{ Hz}$ )],<sup>(5)</sup> an oxygenated methine [ $\delta$  3.45 (dd,  $J=11.7, 4.2\text{ Hz}$ , H-3 $\alpha$ )] on the aglycone part, and two anomeric protons [ $\delta$  4.88 (d,  $J=7.8\text{ Hz}$ , Glc 1'-H), 5.53 (d,  $J=7.8\text{ Hz}$ , Glc 1''-H)] on the saccharide part, similar to those in **1**.

A detailed comparison of the  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR assignments (Tables 1, 2), along with the HMBC correlations observed between each of 29- $\text{H}_3$  and 30- $\text{H}_3$ /each of 21-C and 22-C and between 21 $\beta$ -H ( $\delta$  2.23, d,  $J=6.6\text{ Hz}$ )/each of 17-C and 18-C, substantiated that **2** has the same structure as **1**, except for the presence of 17 $\beta$ -OH ( $\delta_{\text{C}}$  77.9) in **2** instead of 17 $\beta$ -H ( $\delta_{\text{C}}$  50.1) in **1**. Based on accumulated proof, diplazioside VI is defined as 3 $\beta$ -O- $[\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 2)]- $\beta$ -D-glucopyranosyl-17,24-dihydroxyhopane-28,22-olide (**2**).

Diplazioside VII (**3**), colorless needles of mp 286–288 °C,  $[\alpha]_{\text{D}} -8.0^\circ$  ( $c=0.5$ , pyridine) also gave lactone carbonyl and hydroxyl bands at  $1734$  and  $3396\text{ cm}^{-1}$ , respectively, in the IR spectrum. The neg. FAB-MS study, followed by the HR mode, revealed that **3** possesses a molecular formula ( $\text{C}_{42}\text{H}_{68}\text{O}_{15}$ ), the same as that of **2** and larger by one oxygen-atom unit than that of **1**. The  $^1\text{H}$ -NMR spectrum (Table 1) showed the presence of five *tert* methyls, two hydroxymethylenes [ $\delta$  4.32 (H-24a; d,  $J=10.8\text{ Hz}$ ), 3.38 (H-24b; dd,  $J=10.8, 11.4\text{ Hz}$ ), 4.35 (OH-24; d,  $J=11.4\text{ Hz}$ )],<sup>(5)</sup>  $\delta$  3.76 (2H, s, H<sub>2</sub>-30)] in the aglycone part and two anomeric protons [ $\delta$  4.88 (d,  $J=7.8\text{ Hz}$ , Glc 1'-H), 5.53 (d,  $J=7.8\text{ Hz}$ , Glc 1''-H)] in the sugar part.

A detailed comparison of **3** with **1** in their  $^{13}\text{C}$ -NMR assignments (Table 2) indicated that **3** is assigned to the 30-hydroxy derivative of **1**, that is, the methyl group of C-30 in **1** is replaced with the hydroxymethylene group in **3**. The validity of the established structure (**3**) was further corroborated by the presence of the HMBC (between 30- $\text{H}_2$ /each of 21-C, 22-C, and 29-C and between 29- $\text{H}_3$ /each of 17-C and 18-C) and NOESY (between 30- $\text{H}_2$ /29- $\text{H}_3$ ; between 29- $\text{H}_3$ /each of 21-H and 16 $\alpha$ -H; between 21-H/17-H) correlations; thus, diplazioside VII is defined as (22*R*)-3 $\beta$ -O- $[\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 2)]- $\beta$ -D-glucopyranosyl-24,30-dihydroxyhopane-28,22-olide (**3**).<sup>(7)</sup>

The fourth new compound (**4**), colorless needles of mp 286–287 °C,  $[\alpha]_{\text{D}} -7.5^\circ$  ( $c=1.0$ , pyridine) gave an acetyl signal [ $\delta$  2.07 (3H, s)] in addition to signals ascribed to five *tert* methyls and two hydroxymethylenes [ $\delta$  4.34 (H-24a; d,  $J=10.8\text{ Hz}$ ), 3.50 (H-24b; dd,  $J=10.8, 11.4\text{ Hz}$ ), 3.69 (OH-24; d,  $J=11.4\text{ Hz}$ )],<sup>(5)</sup>  $\delta$  3.76 (2H, s, H<sub>2</sub>-30)] on the aglycone part, as well as two anomeric protons [ $\delta$  4.89 (d,  $J=7.8\text{ Hz}$ , Glc 1'-H), 5.55 (d,  $J=7.8\text{ Hz}$ , Glc 1''-H)] on the disaccharide part in the  $^1\text{H}$ -NMR spectrum. The presence of an acetyl residue in **4** was also confirmed by  $^{13}\text{C}$ -NMR ( $\delta_{\text{C}}$  171.2, 21.0) and IR ( $1734\text{ cm}^{-1}$ ; an acetyl and lactone carbonyl absorption) spectra.

A detailed comparison of the  $^{13}\text{C}$ -NMR data of **4** with those of **3** (Table 2), in conjunction with the neg. FAB-MS evidence  $[(\text{M}-\text{H})^-; \text{C}_{44}\text{H}_{69}\text{O}_{16}]$ , suggested that **4** corresponds to the 6''-O-acetyl derivative of **3**. Furthermore, the acetylated position in **4** was also confirmed to be at 6-OH on the terminal glucosyl moiety by the HMBC correlation between 6''- $\text{H}_2$  [ $\delta$  5.08 (1H, dd,  $J=11.4, 1.8\text{ Hz}$ ), 4.87 (1H, dd,  $J=11.4, 6.6\text{ Hz}$ )] and the acetyl carbonyl carbon ( $\delta_{\text{C}}$  171.2).

Glycosides of **1**–**3** were all new compounds; moreover, they also carried new hopane-triterpenes as their aglycones,

respectively.

In the course of the  $^1\text{H}$ -NMR studies toward the structural elucidation of **1**–**4**, we encountered an interesting and novel example on the coupling constant, closely related to the conformational analysis of the terpenic 24-hydroxymethylene in **1**–**4**, as follows. In each of compounds of **1**–**4**, the 24-hydroxy proton coupled with one (H-24b) of the 24-methylene protons with a large  $J$ -value (11.4 Hz in every of **1**–**4**) in pyridine- $d_5$  solutions, suggesting that a dihedral angle between these two protons in the coupling must be estimated to be near  $180^\circ$ , hence, these two protons are in anti-parallel relation with each other. This finding also shows that both of the  $\text{C}_{(4)}\text{--C}_{(24)}$  and  $\text{C}_{(24)}\text{--O}$  bonds in the 24-hydroxymethylene of **1**–**4** are not in free rotation but in a rigid conformation, fixed by any steric interaction. As a candidate steric factor in this case, an intramolecular hydrogen-bond is assumed to be most conceivable, although we have no chemical proof in favor of this presumption at present. Among **1**–**4**, especially in **4**, the presence of a hydrogen-bond between the terpenic 24-OH and the 6''-*O*-acetyl carbonyl group on the terminal glucose is reasonably presumed based on a Dreiding model inspection. Furthermore, the 24-OH group in **1**–**3** appears to also be fixed in its conformation by a hydrogen-bond interaction analogous to that in **4**. In the case of the diglucosides **1**–**3**, we could not exactly determine the partner of the 24-OH in a hydrogen-bond interaction, but the first candidate was deduced to be the primary alcohol (6''-OH) on the terminal glucose based on a Dreiding model inspection.

As described above, in the diglucosides **1**–**3** and the 6''-*O*-acetyl diglucoside **4**, the 24-hydroxymethylene moiety gave a characteristic coupling pattern involving a coupling with a large  $J$ -value (11.4 Hz) between the hydroxy proton and one of the methylene protons in the  $^1\text{H}$ -NMR spectra. As a reason for this unique coupling pattern, it was presumed that the conformation of the 24-primary alcohol group must be fixed through the intramolecular hydrogen-bond with the 6''-OH in **1**–**3** or with 6''-*O*-acetyl carbonyl in **4**.

To our knowledge, such a large coupling constant of a hydroxy proton with the proton on the adjacent carbon atom, as observed in the 24-primary alcohol ( $J=11.4$  Hz) of **1**–**4**, has not previously been known in naturally occurring compounds. Therefore, this report must provide the first or rare instance of such.<sup>8)</sup>

In our subsequent research, we examined a coupling pattern of the 24-hydroxymethylene in both the 3-*O*-monoglucoside (**2a**) and the aglycone (**2b**), previously derived from one (**2**) of the diglucosides by acid hydrolysis, based on the  $^1\text{H}$ -NMR spectra in pyridine- $d_5$  solutions. In **2a**, the 24-hydroxymethylene moiety gave a series of signals,  $\delta$  4.38 (H-24a; d,  $J=10.8$  Hz), 3.63 (H-24b; dd,  $J=10.8, 10.2$  Hz), and 4.71 (OH-24; d,  $J=10.2$  Hz), and according to these data involving the large coupling constant for the 24-OH, the hydroxymethylene group appears to be in a sterically fixed and preferable conformation in this molecule. In addition, the Dreiding model inspection, in consideration of the existence of cross peaks between the glucosyl anomeric proton [ $\delta$  5.06 (d,  $J=7.8$  Hz)] and each of the terpenic  $3\alpha\text{-H}$  [ $\delta$  3.61 (dd,  $J=11.7, 4.2$  Hz)],  $23\text{-H}_3$  [ $\delta$  1.54 (s)] in the NOESY spectrum, led us to suppose that the 24-hydroxy group formed a hydrogen-bond with the 2'-hydroxy group of the glucosyl moiety in **2a**. On the other hand, in the  $^1\text{H}$ -NMR spectrum of **2b**, the

24-hydroxymethylene group showed a novel coupling pattern [ $\delta$  4.50 (H-24a; dd,  $J=10.8, 2.2$  Hz), 3.69 (H-24b; dd,  $J=10.8, 7.8$  Hz), 5.28 (OH-24; dd,  $J=7.8, 2.2$  Hz)]. That is to say, in this case of the aglycone **2b**, the coupling pattern (dd,  $J=7.8, 2.2$  Hz) of the 24-hydroxy proton seems to be slightly different from those in the cases of the diglucoside **2** (d,  $J=11.4$  Hz) and monoglucoside **2a** (d,  $J=10.2$  Hz) mentioned before. However, based on a Dreiding model inspection and the existence of a strong NOESY peak between 24b-H and  $25\text{-H}_3$  [ $\delta$  0.87 (s)], it was also deduced that the two hydroxy groups at C-3 and C-24 in **2b** are favorably located so that they must form an intramolecular hydrogen-bond. As mentioned above, the 24-hydroxymethylene group in the monoglucoside **2a** and the aglycone **2b**, as well as in the diglucosides **1**–**3** and the 6''-*O*-acetyl diglucoside **4**, also showed characteristic coupling, with a large  $J$ -value between the 24-OH and 24b-H (10.2 Hz in **2a** and 7.8 Hz in **2b**); hence, in **2a** and **2b** it was also deduced that the 24-hydroxymethylene groups must be in preferred conformations through the intramolecular hydrogen-bond interactions in pyridine- $d_5$  solutions.

## Experimental

**General Remarks** All melting points were recorded on a Yanagimoto melting point apparatus without correction. IR spectra were measured with a JASCO A-302 spectrometer in KBr discs, and  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra with a GE-Omega 600 ( $^1\text{H}$  at 600 and  $^{13}\text{C}$  at 150 MHz, respectively) spectrometer, with pyridine- $d_5$  as a solvent and tetramethylsilane (TMS) as an standard. FAB- and HR-FAB-MS spectra in a negative mode (matrix, triethanolamine), along with electron impact (EI)- and HR-EI-MS spectra, were obtained with JEOL JMS-DX 300, JMS-700T, and JMS-HX 110/110 spectrometers. Optical rotations were determined for solutions in pyridine on a JASCO DIP-140 polarimeter. GLC was carried out on a Shimadzu GC-7AG instrument. For column chromatography, Kieselgel 60 (Merck) and Chromatorex ODS DM1020T (Fuji Silysia) were used. Preparative HPLC was performed on a JAI LC-908 instrument with a JAIGEL-ODS, S-343-15 column (20 i.d.  $\times$  250 mm).

**Extraction and Isolation** Wild *Diplazium subsinuatum* was collected in the Awa District, Chiba Prefecture, Japan in 1994. The whole plant (30 g), air-dried and cut, was extracted twice with boiling water (700 ml) for 1 h. After filtration, the resulting aqueous solution was extracted three times with 1-butanol (1.2 l) and the solvent was taken off *in vacuo* to give a butanol extract. Repetition of a similar extraction procedure afforded a total of 82.2 g of the butanol extract from 2.73 kg of the plant material. The whole of the obtained butanol extract was chromatographed on silica gel, and eluted successively with the lower phase of  $\text{CHCl}_3\text{--MeOH--H}_2\text{O}$  (9:3:1, 7:3:1) and a mixture solvent of  $\text{CHCl}_3\text{--MeOH--H}_2\text{O}$  (6:4:1) to give 13 fractions (Nos. 1 to 13). Fraction No. 5 (765 mg) was subjected to reversed-phase (ODS) column chromatography (eluting with 70% aqueous MeOH), followed by preparative HPLC (70% aqueous MeOH) to afford **1** (16.9 mg) and **4** (31.8 mg) in that order. Fraction No. 7 (1.04 g) was further column-chromatographed over silica gel [the lower phase of  $\text{CHCl}_3\text{--MeOH--H}_2\text{O}$  (7:3:1)] and ODS (70% aqueous MeOH), successively, followed by preparative HPLC separation (70% aqueous MeOH) to give **2** (34.6 mg) and **3** (12.2 mg).

Diplazioside V (**1**): Colorless needles from MeOH, mp 297–298  $^\circ\text{C}$ ,  $[\alpha]_D^{25} -1.7^\circ$  ( $c=0.69$ , pyridine). FAB- and HR-FAB-MS (negative mode)  $m/z$ : 795.4525 (Calcd for  $\text{C}_{42}\text{H}_{67}\text{O}_{14}$ ,  $[\text{M}-\text{H}]^-$ : 795.4531), 633  $[\text{M}-\text{H}-\text{Glc}]^-$ , 471  $[\text{M}-\text{H}-2\text{Glc}]^-$ . IR  $\text{cm}^{-1}$ : 3420 (OH), 1732 ( $\delta$ -lactone), 1078, 1040.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR: Given in Tables 1 and 2, respectively.

Diplazioside VI (**2**): Colorless needles from MeOH, mp 293–295  $^\circ\text{C}$ ,  $[\alpha]_D^{25} +2.9^\circ$  ( $c=1.0$ , pyridine). FAB- and HR-FAB-MS (negative mode)  $m/z$ : 811.4469 (Calcd for  $\text{C}_{42}\text{H}_{67}\text{O}_{15}$ ,  $[\text{M}-\text{H}]^-$ : 811.4480), 649  $[\text{M}-\text{H}-\text{Glc}]^-$ , 487  $[\text{M}-\text{H}-2\text{Glc}]^-$ . IR  $\text{cm}^{-1}$ : 3420 (OH), 1732 ( $\delta$ -lactone), 1078, 1040.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR: Given in Tables 1 and 2, respectively.

Diplazioside VII (**3**): Colorless needles from MeOH, mp 286–288  $^\circ\text{C}$ ,  $[\alpha]_D^{25} -8.0^\circ$  ( $c=0.5$ , pyridine). FAB- and HR-FAB-MS (negative mode)  $m/z$ : 811.4487 (Calcd for  $\text{C}_{42}\text{H}_{67}\text{O}_{15}$ ,  $[\text{M}-\text{H}]^-$ : 811.4480), 649  $[\text{M}-\text{H}-\text{Glc}]^-$ . IR  $\text{cm}^{-1}$ : 3396 (OH), 1734 ( $\delta$ -lactone), 1078, 1040.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR:

Given in Tables 1 and 2, respectively.

6''-O-Acetyl Diplazioside VII (**4**): Colorless needles from MeOH, mp 286—287 °C,  $[\alpha]_D^{25} -7.5^\circ$  ( $c=1.0$ , pyridine). FAB- and HR-FAB-MS (negative mode)  $m/z$ : 853.4572 (Calcd for  $C_{44}H_{69}O_{16}$ ,  $[M-H]^-$ : 853.4585), 649  $[M-H-Glc-Ac]^-$ . IR  $cm^{-1}$ : 3420 (OH), 1734 ( $\delta$ -lactone, acetyl), 1079, 1040.  $^1H$ - and  $^{13}C$ -NMR: Given in Tables 1 and 2, respectively.

**Acid Hydrolysis of the Diglucoside (**2**) Giving the Corresponding Monoglucoside (**2a**) and Aglycone (**2b**)** A solution of **2** (10 mg) in 1 N HCl–MeOH (1 : 1, 4 ml) was heated under reflux for 7 h. In this reaction, in order to efficiently obtain both hydrolysates **2a** and **2b**, the best reaction time was investigated under TLC monitoring and determined to be 7 h. The reaction mixture was diluted with water and extracted with a mixture of AcOEt and *n*-BuOH (1 : 1). After the usual work-up of the extract, the product obtained was subjected to HPLC separation with 80% aqueous MeOH as an eluting agent to give **2** (0.7 mg), **2a** (1.2 mg), and **2b** (3.0 mg) in that order.

**2a**: Colorless needles of mp 294—296 °C (from MeOH),  $[\alpha]_D^{25} +8.1^\circ$  ( $c=0.088$ , pyridine). FAB- and HR-FAB-MS (negative mode)  $m/z$ : 649.3956 (Calcd for  $C_{36}H_{57}O_{10}$ ,  $[M-H]^-$ : 649.3952).  $^1H$ -NMR (in  $C_5D_5N$ )  $\delta$ : 0.76 (3H, s,  $H_3$ -25), 1.15 (3H, s,  $H_3$ -26), 1.23 (3H, s,  $H_3$ -27), 1.27 (3H, s,  $H_3$ -30), 1.51 (3H, s,  $H_3$ -29), 1.54 (3H, s,  $H_3$ -23), 3.61 (1H, dd,  $J=11.7$ , 4.2 Hz, H-3 $\alpha$ ), 3.63 (1H, dd,  $J=10.8$ , 10.2 Hz, H-24b), 4.38 (1H, d,  $J=10.8$  Hz, H-24a), 4.71 (1H, d,  $J=10.2$  Hz, OH-24), 4.01 (1H, dd,  $J=7.8$ , 9.0 Hz, H-Glc 2'), 4.42 (1H, dd,  $J=11.4$ , 5.4 Hz, H-Glc 6'), 4.63 (1H, dd,  $J=11.4$ , 2.4 Hz, H-Glc 6'), 5.06 (1H, d,  $J=7.8$  Hz, H-Glc 1').  $^{13}C$ -NMR: Given in Table 2.

**2b**: Colorless needles of mp >300 °C (from MeOH),  $[\alpha]_D^{25} +52.63^\circ$  ( $c=0.25$ , pyridine). EI-MS  $m/z$  (%): 488 ( $M^+$ , 44.8), 205 (100) and HR-EI-MS  $m/z$ : 488.3509 (Calcd for  $C_{30}H_{48}O_5$ ,  $M^+$ : 488.3502).  $^1H$ -NMR (in  $C_5D_5N$ )  $\delta$ : 0.87 (3H, s,  $H_3$ -25), 1.18 (3H, s,  $H_3$ -26), 1.20 (3H, s,  $H_3$ -27), 1.27 (3H, s,  $H_3$ -30), 1.51 (3H, s,  $H_3$ -29), 1.54 (3H, s,  $H_3$ -23), 3.65 (1H, ddd,  $J=11.7$ , 4.8, 4.2 Hz, H-3 $\alpha$ ), 3.69 (1H, dd,  $J=10.8$ , 7.8 Hz, H-24b), 4.50 (1H, dd,  $J=10.8$ , 2.2 Hz, H-24a), 5.28 (1H, dd,  $J=7.8$ , 2.2 Hz, OH-24), 6.63 (1H, d,  $J=4.8$  Hz, OH-3 $\beta$ ).  $^{13}C$ -NMR: Given in Table 2.

**Determination of Configurations of Glucosyl Moieties in **1**—**3****<sup>6)</sup> A solution of each of **1**—**3** (each 1.0 mg) in 2 M HCl–EtOH (1 : 1; 0.5 ml) was refluxed for 3 h. The mixture solution was extracted with EtOAc and the residual aqueous layer was neutralized with  $Ag_2CO_3$ . After centrifugation of the inorganic precipitate, the supernatant was concentrated *in vacuo* to afford a glucosyl residue. The residue was subjected to the preparation of the corresponding thiazolidine derivative, followed by trimethylsilylation and GLC analysis, according to the reported procedure.<sup>6)</sup> The D-configuration for glucose obtained from each of **1**—**3** was decided, based on direct comparison

with the D- and L-standards of glucose ( $t_R$ : D-Glc, 14 min 25 s; L-Glc, 15 min 10 s). In the case of **4** containing a 6-O-acetyl glucosyl moiety, saponification of **4** in 2 M aqueous NaOH–MeOH (1 : 1; 0.5 ml) under reflux for 2 h gave the corresponding diglucoside (identified with **3** by TLC), which was treated in a manner similar to the case of **1**—**3** to confirm D-glucose as the component sugar. GC conditions: capillary column, TC-1 (0.32 mm i.d.  $\times$  30 m, GL Sciences Inc.); detector, hydrogen flame ionization detector; column temperature, 230 °C; injection temperature, 250 °C; carrier  $N_2$  gas.

## References and Notes

- 1) This paper constitutes Part III in the series of "Constituents of a Fern, *Diplazium subsinuatum*." For Part II, see the following literature: Nakanishi T., Inatomi Y., Nishi M., Murata H., Inada A., *Chem. Pharm. Bull.*, **45**, 8—12 (1997).
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- 3) Tanaka N., Yamauchi K., Murakami T., Saiki Y., Chen C. M., *Chem. Pharm. Bull.*, **30**, 3632—3639 (1982).
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- 5) On the addition of a few drops of  $D_2O$  to the measured solution, the signal and the coupling due to the 24-hydroxy proton disappeared.
- 6) The absolute configuration of each component glucose in the diglucosides **1**—**4** was determined to be D (see Experimental), according to the reported procedure: Hara S., Okabe H., Mihashi K., *Chem. Pharm. Bull.*, **34**, 1843—1845 (1986).
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# Optically Active Antifungal Azoles. X.<sup>1)</sup> Synthesis and Antifungal Activity of *N*-[4-(Azolyl)phenyl]- and *N*-[4-(Azolylmethyl)phenyl]-*N'*-[(1*R*,2*R*)-2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-azolones

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New optically active antifungal azoles, *N*-[4-(azolyl)phenyl]- and *N*-[4-(azolylmethyl)phenyl]-*N'*-[(1*R*,2*R*)-2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]azolones (**1**, **2**, **3**), were prepared in a stereocontrolled manner. Compounds **1**–**3** showed strong antifungal activity against *Candida albicans* *in vitro*. Among them, the imidazolidinones **3** showed a broad antifungal spectrum *in vitro* as well as potent *in vivo* activity against candidiasis and aspergillosis in mice. The imidazolidinones (**3i**, **j**, **k**) having 1*H*-1,2,3-triazol-1-yl, 2*H*-2-tetrazolyl and 1*H*-1-tetrazolyl moieties were found to exert strong protective effect against aspergillosis.

**Key words** optically active antifungal azole; 1,2,3-trisubstituted-2-butanol; triazolone; imidazolone; imidazolidinone; stereocontrolled synthesis; antifungal activity

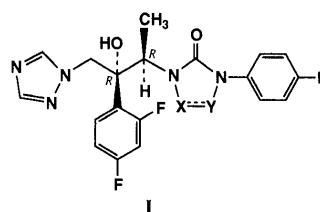
Over the past two decades, the incidence of systemic fungal infections has been increasing due to an increase in the number of immunocompromised hosts. Patients undergoing organ transplants, anticancer chemotherapy or long-term treatment with antimicrobial agents and patients with AIDS are immunosuppressed and susceptible to life threatening systemic fungal infections such as candidiasis, cryptococcosis and aspergillosis. Orally active antifungal azoles, fluconazole and itraconazole, which are strong inhibitors of lanosterol 14 $\alpha$ -demethylase (cytochrome P450<sub>14DM</sub>), have been widely used in antifungal chemotherapy. However, the development of resistance to currently available antifungal azoles in *Candida* spp. as well as clinical failures in the treatment of fungal infections have been reported in recent years.<sup>2)</sup> Furthermore, invasive aspergillosis still remains resistant to antifungal chemotherapy, although injectable amphotericin B has been used for this purpose. Therefore, there is still the medical need for new and more effective antifungal agents with a broad antifungal spectrum.

In the course of our search for therapeutically useful antifungal azoles, we designed optically active azolone-based triazole derivatives depicted by the general formula I (Chart 1).<sup>3f,h)</sup> We previously described the stereocontrolled synthesis of the three types of azolones; triazolones (**1a**),<sup>3f)</sup> imidazolones (**1b**)<sup>3h)</sup> and imidazolidinones (**1c**)<sup>3h)</sup> bearing a phenyl group substituted with the metabolically stable fluorine-containing groups. These azolones revealed strong growth inhibitory activity against *Candida albicans* (*C. albicans*) *in vitro* as well as potent protective effects against candidiasis in mice. From these azolones, 2-[(1*R*,2*R*)-2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-4-[4-(2,2,3,3-tetrafluoropropoxy)phenyl]-3-(2*H*,4*H*)-1,2,4-triazolone (TAK-187)<sup>3g)</sup> was selected as a candidate for clinical trials. Furthermore, we recently reported that the imidazolidinones (**1c**) bearing tetrafluoroethoxy- and tetrafluoropropoxy-phenyl groups at the nitrogen atom exert strong *in vitro* antifungal activity against not only yeasts such as *C. albicans*

and *Cryptococcus neoformans* (*C. neoformans*), but also against molds such as *Aspergillus fumigatus* (*A. fumigatus*).<sup>3h)</sup>

As an extension of our study on the azolone-based antifungal triazoles, we planned to modify the physicochemical properties of this series of derivatives in order to improve the *in vitro* and the *in vivo* antifungal activities. For this purpose, we chose five-membered aromatic heterocycles (Az: azoles), i.e., thiazole, oxazole, 1,2,3-triazole, pyrazole, imidazole, 1,2,4-triazole and tetrazole, as the substituent R in place of the fluoroalkoxy group in **1a**–**c** and designed *N*-[4-(azolyl)phenyl]- and *N*-[4-(azolylmethyl)phenyl]-*N'*-[(1*R*,2*R*)-2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]azolones (**1**–**3**) depicted in Chart 2. Compounds **1**–**3** were expected to have potent antifungal activity because of their structural similarity to **1**, although they possess a variety of Az structures.

In this paper, we describe the synthesis of the triazolones (**1a**–**j**), the imidazolones (**2a**–**k**) and the imidazolidinones (**3e**–**k**) bearing a phenyl group substituted with 2-methyl-4-thiazolyl (**a**), 2-methyl-4-oxazolyl (**b**), 2*H*-1,2,3-triazol-2-ylmethyl (**c**), 1*H*-1,2,3-triazol-1-ylmethyl (**d**), 1*H*-1-pyrazolyl (**e**), 1*H*-1-imidazolyl (**f**), 1*H*-1,2,4-triazol-1-yl (**g**), 2*H*-1,2,3-triazol-2-yl (**h**), 1*H*-1,2,3-triazol-1-yl (**i**), 2*H*-2-tetrazolyl (**j**)



**a:** X, Y = N, CH  
(TAK-187: X = N, Y = CH, R = OCH<sub>2</sub>CF<sub>2</sub>CF<sub>2</sub>H)  
**b:** X = Y = CH  
**c:** X = Y = CH<sub>2</sub>  
R = OCH<sub>2</sub>CF<sub>2</sub>CF<sub>2</sub>H, OCF<sub>2</sub>CF<sub>2</sub>H

Chart 1

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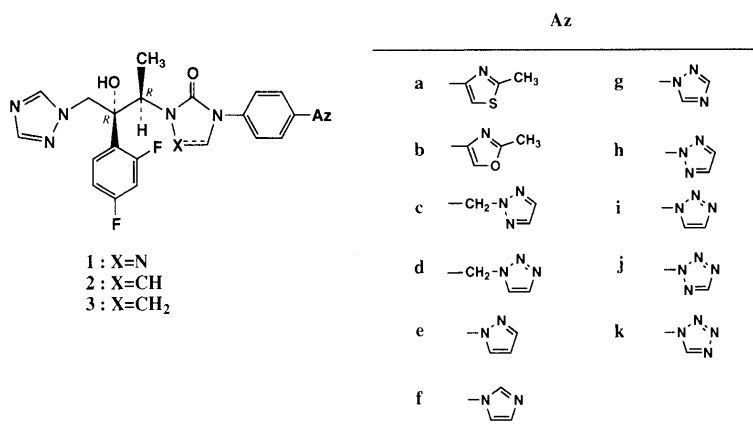


Chart 2

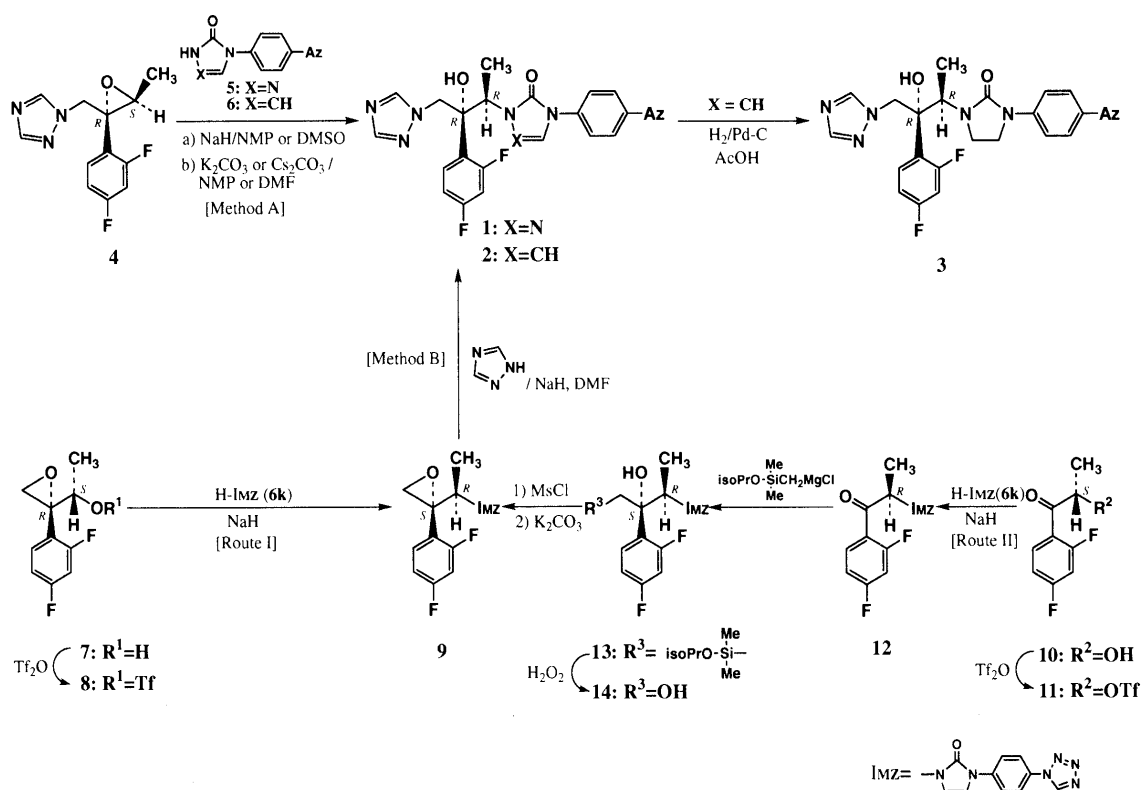


Chart 3

and 1*H*-1-tetrazolyl (**k**) groups, as well as their antifungal activities *in vitro* and *in vivo*.

**Chemistry** We previously established the route for the stereocontrolled synthesis of (2*R*,3*S*)-2-(2,4-difluorophenyl)-3-methyl-2-(1*H*-1,2,4-triazol-1-yl)methyloxirane (**4**),<sup>3a)</sup> (1*S*)-1-[(2*R*)-2-(2,4-difluorophenyl)-2-oxiranyl]ethanol (**7**)<sup>3f,g,h)</sup> and (2*S*)-2',4'-difluoro-2-hydroxypropiphenone (**10**)<sup>1)</sup> starting from the esters of (*R*)- or (*S*)-lactic acid. Compounds **4**, **7** and **10** were the key synthetic intermediates for the preparation of a variety of optically active antifungal azoles with the 1,2,3-trisubstituted 2-butanol skeleton.<sup>3)</sup> We exploited these synthetic intermediates for the preparation of the newly designed triazolone (**1**), imidazolone (**2**) and imidazolidinone (**3**) derivatives shown in Chart 2. The synthetic route for the new azolones is illustrated in Chart 3.

The oxirane **4** was allowed to react with *N*-(4-substituted

phenyl)-triazolones (**5a—j**; Table 2) and -imidazolones (**6a—j**; Table 3) in the presence of base [sodium hydride (NaH), potassium carbonate (K<sub>2</sub>CO<sub>3</sub>) or cesium carbonate (Cs<sub>2</sub>CO<sub>3</sub>)] in aprotic polar solvent [dimethylsulfoxide (DMSO), *N*-methyl-2-pyrrolidone (NMP) or *N,N*-dimethylformamide (DMF)]. The corresponding triazolones (**1a—j**; Table 1) and imidazolones (**2a—j**; Table 1) were obtained in 9—41% isolated yields [Method A]. In the case of the synthesis of the 1-[4-(1*H*-1-tetrazolyl)phenyl]-2(1*H*,3*H*)-imidazolone derivative (**2k**; Table 1), the oxiranylethanol (**7**) was used as the starting material [Route I]. Compound **7** was converted to the triflate **8** by treatment with trifluoromethanesulfonic anhydride (Tf<sub>2</sub>O) in the presence of diisopropylethylamine (isoPr<sub>2</sub>NET) and then allowed to react with the sodium salt of 1-[4-(1*H*-1-tetrazolyl)phenyl]-2(1*H*,3*H*)-imidazolone (H-IMZ, **6k**) to obtain the *S*<sub>N</sub>2 reaction product **9**.<sup>4)</sup> Consider-

able decomposition of the triflate was observed during the course of the reaction and the isolated yield of **9** was low (7.7% based on **6k**). The oxirane **9** was reacted with 1*H*-1,2,4-triazole in the presence of NaH in DMF to give the imidazolone derivative **2k** in 63% yield [Method B]. The preparation of the precursor **9** for the synthesis of **2k** was carried out by an alternative route starting from the hydroxypropio-phenone **10** [Route II]. Compound **10** was converted to the triflate **11**, and the resulting triflate **11** was then allowed to react with the sodium salt of **6k** at  $-20$ — $-30$  °C in a mixture of tetrahydrofuran (THF) and NMP (2:3, v/v) to give the propiophenone **12**. Compound **12** was reacted with (dimethylisopropoxysilyl)methylmagnesium chloride [ $\text{iso-PrOSi}(\text{Me}_2)\text{CH}_2\text{MgCl}$ ]<sup>5</sup> in THF to obtain the silyl alcohol **13** in 20% isolated yield. Oxidative desilylation of **13** with hydrogen peroxide ( $\text{H}_2\text{O}_2$ )<sup>5</sup> in the presence of sodium bicarbonate ( $\text{NaHCO}_3$ ) afforded the (1*R*,2*S*)-diol **14** in 79% yield, which was then converted to the corresponding mesylate by treatment with methanesulfonyl chloride (MsCl). Subsequent oxirane ring formation with  $\text{K}_2\text{CO}_3$  afforded **9** in 62% isolated yield. Compound **9** prepared by this route was identical to that obtained by Route I.

The imidazolidinones **3e—k** (Table 1) were prepared from the corresponding imidazolones (**2e—k**) by catalytic hydrogenation on palladium on carbon (Pd-C) in acetic acid (AcOH). The structural confirmation of these triazolone, imidazolone and imidazolidinone derivatives (**1—3**) was done by assignment of the analytical results shown in Table 1.

The triazolones **5** and the imidazolones **6**, which were used in the above synthesis, were prepared by the methods shown in Chart 4.

4-Substituted nitrobenzenes (**17c—k**)<sup>7</sup> were synthesized from 4-chloromethylnitrobenzene (**15**) or 4-fluoronitrobenzene (**16**) by displacement reaction with the commercially available azoles (H-Az). In the case of the reaction of **15** and **16** with 1*H*-1,2,3-triazole or 1*H*-tetrazole, mixtures of the substitution position isomers (**17c, d**; **17h, i** and **17j, k**) at the azole nitrogen atom were obtained. Compounds **17c, d** and **17h, i** were separated into each isomer by crystallization and/or silica gel column chromatography. The substitution position on the 1,2,3-triazole moiety in these two regioisomers (**17c, d** and **17h, i**) was determined by <sup>1</sup>H-NMR measurement; the two nonequivalent protons of the 1*H*-1,2,3-triazole moiety in **17d** and **17i** appeared as two singlets and as two doublets, respectively, while the two equivalent protons in **17c** and **17h** appeared as one singlet. The nitrobenzenes (**17c—i**) were reduced to the corresponding anilines (**18c—i**) by catalytic hydrogenation or with hydrazine hydrate ( $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$ )–ferric chloride ( $\text{FeCl}_3$ ). The mixture of 4-tetrazolylnitrobenzenes (**17j, k**) was submitted to the next reduction step without separation and the resulting isomeric 4-tetrazolyl anilines **18j** and **18k**<sup>8</sup> were separated. The anilines containing thiazole and oxazole moieties, **18a** and **18b**, were prepared by catalytic hydrogenation with Pd-C from the corresponding 4-nitrobenzenes (**17a, b**)<sup>10</sup>). All anilines (**18a—k**) were allowed to react with phenyl chloroformate (PhO-COCl) to afford the phenyl carbamates **19a—k**. The phenyl carbamates **19a—j** were converted to the corresponding 2-(4-substituted phenyl)-3(2*H*,4*H*)-1,2,4-triazolones **5a—j** (Table 2) by treatment with  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$  affording the semicarbazides **20a—j** followed by cyclization with formamidine ac-

etate ( $\text{HN}=\text{CH}-\text{NH}_2 \cdot \text{AcOH}$ ). The 1-(4-substituted phenyl)-2(1*H*,3*H*)-imidazolones (**6a—k**; Table 3) were prepared from the corresponding phenylcarbamates **19a—k** via two reaction steps: conversion of **19a—k** to the ureas **21a—k** by treatment with 2,2-diethoxyethylamine [ $(\text{EtO})_2\text{CHCH}_2\text{NH}_2$ ] and the subsequent cyclization with hydrochloric acid (HCl).

**Antifungal Activities** *In vitro* antifungal activities of the triazolone, imidazolone and imidazolidinone derivatives (**1—3**) against *C. albicans* TA are shown in Table 1. The *in vitro* assay using *C. albicans* TA was carried out by an agar-dilution method on RPMI 1640 medium under 20%  $\text{CO}_2$ .<sup>11</sup> The *in vitro* activity is expressed as the minimum inhibitory concentration (MIC,  $\mu\text{g/ml}$ ). Compounds **1—3**, except **1d, 2d** which showed moderate activity (MIC, 0.13—0.5  $\mu\text{g/ml}$ ) comparable to fluconazole, had strong growth-inhibitory activity against *C. albicans* TA in agar-dilution assay (MIC,  $<0.001$ —0.03  $\mu\text{g/ml}$ ). In particular, the imidazolidinone derivatives **3** mostly showed lower MIC values for *C. albicans* TA compared with those of the corresponding triazolone and imidazolone derivatives (**1** and **2**). The order of inhibitory potency was the imidazolidinones (**3**)  $\geq$  the imidazolones (**2**)  $\geq$  the triazolones (**1**).

Selected compounds **3e, g, i—k**, which had potent activity against *C. albicans* TA, were evaluated for their *in vitro* antifungal spectrum against *C. albicans* (TIMM1756, TIMM1850), *C. neoformans* (TIMM1740, TIMM1855) and *A. fumigatus* (437, TIMM1728, IFO6344), and for *in vivo* activity against *C. albicans* and *A. fumigatus* in mice. The results are shown in Table 4. The MIC values for yeast type fungi such as *C. albicans* and *C. neoformans* were determined by an agar-dilution method on RPMI 1640 medium under 20%  $\text{CO}_2$ , and MIC values for *A. fumigatus* were measured using the same medium but under air. *C. albicans* TA infected mice and *A. fumigatus* 437 infected neutropenic mice were used for the *in vivo* assay. In the *in vivo* assay against *C. albicans* TA, the test compounds were administered orally once immediately after infection. On the other hand, in the case of the *in vivo* assay against *A. fumigatus* 437, the test compounds were administered orally, once on the day of infection and twice daily on the following 2 d. The *in vivo* activity is expressed in terms of  $\text{ED}_{50}$  (mg/kg, the dose of the test compound which allows 50% survival of the infected mice).

In the *in vitro* assay with two strains of *C. albicans*, TIMM1756 and TIMM1850, the selected imidazolidinone derivatives (**3e, g, i—k**) showed low MIC values of  $\leq 0.001$ —0.016  $\mu\text{g/ml}$ , which were lower than those of fluconazole and itraconazole. Moreover, these compounds had strong inhibitory activity against *C. neoformans* (TIMM1740, TIMM1855: MIC, 0.016—0.13  $\mu\text{g/ml}$ ) as well as against *A. fumigatus* (437, TIMM1728, IFO6344: MIC, 0.25—1  $\mu\text{g/ml}$ ). These potencies are superior to those of fluconazole and are comparable to itraconazole. In the *in vivo* assay, compounds **3g, i—k** were found to have strong protective effects against candidiasis. The activity ( $\text{ED}_{50}$ , 0.18—0.89 mg/kg) against *C. albicans* TA infection in mice was comparable with that of fluconazole ( $\text{ED}_{50}$ , 0.22—0.35 mg/kg), although **3e** showed higher  $\text{ED}_{50}$  value (2.0 mg/kg).<sup>13</sup> Furthermore, compounds **3e, g, i—k** showed potent therapeutic effects against aspergillosis ( $\text{ED}_{50}$ , 4.4—17.7 mg/kg) in neutropenic mice. In particular, compounds **3i, j, k** showed lower  $\text{ED}_{50}$

Table 1. Physicochemical Properties and *In Vitro* Antifungal Activity of *N*-[4-(Azolyl)phenyl]- and *N*-[4-(Azolylmethyl)phenyl]-*N'*-[(1*R*,2*R*)-2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]azolones (**1**—**3**)

| Compd.    | Yield <sup>(a)</sup><br>(%)<br>[Solv.<br>/Base] <sup>(b)</sup> | mp (°C)<br>(Solv.) <sup>(c)</sup> | Analysis (%)                                                                                                                                    |                                                                                                                                                                                                                                                                                                                                           |                                                | <sup>1</sup> H-NMR (in CDCl <sub>3</sub> ) δ | IR (KBr)<br>cm <sup>−1</sup> | [α] <sub>D</sub><br>{°C}<br>MeOH | MIC (μg/ml) <sup>(d)</sup><br><i>C. albicans</i> TA <sup>(e)</sup> |
|-----------|----------------------------------------------------------------|-----------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------|----------------------------------------------|------------------------------|----------------------------------|--------------------------------------------------------------------|
|           |                                                                |                                   | Calcd                                                                                                                                           | (Found)                                                                                                                                                                                                                                                                                                                                   |                                                |                                              |                              |                                  |                                                                    |
|           |                                                                |                                   | C                                                                                                                                               | H                                                                                                                                                                                                                                                                                                                                         | N                                              |                                              |                              |                                  |                                                                    |
| <b>1a</b> | 25<br>[DMF<br>/K <sub>2</sub> CO <sub>3</sub> ]                | 195—197<br>(EA-IPE)               | C <sub>24</sub> H <sub>21</sub> F <sub>2</sub> N <sub>7</sub> O <sub>2</sub> S<br>56.57 4.15 19.24<br>(56.42 4.19 19.11)                        | 1.31 (3H, d, <i>J</i> =7 Hz), 2.79 (3H, s), 4.37 (1H, d, <i>J</i> =14 Hz), 5.04 (1H, d, <i>J</i> =14 Hz), 5.11 (1H, q, <i>J</i> =7 Hz), 5.47 (1H, s), 6.77—6.90 (2H, m), 7.39 (1H, s), 7.50—7.70 (4H, m), 7.85—8.05 (4H, m)                                                                                                               | 3468, 1698, 1559, 1503, 1395, 1273             | −21.0°<br>{20}<br>(1.0)                      | 0.002                        |                                  |                                                                    |
| <b>1b</b> | 33<br>[DMF-<br>MP<br>/K <sub>2</sub> CO <sub>3</sub> ]         | 166—168<br>(EA-IPE)               | C <sub>24</sub> H <sub>21</sub> F <sub>2</sub> N <sub>7</sub> O <sub>3</sub><br>58.42 4.29 19.87<br>(58.11 4.43 19.67)                          | 1.31 (3H, d, <i>J</i> =7 Hz), 2.54 (3H, s), 4.36 (1H, d, <i>J</i> =14 Hz), 5.04 (1H, d, <i>J</i> =14 Hz), 5.10 (1H, q, <i>J</i> =7 Hz), 5.47 (1H, s), 6.77—6.88 (2H, m), 7.51—7.69 (4H, m), 7.85 (1H, s), 7.86 (2H, d, <i>J</i> =8 Hz), 7.88 (1H, s), 7.96 (1H, s)                                                                        | 1314, 1705, 1559, 1510, 1431, 1395             | −22.0°<br>{20}<br>(1.1)                      | 0.004                        |                                  |                                                                    |
| <b>1c</b> | 18<br>[NMP<br>/NaH]                                            | 142—143<br>(EA-IPE)               | C <sub>23</sub> H <sub>21</sub> F <sub>2</sub> N <sub>6</sub> O <sub>2</sub><br>55.98 4.29 25.55<br>(55.87 4.18 25.42)                          | 1.29 (3H, d, <i>J</i> =7 Hz), 4.34 (1H, d, <i>J</i> =14.4 Hz), 5.02 (1H, d, <i>J</i> =14.4 Hz), 5.08 (1H, q, <i>J</i> =7 Hz), 5.44 (1H, s), 5.66 (2H, s), 6.73—6.87 (2H, m), 7.46 (2H, d, <i>J</i> =8.6 Hz), 7.50—7.62 (1H, m), 7.58 (2H, d, <i>J</i> =8.6 Hz), 7.66 (2H, s), 7.68 (1H, s), 7.78 (1H, s), 7.94 (1H, s)                    | 3532, 1678, 1618, 1563, 1518, 1503             | −25.1°<br>{20}<br>(1.2)                      | 0.03                         |                                  |                                                                    |
| <b>1d</b> | 24<br>[DMF<br>/K <sub>2</sub> CO <sub>3</sub> ]                | 168—169<br>(AC-EA)                | C <sub>23</sub> H <sub>21</sub> F <sub>2</sub> N <sub>6</sub> O <sub>2</sub><br>55.98 4.29 25.55<br>(55.82 4.44 25.49)                          | 1.30 (3H, d, <i>J</i> =7 Hz), 4.36 (1H, d, <i>J</i> =14 Hz), 5.00 (1H, d, <i>J</i> =14 Hz), 5.08 (1H, q, <i>J</i> =7 Hz), 5.41 (1H, s), 5.63 (2H, s), 6.75—6.90 (2H, m), 7.40—7.64 (6H, m), 7.69 (1H, s), 7.76 (1H, s), 7.80 (1H, s), 7.94 (1H, s)                                                                                        | 3517, 1682, 1563, 1520, 1503, 1400             | −23.4°<br>{20}<br>(1.1)                      | 0.5                          |                                  |                                                                    |
| <b>1e</b> | 19<br>[NMP<br>/NaH]                                            | 210—212<br>(EA-IPE)               | C <sub>23</sub> H <sub>20</sub> F <sub>2</sub> N <sub>8</sub> O <sub>2</sub><br>57.94 4.21 23.42<br>(57.47 4.17 23.45)                          | 1.32 (3H, d, <i>J</i> =7 Hz), 4.38 (1H, d, <i>J</i> =14.4 Hz), 5.05 (1H, d, <i>J</i> =14.4 Hz), 5.11 (1H, q, <i>J</i> =7 Hz), 5.45 (1H, s), 6.52—6.54 (1H, m), 6.76—6.90 (2H, m), 7.50—7.65 (1H, m), 7.65—7.93 (7H, m), 7.96 (1H, s), 7.98 (1H, d, <i>J</i> =2.6 Hz)                                                                      | 3340, 1695, 1560, 1522, 1500, 1384             | −24.5°<br>{20}<br>(0.4)                      | 0.002                        |                                  |                                                                    |
| <b>1f</b> | 21<br>[NMP<br>/NaH]                                            | Amorphous<br>powder               | C <sub>23</sub> H <sub>20</sub> F <sub>2</sub> N <sub>8</sub> O <sub>2</sub><br>· 1/2H <sub>2</sub> O<br>56.94 4.34 22.99<br>(56.73 4.34 22.71) | 1.32 (3H, d, <i>J</i> =7 Hz), 4.40 (1H, d, <i>J</i> =14 Hz), 5.03 (1H, d, <i>J</i> =14 Hz), 5.11 (1H, q, <i>J</i> =7 Hz), 5.42 (1H, s), 6.73—6.88 (2H, m), 7.26 (1H, s), 7.33 (1H, s), 7.51—7.65 (1H, m), 7.57 (1H, d, <i>J</i> =9 Hz), 7.71 (1H, s), 7.76 (2H, d, <i>J</i> =9 Hz), 7.86 (1H, s), 7.91 (1H, s), 7.95 (1H, s)              | 3400, 1700, 1612, 1554, 1521, 1498             | −21.1°<br>{20}<br>(1.0)                      | 0.016                        |                                  |                                                                    |
| <b>1g</b> | 28<br>[DMSO<br>/NaH]                                           | 182—184<br>(DCM<br>-EE)           | C <sub>22</sub> H <sub>19</sub> F <sub>2</sub> N <sub>9</sub> O <sub>2</sub><br>55.11 3.99 26.29<br>(55.05 4.01 26.14)                          | 1.32 (3H, d, <i>J</i> =7 Hz), 4.40 (1H, d, <i>J</i> =14.4 Hz), 5.03 (1H, d, <i>J</i> =14.4 Hz), 5.11 (1H, q, <i>J</i> =7 Hz), 5.41 (1H, s), 6.75—6.90 (2H, m), 7.50—7.65 (1H, m), 7.69 (1H, s), 7.79 (2H, d, <i>J</i> =9 Hz), 7.88 (2H, d, <i>J</i> =9 Hz), 7.92 (1H, s), 7.96 (1H, s), 8.14 (1H, s), 8.65 (1H, s)                        | 1714, 1618, 1556, 1527, 1394                   | −23.4°<br>{20}<br>(1.0)                      | 0.03                         |                                  |                                                                    |
| <b>1h</b> | 34<br>[DMSO<br>/NaH]                                           | 213—215<br>(EA-IPE)               | C <sub>22</sub> H <sub>19</sub> F <sub>2</sub> N <sub>9</sub> O <sub>2</sub><br>55.11 3.99 26.29<br>(54.97 3.96 26.29)                          | 1.32 (3H, d, <i>J</i> =7 Hz), 4.38 (1H, d, <i>J</i> =14.2 Hz), 5.04 (1H, d, <i>J</i> =14.2 Hz), 5.11 (1H, q, <i>J</i> =7 Hz), 5.42 (1H, s), 6.75—6.90 (2H, m), 7.50—7.64 (1H, m), 7.69 (1H, s), 7.74 (2H, d, <i>J</i> =9 Hz), 7.85 (2H, s), 7.86 (1H, s), 7.95 (1H, s), 8.25 (2H, d, <i>J</i> =9 Hz)                                      | 1697, 1623, 1602, 1564, 1519, 1510             | −23.1°<br>{20}<br>(0.4)                      | <0.016                       |                                  |                                                                    |
| <b>1i</b> | 14<br>[DMSO<br>/NaH]                                           | 219—220<br>(DCM-IPE)              | C <sub>22</sub> H <sub>19</sub> F <sub>2</sub> N <sub>9</sub> O <sub>2</sub><br>55.11 3.99 26.29<br>(54.91 3.97 26.26)                          | 1.32 (3H, d, <i>J</i> =7 Hz), 4.40 (1H, d, <i>J</i> =14.2 Hz), 5.03 (1H, d, <i>J</i> =14.2 Hz), 5.10 (1H, q, <i>J</i> =7 Hz), 5.38 (1H, s), 6.75—6.90 (2H, m), 7.50—7.65 (1H, m), 7.70 (1H, s), 7.82 (2H, d, <i>J</i> =9 Hz), 7.88 (1H, s), 7.90 (1H, s), 7.94 (2H, d, <i>J</i> =9 Hz), 7.94 (1H, s), 8.05 (1H, s)                        | 1700, 1975, 1618, 1556, 1527, 1502             | −23.8°<br>{20}<br>(1.0)                      | 0.03                         |                                  |                                                                    |
| <b>1j</b> | 9<br>[NMP<br>/NaH]                                             | 165—166<br>(ME-W)                 | C <sub>21</sub> H <sub>18</sub> F <sub>2</sub> N <sub>10</sub> O <sub>2</sub><br>52.50 3.78 29.15<br>(52.36 3.85 29.02)                         | 1.33 (3H, d, <i>J</i> =7 Hz), 4.40 (1H, d, <i>J</i> =14 Hz), 5.04 (1H, d, <i>J</i> =14 Hz), 5.11 (1H, q, <i>J</i> =7 Hz), 5.37 (1H, s), 6.77—6.88 (2H, m), 7.52—7.64 (1H, m), 7.71 (1H, s), 7.87 (2H, d, <i>J</i> =9 Hz), 7.92 (1H, s), 7.95 (1H, s), 8.34 (2H, d, <i>J</i> =9 Hz), 8.71 (1H, s)                                          | 3420, 1700, 1618, 1562, 1517, 1502             | −22.2°<br>{20}<br>(1.0)                      | 0.002                        |                                  |                                                                    |
| <b>2a</b> | 13<br>[NMP<br>/NaH]                                            | 147—149<br>(EA-IPE)               | C <sub>25</sub> H <sub>22</sub> F <sub>2</sub> N <sub>6</sub> O <sub>2</sub> S<br>59.05 4.36 16.53<br>(59.01 4.46 16.48)                        | 1.21 (3H, d, <i>J</i> =7 Hz), 2.78 (3H, s), 4.22 (1H, d, <i>J</i> =14 Hz), 4.98 (1H, q, <i>J</i> =7 Hz), 5.12 (1H, d, <i>J</i> =14 Hz), 5.60 (1H, br), 6.70—6.85 (4H, m), 7.33 (1H, s), 7.40—7.55 (1H, m), 7.69—8.00 (6H, m)                                                                                                              | 3411, 1655, 1615, 1635, 1501, 1427             | −13.0°<br>{20}<br>(1.2)                      | 0.004                        |                                  |                                                                    |
| <b>2b</b> | 41<br>[NMP<br>/NaH]                                            | 110—111<br>(EA-IPE)               | C <sub>25</sub> H <sub>22</sub> F <sub>2</sub> N <sub>6</sub> O <sub>3</sub><br>60.97 4.50 17.06<br>(60.69 4.56 17.05)                          | 1.21 (3H, d, <i>J</i> =7 Hz), 2.53 (3H, s), 4.21 (1H, d, <i>J</i> =14 Hz), 4.97 (1H, q, <i>J</i> =7 Hz), 5.12 (1H, d, <i>J</i> =14 Hz), 5.55 (1H, br), 6.69—6.86 (4H, m), 7.42—7.55 (1H, m), 7.69 (2H, d, <i>J</i> =9 Hz), 7.73 (1H, s), 7.80 (2H, d, <i>J</i> =9 Hz), 7.84 (1H, s), 7.86 (1H, s)                                         | 3137, 1672, 1617, 1588, 1503, 1429, 1395       | −15.3°<br>{20}<br>(1.2)                      | ≤0.001                       |                                  |                                                                    |
| <b>2c</b> | 30<br>[NMP<br>/NaH]                                            | Amorphous<br>powder               | C <sub>24</sub> H <sub>22</sub> F <sub>2</sub> N <sub>8</sub> O <sub>2</sub><br>· 1/2H <sub>2</sub> O<br>57.48 4.62 22.34<br>(57.48 4.54 22.31) | 1.19 (3H, d, <i>J</i> =7 Hz), 4.17 (1H, d, <i>J</i> =14.4 Hz), 4.95 (1H, q, <i>J</i> =7 Hz), 5.09 (1H, d, <i>J</i> =14.4 Hz), 5.55 (1H, br), 5.63 (2H, s), 6.63 (1H, d, <i>J</i> =3.2 Hz), 6.70—6.86 (3H, m), 7.40—7.55 (1H, m), 7.42 (2H, d, <i>J</i> =8.6 Hz), 7.64 (2H, s), 7.64 (2H, d, <i>J</i> =8.6 Hz), 7.73 (1H, s), 7.85 (1H, s) | 3405, 1686, 1615, 1520, 1431, 1254             | −17.5°<br>{20}<br>(1.2)                      | 0.016                        |                                  |                                                                    |
| <b>2d</b> | 30<br>[NMP<br>/NaH]                                            | Amorphous<br>powder               | C <sub>24</sub> H <sub>22</sub> F <sub>2</sub> N <sub>8</sub> O <sub>2</sub><br>· 1/2H <sub>2</sub> O<br>57.48 4.62 22.34<br>(57.71 4.55 22.22) | 1.20 (3H, d, <i>J</i> =7 Hz), 4.19 (1H, d, <i>J</i> =14 Hz), 4.97 (1H, q, <i>J</i> =7 Hz), 5.09 (1H, d, <i>J</i> =14 Hz), 5.55 (1H, br), 5.59 (2H, s), 6.65 (1H, d, <i>J</i> =3.2 Hz), 6.75—6.90 (3H, m), 7.35—7.55 (4H, m), 7.66—7.75 (4H, m), 7.84 (1H, s)                                                                              | 3382, 3125, 1684, 1615, 1520, 1501, 1431, 1254 | −16.9°<br>{20}<br>(1.1)                      | 0.13                         |                                  |                                                                    |

Table 1. (continued)

| Compd.    | Yield <sup>(a)</sup><br>(%)<br>[Solv.<br>/Base] <sup>(b)</sup> | mp (°C)<br>(Solv.) <sup>(c)</sup> | Analysis (%)                                                                                                                                    |       |   | <sup>1</sup> H-NMR (in CDCl <sub>3</sub> ) $\delta$                                                                                                                                                                                                                                                                                                                       | IR (KBr)<br>cm <sup>-1</sup>                   | [ $\alpha$ ] <sub>D</sub><br>{°C}<br>MeOH | MIC ( $\mu$ g/ml) <sup>(d)</sup><br><i>C. albicans</i> TA <sup>(e)</sup> |
|-----------|----------------------------------------------------------------|-----------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|-------|---|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------|-------------------------------------------|--------------------------------------------------------------------------|
|           |                                                                |                                   | Calcd                                                                                                                                           | Found |   |                                                                                                                                                                                                                                                                                                                                                                           |                                                |                                           |                                                                          |
|           |                                                                |                                   | C                                                                                                                                               | H     | N |                                                                                                                                                                                                                                                                                                                                                                           |                                                |                                           |                                                                          |
| <b>2e</b> | 32<br>[NMP<br>/NaH]                                            | 100—110<br>(AC-EE-<br>IPE)        | C <sub>24</sub> H <sub>21</sub> F <sub>2</sub> N <sub>7</sub> O <sub>2</sub><br>60.37 4.43 20.53<br>(60.29 4.42 20.50)                          |       |   | 1.21 (3H, d, <i>J</i> =7 Hz), 4.22 (1H, d, <i>J</i> =14.4 Hz), 4.98 (1H, q, <i>J</i> =7 Hz), 5.12 (1H, d, <i>J</i> =14.4 Hz), 5.56 (1H, br), 6.47—6.54 (1H, m), 6.68—6.88 (4H, m), 7.40—7.56 (1H, m), 7.70—7.85 (6H, m), 7.85 (1H, s), 7.94 (1H, d, <i>J</i> =2.4 Hz)                                                                                                     | 3320, 1660, 1615, 1510, 1430, 1390             | −16.0°<br>{20}<br>(1.0)                   | 0.004                                                                    |
| <b>2f</b> | 24<br>[NMP<br>/NaH]                                            | Amorphous<br>powder               | C <sub>24</sub> H <sub>21</sub> F <sub>2</sub> N <sub>7</sub> O <sub>2</sub><br>· 1/2H <sub>2</sub> O<br>59.25 4.56 20.15<br>(59.13 4.42 19.96) |       |   | 1.21 (3H, d, <i>J</i> =7 Hz), 4.22 (1H, d, <i>J</i> =14 Hz), 5.00 (1H, q, <i>J</i> =7 Hz), 5.12 (1H, d, <i>J</i> =14 Hz), 5.56 (1H, br), 6.70 (1H, d, <i>J</i> =3 Hz), 6.76—6.86 (3H, m), 7.23 (1H, s), 7.30 (1H, s), 7.42—7.54 (1H, m), 7.49 (2H, d, <i>J</i> =8 Hz), 7.75 (1H, s), 7.78 (1H, s), 7.84 (2H, d, <i>J</i> =8 Hz), 7.86 (1H, s)                             | 3400, 1685, 1612, 1521, 1498, 1428             | −15.7°<br>{20}<br>(1.0)                   | 0.004                                                                    |
| <b>2g</b> | 22<br>[DMF<br>/Cs <sub>2</sub> CO <sub>3</sub> ]               | Amorphous<br>powder               | C <sub>23</sub> H <sub>20</sub> F <sub>2</sub> N <sub>8</sub> O <sub>2</sub><br>· 1/2H <sub>2</sub> O<br>56.67 4.34 22.99<br>(56.88 4.67 22.61) |       |   | 1.21 (3H, d, <i>J</i> =7 Hz), 4.22 (1H, d, <i>J</i> =14.2 Hz), 5.01 (1H, q, <i>J</i> =7 Hz), 5.12 (1H, d, <i>J</i> =14.2 Hz), 5.50 (1H, br), 6.72 (1H, d, <i>J</i> =3.2 Hz), 6.73—6.90 (2H, m), 6.83 (1H, d, <i>J</i> =3.2 Hz), 7.40—7.55 (1H, m), 7.75 (1H, s), 7.78 (2H, d, <i>J</i> =9.4 Hz), 7.86 (1H, s), 7.86 (2H, d, <i>J</i> =9.4 Hz), 8.13 (1H, s), 8.59 (1H, s) | 3400, 3118, 1683, 1616, 1527, 1500             | −15.0°<br>{20}<br>(1.0)                   | <0.016                                                                   |
| <b>2h</b> | 29<br>[NMP<br>/NaH]                                            | 182—185<br>(ME-W)                 | C <sub>23</sub> H <sub>20</sub> F <sub>2</sub> N <sub>8</sub> O <sub>2</sub><br>57.74 4.21 23.42<br>(57.67 4.20 23.59)                          |       |   | 1.22 (3H, d, <i>J</i> =7 Hz), 4.22 (1H, d, <i>J</i> =14.4 Hz), 4.99 (1H, q, <i>J</i> =7 Hz), 5.01 (1H, d, <i>J</i> =14.4 Hz), 5.13 (1H, br), 6.70—6.88 (4H, m), 7.40—7.56 (1H, m), 7.75 (1H, s), 7.81 (2H, d, <i>J</i> =9.2 Hz), 7.84 (2H, s), 7.86 (1H, s), 8.18 (2H, d, <i>J</i> =9.2 Hz)                                                                               | 3328, 1664, 1614, 1519, 1430, 1384             | −17.4°<br>{20}<br>(1.0)                   | <0.008                                                                   |
| <b>2i</b> | 38<br>[NMP<br>/NaH]                                            | 178—181<br>(AC-EA-<br>IPE)        | C <sub>23</sub> H <sub>20</sub> F <sub>2</sub> N <sub>8</sub> O <sub>2</sub><br>57.74 4.21 23.42<br>(57.46 4.25 23.30)                          |       |   | 1.22 (3H, d, <i>J</i> =7 Hz), 4.22 (1H, d, <i>J</i> =14.4 Hz), 5.01 (1H, q, <i>J</i> =7 Hz), 5.12 (1H, d, <i>J</i> =14.4 Hz), 5.38 (1H, br), 6.70—6.88 (4H, m), 7.40—7.55 (1H, m), 7.76 (1H, s), 7.80—7.93 (6H, m), 8.03 (1H, s)                                                                                                                                          | 1691, 1656, 1619, 1527, 1502, 1430             | −16.0°<br>{20}<br>(1.0)                   | <0.008                                                                   |
| <b>2j</b> | 17<br>[NMP<br>/NaH]                                            | 135—137<br>(ME-W)                 | C <sub>22</sub> H <sub>19</sub> F <sub>2</sub> N <sub>9</sub> O <sub>2</sub><br>· 1/2H <sub>2</sub> O<br>54.10 4.13 25.81<br>(53.89 4.18 25.82) |       |   | 1.21 (3H, d, <i>J</i> =7 Hz), 4.22 (1H, d, <i>J</i> =14 Hz), 5.02 (1H, q, <i>J</i> =7 Hz), 5.12 (1H, d, <i>J</i> =14 Hz), 5.49 (1H, br), 6.75 (3H, d, <i>J</i> =3 Hz), 6.75—6.85 (2H, m), 6.85 (1H, d, <i>J</i> =3 Hz), 7.42—7.54 (1H, m), 7.76 (1H, s), 7.85 (1H, s), 7.93 (2H, d, <i>J</i> =9 Hz), 8.25 (2H, d, <i>J</i> =9 Hz), 8.68 (1H, s)                           | 3400, 3120, 1691, 1670, 1611, 1518, 1500, 1426 | −14.3°<br>{20}<br>(1.0)                   | 0.002                                                                    |
| <b>2k</b> | 63                                                             | 198—200<br>(EA)                   | C <sub>22</sub> H <sub>19</sub> F <sub>2</sub> N <sub>9</sub> O <sub>2</sub><br>55.11 3.99 26.29<br>(54.92 4.04 26.05)                          |       |   | 1.21 (3H, d, <i>J</i> =7 Hz), 4.22 (1H, d, <i>J</i> =14 Hz), 5.03 (1H, q, <i>J</i> =7 Hz), 5.13 (1H, d, <i>J</i> =14 Hz), 5.45 (1H, br), 6.74—6.88 (4H, m), 7.42—7.55 (1H, m), 7.77 (1H, s), 7.82 (2H, d, <i>J</i> =9 Hz), 7.86 (1H, s), 7.96 (2H, d, <i>J</i> =9 Hz), 9.06 (1H, s)                                                                                       | 3420, 3115, 1678, 1610, 1520, 1498, 1422       | −15.6°<br>{20}<br>(0.40)                  | 0.008                                                                    |
| <b>3e</b> | 73                                                             | 142—144<br>(EE-IPE)               | C <sub>24</sub> H <sub>23</sub> F <sub>2</sub> N <sub>7</sub> O <sub>2</sub><br>60.12 4.83 20.45<br>(60.02 4.95 20.34)                          |       |   | 1.08 (3H, d, <i>J</i> =7 Hz), 3.68—4.10 (4H, m), 4.53 (1H, d, <i>J</i> =14.2 Hz), 4.55—4.75 (1H, m), 5.12 (1H, d, <i>J</i> =14.2 Hz), 5.45 (1H, br), 6.46—6.48 (1H, m), 6.70—6.85 (2H, m), 7.35—7.50 (1H, m), 7.60—7.75 (5H, m), 7.76 (1H, s), 7.88 (1H, s), 7.90 (1H, d, <i>J</i> =2.6 Hz)                                                                               | 3130, 1690, 1660, 1520, 1500, 1420             | −60.5°<br>{20}<br>(1.0)                   | 0.002                                                                    |
| <b>3f</b> | 63                                                             | Amorphous<br>powder               | C <sub>24</sub> H <sub>23</sub> F <sub>2</sub> N <sub>7</sub> O <sub>2</sub><br>· H <sub>2</sub> O<br>59.01 4.95 20.07<br>(58.87 4.86 19.93)    |       |   | 1.08 (3H, d, <i>J</i> =7 Hz), 3.70—4.08 (4H, m), 4.52 (1H, d, <i>J</i> =14 Hz), 4.55—4.76 (1H, m), 5.11 (1H, d, <i>J</i> =14 Hz), 5.40 (1H, br), 6.73—6.84 (2H, m), 7.20 (1H, s), 7.26 (1H, s), 7.36—7.50 (1H, m), 7.39 (2H, d, <i>J</i> =9 Hz), 7.69 (2H, d, <i>J</i> =9 Hz), 7.76 (1H, s), 7.82 (1H, s), 7.87 (1H, s)                                                   | 3400, 1690, 1610, 1519, 1492, 1480             | −58.4°<br>{20}<br>(1.0)                   | 0.004                                                                    |
| <b>3g</b> | 74                                                             | 205—206<br>(EA-IPE)               | C <sub>23</sub> H <sub>22</sub> F <sub>2</sub> N <sub>8</sub> O <sub>2</sub><br>57.50 4.62 23.32<br>(57.46 4.47 23.19)                          |       |   | 1.08 (3H, d, <i>J</i> =7.2 Hz), 3.68—4.18 (4H, m), 4.52 (1H, d, <i>J</i> =14 Hz), 4.58—4.80 (1H, m), 5.12 (1H, d, <i>J</i> =14 Hz), 5.38 (1H, br), 6.70—6.86 (2H, m), 7.35—7.50 (1H, m), 7.66 (2H, dt, <i>J</i> =9.4 Hz, 2.4 Hz), 7.75 (2H, dt, <i>J</i> =9.4 Hz, 2.4 Hz), 7.77 (1H, s), 7.87 (1H, s), 8.11 (1H, s), 8.53 (1H, s)                                         | 3390, 3106, 1677, 1614, 1523, 1484             | −60.2°<br>{20}<br>(1.0)                   | ≤0.001                                                                   |
| <b>3h</b> | 87                                                             | 196—197<br>(EA-IPE)               | C <sub>23</sub> H <sub>22</sub> F <sub>2</sub> N <sub>8</sub> O <sub>2</sub><br>57.50 4.62 23.32<br>(57.42 4.54 23.25)                          |       |   | 1.08 (3H, d, <i>J</i> =7.4 Hz), 3.68—4.12 (4H, m), 4.53 (1H, d, <i>J</i> =14 Hz), 4.58—4.76 (1H, m), 5.13 (1H, d, <i>J</i> =14 Hz), 5.42 (1H, br), 6.70—6.85 (2H, m), 7.36—7.50 (1H, m), 7.71 (2H, d, <i>J</i> =9 Hz), 7.76 (1H, s), 7.81 (2H, s), 7.87 (1H, s), 8.07 (2H, d, <i>J</i> =9 Hz)                                                                             | 3426, 1687, 1658, 1616, 1517, 1484             | −61.6°<br>{20}<br>(1.0)                   | <0.008                                                                   |
| <b>3i</b> | 86                                                             | 193—195<br>(ET-IPE)               | C <sub>23</sub> H <sub>22</sub> F <sub>2</sub> N <sub>8</sub> O <sub>2</sub><br>57.50 4.62 23.32<br>(57.38 4.59 23.41)                          |       |   | 1.08 (3H, d, <i>J</i> =7 Hz), 3.70—4.14 (4H, m), 4.52 (1H, d, <i>J</i> =14.2 Hz), 4.60—4.78 (1H, m), 5.12 (1H, d, <i>J</i> =14.2 Hz), 5.38 (1H, br), 6.70—6.86 (2H, m), 7.35—7.50 (1H, m), 7.68—7.82 (4H, m), 7.77 (1H, s), 7.86 (2H, s), 7.97 (1H, s)                                                                                                                    | 1697, 1664, 1618, 1527, 1502, 1427             | −61.6°<br>{20}<br>(1.0)                   | 0.008                                                                    |
| <b>3j</b> | 66                                                             | 165—166<br>(EA-IPE)               | C <sub>22</sub> H <sub>21</sub> F <sub>2</sub> N <sub>9</sub> O <sub>2</sub><br>54.88 4.40 26.18<br>(54.62 4.55 26.19)                          |       |   | 1.08 (3H, d, <i>J</i> =7 Hz), 3.69—3.81 (1H, m), 3.94—4.10 (3H, m), 4.52 (1H, d, <i>J</i> =14 Hz), 4.62—4.80 (1H, m), 5.13 (1H, d, <i>J</i> =14 Hz), 5.25—5.50 (1H, br), 6.72—6.84 (2H, m), 7.36—7.49 (1H, m), 7.77 (1H, s), 7.80 (2H, d, <i>J</i> =9 Hz), 7.86 (1H, s), 8.13 (2H, d, <i>J</i> =9 Hz), 8.64 (1H, s)                                                       | 3420, 1695, 1610, 1518, 1500, 1482             | −61.1°<br>{20}<br>(1.0)                   | ≤0.001                                                                   |

Table 1. (continued)

| Compd.      | Yield <sup>(a)</sup><br>(%)<br>[Solv.<br>/Base] <sup>(b)</sup> | mp (°C)<br>(Solv.) <sup>(c)</sup> | Analysis (%)                                                                                                           |   |   | <sup>1</sup> H-NMR (in CDCl <sub>3</sub> ) δ                                                                                                                                                                                                                                                | IR (KBr)<br>cm <sup>−1</sup>             | [α] <sub>D</sub><br>{°C}<br>MeOH | MIC (μg/ml) <sup>(d)</sup><br><i>C. albicans</i> TA <sup>(e)</sup> |
|-------------|----------------------------------------------------------------|-----------------------------------|------------------------------------------------------------------------------------------------------------------------|---|---|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------|----------------------------------|--------------------------------------------------------------------|
|             |                                                                |                                   | Calcd (Found)                                                                                                          | C | H |                                                                                                                                                                                                                                                                                             |                                          |                                  |                                                                    |
| 3k          | 48                                                             | 211—213<br>(ET)                   | C <sub>22</sub> H <sub>21</sub> F <sub>2</sub> N <sub>9</sub> O <sub>2</sub><br>54.88 4.40 26.18<br>(54.79 4.42 26.00) |   |   | 1.08 (3H, d, <i>J</i> =7 Hz), 3.69—4.14 (4H, m), 4.52 (1H, d, <i>J</i> =14 Hz), 4.65—4.80 (1H, m), 5.12 (1H, d, <i>J</i> =14 Hz), 5.35 (1H, br), 6.74—6.84 (2H, m), 7.36—7.49 (1H, m), 7.68 (2H, d, <i>J</i> =9 Hz), 7.77 (1H, s), 7.82 (2H, d, <i>J</i> =9 Hz), 7.87 (1H, s), 8.98 (1H, s) | 3400, 3120, 1680, 1610, 1520, 1498, 1480 | −60.6°<br>{20}<br>(1.0)          | 0.002                                                              |
| Fluconazole |                                                                |                                   |                                                                                                                        |   |   |                                                                                                                                                                                                                                                                                             |                                          |                                  | 0.13                                                               |

a) 1a—j, 2a—j: yields based on the oxirane (4); 2k: yields from the oxirane (9); 3e—k: yields from the imidazolones (2). b) Reaction solvent: NMP, *N*-methyl-2-pyrrolidone; DMSO, dimethylsulfoxide; DMF, *N,N*-dimethylformamide. c) Recrystallization solvent: DCM, dichloromethane; EE, diethyl ether; EA, ethyl acetate; IPE, diisopropyl ether; ME, methanol; W, water; AC, acetone; H, hexane; ET, ethanol. d) Medium: RPMI 1640 containing 1.0% agar. e) Determined under 20% CO<sub>2</sub>.

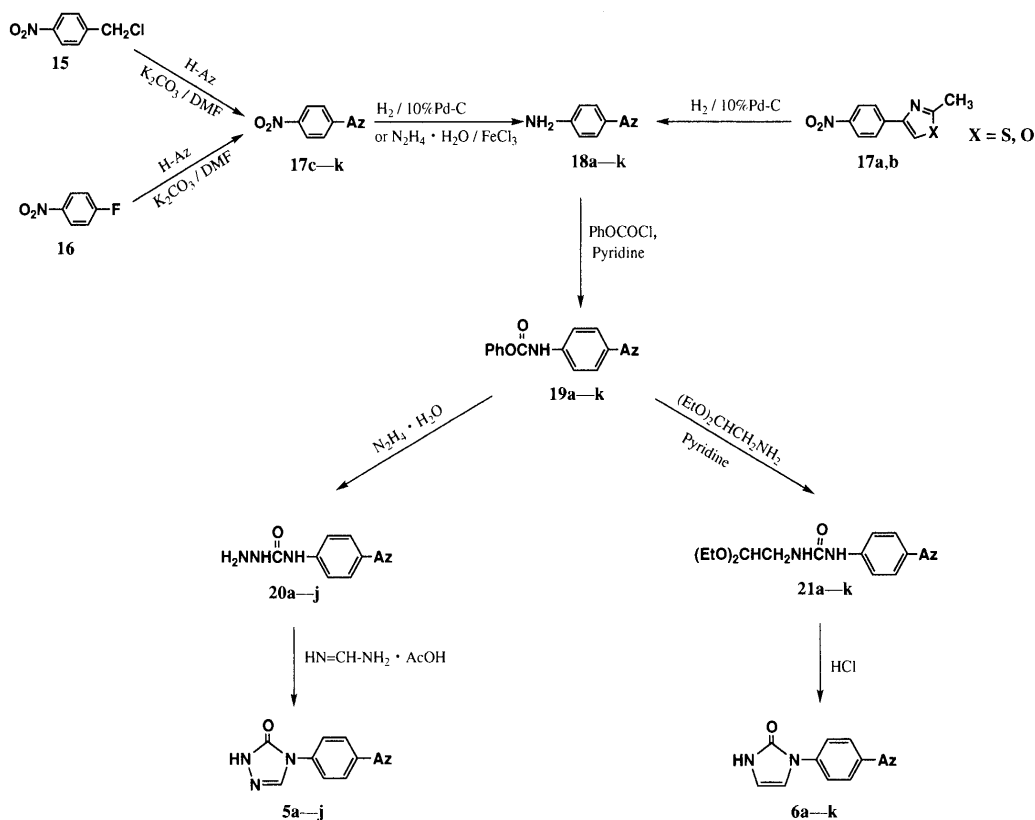


Chart 4

values (ca. 4 mg/kg) than that of itraconazole.

In conclusion, we found that the optically active imidazolidinone derivatives (3) showed potent *in vitro* antifungal activity against not only yeasts such as *C. albicans* and *C. neoformans* but also against molds such as *A. fumigatus*. It was particularly noteworthy that the imidazolidinones having 1*H*-1,2,3-triazol-1-yl, 2*H*-2-tetrazolyl and 1*H*-1-tetrazolyl moieties, 3i, j, k, exhibited excellent therapeutic effects against candidiasis as well as aspergillosis. Further biological evaluations of this series of derivatives are in progress.

#### Experimental

Melting points were determined using a Yanagimoto melting point apparatus and are uncorrected. IR spectra were measured with a JASCO IR-810 spectrometer. <sup>1</sup>H-NMR spectra were recorded on a Varian Gemini-200 spectrometer with tetramethylsilane as an internal standard. The following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad. The secondary ion mass spectra (SI-MS) were measured with a

Hitachi M-80A mass spectrometer. The optical rotations were recorded with a JASCO DIP-181 or DIP-370 digital polarimeter.

Reactions were carried out at room temperature unless otherwise noted and followed by TLC on Silica gel 60 F<sub>254</sub> precoated TLC plates (E. Merck) or by HPLC using an octadecyl silica (ODS) column (A-303, 4.6 mm i.d. × 250 mm, YMC Co., Ltd.). Standard work-up procedures were as follows. The reaction mixture was partitioned between the indicated solvent and water. Organic extracts were combined and washed in the indicated order using the following aqueous solutions; water, 1 *N* aqueous sodium hydroxide solution (1 *N* NaOH), 5% aqueous NaHCO<sub>3</sub> solution (aqueous NaHCO<sub>3</sub>), 1 *N* HCl and saturated sodium chloride (NaCl) solution (brine). Extracts were dried over anhydrous magnesium sulfate (MgSO<sub>4</sub>), filtered and evaporated *in vacuo*.

Chromatographic separations were carried out on Silica gel 60 (0.063—0.200 mm, E. Merck) using the indicated eluents.

**2-(4-Nitrobenzyl)-2*H*-1,2,3-triazole (17c) and 1-(4-Nitrobenzyl)-1*H*-1,2,3-triazole (17d)** A mixture of 15 (24.6 g, 143 mmol), 1*H*-1,2,3-triazole (10.9 g, 157 mmol), K<sub>2</sub>CO<sub>3</sub> (65 g, 471 mmol) and DMF (150 ml) was stirred for 3 h. The whole was poured into ice-water and worked up (AcOEt; water). The residue was crystallized from ethyl acetate (AcOEt)—diisopropyl ether

Table 2. 4-(4-Substituted phenyl)-3(2*H*, 4*H*)-1,2,4-triazolones (**5**)

| 5         | Az                                                                                  | Yield (%) | mp (°C)<br>(Solv.) <sup>a)</sup> | Formula                                                       | Analysis (%)                 |                |                  | <sup>1</sup> H-NMR (in DMSO- <i>d</i> <sub>6</sub> ) δ {IR (KBr) cm <sup>-1</sup> }                                                                                                                       |
|-----------|-------------------------------------------------------------------------------------|-----------|----------------------------------|---------------------------------------------------------------|------------------------------|----------------|------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|           |                                                                                     |           |                                  |                                                               | Calcd                        | Found          |                  |                                                                                                                                                                                                           |
|           |                                                                                     |           |                                  |                                                               | C                            | H              | N                |                                                                                                                                                                                                           |
| <b>5a</b> |    | 54        | 282—284<br>(ME-EA)               | C <sub>12</sub> H <sub>10</sub> N <sub>4</sub> OS             | 55.80<br>(55.63)             | 3.90<br>(4.01) | 21.69<br>(21.39) | 2.72 (3H, s), 7.77 (2H, d, <i>J</i> =9 Hz), 7.97 (1H, s), 8.05 (2H, d, <i>J</i> =9 Hz), 8.42 (1H, s), 11.99 (1H, br) {3102, 1705, 1568, 1535, 1508, 1227}                                                 |
| <b>5b</b> |    | 70        | 280—283<br>(DMF-W)               | C <sub>12</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub> | 59.50<br>(59.08)             | 4.16<br>(4.23) | 23.13<br>(22.71) | 2.48 (3H, s), 7.75 (2H, d, <i>J</i> =8.4 Hz), 7.87 (2H, d, <i>J</i> =8.4 Hz), 8.41 (1H, s), 5.51 (1H, s), 12.0 (1H, br) {— <sup>b)</sup> }                                                                |
| <b>5c</b> |    | 49        | 201—205<br>(ME)                  | C <sub>11</sub> H <sub>10</sub> N <sub>6</sub> O              | 54.54<br>(54.44)             | 4.16<br>(4.23) | 34.69<br>(34.96) | 5.69 (2H, s), 7.38 (2H, d, <i>J</i> =8.4 Hz), 7.68 (2H, d, <i>J</i> =8.4 Hz), 7.81 (1H, s), 8.34 (1H, s), 11.98 (1H, br) {3090, 1719, 1572, 1522, 1435, 1221}                                             |
| <b>5d</b> |    | 76        | 250—252<br>(ME)                  | C <sub>11</sub> H <sub>10</sub> N <sub>6</sub> O              | 54.54<br>(54.47)             | 4.16<br>(4.36) | 34.69<br>(34.47) | 5.67 (2H, s), 7.44 (2H, d, <i>J</i> =8.8 Hz), 7.70 (2H, d, <i>J</i> =8.8 Hz), 7.75 (1H, s), 8.21 (1H, s), 8.36 (1H, s), 11.99 (1H, br) {3137, 1717, 1559, 1518, 1439, 1385}                               |
| <b>5e</b> |    | 64        | 269—270<br>(DMF)                 | C <sub>11</sub> H <sub>9</sub> N <sub>5</sub> O               | 58.15<br>(58.01)             | 3.99<br>(4.10) | 30.82<br>(30.74) | 6.58 (1H, t, <i>J</i> =2 Hz), 7.78 (1H, d, <i>J</i> =1.6 Hz), 7.84 (2H, d, <i>J</i> =9 Hz), 7.98 (2H, d, <i>J</i> =9 Hz), 8.44 (1H, s), 8.60 (1H, d, <i>J</i> =2 Hz) {3144, 1717, 1559, 1532, 1397, 1339} |
| <b>5f</b> |    | 46        | 284—286<br>(DMF-W)               | C <sub>11</sub> H <sub>9</sub> N <sub>5</sub> O               | 58.15<br>(57.97)             | 3.99<br>(3.99) | 30.82<br>(30.77) | 7.13 (1H, s), 7.79 (1H, s), 7.80 (2H, d, <i>J</i> =9 Hz), 7.87 (2H, d, <i>J</i> =9 Hz), 8.31 (1H, s), 8.46 (1H, s), 12.1 (1H, br) {3112, 1709, 1530, 1435, 1350, 1316}                                    |
| <b>5g</b> |    | 40        | >300<br>(DMF-W)                  | C <sub>10</sub> H <sub>8</sub> N <sub>6</sub> O               | 52.63<br>(52.66)             | 3.53<br>(3.58) | 36.83<br>(36.79) | 7.91 (2H, d, <i>J</i> =9.4 Hz), 8.00 (2H, d, <i>J</i> =9.4 Hz), 8.25 (1H, s), 8.46 (1H, s), 9.33 (1H, s) {3123, 1717, 1561, 1532, 1426, 1279}                                                             |
| <b>5h</b> |   | 54        | 281—283<br>(DMF-W)               | C <sub>10</sub> H <sub>8</sub> N <sub>6</sub> O               | 52.63<br>(52.34)             | 3.53<br>(3.69) | 36.83<br>(36.55) | 7.92 (2H, d, <i>J</i> =9 Hz), 8.14 (2H, d, <i>J</i> =9 Hz), 8.14 (2H, s), 8.46 (1H, s) {3185, 1732, 1559, 1520, 1416, 1373}                                                                               |
| <b>5i</b> |  | 58        | >300<br>(EA)                     | C <sub>10</sub> H <sub>8</sub> N <sub>6</sub> O               | 52.63<br>(52.16)             | 3.53<br>(3.72) | 36.83<br>(36.39) | 7.96 (2H, d, <i>J</i> =9 Hz), 7.98 (1H, s), 8.06 (2H, d, <i>J</i> =9 Hz), 8.49 (1H, s), 8.87 (1H, d, <i>J</i> =1 Hz) {3115, 1694, 1603, 1559, 1522, 1362}                                                 |
| <b>5j</b> |  | 63        | 245—248<br>(dec.)<br>(DMF-W)     | C <sub>9</sub> H <sub>7</sub> N <sub>7</sub> O                | SIMS (MH <sup>+</sup> ): 230 |                |                  | 8.06 (2H, d, <i>J</i> =9 Hz), 8.25 (2H, d, <i>J</i> =9 Hz), 8.55 (1H, s), 9.28 (1H, s), 12.2 (1H, br) {—}                                                                                                 |

a) Recrystallization solvent: DMF, *N,N*-dimethylformamide; W, water; EA, ethyl acetate; ME, methanol. b) Not measured.

(isoPr<sub>2</sub>O) to give **17d** (9.65 g, 33.2%) as pale yellow needles. The mother liquor was concentrated *in vacuo* and the residue was purified by silica gel column chromatography (hexane-AcOEt, 2:1→AcOEt, v/v). The compound in the first eluted fraction was crystallized from AcOEt-isoPr<sub>2</sub>O to give **17c** (10.1 g, 37%). The compound in the second eluted fraction was crystallized from AcOEt-isoPr<sub>2</sub>O to give an additional amount of **17d** (4.9 g, 16.8%). **17c**<sup>7c)</sup>: mp 112—114 °C. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 5.73 (2H, s), 7.43 (2H, d, *J*=8.8 Hz), 7.69 (2H, s), 8.22 (2H, d, *J*=8.8 Hz). **17d**<sup>7c)</sup>: <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 5.70 (2H, s), 7.40 (2H, d, *J*=8.6 Hz), 7.57 (1H, s), 7.79 (1H, s), 8.24 (2H, d, *J*=8.6 Hz).

**General Procedure for the Preparation of *N*-(Nitrophenyl)azoles (**17e—k**)** A mixture of **16** (1 eq), H-Az (1—1.5 eq), K<sub>2</sub>CO<sub>3</sub> (1—2 eq) in DMF was stirred at 70—80 °C for 3—10 h. The whole was poured into ice-water. The precipitate was collected by filtration and purified by silica gel column chromatography or by recrystallization.

Compounds **17h** and **17i**, which were the substitution position isomers, were separated by silica gel column chromatography [dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>)→CH<sub>2</sub>Cl<sub>2</sub>-acetone, 8:1, v/v] and crystallized from CH<sub>2</sub>Cl<sub>2</sub>-isoPr<sub>2</sub>O. The mixture of **17j** and **17k** was used for the next step without separation.

**17e**<sup>7a,b)</sup> (96%): mp 172—173 °C (recrystallized from DMF-water). *Anal.* Calcd for C<sub>9</sub>H<sub>7</sub>N<sub>3</sub>O<sub>2</sub>: C, 57.14; H, 3.73; N, 22.21. Found: C, 56.97; H, 3.57; N, 22.17. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 6.57 (1H, t, *J*=2 Hz), 7.81 (1H, d, *J*=2 Hz), 7.90 (2H, dt, *J*=9.2 Hz, 2 Hz), 8.05 (1H, d, *J*=2 Hz), 8.36 (2H, dt, *J*=9.2 Hz, 2 Hz). **17f**<sup>7a,b)</sup> (98%): mp 204—206 °C (recrystallized from DMF-water). *Anal.* Calcd for C<sub>9</sub>H<sub>7</sub>N<sub>3</sub>O<sub>2</sub>: C, 57.14; H, 3.73; N, 22.21. Found: C, 57.18; H, 3.69; N, 22.26. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 7.29 (1H, s), 7.39 (1H, s), 7.60 (2H, d, *J*=9.1 Hz), 8.00 (1H, s), 8.40 (2H, d, *J*=9.1 Hz). **17g**<sup>7a—c)</sup> (83%): mp 198—199 °C (recrystallized from AcOEt-isoPr<sub>2</sub>O). *Anal.* Calcd for C<sub>8</sub>H<sub>6</sub>F<sub>4</sub>N<sub>3</sub>O<sub>2</sub>: C, 50.53; H, 3.18; N, 29.46. Found: C, 50.73; H, 3.19; N, 29.31. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 7.93 (2H, dt, *J*=9.4 Hz, 2.4 Hz), 8.05 (1H, s), 8.18 (1H, s), 8.43 (2H, dt, *J*=9.4 Hz, 2.4 Hz). **17h**<sup>14)</sup> (22%): mp 183—184 °C (recrystallized

from CH<sub>2</sub>Cl<sub>2</sub>-isoPr<sub>2</sub>O). *Anal.* Calcd for C<sub>8</sub>H<sub>6</sub>N<sub>4</sub>O<sub>2</sub>: C, 50.53; H, 3.18; N, 29.46. Found: C, 50.76; H, 3.19; N, 29.51. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 7.90 (2H, s), 8.28 (2H, dt, *J*=9.4 Hz, 2.4 Hz), 8.38 (2H, dt, *J*=9.4 Hz, 2.4 Hz). **17i**<sup>15)</sup> (50%): mp 205—206 °C (recrystallized from CH<sub>2</sub>Cl<sub>2</sub>-isoPr<sub>2</sub>O). *Anal.* Calcd for C<sub>8</sub>H<sub>6</sub>N<sub>4</sub>O<sub>2</sub>: C, 50.53; H, 3.18; N, 29.46. Found: C, 50.30; H, 2.94; N, 29.41. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 7.92 (1H, d, *J*=1.4 Hz), 8.00 (2H, dt, *J*=9 Hz, 2.4 Hz), 8.13 (1H, d, *J*=1.4 Hz), 8.44 (2H, dt, *J*=9 Hz, 2.4 Hz).

**1-(4-Aminophenyl)-1*H*-1,2,4-triazole (**18g**)** A solution of **17g** (20 g) in ethanol (EtOH, 760 ml) was hydrogenated over 10% Pd-C (50% wet, 3.0 g) under atmospheric pressure. After absorption of hydrogen stopped, the catalyst was removed by filtration and the filtrate was concentrated *in vacuo*. The residue was crystallized from AcOEt to give **18g**<sup>16a,b)</sup> (15.9 g, 94%) as a colorless crystalline powder. mp 138—139 °C. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 3.85 (2H, bs), 6.76 (2H, dt, *J*=8.8 Hz, 2.2 Hz), 7.40 (2H, dt, *J*=8.8 Hz, 2.2 Hz), 8.05 (1H, s), 8.39 (1H, s). *Anal.* Calcd for C<sub>8</sub>H<sub>8</sub>N<sub>4</sub>: C, 59.99; H, 5.03; N, 34.98. Found: C, 60.12; H, 4.97; N, 34.93.

The anilines (**18a—f, h, i**) were prepared from the corresponding 4-substituted nitrobenzenes (**17a—f, h, i**) by the same method as described above. **18a**<sup>17)</sup> (86%): <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 2.75 (3H, s), 3.74 (2H, br s), 6.71 (2H, dt, *J*=8.4 Hz, 2.6 Hz), 7.10 (1H, s), 7.68 (2H, dt, *J*=8.4 Hz, 2.6 Hz). **18b**<sup>18)</sup> (quant.): <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 2.50 (3H, s), 5.48 (2H, br s), 6.71 (2H, dt, *J*=8.6 Hz, 2.4 Hz), 7.49 (2H, dt, *J*=8.6 Hz, 2.4 Hz), 7.67 (1H, s). **18c** (96%): <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 3.70 (2H, br s), 5.48 (2H, s), 6.64 (2H, d, *J*=8.4 Hz), 7.16 (2H, d, *J*=8.4 Hz), 7.60 (2H, s). **18d** (98%): <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 3.77 (2H, br s), 5.43 (2H, s), 6.66 (2H, d, *J*=7.4 Hz), 7.10 (2H, d, *J*=7.4 Hz), 7.42 (1H, s), 7.68 (1H, s). **18e**<sup>16a,b)</sup> (97%): <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 3.74 (2H, br s), 6.38—6.45 (1H, m), 6.75 (2H, dt, *J*=7.4 Hz, 1.4 Hz), 7.45 (2H, dt, *J*=7.4 Hz, 1.4 Hz), 7.67 (1H, s), 7.75—7.80 (1H, m). **18f**<sup>16a,b)</sup> (73%): mp 145—146 °C (recrystallized from AcOEt). <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 5.29 (2H, s), 6.65 (2H, d, *J*=8.8 Hz), 7.03 (1H, s), 7.23 (2H, d, *J*=8.8 Hz), 7.49 (1H, s), 7.97 (1H, s). **18h**<sup>16a)</sup> (quant.): mp 50—51 °C (recrystallized from AcOEt-hexane). <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 3.80 (2H, br s), 6.76 (2H, dt, *J*=7.1 Hz, 1.6

Table 3. 1-(4-Substituted phenyl)-2(2*H*, 3*H*)-imidazolones (**6**)

| 6  | Az | Yield (%) | mp (°C)<br>(Solv.) <sup>a)</sup> | Formula                                                                    | Analysis (%)                 |                |                  | <sup>1</sup> H-NMR (in DMSO- <i>d</i> <sub>6</sub> ) δ {IR (KBr) cm <sup>-1</sup> }                                                                                                                                    |
|----|----|-----------|----------------------------------|----------------------------------------------------------------------------|------------------------------|----------------|------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|    |    |           |                                  |                                                                            | Calcd (Found)                |                |                  |                                                                                                                                                                                                                        |
|    |    |           |                                  |                                                                            | C                            | H              | N                |                                                                                                                                                                                                                        |
| 6a |    | 60        | 206—208<br>(EA-ME)               | C <sub>13</sub> H <sub>11</sub> N <sub>3</sub> OS<br>· 1/2H <sub>2</sub> O | 58.63<br>(59.12)             | 4.54<br>(4.95) | 15.78<br>(15.38) | 2.73 (3H, s), 6.60 (1H, t, <i>J</i> =2.6 Hz), 7.00 (1H, t, <i>J</i> =2.6 Hz), 7.80 (2H, d, <i>J</i> =8.8 Hz), 7.89 (1H, s), 7.98 (2H, d, <i>J</i> =8.8 Hz), 10.34 (1H, br) {3146, 1674, 1534, 1505, 1429, 1310}        |
| 6b |    | 73        | 216—218<br>(ME-W)                | C <sub>13</sub> H <sub>11</sub> N <sub>3</sub> O <sub>2</sub>              | 64.72<br>(64.56)             | 4.60<br>(4.84) | 17.40<br>(17.28) | 2.47 (3H, s), 6.60 (1H, t, <i>J</i> =2.8 Hz), 7.00 (1H, t, <i>J</i> =2.8 Hz), 7.79 (4H, s), 8.45 (1H, s), 10.30 (1H, br) {— <sup>b)</sup> }                                                                            |
| 6c |    | 81        | 190—191<br>(ME)                  | C <sub>12</sub> H <sub>11</sub> N <sub>5</sub> O                           | 59.74<br>(59.65)             | 4.60<br>(4.63) | 29.03<br>(29.00) | 5.66 (2H, s), 6.55—6.64 (1H, m), 6.90—7.00 (1H, m), 7.33 (2H, d, <i>J</i> =8.6 Hz), 7.70 (2H, d, <i>J</i> =8.6 Hz), 7.81 (2H, s), 10.32 (1H, br) {3164, 1686, 1520, 1433, 1410, 1339}                                  |
| 6d |    | 82        | 213—215<br>(ME)                  | C <sub>12</sub> H <sub>11</sub> N <sub>5</sub> O                           | 59.74<br>(59.64)             | 4.60<br>(4.74) | 29.03<br>(28.93) | 5.63 (2H, s), 6.57—6.60 (1H, m), 6.93—6.96 (1H, m), 7.37 (2H, d, <i>J</i> =8.6 Hz), 7.73 (2H, d, <i>J</i> =8.6 Hz), 7.75 (1H, s), 8.20 (1H, s), 10.33 (1H, br) {3129, 1699, 1520, 1429, 1314, 1219}                    |
| 6e |    | 98        | 239—240<br>(ME-W)                | C <sub>12</sub> H <sub>10</sub> N <sub>4</sub> O                           | 63.71<br>(63.45)             | 4.46<br>(4.51) | 24.76<br>(24.77) | 6.55 (1H, t, <i>J</i> =2.5 Hz), 6.62 (1H, t, <i>J</i> =2.5 Hz), 7.02 (1H, t, <i>J</i> =2 Hz), 7.75 (1H, d, <i>J</i> =1.6 Hz), 7.88 (4H, br s), 8.50 (1H, d, <i>J</i> =2 Hz) {3142, 1678, 1526, 1429, 1393, 1323}       |
| 6f |    | 57        | 210—211<br>(ME-W)                | C <sub>12</sub> H <sub>10</sub> N <sub>4</sub> O                           | SIMS (MH <sup>+</sup> ): 227 |                |                  | 6.65 (1H, t, <i>J</i> =3 Hz), 7.09 (1H, t, <i>J</i> =3 Hz), 7.54 (1H, s), 7.80 (2H, d, <i>J</i> =9 Hz), 7.97 (2H, d, <i>J</i> =9 Hz), 8.06 (1H, s), 9.07 (1H, s), 10.5 (1H, br) {3094, 1709, 1686, 1526, 1424, 1310}   |
| 6g |    | 92        | 294—296<br>(ME-W)                | C <sub>11</sub> H <sub>9</sub> N <sub>5</sub> O                            | 58.15<br>(57.91)             | 3.99<br>(3.85) | 30.83<br>(30.56) | 6.65 (1H, t, <i>J</i> =2.8 Hz), 7.08 (1H, dd, <i>J</i> =2.8 Hz, 2 Hz), 7.91 (2H, d, <i>J</i> =9.6 Hz), 7.97 (2H, d, <i>J</i> =9.6 Hz), 8.25 (1H, s), 9.33 (1H, s), 10.42 (1H, br) {3142, 1682, 1518, 1429, 1304, 1238} |
| 6h |    | 85        | >300<br>(ME-W)                   | C <sub>11</sub> H <sub>9</sub> N <sub>5</sub> O                            | 58.15<br>(57.80)             | 3.99<br>(4.01) | 30.83<br>(30.68) | 6.64 (1H, t, <i>J</i> =2.8 Hz), 7.05 (1H, t, <i>J</i> =2.8 Hz), 7.95 (2H, d, <i>J</i> =9.4 Hz), 8.07 (2H, d, <i>J</i> =9.4 Hz), 8.11 (2H, s) {3135, 1703, 1520, 1439, 1414, 1325}                                      |
| 6i |    | 86        | 255—257<br>(dec.)<br>(ME-W)      | C <sub>11</sub> H <sub>9</sub> N <sub>5</sub> O                            | 58.15<br>(58.01)             | 3.99<br>(3.85) | 30.83<br>(30.82) | 6.65 (1H, t, <i>J</i> =2.8 Hz), 7.09 (1H, t, <i>J</i> =2.8 Hz), 7.90—8.03 (5H, m), 8.83 (1H, d, <i>J</i> =1 Hz) {3140, 1690, 1524, 1310, 1231, 1038}                                                                   |
| 6j |    | 62        | 235—240<br>(dec.)<br>(DMF)       | C <sub>10</sub> H <sub>8</sub> N <sub>6</sub> O                            | 52.63<br>(52.34)             | 3.53<br>(3.57) | 36.83<br>(36.65) | 6.69 (1H, d, <i>J</i> =3 Hz), 7.13 (1H, d, <i>J</i> =3 Hz), 8.09 (2H, d, <i>J</i> =9 Hz), 8.17 (2H, d, <i>J</i> =9 Hz), 9.25 (1H, s), 10.5 (1H, br) {3144, 1705, 1607, 1516, 1422, 1310}                               |
| 6k |    | 79        | 251—255<br>(dec.)<br>(DMF)       | C <sub>10</sub> H <sub>8</sub> N <sub>6</sub> O                            | 52.63<br>(52.33)             | 3.53<br>(3.70) | 36.83<br>(36.46) | 6.67 (1H, s), 7.12 (1H, s), 7.96 (2H, d, <i>J</i> =9 Hz), 8.06 (2H, d, <i>J</i> =9 Hz), 10.1 (1H, s), 10.5 (1H, br) {3283, 1713, 1694, 1524, 1395, 1221}                                                               |

a) Recrystallization solvent: DMF, *N,N*-dimethylformamide; W, water; EA, ethyl acetate; ME, methanol. b) Not measured.

Table 4. Antifungal Activity of Compound **3**

| Compd.       | MIC (μg/ml) <sup>a)</sup>        |          |                                    |          |                                   |          |         | ED <sub>50</sub> (mg/kg)<br><i>p.o.</i> <sup>d)</sup> |                     |
|--------------|----------------------------------|----------|------------------------------------|----------|-----------------------------------|----------|---------|-------------------------------------------------------|---------------------|
|              | <i>C. albicans</i> <sup>b)</sup> |          | <i>C. neoformans</i> <sup>b)</sup> |          | <i>A. fumigatus</i> <sup>c)</sup> |          |         | <i>C. albicans</i>                                    | <i>A. fumigatus</i> |
|              | TIMM1756                         | TIMM1850 | TIMM1740                           | TIMM1855 | 437                               | TIMM1728 | IFO6344 | TA                                                    | 437                 |
| <b>3e</b>    | 0.004                            | 0.004    | 0.016                              | 0.06     | 0.5                               | 0.25     | 0.25    | 2.0                                                   | 17.7                |
| <b>3g</b>    | 0.002                            | ≤0.001   | 0.06                               | 0.13     | 1                                 | 0.5      | 1       | 0.32                                                  | 8.84                |
| <b>3i</b>    | 0.016                            | 0.016    | 0.13                               | 0.13     | 0.5                               | 0.5      | 0.5     | 0.71                                                  | 4.42                |
| <b>3j</b>    | 0.004                            | 0.002    | 0.03                               | 0.06     | 0.5                               | 0.25     | 0.25    | 0.18                                                  | 4.8                 |
| <b>3k</b>    | 0.008                            | 0.004    | 0.03                               | 0.06     | 1                                 | 0.5      | 0.5     | 0.89                                                  | 4.4                 |
| Fluconazole  | 0.5                              | 0.25     | 4                                  | 8        | >64                               | >64      | >64     | 0.22—0.35 <sup>e)</sup>                               | 179 <sup>e)</sup>   |
| Itraconazole | 0.03                             | 0.016    | 0.06                               | 0.13     | 0.5                               | 0.5      | 0.25    | 5.2 <sup>f)</sup>                                     | 19 <sup>f)</sup>    |

a) Medium: RPMI 1640 containing 1.0% agar. b) Determined under 20% CO<sub>2</sub>. c) Determined under air. d) Administered in the form of a 0.5% carboxymethylcellulose (CMC) suspension except fluconazole and itraconazole. e) Administered as an aqueous solution. f) Administered as a 2-hydroxypropyl β-cyclodextrin solution.<sup>12)</sup>

Hz), 7.75 (2H, s), 7.84 (2H, dt, *J*=7.1 Hz, 1.6 Hz). *Anal.* Calcd for C<sub>8</sub>H<sub>8</sub>N<sub>4</sub>: C, 59.99; H, 5.03; N, 34.98. Found: C, 59.77; H, 4.95; N, 34.87. **18i**<sup>16a)</sup> (96%): mp 121—122 °C (recrystallized from AcOEt). <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 3.93 (2H, br s), 6.77 (2H, dt, *J*=9 Hz, 2.2 Hz), 7.48 (2H, dt, *J*=9 Hz, 2.2 Hz), 7.81 (1H, s), 7.87 (1H, s). *Anal.* Calcd for C<sub>8</sub>H<sub>8</sub>N<sub>4</sub>: C, 59.99; H, 5.03; N, 34.98. Found: C, 60.02; H, 5.08; N, 34.66.

**2-(4-Aminophenyl)-2*H*-tetrazole (**18j**) and 1-(4-Aminophenyl)-1*H*-tetrazole (**18k**)** A mixture of **16** (50.4 g, 357 mmol), 1*H*-tetrazole (25 g, 357 mmol), K<sub>2</sub>CO<sub>3</sub> (50 g, 357 mmol), and DMF (350 ml) was stirred at 70—75 °C for 10 h. The mixture was cooled and poured into water (2.5 l). The resulting precipitate was collected by filtration and washed with water (500 ml) to give a mixture of **17j** and **17k** as a pale yellow wet solid. N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O

Table 5. Phenyl(4-Substituted phenyl)carbamates (**19**), 4-(4-Substituted phenyl)semicarbazides (**20**) and *N*-(2,2-Diethoxyethyl)-*N'*-(4-substituted phenyl)ureas (**21**)

| Compd.     | Yield (%)                                                        | mp (°C)<br>(Solv.) <sup>a)</sup> | Analysis (%)                                                    |       |        | <sup>1</sup> H-NMR (in DMSO- <i>d</i> <sub>6</sub> ) $\delta$ |                                                                                                                                                                                               |
|------------|------------------------------------------------------------------|----------------------------------|-----------------------------------------------------------------|-------|--------|---------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|            |                                                                  |                                  | Calcd                                                           | Found |        |                                                               |                                                                                                                                                                                               |
|            |                                                                  |                                  | C                                                               | H     | N      |                                                               |                                                                                                                                                                                               |
| <b>19a</b> | 94                                                               | — <sup>b)</sup>                  | C <sub>17</sub> H <sub>14</sub> N <sub>2</sub> O <sub>2</sub> S | 65.79 | 4.55   | 9.03                                                          | 2.77 (3H, s), 7.02 (1H, br), 7.18—7.26 (4H, m), 7.28—7.53 (4H, m), 7.68 (2H, d, <i>J</i> =8.4 Hz) (in CDCl <sub>3</sub> )                                                                     |
|            |                                                                  |                                  | (—)                                                             |       |        |                                                               |                                                                                                                                                                                               |
| <b>19b</b> | 56                                                               | 181—183<br>(EA-H)                | C <sub>17</sub> H <sub>14</sub> N <sub>2</sub> O <sub>3</sub>   | 69.38 | 4.79   | 9.52                                                          | 2.51 (3H, s), 7.04 (1H, br), 7.18—7.28 (3H, m), 7.37—7.51 (4H, m), 7.69 (2H, d, <i>J</i> =8.6 Hz), 7.78 (1H, s) (in CDCl <sub>3</sub> )                                                       |
|            |                                                                  |                                  | (—)                                                             |       |        |                                                               |                                                                                                                                                                                               |
| <b>19c</b> | 95                                                               | 161—162<br>(EA-IPE)              | C <sub>16</sub> H <sub>14</sub> N <sub>4</sub> O <sub>2</sub>   | 65.30 | 4.79   | 19.04                                                         | 5.58 (2H, s), 6.98 (1H, br), 7.13—7.50 (9H, m), 7.63 (2H, s) (in CDCl <sub>3</sub> )                                                                                                          |
|            |                                                                  |                                  | (—)                                                             |       |        |                                                               |                                                                                                                                                                                               |
| <b>19d</b> | 91                                                               | —                                | C <sub>16</sub> H <sub>14</sub> N <sub>4</sub> O <sub>2</sub>   | 65.30 | 4.79   | 19.04                                                         | 5.54 (2H, s), 7.10 (1H, br), 7.15—7.55 (10H, m), 7.72 (1H, s) (in CDCl <sub>3</sub> )                                                                                                         |
|            |                                                                  |                                  | (—)                                                             |       |        |                                                               |                                                                                                                                                                                               |
| <b>19e</b> | 95                                                               | 174—176<br>(EA-IPE)              | C <sub>16</sub> H <sub>13</sub> N <sub>3</sub> O <sub>2</sub>   | 68.81 | 4.69   | 15.04                                                         | 6.53 (1H, t, <i>J</i> =2.4 Hz), 7.23—7.49 (5H, m), 7.63 (2H, d, <i>J</i> =9 Hz), 7.72 (1H, d, <i>J</i> =2.4 Hz), 7.80 (2H, d, <i>J</i> =9 Hz), 8.42 (1H, d, <i>J</i> =2.4 Hz), 10.38 (1H, br) |
|            |                                                                  |                                  | (69.05                                                          | 4.40  | 15.18) |                                                               |                                                                                                                                                                                               |
| <b>19f</b> | 98                                                               | 158—160<br>(EA)                  | C <sub>16</sub> H <sub>13</sub> N <sub>3</sub> O <sub>2</sub>   | 68.81 | 4.69   | 15.04                                                         | 7.18—7.32 (4H, m), 7.42—7.46 (2H, m), 7.65 (4H, s), 7.74 (1H, s), 8.33 (1H, s), 10.5 (1H, s)                                                                                                  |
|            |                                                                  |                                  | (68.62                                                          | 4.83  | 14.94) |                                                               |                                                                                                                                                                                               |
| <b>19g</b> | 96                                                               | 157—160<br>(EA-IPE)              | C <sub>15</sub> H <sub>12</sub> N <sub>4</sub> O <sub>2</sub>   | 64.28 | 4.31   | 19.98                                                         | 7.24—7.49 (5H, m), 7.68 (2H, d, <i>J</i> =9 Hz), 7.82 (2H, d, <i>J</i> =9 Hz), 8.22 (1H, s), 9.22 (1H, s), 10.48 (1H, br)                                                                     |
|            |                                                                  |                                  | (64.24                                                          | 4.31  | 19.95) |                                                               |                                                                                                                                                                                               |
| <b>19h</b> | 90                                                               | 143—144<br>(DCM-IPE)             | C <sub>15</sub> H <sub>12</sub> N <sub>4</sub> O <sub>2</sub>   | 64.28 | 4.31   | 19.98                                                         | 7.22—7.49 (5H, m), 7.70 (2H, d, <i>J</i> =9 Hz), 8.00 (2H, d, <i>J</i> =9 Hz), 8.09 (2H, s), 10.49 (1H, br)                                                                                   |
|            |                                                                  |                                  | (64.09                                                          | 4.29  | 19.99) |                                                               |                                                                                                                                                                                               |
| <b>19i</b> | 97                                                               | 195—200<br>(EA)                  | C <sub>15</sub> H <sub>12</sub> N <sub>4</sub> O <sub>2</sub>   | 64.28 | 4.31   | 19.98                                                         | 7.24—7.49 (5H, m), 7.72 (2H, d, <i>J</i> =9 Hz), 7.88 (2H, d, <i>J</i> =9 Hz), 7.96 (1H, s), 8.76 (1H, s), 10.53 (1H, br)                                                                     |
|            |                                                                  |                                  | (64.38                                                          | 4.32  | 20.02) |                                                               |                                                                                                                                                                                               |
| <b>19j</b> | 89                                                               | 164—165<br>(EA-IPE)              | C <sub>14</sub> H <sub>11</sub> N <sub>3</sub> O <sub>2</sub>   | 59.78 | 3.94   | 24.90                                                         | 7.26—7.33 (3H, m), 7.43—7.51 (2H, m), 7.81 (2H, d, <i>J</i> =9 Hz), 8.10 (2H, d, <i>J</i> =9 Hz), 9.21 (1H, s), 10.7 (1H, s)                                                                  |
|            |                                                                  |                                  | (59.64                                                          | 4.03  | 24.84) |                                                               |                                                                                                                                                                                               |
| <b>19k</b> | 88                                                               | 186—189<br>(EA)                  | C <sub>14</sub> H <sub>11</sub> N <sub>3</sub> O <sub>2</sub>   | 59.78 | 3.94   | 24.90                                                         | 7.25—7.34 (3H, m), 7.42—7.50 (2H, m), 7.77 (2H, d, <i>J</i> =9 Hz), 7.89 (2H, d, <i>J</i> =9 Hz), 10.0 (1H, s), 10.6 (1H, s)                                                                  |
|            |                                                                  |                                  | (59.56                                                          | 3.99  | 24.89) |                                                               |                                                                                                                                                                                               |
| <b>20a</b> | 84                                                               | —                                | C <sub>11</sub> H <sub>12</sub> N <sub>4</sub> O <sub>8</sub>   | 53.21 | 4.87   | 22.56                                                         | 2.70 (3H, s), 4.35 (2H, br), 7.42 (1H, br), 7.69 (2H, d, <i>J</i> =8.4 Hz), 7.72 (1H, s), 7.80 (2H, d, <i>J</i> =8.4 Hz), 8.71 (1H, br)                                                       |
|            |                                                                  |                                  | (—)                                                             |       |        |                                                               |                                                                                                                                                                                               |
| <b>20b</b> | 92                                                               | 251—253<br>(ET-W)                | C <sub>11</sub> H <sub>12</sub> N <sub>4</sub> O <sub>2</sub>   | 56.89 | 5.21   | 24.12                                                         | 2.45 (3H, s), 4.38 (2H, br), 7.47 (1H, br), 7.59 (4H, s), 8.32 (1H, s), 8.72 (1H, br s)                                                                                                       |
|            |                                                                  |                                  | (—)                                                             |       |        |                                                               |                                                                                                                                                                                               |
| <b>20c</b> | 98                                                               | 243—244<br>(ET)                  | C <sub>10</sub> H <sub>12</sub> N <sub>6</sub> O                | 51.72 | 5.21   | 36.19                                                         | 4.33 (2H, br), 5.54 (2H, s), 7.16 (2H, d, <i>J</i> =8.4 Hz), 7.40 (1H, br), 7.49 (2H, d, <i>J</i> =8.4 Hz), 7.78 (2H, s), 8.65 (1H, br s)                                                     |
|            |                                                                  |                                  | (—)                                                             |       |        |                                                               |                                                                                                                                                                                               |
| <b>20d</b> | 97                                                               | —                                | C <sub>10</sub> H <sub>12</sub> N <sub>6</sub> O                | 51.72 | 5.21   | 36.19                                                         | 4.33 (2H, br), 5.51 (2H, s), 7.20 (2H, d, <i>J</i> =8.6 Hz), 7.42 (1H, br), 7.51 (2H, d, <i>J</i> =8.6 Hz), 7.71 (1H, d, <i>J</i> =1 Hz), 8.12 (1H, d, <i>J</i> =1 Hz), 8.67 (1H, br s)       |
|            |                                                                  |                                  | (—)                                                             |       |        |                                                               |                                                                                                                                                                                               |
| <b>20e</b> | 95                                                               | 259—261<br>(ET)                  | C <sub>10</sub> H <sub>11</sub> N <sub>5</sub> O                | 55.29 | 5.10   | 32.24                                                         | 4.37 (2H, br), 6.49 (1H, t, <i>J</i> =2.4 Hz), 7.47 (1H, br), 7.61—7.71 (5H, m), 8.36 (1H, d, <i>J</i> =2.4 Hz), 8.77 (1H, br)                                                                |
|            |                                                                  |                                  | (55.26                                                          | 4.88  | 32.31) |                                                               |                                                                                                                                                                                               |
| <b>20f</b> | 78                                                               | 202—203<br>(ET-W)                | C <sub>10</sub> H <sub>11</sub> N <sub>5</sub> O                | 55.29 | 5.10   | 32.24                                                         | 4.41 (2H, br), 7.09 (1H, s), 7.50 (2H, d, <i>J</i> =9 Hz), 7.52 (1H, s), 7.65 (1H, s), 7.69 (2H, d, <i>J</i> =9 Hz), 8.15 (1H, s), 8.85 (1H, s)                                               |
|            |                                                                  |                                  | (55.05                                                          | 5.08  | 32.23) |                                                               |                                                                                                                                                                                               |
| <b>20g</b> | 95                                                               | 228—232<br>(ET)                  | C <sub>9</sub> H <sub>10</sub> N <sub>6</sub> O                 | 49.54 | 4.62   | 38.51                                                         | 4.42 (2H, br), 7.54 (1H, br), 7.72 (4H, br), 8.18 (1H, s), 8.89 (1H, s), 9.16 (1H, s)                                                                                                         |
|            |                                                                  |                                  | (49.72                                                          | 4.49  | 38.41) |                                                               |                                                                                                                                                                                               |
| <b>20h</b> | 96                                                               | 275—277<br>(ET)                  | C <sub>9</sub> H <sub>10</sub> N <sub>6</sub> O                 | 49.54 | 4.62   | 38.51                                                         | 4.39 (2H, br), 7.53 (1H, br), 7.73 (2H, d, <i>J</i> =9 Hz), 7.88 (2H, d, <i>J</i> =9 Hz), 8.04 (2H, s), 8.88 (1H, br)                                                                         |
|            |                                                                  |                                  | (49.67                                                          | 4.48  | 38.47) |                                                               |                                                                                                                                                                                               |
| <b>20i</b> | 98                                                               | 225—234<br>(ET)                  | C <sub>9</sub> H <sub>10</sub> N <sub>6</sub> O                 | 49.54 | 4.62   | 38.51                                                         | 4.40 (2H, br), 7.55 (1H, br), 7.76 (4H, br), 7.92 (1H, s), 8.70 (1H, s), 8.92 (1H, s)                                                                                                         |
|            |                                                                  |                                  | (49.73                                                          | 4.64  | 38.74) |                                                               |                                                                                                                                                                                               |
| <b>20j</b> | 98                                                               | 203—210<br>(dec.)                | C <sub>8</sub> H <sub>9</sub> N <sub>7</sub> O                  | 43.83 | 4.14   | 44.73                                                         | 4.42 (2H, br), 7.62 (1H, br), 7.84 (2H, d, <i>J</i> =9 Hz), 7.97 (2H, d, <i>J</i> =9 Hz), 9.06 (1H, s), 9.18 (1H, s)                                                                          |
|            |                                                                  | (ET-W)                           | (43.75                                                          | 4.25  | 44.85) |                                                               |                                                                                                                                                                                               |
| <b>21a</b> | [This compound was used for the next step without purification.] |                                  |                                                                 |       |        |                                                               |                                                                                                                                                                                               |

Table 5. (continued)

| Compd.     | Yield (%) | mp (°C)<br>(Solv.) <sup>a)</sup> | Analysis (%)                                                                                                                    |                                                                                                                                                                                                                                                                                 |   | <sup>1</sup> H-NMR (in DMSO- <i>d</i> <sub>6</sub> ) δ |
|------------|-----------|----------------------------------|---------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---|--------------------------------------------------------|
|            |           |                                  | Calcd                                                                                                                           | (Found)                                                                                                                                                                                                                                                                         |   |                                                        |
|            |           |                                  | C                                                                                                                               | H                                                                                                                                                                                                                                                                               | N |                                                        |
| <b>21b</b> | 88        | 124—126<br>(ET-IPE)              | C <sub>18</sub> H <sub>25</sub> N <sub>3</sub> O <sub>4</sub><br>62.23 7.25 12.10<br>(—)                                        | 1.15 (6H, t, <i>J</i> =7 Hz), 2.44 (3H, s), 3.19 (2H, t, <i>J</i> =6 Hz), 3.42—3.72 (4H, m), 4.50 (1H, t, <i>J</i> =6 Hz), 6.15 (1H, t, <i>J</i> =6 Hz), 7.42 (2H, d, <i>J</i> =8.6 Hz), 7.60 (2H, d, <i>J</i> =8.6 Hz), 8.31 (1H, s), 7.70 (1H, s)                             |   |                                                        |
| <b>21c</b> | 91        | 111—112<br>(IPE-H)               | C <sub>17</sub> H <sub>25</sub> N <sub>3</sub> O <sub>3</sub><br>58.77 7.25 20.16<br>(—)                                        | 1.21 (6H, t, <i>J</i> =7 Hz), 3.30—3.80 (6H, m), 4.53 (1H, t, <i>J</i> =5 Hz), 5.13 (1H, br), 5.55 (2H, s), 6.93 (1H, br), 7.20—7.30 (4H, m), 7.62 (2H, s) (in CDCl <sub>3</sub> )                                                                                              |   |                                                        |
| <b>21d</b> | 98        | 89—91<br>(IPE)                   | C <sub>17</sub> H <sub>25</sub> N <sub>3</sub> O <sub>3</sub><br>58.77 7.25 20.16<br>(—)                                        | 1.21 (6H, t, <i>J</i> =7 Hz), 3.34—3.80 (7H, m), 4.55 (1H, t, <i>J</i> =5 Hz), 5.47 (1H, br), 5.48 (2H, s), 7.15 (2H, d, <i>J</i> =8.6 Hz), 7.32 (2H, d, <i>J</i> =8.6 Hz), 7.40 (1H, br), 7.49 (1H, s), 7.71 (1H, s) (in CDCl <sub>3</sub> )                                   |   |                                                        |
| <b>21e</b> | 97        | 132—133<br>(IPE)                 | C <sub>16</sub> H <sub>22</sub> N <sub>4</sub> O <sub>3</sub><br>60.36 6.96 15.08<br>(60.28 6.88 17.68)                         | 1.15 (6H, t, <i>J</i> =7 Hz), 3.20 (2H, t, <i>J</i> =5.4 Hz), 3.35—3.68 (4H, m), 4.51 (1H, t, <i>J</i> =5.4 Hz), 6.14 (1H, t, <i>J</i> =5.4 Hz), 6.48 (1H, t, <i>J</i> =2.4 Hz), 7.50 (2H, d, <i>J</i> =9 Hz), 7.66—7.71 (3H, m), 8.36 (1H, d, <i>J</i> =2.4 Hz), 8.74 (1H, br) |   |                                                        |
| <b>21f</b> | 82        | 148—151<br>(ET-IPE)              | C <sub>16</sub> H <sub>22</sub> N <sub>4</sub> O <sub>3</sub><br>·1/2H <sub>2</sub> O<br>58.70 7.08 17.11<br>(58.31 6.77 16.81) | 1.15 (6H, t, <i>J</i> =7 Hz), 3.20 (2H, t, <i>J</i> =6 Hz), 3.43—3.70 (4H, m), 4.51 (1H, t, <i>J</i> =6 Hz), 6.21 (1H, t, <i>J</i> =6 Hz), 7.08 (1H, s), 7.51 (4H, s), 7.63 (1H, s), 8.13 (1H, s), 8.85 (1H, s)                                                                 |   |                                                        |
| <b>21g</b> | 94        | 139—140<br>(IPE-PE)              | C <sub>15</sub> H <sub>21</sub> N <sub>5</sub> O <sub>3</sub><br>56.41 6.63 21.93<br>(56.36 6.68 21.95)                         | 1.25 (6H, t, <i>J</i> =7.2 Hz), 3.43 (2H, t, <i>J</i> =5 Hz), 3.52—3.85 (4H, m), 4.57 (1H, t, <i>J</i> =5 Hz), 5.08—5.18 (1H, m), 7.16 (1H, br), 7.49 (2H, d, <i>J</i> =9.4 Hz), 7.57 (2H, d, <i>J</i> =9 Hz), 8.08 (1H, s), 8.48 (1H, s) (CDCl <sub>3</sub> )                  |   |                                                        |
| <b>21h</b> | 84        | 175—176<br>(IPE-H)               | C <sub>15</sub> H <sub>21</sub> N <sub>5</sub> O <sub>3</sub><br>56.41 6.63 21.93<br>(56.10 6.41 21.85)                         | 1.15 (6H, t, <i>J</i> =7 Hz), 3.21 (2H, t, <i>J</i> =5 Hz), 3.30—3.69 (4H, m), 4.52 (1H, t, <i>J</i> =5 Hz), 6.16—6.21 (1H, m), 7.57 (2H, d, <i>J</i> =9 Hz), 7.89 (2H, d, <i>J</i> =9 Hz), 8.05 (1H, s), 8.86 (1H, s)                                                          |   |                                                        |
| <b>21i</b> | 95        | 194—196<br>(ET)                  | C <sub>15</sub> H <sub>21</sub> N <sub>5</sub> O <sub>3</sub><br>56.41 6.63 21.93<br>(56.59 6.58 22.02)                         | 1.15 (6H, t, <i>J</i> =7 Hz), 3.21 (2H, t, <i>J</i> =5 Hz), 3.30—3.74 (4H, m), 4.52 (1H, t, <i>J</i> =5 Hz), 6.15—6.28 (1H, m), 7.58 (2H, d, <i>J</i> =9 Hz), 7.74 (2H, d, <i>J</i> =9 Hz), 7.91 (1H, s), 7.87 (1H, s), 8.89 (1H, s) (in CDCl <sub>3</sub> )                    |   |                                                        |
| <b>21j</b> | 92        | 116—117<br>(ET-IPE)              | C <sub>14</sub> H <sub>20</sub> N <sub>6</sub> O <sub>3</sub><br>52.49 6.29 26.23<br>(52.37 6.34 26.26)                         | 1.15 (6H, t, <i>J</i> =7 Hz), 3.23 (2H, t, <i>J</i> =6 Hz), 3.47—3.73 (4H, m), 4.53 (1H, t, <i>J</i> =6 Hz), 6.26 (1H, t, <i>J</i> =6 Hz), 7.67 (2H, d, <i>J</i> =9 Hz), 7.97 (2H, d, <i>J</i> =9 Hz), 9.03 (1H, s), 9.18 (1H, s)                                               |   |                                                        |
| <b>21k</b> | 99        | 169—170<br>(ET-IPE)              | C <sub>14</sub> H <sub>20</sub> N <sub>6</sub> O <sub>3</sub><br>52.49 6.29 26.23<br>(52.46 6.19 26.32)                         | 1.15 (6H, t, <i>J</i> =7 Hz), 3.22 (2H, t, <i>J</i> =6 Hz), 3.40—3.70 (4H, m), 4.52 (1H, t, <i>J</i> =6 Hz), 6.25 (1H, t, <i>J</i> =6 Hz), 7.64 (2H, d, <i>J</i> =9 Hz), 7.75 (2H, d, <i>J</i> =9 Hz), 8.98 (1H, s), 9.18 (1H, s)                                               |   |                                                        |

a) Recrystallization solvent: IPE, diisopropyl ether; DCM, dichloromethane; W, water; EA, ethyl acetate; ME, methanol; PE, petroleum ether; H, hexane. b) Not measured.

(27 g, 539 mmol) was added dropwise over a period of 15 min at 50 °C to a stirred mixture of the wet solid (**17j** and **17k**), FeCl<sub>3</sub> (0.33 g), activated carbon (3.3 g), methanol (MeOH, 280 ml) and THF (420 ml). The resulting mixture was refluxed with stirring for 10 h and cooled. The activated carbon was filtered off and washed with MeOH (200 ml). The filtrate and the washing were combined and evaporated *in vacuo*. The residue was purified by column chromatography on silica gel (THF–hexane, 2 : 3→THF–hexane–AcOEt, 1 : 1 : 1, v/v). The first eluted fraction was evaporated *in vacuo* and the residue was crystallized from hexane to give **18j** (14.5 g, 25% based on **16**) as a pale yellow crystalline powder. The second eluted fraction was evaporated *in vacuo* and the residue was crystallized from isoPr<sub>2</sub>O to give **18k** (25.3 g, 44% based on **16**) as a pale yellow crystalline powder. **18j**<sup>[6a]</sup>, mp 124—125 °C (recrystallized from AcOEt–isoPr<sub>2</sub>O). <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ: 5.76 (2H, s), 6.76 (2H, d, *J*=8.8 Hz), 7.74 (2H, d, *J*=8.8 Hz), 9.08 (1H, s). *Anal.* Calcd for C<sub>7</sub>H<sub>7</sub>N<sub>5</sub>: C, 52.17; H, 4.38; N, 43.45. Found: C, 52.01; H, 4.44; N, 43.41. **18k**<sup>[6a]</sup>, mp 142—143 °C (recrystallized from AcOEt–isoPr<sub>2</sub>O). <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ: 5.65 (2H, s), 6.73 (2H, d, *J*=8.8 Hz), 7.48 (2H, d, *J*=8.8 Hz), 9.83 (1H, s). *Anal.* Calcd for C<sub>7</sub>H<sub>7</sub>N<sub>5</sub>: C, 52.17; H, 4.38; N, 43.45. Found: C, 51.88; H, 4.38; N, 43.62.

**Phenyl 4-(1H-1,2,4-Triazol-1-yl)phenylcarbamate (19g; Table 5)** PhOCOCl (14.6 g, 93.5 mmol) was added dropwise to a stirred mixture of **18g** (13.6 g, 85 mmol), pyridine (7.4 g, 93.5 mmol) and CH<sub>2</sub>Cl<sub>2</sub> (200 ml) at 0 °C. After having been stirred for 30 min, the mixture was washed (water, brine) and concentrated *in vacuo*. The deposited crystals were collected by filtration and washed with AcOEt–isoPr<sub>2</sub>O to give **19g**<sup>[9]</sup> (22.8 g, 96%) as pale yellow crystals.

The phenyl carbamates (**19a—f, h—k**; Table 5) were prepared from the corresponding 4-substituted anilines (**18a—f, h—k**) by the same method as described above.

**4-[4-(1H-1,2,4-Triazol-1-yl)phenyl]semicarbazide (20g; Table 5)** A mixture of **19g** (22.8 g, 81.4 mmol), N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O (8.14 g, 163 mmol) and EtOH (150 ml) was stirred at 80 °C for 1 h. After having been cooled, the precipitate was collected by filtration and washed with cooled EtOH to give

**20g** (16.8 g, 95%) as colorless crystals.

The semicarbazides (**20a—f, h—j**; Table 5) were prepared from the corresponding phenyl carbamates (**19a—f, h—j**) by the same method as described above.

**1-(2,2-Diethoxyethyl)-3-[4-(1H-1,2,4-triazol-1-yl)phenyl]urea (21g; Table 5)** A mixture of **19g** (13 g), pyridine (3.67 g) and (EtO)<sub>2</sub>CHCH<sub>2</sub>NH<sub>2</sub> (7.4 g) was heated at 50 °C for 3 h. After having been cooled, the precipitate was collected by filtration and washed with a mixture of isoPr<sub>2</sub>O–petroleum ether (1 : 1, v/v; 100 ml×2) to give **21g** (14.5 g, 94%) as a colorless crystalline powder.

The ureas (**21a—f, h—k**; Table 5) were prepared from the corresponding phenyl carbamates (**19a—f, h—k**) by the same method as described above.

**4-(1H-1,2,4-Triazol-1-yl)phenyl-3-(2H,4H)-1,2,4-triazolone (5g; Table 2)** A mixture of HN=CH–NH<sub>2</sub>·AcOH (19 g, 183 mmol), **20g** (8 g, 36.6 mmol) and DMF (200 ml) was stirred for 2 h. AcOH (19 g, 183 mmol) was added, and the resulting mixture was heated at 80 °C for 9 h. The whole was evaporated *in vacuo*, and the residue was poured into water. The precipitate was collected by filtration and recrystallized from DMF–water to give **5g** (3.34 g, 40%) as colorless needles.

The triazolones **5a—f, h—j** (Table 2) were prepared from the corresponding semicarbazides (**20a—f, h—j**) by the same method as described above.

**1-[4-(1H-1,2,4-Triazol-1-yl)phenyl]-2-(1H,3H)-imidazolone (6g; Table 3)** Compound **21g** (14.5 g, 43.5 mmol) was dissolved in a mixture of MeOH (214 ml) and water (85 ml), and then 0.48 N HCl (104 ml) was added to the solution. The mixture was stirred for 14 h, and the resulting precipitate was collected by filtration to give **6g** (9.01 g, 92%) as a colorless crystalline powder.

The imidazolones **6a—f, h—k** (Table 3) were prepared from the corresponding carbamates (**19a—f, h—k**) by the same method as described above.

**2-[(1R,2R)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1H-1,2,4-triazol-1-yl)propyl]-4-[4-(2-methyl-4-oxazolyl)phenyl]-3-(2H,4H)-1,2,4-triazolone (1b; Table 1)** A mixture of **4** (0.5 g), **5b** (0.56 g), K<sub>2</sub>CO<sub>3</sub> (powder:

1.38 g), NMP (5 ml) and DMF (4 ml) was stirred at 90 °C for 2 h. After having been cooled, the whole was worked up (AcOEt; water, 0.5 N NaOH, 1 N HCl, brine). The residue was purified by silica gel column chromatography (AcOEt→AcOEt–MeOH, 9:1, v/v) to give **1b** (0.33 g, 33%) as a colorless crystalline powder.

**2-[(1R,2R)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1H-1,2,4-triazol-1-yl)propyl]-4-[4-(1H-1,2,3-triazol-1-ylmethyl)phenyl]-3(2H,4H)-1,2,4-triazolone (1d: Table 1)** A mixture of **4** (1.25 g, 5.0 mmol), **5d** (1.44 g, 6.0 mmol), K<sub>2</sub>CO<sub>3</sub> (powder: 3.0 g, 21 mmol) and DMF (60 ml) was heated at 80 °C with stirring for 26 h. After having been cooled, the mixture was filtered to remove the insoluble substance. The filtrate was concentrated *in vacuo* and AcOEt (300 ml) was added. The resulting precipitate was filtered off, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt–MeOH, 20:1, v/v) to give **1d** (0.6 g, 24%) as a colorless crystalline powder.

The reaction of **4** with **5a** was carried out by the same method as described above to obtain **1a** (Table 1).

**2-[(1R,2R)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1H-1,2,4-triazol-1-yl)propyl]-4-[4-(1H-1-imidazolyl)phenyl]-3(2H,4H)-1,2,4-triazolone (1f: Table 1)** NaH (70% oil dispersion, 150 mg, 4.5 mmol) was added to a mixture of **5f** (1140 mg, 5 mmol) and NMP (30 ml). The mixture was stirred for 1.5 h, and then **4** (1000 mg, 4.0 mmol) was added. The resulting mixture was stirred at 80 °C for 19 h under an argon atmosphere. After having been cooled, the whole was worked up (AcOEt; water). The residue was purified by silica gel column chromatography [AcOEt–MeOH, 4:1, v/v] to give **1f** (400 mg, 21%) as a colorless amorphous powder.

The reaction of **4** with **5c**, **e**, **j** was carried out by the same method as described above to obtain the corresponding triazolone derivatives (**1c**, **e**, **j**; Table 1).

**2-[(1R,2R)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1H-1,2,4-triazol-1-yl)propyl]-4-[4-(1H-1,2,4-triazol-1-yl)phenyl]-3(2H,4H)-1,2,4-triazolone (1g: Table 1)** A mixture of NaH (70% oil dispersion, 160 mg, 4.0 mmol) and DMSO (40 ml) was stirred at 80 °C for 30 min. Compound **5g** (910 mg, 4 mmol) was added, and the mixture was stirred for 5 min. Next, compound **4** (1000 mg, 4.0 mmol) was added and the resulting mixture was stirred at 80 °C for 24 h under an argon atmosphere. After having been cooled, the whole was worked up (AcOEt; water, brine). The residue was purified by silica gel column chromatography (CH<sub>2</sub>Cl<sub>2</sub>–MeOH, 10:1, v/v) to give **1g** (540 mg, 28%) as a colorless crystalline powder.

The reaction of **4** with **5h** and **5i** was carried out by the same method as described above to obtain the corresponding triazolone derivatives (**1h**, **i**; Table 1).

**1-[(1R,2R)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1H-1,2,4-triazol-1-yl)propyl]-3-[4-(1H-1,2,4-triazol-1-yl)phenyl]-2(1H,3H)-imidazolone (2g: Table 1)** A mixture of **4** (2.5 g), **6a** (2.72 g), Cs<sub>2</sub>CO<sub>3</sub> (powder: 9.7 g) and DMF (150 ml) was stirred at 80 °C for 9.5 h. After having been cooled, the whole was worked up (AcOEt; water, 0.5 N NaOH, 1 N HCl, brine). The residue was purified by silica gel column chromatography (AcOEt–acetone, 2:1, v/v) to give **2g** (1.03 g, 22%) as a pale yellow amorphous powder.

**1-[(1R,2R)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1H-1,2,4-triazol-1-yl)propyl]-3-[4-(1H-1,2,3-triazol-1-yl)phenyl]-2(1H,3H)-imidazolone (2i: Table 1)** Compound **4** (2.51 g) was allowed to react with **6i** (2.72 g) in the presence of NaH (70% oil dispersion, 0.4 g) in a manner similar to that described for the synthesis of **1f**, and the product was purified by silica gel column chromatography (AcOEt→AcOEt–acetone, 5:1, v/v) followed by crystallization from acetone–AcOEt–isoPr<sub>2</sub>O to give **2i** (1.82 g, 38%) as a pale yellow crystalline powder.

The reaction of **4** with **6a–f**, **h**, **j** was carried out in the same manner as described above to obtain the corresponding imidazolone derivatives (**2a–f**, **h**, **j**; Table 1).

**1-[(1R,2S)-2-(2,4-Difluorophenyl)-2,3-epoxy-1-methylpropyl]-3-[4-(1H-1-tetrazolyl)phenyl]-2(1H,3H)-imidazolone (9)** Route I: Tf<sub>2</sub>O (0.49 ml) was added dropwise to a stirred solution of **7<sup>3(g)</sup>** (1.20 g) and isoPr<sub>2</sub>NET (1.15 ml) in CH<sub>2</sub>Cl<sub>2</sub> (26 ml) over a period of 5 min at –78 °C under a nitrogen atmosphere. The resulting mixture was stirred for 20 min at –78 °C and then for 15 min at –20 °C. The mixture was diluted with hexane (26 ml), and the whole was evaporated to about 9 ml *in vacuo* at –10 °C. The residue was submitted to flash chromatography on silica gel (CH<sub>2</sub>Cl<sub>2</sub>–hexane, 1:1, v/v). The eluates containing the triflate **8<sup>3(e–h)</sup>** were combined and concentrated to about 20 ml. This solution was added to a stirred mixture of **6k** (0.94 g), NaH (72% in oil, 0.126 g), DMF (20 ml), DMSO (10 ml) and THF (10 ml) at –30 °C. The resulting mixture was stirred at –30 °C for 20 min and at 0 °C for further 40 min. The whole was worked up (AcOEt; water,

brine) and the residue was purified by chromatography on silica gel (hexane–AcOEt, 1:3, v/v) to give **9** (0.13 g, 7.7% based on **6k**) as a colorless crystalline powder. mp 205–207 °C (recrystallized from hexane–AcOEt). <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 1.39 (3H, d, *J*=7.2 Hz), 2.74 (1H, d, *J*=4.6 Hz), 2.83 (1H, d, *J*=4.6 Hz), 5.10 (1H, q, *J*=7.2 Hz), 6.53 (1H, d, *J*=3.2 Hz), 6.67 (1H, d, *J*=3.2 Hz), 6.83–6.96 (2H, m), 7.40–7.48 (1H, m), 7.79 (2H, d, *J*=9 Hz), 7.95 (2H, d, *J*=9 Hz), 9.03 (1H, s). SIMS (MH<sup>+</sup>): 411. IR (KBr): 3080, 1678, 1615, 1520, 1500, 1420 cm<sup>–1</sup>. [α]<sub>D</sub><sup>25</sup> = –22.6° (*c*=0.36, MeOH).

Route II: A mixture of **6k** (23.1 g), NaH (72% in oil, 3.3 g) and NMP (300 ml) was stirred for 1 h. The resulting solution was cooled in an ice bath and added dropwise to a solution of **11<sup>3(h)</sup>** (32.3 g) in THF (200 ml) over a period of 20 min at –30 °C under a nitrogen atmosphere. After having been stirred for 30 min at –20 °C, the whole was worked up (AcOEt; water, 1 N HCl, brine). The residue was purified by silica gel column chromatography (hexane–AcOEt–AcOH, 1:2:0.03→1:3:0.04, v/v) and crystallized from AcOEt to give **12** (13.8 g, 34%) as a colorless crystalline powder. mp 140–141 °C. Anal. Calcd for C<sub>19</sub>H<sub>14</sub>F<sub>2</sub>N<sub>6</sub>O<sub>2</sub>: C, 57.58; H, 3.56; N, 21.20. Found: C, 57.40; H, 3.58; N, 21.03. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 1.66 (3H, d, *J*=7.2 Hz), 5.77 (1H, q, *J*=7.2 Hz), 6.66 (1H, d, *J*=3.2 Hz), 6.73 (1H, d, *J*=3.2 Hz), 6.88–7.05 (2H, m), 7.76 (2H, d, *J*=8.8 Hz), 7.90 (2H, d, *J*=8.8 Hz), 7.95–8.07 (1H, m), 8.99 (1H, s). [α]<sub>D</sub><sup>25</sup> = +11.8° (*c*=1.0, THF).

A mixture of isoPrOSi(Me<sub>2</sub>)CH<sub>2</sub>Cl (8.34 g), magnesium (Mg, turnings, 1.22 g) and THF (50 ml) was refluxed. After having been cooled, the mixture was diluted with THF (12.5 ml) to obtain a 0.8 M solution of the Grignard reagent. This 0.8 M solution (6.31 ml) was added dropwise to a solution of **12** (1.00 g) in THF (20 ml) over a period of 5 min at –5 °C. The resulting mixture was stirred for 3 h at –5 °C and diluted with a cooled saturated aqueous solution of ammonium chloride (aq. NH<sub>4</sub>Cl, 20 ml) and ice water (20 ml). The whole was worked up (AcOEt; brine) and the residue was purified by silica gel column chromatography (hexane–AcOEt, 3:1→2:1, v/v). The product was recrystallized from isoPr<sub>2</sub>O–hexane (1:2, v/v, 15 ml) to obtain **13** (0.27 g, 20%) as colorless needles. mp 124–135 °C. Anal. Calcd for C<sub>24</sub>H<sub>30</sub>F<sub>2</sub>N<sub>6</sub>O<sub>3</sub>Si: C, 56.80; H, 5.72; N, 15.90. Found: C, 56.48; H, 5.79; N, 15.00. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ: –0.27 (3H, s), –0.26 (3H, s), 0.96–1.07 (10H, m), 1.57 (1H, d, *J*=17 Hz), 3.81 (1H, quintet, *J*=6 Hz), 4.70 (1H, q, *J*=7 Hz), 5.27 (1H, br s), 6.89 (1H, d, *J*=3 Hz), 7.13–7.30 (2H, m), 7.28 (1H, d, *J*=3 Hz), 7.65–7.78 (1H, m), 8.02 (2H, d, *J*=9 Hz), 8.12 (2H, d, *J*=9 Hz), 10.13 (1H, s). IR (KBr): 1670, 1610, 1520, 1500 cm<sup>–1</sup>.

A 30% aqueous solution of H<sub>2</sub>O<sub>2</sub> (3.86 ml) and NaHCO<sub>3</sub> (0.32 g) were added to a solution of **13** (2.00 g) in MeOH–THF (1:1, v/v, 20 ml). The mixture was heated for 3 h at 50 °C. After having been cooled, the whole was worked up [AcOEt; water, aqueous solution of sodium thiosulfate (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>), brine]. The residue was purified by silica gel column chromatography (hexane–AcOEt, 1:2→1:3, v/v), and crystallized from isoPr<sub>2</sub>O to give **14** (1.28 g, 79%) as a colorless crystalline powder. mp 204–210 °C. Anal. Calcd for C<sub>20</sub>H<sub>18</sub>F<sub>2</sub>N<sub>6</sub>O<sub>3</sub>: C, 56.07; H, 4.24; N, 19.62. Found: C, 55.73; H, 4.30; N, 19.05. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 1.27 (3H, d, *J*=7 Hz), 2.35 (1H, t, *J*=6 Hz), 3.80 (1H, dd, *J*=12 Hz, 6 Hz), 3.97 (1H, dd, *J*=12 Hz, 6 Hz), 4.40–4.65 (1H, br), 4.86 (1H, q, *J*=7 Hz), 6.61 (1H, d, *J*=3.2 Hz), 6.70 (1H, d, *J*=3.2 Hz), 6.79–7.00 (2H, m), 7.69–7.83 (1H, m), 7.80 (1H, d, *J*=9 Hz), 7.94 (2H, d, *J*=9 Hz), 9.01 (1H, s).

MsCl (0.39 ml) and triethylamine (Et<sub>3</sub>N, 0.51 g) were added dropwise to a mixture of **14** (1.31 g), AcOEt (10 ml) and THF (50 ml) at 0 °C. After having been stirred at 0 °C for 30 min, the whole was worked up (AcOEt; water, brine). The residue was dissolved in DMF (15 ml). K<sub>2</sub>CO<sub>3</sub> (0.70 g) was added to the solution, and the mixture was heated at 40 °C for 1 h. After having been cooled, the whole was worked up (AcOEt–THF; aq. NH<sub>3</sub>Cl, brine). The residue was purified by silica gel column chromatography (hexane–AcOEt, 1:1→2:3, v/v) and crystallized from AcOEt–Et<sub>2</sub>O to give **9** (0.78 g, 62%) as a colorless crystalline powder, which was identical with the authentic sample prepared above.

**1-[(1R,2R)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1H-1,2,4-triazol-1-yl)propyl]-3-[4-(1H-1-tetrazolyl)phenyl]-2(1H,3H)-imidazolone (2k: Table 1)** Method B: 1H-1,2,4-Triazole (42 mg) was added to a stirred mixture of NaH (72% in oil, 17 mg) and DMF (3 ml) at 0 °C, and the mixture was stirred for 40 min. A solution of **9** (0.205 g) in DMF (2 ml) was added and the resulting mixture was stirred for 6 h at 50 °C. The whole was worked up (AcOEt; water, brine) and the residue was chromatographed on silica gel (AcOEt) to give **2k** (0.15 g, 63%).

**1-[(1R,2R)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1H-1,2,4-triazol-1-yl)propyl]-3-[4-(1H-1,2,4-triazol-1-yl)phenyl]-2-imidazolidinone (3g: Table 1)** A solution of **2g** (0.5 g) in AcOH (25 ml) was hydrogenated over 10% Pd–C (0.2 g) under atmospheric pressure for 3 h and then at 50 °C

for a further 3 h. The catalyst was removed by filtration, and the filtrate was concentrated *in vacuo*. The residue was purified by chromatography on silica gel (AcOEt–acetone, 5 : 1→2 : 1, v/v) to give **3g** (0.37 g, 74%) as a colorless crystalline powder.

Catalytic hydrogenation of **2e**, **f**, **h**–**k** was carried out by the same method as described above to obtain the corresponding imidazolidinones (**3e**, **f**, **h**–**k**; Table 1).

**Antifungal Activity** *In vitro* antifungal activities against *C. albicans* and *C. neoformans* were measured by the following method: MICs were determined by an agar dilution method using RPMI-1640 medium (Gibco BRL, Grand Island, N.Y.). A double concentration of RPMI-1640 medium was prepared with 0.3 M morpholinepropanesulfonic acid (MOPS; Dojindo, Tokyo, Japan) buffer (pH 7.0), sterilized by filtration through a membrane filter (pore size, 0.45  $\mu$ m), and mixed with an equal volume of 2.0% agar (Wako, Osaka, Japan) which had been autoclaved at 121 °C for 15 min and kept at 55 °C. The agar medium (9.9 ml) was then poured into petri dishes containing 0.1 ml of serial dilutions of antifungal agents dissolved in DMSO (Wako) and allowed to solidify. About 10<sup>3</sup> CFU of fungal cells suspended in saline was inoculated with a multiple inoculator (Sakuma, Tokyo, Japan) onto the agar plates prepared as described above. The plates were then incubated in a CO<sub>2</sub> incubator at 35 °C for 20 h. After the MICs for *C. albicans* were determined, the plates were incubated in a CO<sub>2</sub> incubator for an additional 48 h to determine the MIC for *C. neoformans*. The MIC was defined as the lowest concentration of antifungal agent giving no visible growth or causing almost complete inhibition of growth.

*In vitro* antifungal activity against *A. fumigatus* was measured by the following method: MICs were determined by an agar dilution method using RPMI-1640 medium. A double concentration of RPMI-1640 medium was prepared with 0.3 M MOPS buffer (pH 7.0), sterilized by filtration through a membrane filter (pore size, 0.45  $\mu$ m), and mixed with an equal volume of 2.0% agar which had been autoclaved at 121 °C for 15 min and kept at 55 °C. The agar medium (9.9 ml) was then poured into petri dishes containing 0.1 ml of serial dilutions of antifungal agents dissolved in DMSO and allowed to solidify. About 10<sup>3</sup> CFU of fungal cells suspended in saline was inoculated with a multiple inoculator onto the agar plates prepared as described above. The plates were then incubated in an ordinary incubator at 35 °C for 20 h. The MIC was defined as the lowest concentration of antifungal agent giving no visible growth.

ED<sub>50</sub> values of the compounds against candidiasis were determined by the method described in our preceding report.<sup>3a)</sup>

*In vivo* antifungal activity against *A. fumigatus* was measured by the following method: six-week-old CDF<sub>1</sub> female mice were infected intravenously with 2×10<sup>4</sup>–5×10<sup>4</sup> CFU of *A. fumigatus* 437 per mouse 4 d after the intraperitoneal administration of 200 mg/kg of cyclophosphamide. The test compound was administered orally (*p.o.*) 4 h after infection and twice daily on the following 2 d. ED<sub>50</sub> values were calculated by the method of Reed and Muench<sup>20)</sup> from survival rates on day 10 after infection.

**Acknowledgments** We thank Dr. S. Kishimoto and Dr. A. Miyake for their encouragement throughout this work. We also thank Ms. J. Obita, Ms. N. Kitamoto and Mr. T. Yoshida for the biological assays.

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# Optically Active Antifungal Azoles. XI.<sup>1)</sup> An Alternative Synthetic Route for 1-[(1*R*,2*R*)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-3-[4-(1*H*-1-tetrazolyl)phenyl]-2-imidazolidinone (TAK-456) and Its Analog

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Received July 3, 2000; accepted August 28, 2000

New routes for the synthesis of the optically active antifungal triazoles 1-[(1*R*,2*R*)-2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-3-[4-(1*H*-1-tetrazolyl)phenyl]-2-imidazolidinone (**1b**) and the 3-[4-(1*H*-1,2,3-triazol-1-yl)phenyl]-2-imidazolidinone analog (**1a**) that possess an imidazolidine nucleus were established. The key synthetic intermediates, (2*R*,3*R*)-3-(2,2-diethoxyethyl)amino-2-(2,4-difluorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)-2-butanol (**8**) and (2*R*,3*R*)-2-(2,4-difluorophenyl)-3-(2-hydroxyethyl)amino-1-(1*H*-1,2,4-triazol-1-yl)-2-butanol (**14**), were prepared by the ring-opening reaction of the oxirane (**2**) with the corresponding 2-substituted ethylamines. The acetal (**8**) was converted to the imidazolidinones (**1a**, **b**) by condensation with the carbamates (**10a**, **b**) followed by treatment with hydrochloric acid and subsequent catalytic hydrogenation. The candidate selected for the clinical trials, **1b** (TAK-456), was alternatively prepared from the hydroxyethylamino intermediate (**14**) via two reaction steps: condensation with the carbamate (**10b**) to the urea (**15**) and subsequent cyclization to the imidazolidinones. This newly developed synthetic route could be applied to a large scale preparation of **1b**.

**Key words** TAK-456; optically active antifungal azoles; 1,2,3-trisubstituted-2-butanol; imidazolidinone; practical synthesis

We have recently reported the synthesis and antifungal activity of imidazolidinone derivatives with the general formula **1** (Chart 1).<sup>1)</sup> These compounds exhibited strong growth inhibitory activity against not only yeasts such as *Candida albicans* and *Cryptococcus neoformans* but also against molds such as *Aspergillus fumigatus*, as well as potent protective effects against candidiasis and aspergillosis in mice. From these imidazolidinones, 1-[(1*R*,2*R*)-2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-3-[4-(1*H*-1,2,3-triazol-1-yl)phenyl]-2-imidazolidinone (**1a**) and 1-[(1*R*,2*R*)-2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-3-[4-(1*H*-1-tetrazolyl)phenyl]-2-imidazolidinone (**1b**) were selected for further biological evaluation to determine a candidate for clinical trials.

As we have previously described,<sup>1)</sup> compounds **1a** and **1b** were prepared from the optically active synthetic intermediates, **2**, **4** and **5**, which were derived from (*R*)- and (*S*)-lactic acid esters as shown in Chart 2. Compound **1a** was obtained in an overall yield of 33% from the oxirane **2** by ring opening with **7a** followed by catalytic reduction, and **1b** was obtained via *S<sub>N</sub>2* displacement with the anion derived from **7b** in either four steps from the oxiraneethanol **4** or seven steps from the hydroxypropiophenone **5** in overall yields of 1.7% and 0.9%, respectively. Although these procedures were useful for the synthesis of a wide variety of optically active 1,2,3-trisubstituted-2-butanols,<sup>1-3)</sup> the overall yields for the synthesis of **1a** and **1b** by these routes are low. We therefore needed to develop a higher-yielding synthetic procedure to supply sufficient quantities required for the detailed biological evaluation of **1a** and **1b**. In this paper we describe a new route for the efficient synthesis of the novel antifungal agents, **1a** and **1b**, starting from the oxirane **2**.

The main cause of the low yield in our previous synthesis was considered to be the low reactivity of the anion of the imidazolones **7a** and **7b** to nucleophilic substitution. Therefore, we devised an alternative retrosynthetic pathway, in which the imidazolone- or imidazolidinone ring is formed by intramolecular cyclization after introduction of the appropriate functionality into the butanol framework (Chart 3). Thus the 2-substituted ethylamine moiety is first introduced into the 3-position of the oxirane **2** to give the aminoalcohol III whose secondary amino group is sufficiently basic to allow addition of an *N*-substituted carbamoyl group to give the urea II. Intramolecular cyclization utilizing the functional groups (*R*<sup>1</sup> and *R*<sup>2</sup>) then allows synthesis of the desired skeleton I.

The synthetic routes initially investigated are shown in Chart 4. The oxirane **2** was allowed to react with 2,2-diethoxyethylamine [H<sub>2</sub>NCH<sub>2</sub>CH(OEt)<sub>2</sub>] in the presence of titanium tetrakisopropoxide [Ti(O-isoPr)<sub>4</sub>]<sup>4)</sup> in propanol (PrOH)

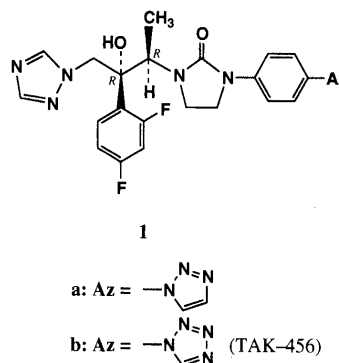


Chart 1

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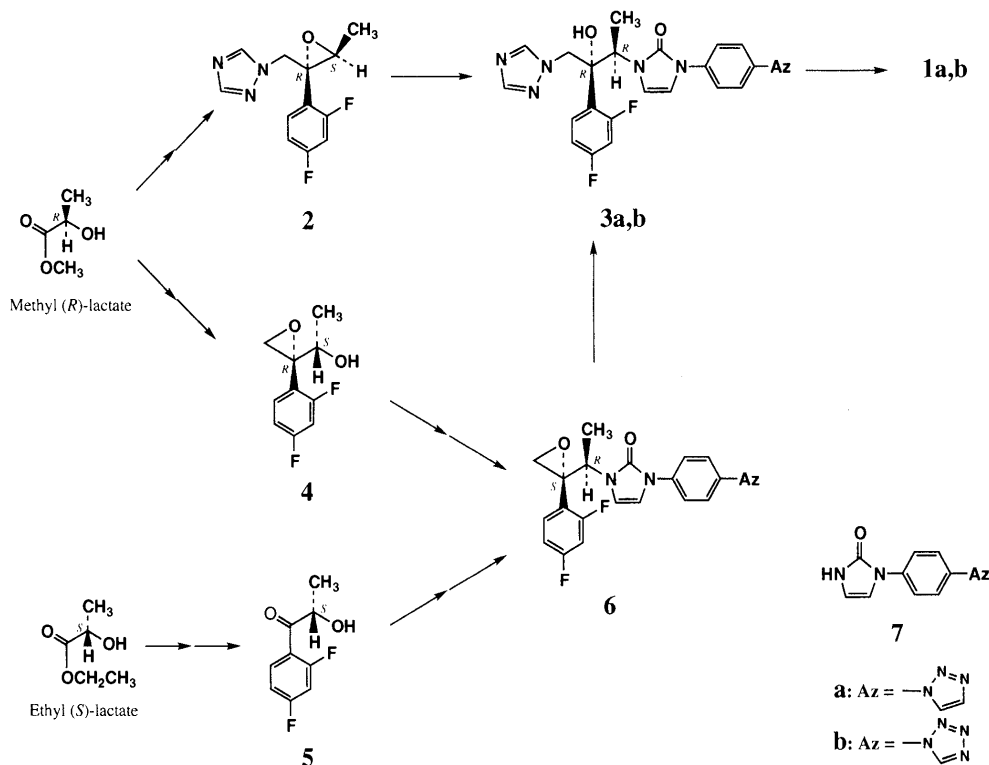


Chart 2

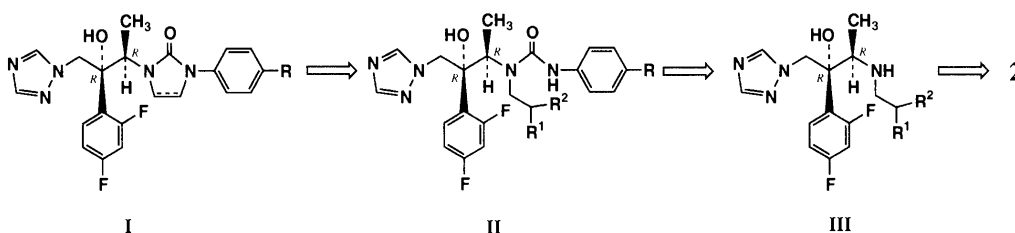


Chart 3

to give the ring-opened product **8**, which is the key intermediate in this synthetic route, as an oil in 81% yield. For the synthesis of **1a**, compound **8** was reacted with phenyl 4-(1*H*-1,2,3-triazol-1-yl)phenylcarbamate (**10a**) at 110 °C in *N,N*-dimethylformamide (DMF) to give the urea **9a** (94% isolated yield). Intramolecular cyclization was achieved by heating **9a** in the presence of methanolic hydrochloric acid (HCl–MeOH) to form the imidazolone **3a** in 84% yield. The synthesis could also be carried out without purification of the intermediate **9a**, resulting in a slightly improved overall yield of **3a** from **8** (83%). Catalytic hydrogenation of **3a** over palladium on carbon (Pd–C) in acetic acid (AcOH) using the conditions described in our preceding report afforded **1a**. This synthetic route, which allowed the preparation of **1a** in 58% overall yield from the starting material **2**, was then applied to the synthesis of the 1-tetrazolylphenylimidazolone derivative **1b**. Thus compound **8** was reacted with phenyl 4-(1*H*-1-tetrazolyl)phenylcarbamate (**10b**) and then cyclized and hydrogenated as described above to give **1b** in 40% overall yield from **2**.

The synthesis of **1b** was also carried out by an alternative route using commercially available 4-nitrophenyl isocyanate (4-NO<sub>2</sub>PhNCO) instead of **10b**. Room temperature reaction

of **8** with 4-NO<sub>2</sub>PhNCO gave the nitrophenylurea **11** in 81% isolated yield, which was then cyclized to the imidazolone **12** (86%). When the synthesis was carried out without purification of **11**, the overall yield of **12** from **8** was improved slightly to 80%. The nitro group and the double bond were simultaneously reduced by catalytic hydrogenation on Pd–C to afford the aminophenylimidazolidinone **13** in a moderate yield (52%). The amino group of **13** was converted into a tetrazole ring in 55% isolated yield by reaction with sodium azide (NaN<sub>3</sub>) and ethyl orthoformate [CH(OEt)<sub>3</sub>] in the presence of AcOH. Using this route, **1b** was obtained in 19% overall yield from **2**.

On the basis of results of detailed biological studies carried out on compounds **1a** and **1b**, compound **1b** (TAK-456) was selected as the candidate for clinical trials. Therefore, a more practical pathway for the large scale synthesis of **1b** was now required, since the previous synthetic routes involved a moderate yielding conversion of imidazolone to imidazolidinone, i.e., **3b**→**1b** (64%) and **12**→**13** (52%), by catalytic hydrogenation. Thus we devised an alternative route to synthesize the imidazolidinone ring by direct cyclization of the hydroxyethyl precursor **II** (R<sup>1</sup>=H, R<sup>2</sup>=OH; Chart 3).

The route for the alternative synthesis for **1b** is shown in

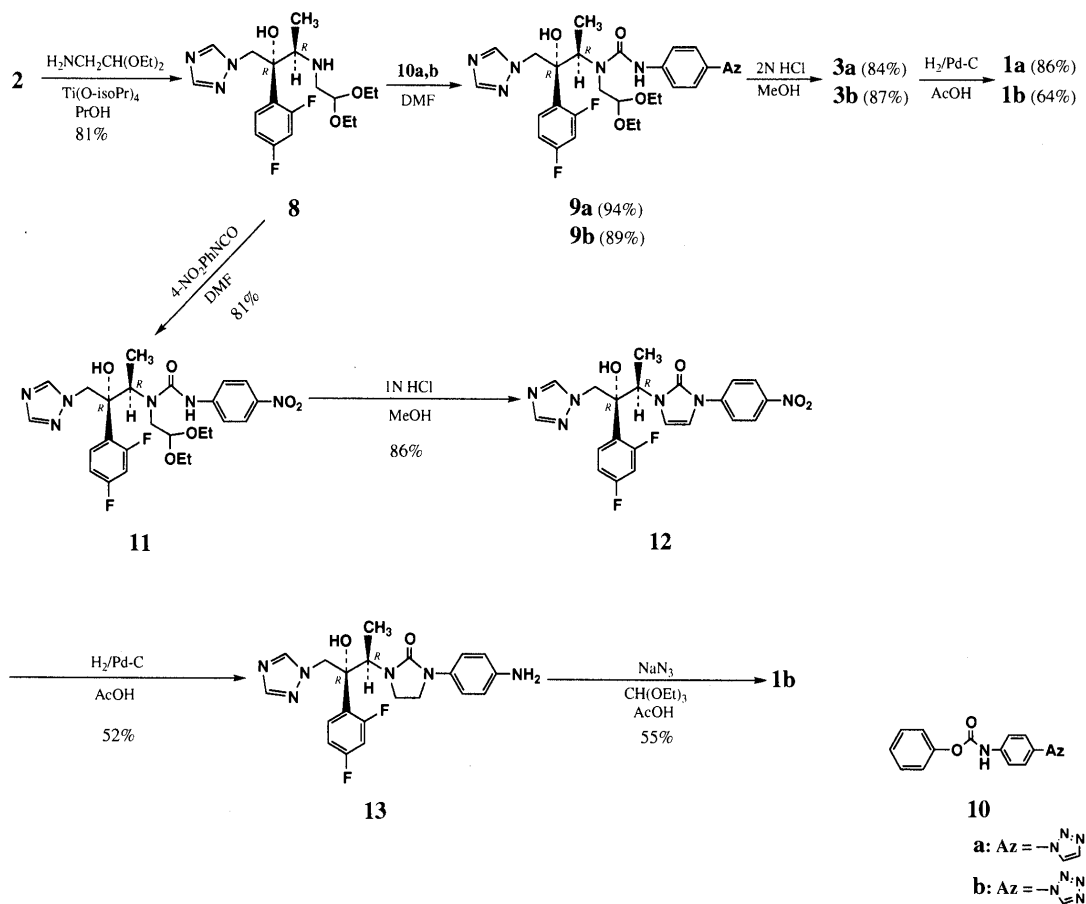


Chart 4

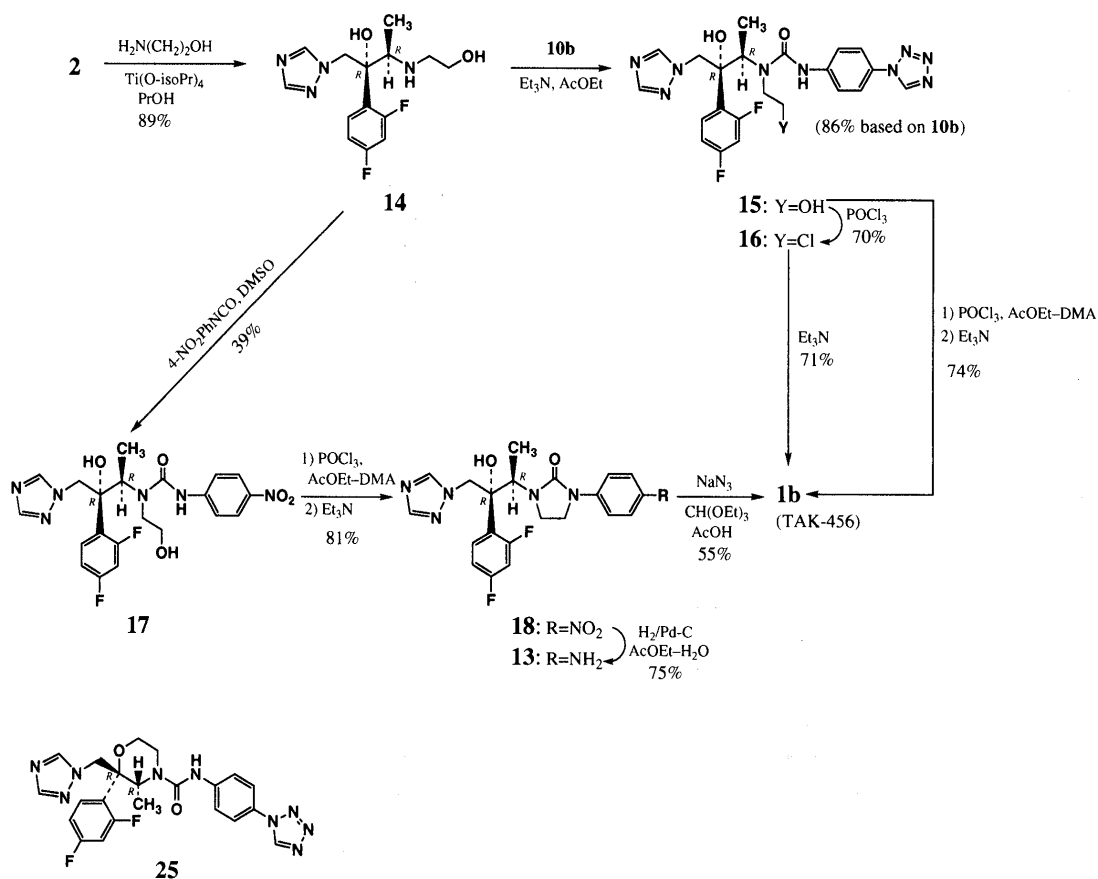


Chart 5

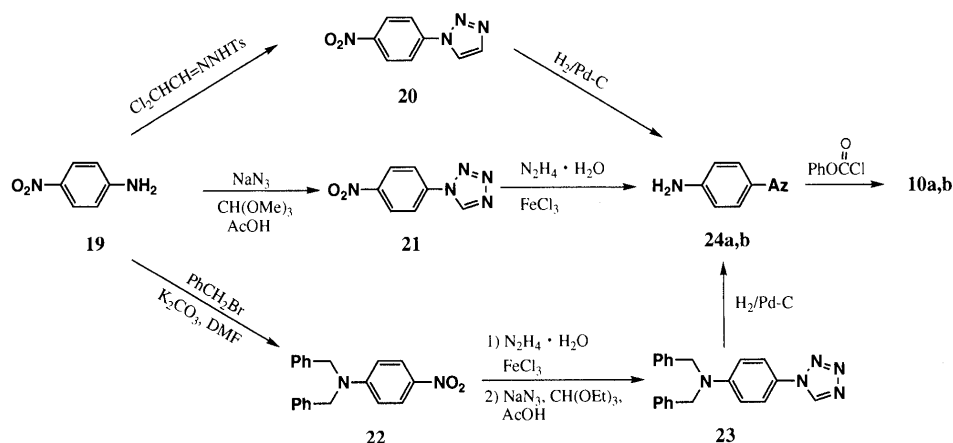


Chart 6

Chart 5. The oxirane ring-opening reaction of **2** with 2-aminoethanol [ $\text{H}_2\text{N}(\text{CH}_2)_2\text{OH}$ ] was successfully carried out using similar reaction conditions to those used in the synthesis of **8**, to give the hydroxyethylaminobutanol **14** as an oil in 89% yield. Condensation of **14** with the carbamate **10b** (0.8 eq) in the presence of triethylamine ( $\text{Et}_3\text{N}$ ) in ethyl acetate ( $\text{AcOEt}$ ) at reflux resulted in the precipitation of **15** from the reaction mixture (86% yield from **10b**). Several reaction conditions were examined for the ring closure of **15** to give the imidazolidinone and initially it seemed that the Mitsunobu reaction<sup>5)</sup> using diethyl azodicarboxylate (DEAD) and triphenylphosphine ( $\text{Ph}_3\text{P}$ ) would be able to affect direct ring closure. However, this reaction was found to proceed *via* 2 pathways, which in addition to giving the desired product **1b** (67% yield), also produced the undesired morpholine derivative **25**<sup>6)</sup> (12% yield) as a byproduct. We next examined a 2-step cyclization *via* initial conversion of **15** to the chloride **16** followed by dehydrochlorination with base to give **1b**. Treatment of **15** with thionyl chloride ( $\text{SOCl}_2$ ) afforded the chloride **16** in 43% yield, but an improved isolated yield (70%) was obtained by using phosphorous oxychloride ( $\text{POCl}_3$ ), and dehydrochlorination of **16** with  $\text{Et}_3\text{N}$  gave the imidazolidinone **1b** in 71% yield. Furthermore, this two-step cyclization reaction could be carried out as a one-pot process. Thus  $\text{POCl}_3$  was added to a solution of **15** in  $\text{AcOEt}$  and *N,N*-dimethylacetamide (DMA), and the subsequent addition of  $\text{Et}_3\text{N}$  gave the product **1b** in 74% overall yield from **15**.

As described above (Chart 4), **1b** was also obtained by tetrazole ring formation at the amino group of **13**, although the yield of the preceding hydrogenation step to synthesize **13** was moderate (52%). Therefore, in order to improve the yield of **13**, we investigated its synthesis from **14** *via* the imidazolidinone cyclization route we have established. Ring closure of **17** using  $\text{POCl}_3\text{-Et}_3\text{N}$  to give **18** and subsequent catalytic hydrogenation to give **13** proceeded successfully in 81% yield and in 75% yield, respectively. However the initial step to synthesize **17** by introduction of the *N*-nitrophenyl-carbamoyl moiety onto the secondary amino group of **14** proceeded in a poor yield (39%), probably due to the competing reaction of the primary hydroxyl group of **14** with the isocyanate.

Our synthesis of **1a** and **1b** above used the carbamate **10a** and **10b** as key synthetic intermediates. In our preceding report, **10a** and **10b** were prepared from the corresponding ani-

lines **24a** and **24b**, which in turn were synthesized by reduction of the corresponding 4-azolylnitrobenzenes prepared by nucleophilic substitution of 4-fluoronitrobenzene with the appropriate azoles, *i.e.*, 1,2,3-triazole and tetrazole. However, in both cases, an isomeric mixture of 1-substituted and 2-substituted azoles were obtained, which required separation at either the 4-azolylnitrobenzene or the 4-azolylaniline stage.<sup>1)</sup> Clearly for the efficient large scale synthesis of **1a** and **1b**, we needed a method to selectively prepare the 1-substituted azole isomer. Thus a new route was devised for the synthesis of the carbamates **10a** and **10b** using *p*-nitroaniline (**19**) as the starting material (Chart 6), in which the 1-substituted 1,2,3-triazole derivative **20** was selectively synthesized by reaction with chloroacetaldehyde tosylhydrazone ( $\text{ClCH}_2\text{-CH=NNHTs}$ ) according to the method described in a patent.<sup>7)</sup> This enabled **20** to be obtained as crystals in 67% yield from the hydrazone, after which **10a** could be obtained by reduction to **24a** followed by treatment with phenyl chlorocarbonate ( $\text{PhOCOCl}$ ) [overall yield from **20**, 80%]. For the synthesis of the 1-substituted tetrazole derivative **10b**, compound **19** was first converted quantitatively to **21** according to the method reported by Gaponik *et al.*<sup>8)</sup> Subsequent reduction of **21** to **24b** with hydrazine hydrate ( $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$ ) in the presence of ferric chloride ( $\text{FeCl}_3$ ) followed by treatment with  $\text{PhOCOCl}$  afforded the carbamate **10b**, in 82% overall yield from **19**. In alternative approach, compound **19** was dibenzylated with benzyl bromide ( $\text{PhCH}_2\text{Br}$ ) in the presence of potassium carbonate ( $\text{K}_2\text{CO}_3$ ) to give **22** (82% yield), and the nitro group was reduced to amino group and then reacted with  $\text{NaN}_3$  and  $\text{CH}(\text{OEt})_3$  to form the 1-substituted tetrazole **23** (63% yield). The dibenzyl group was removed by catalytic hydrogenolysis, and the resulting aniline **24b** was converted to the carbamate **10b** in 91% yield from **23**.

In conclusion, we have established an efficient and practical route for the synthesis of the imidazolidinone-based antifungal triazoles, **1a** and **1b**. This route is suitable for application to large-scale production, since each reaction step proceeded in a good yield under mild conditions, and in addition all the synthetic intermediates were stable and easily purified. Pre-clinical studies on the selected candidate **1b** (TAK-456) are currently in progress and detailed profiles of the biological activity of **1b** will be reported in due course.

## Experimental

Melting points were determined using a Yanagimoto melting point apparatus and are uncorrected. IR spectra were measured with JASCO IR-810 spectrometer. <sup>1</sup>H-NMR spectra were recorded on a Varian Gemini-200 spectrometer with tetramethylsilane as an internal standard. The following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet and br=broad. The secondary ion mass spectra (SI-MS) were measured with a Hitachi M-80A mass spectrometer. The optical rotations were recorded with a Jasco DIP-181 or DIP-370 digital polarimeter.

Reactions were carried out at room temperature unless otherwise noted and followed by TLC on Silica gel 60 F<sub>254</sub> precoated TLC plates (E. Merck) or by HPLC using an octadecyl silica (ODS) column (A-303, 4.6 mm i.d. × 250 nm, YMC Co., Ltd.). Standard work-up procedures were as follows. The reaction mixture was partitioned between the indicated solvent and water. Organic extracts were combined and washed in the indicated order using the following aqueous solutions: water, 5% aqueous sodium carbonate solution (aqueous NaHCO<sub>3</sub>), saturated sodium chloride (NaCl) solution (brine), 1 N aqueous sodium hydroxide solution (1 N NaOH), 1 N hydrochloric acid (1 N HCl) and 5% aqueous phosphoric acid solution (5% H<sub>3</sub>PO<sub>4</sub>). Extracts were dried over anhydrous magnesium sulfate (MgSO<sub>4</sub>), filtered and evaporated *in vacuo*. Chromatographic separations were carried out on Silica gel 60 (0.063–0.200 mm, E. Merck) using the indicated eluents.

**(2R,3R)-3-(2,2-Diethoxyethyl)amino-2-(2,4-difluorophenyl)-1-(1H-1,2,4-triazol-1-yl)-2-butanol (8)** A mixture of **2** (40 g), Ti(O-isoPr)<sub>4</sub> (68 g), H<sub>2</sub>NCH<sub>2</sub>CH(OEt)<sub>2</sub> (464 ml) and PrOH (400 ml) was stirred under reflux for 24 h under a nitrogen atmosphere. The solvent and H<sub>2</sub>NCH<sub>2</sub>CH(OEt)<sub>2</sub> were distilled off under reduced pressure. The residue was diluted with AcOEt (800 ml). 1 N NaOH-brine (1 : 1, v/v, 520 ml) was added and the resulting mixture was stirred for 1 h. A milky precipitate was filtered off and washed with AcOEt (250 ml). The filtrate and the washing were combined. The organic layer was extracted with 1 N HCl (150 ml × 4). The aqueous extracts were combined and neutralized with 2 N NaOH (300 ml) at 0 °C. The whole was worked up (AcOEt; 1 N NaOH, brine). Compound **8** (49.4 g, 81%) was obtained as a pale yellow oil. *Anal.* Calcd for C<sub>18</sub>H<sub>26</sub>F<sub>2</sub>N<sub>4</sub>O<sub>3</sub>: C, 56.24; H, 6.82; N, 14.57. Found: C, 55.88; H, 6.74; N, 14.50. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 0.91 (3H, d, J=6.6 Hz), 1.0–1.2 (1H, br), 1.23 (6H, t, J=7 Hz), 2.66 (1H, dd, J=12 Hz, 4.6 Hz), 2.95 (1H, dd, J=12, 6.4 Hz), 3.14 (1H, q, J=6.6 Hz), 3.48–3.81 (4H, m), 4.56 (1H, dd, J=6.4, 4.6 Hz), 4.75 (1H, d, J=14 Hz), 4.88 (1H, s), 4.89 (1H, d, J=14 Hz), 6.69–6.80 (2H, m), 7.33–7.45 (1H, m), 7.76 (1H, s), 7.93 (1H, s). IR (neat): 2976, 1617, 1501, 1273, 1136, 1100, 1063, 964 cm<sup>-1</sup>. [α]<sub>D</sub><sup>20</sup>: –60.4° (c=0.28, MeOH). SI-MS (m/z): 385 (MH<sup>+</sup>).

**1-(2,2-Diethoxyethyl)-1-[(1R,2R)-2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1H-1,2,4-triazol-1-yl)propyl]-3-[4-(1H-1,2,3-triazol-1-yl)-phenyl]urea (9a)** A mixture of **8** (188 mg), **10a** (137 mg) and DMF (2 ml) was stirred for 2 h at 110 °C under an argon atmosphere. The reaction mixture was cooled and worked up (AcOEt; water, brine). The residue was purified by silica gel column chromatography (AcOEt-hexane, 3 : 1, v/v → AcOEt) to give **9a** (262 mg, 94%) as a white powder. *Anal.* Calcd for C<sub>27</sub>H<sub>32</sub>F<sub>2</sub>N<sub>6</sub>O<sub>4</sub>: C, 56.83; H, 5.65; N, 19.64. Found: C, 56.71; H, 5.76; N, 14.44. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 1.07 (1.2H, d, J=7 Hz), 1.23 (1.8H, d, J=7 Hz), 1.34 (6H, t, J=7 Hz), 3.51–4.07 (7H, m), 4.39 (0.4H, d, J=14 Hz), 4.54 (0.6H, d, J=14 Hz), 4.74 (0.6H, m), 4.94–5.01 (1H, m), 5.24–5.41 (1.4H, m), 6.72–6.82 (2H, m), 7.37–7.84 (8.2H, m), 7.95 (0.6H, s), 8.24 (0.4H, s), 8.75 (0.4H, br), 9.05 (0.4H, s). IR (KBr): 3350, 3160, 2970, 1630, 1540, 1510, 1460, 1220, 1150 cm<sup>-1</sup>. [α]<sub>D</sub><sup>20</sup>: –107.1° (c=1.0, MeOH).

**1-[(1R,2R)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1H-1,2,4-triazol-1-yl)propyl]-3-[4-(1H-1,2,3-triazol-1-yl)phenyl]-2-(1H,3H)-imidazolone (3a)** 1) 2 N HCl (2.4 ml) was added to a solution of **9a** (228 mg) in MeOH (2.4 ml). The mixture was stirred for 4 h at 60 °C and cooled. After having been neutralized with 1 N NaOH (4.8 ml), the whole was concentrated *in vacuo* and worked up (AcOEt; water, brine). The residue was crystallized from AcOEt-diisopropyl ether (isoPr<sub>2</sub>O) to give **3a** (161 mg, 84%) as white powdery crystals.

2) A mixture of **8** (16 g), **10a** (11.66 g) and DMF (160 ml) was stirred for 2 h at 110 °C under an argon atmosphere. The mixture was cooled and worked up (AcOEt; water, brine). The residue was dissolved in MeOH (210 ml), and then 2 N HCl (210 ml) was added to the solution. The mixture was stirred for 6 h at 60 °C and cooled. After having been concentrated *in vacuo*, the remaining aqueous solution was washed with isoPr<sub>2</sub>O (200 ml) and neutralized with 1 N NaOH (420 ml). The whole was extracted with AcOEt and the extract was washed with brine. The organic layer was dried over MgSO<sub>4</sub> and concentrated *in vacuo*. The residue was crystallized from AcOEt-isoPr<sub>2</sub>O to give **3a** (18 g), which was then recrystallized from AcOEt to give

white powdery crystals (16.5 g, 83% from **8**).

Compound **3a** was hydrogenated to **1a** in the same manner as that described in our preceding report.<sup>1)</sup> Compounds **3a** and **1a** were identical to the authentic sample<sup>1)</sup> upon direct comparison.

**1-(2,2-Diethoxyethyl)-1-[(1R,2R)-2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1H-1,2,4-triazol-1-yl)propyl]-3-[4-(1H-1-tetrazolyl)phenyl]urea (9b)** A mixture of **8** (30 g), **10b** (21.9 g) and DMF (300 ml) was stirred for 5 h at 80 °C. The reaction mixture was cooled and worked up (AcOEt; water). The residue was crystallized from AcOEt-isoPr<sub>2</sub>O to give **9b** (35.7 g) as white powdery crystals. The mother liquor was concentrated *in vacuo* and the residue was purified by silica gel column chromatography (AcOEt-hexane, 3 : 1, v/v → AcOEt) to obtain an additional amount of **9b** (4 g). Yield: 39.7 g (89%). mp 180–182 °C. *Anal.* Calcd for C<sub>26</sub>H<sub>31</sub>F<sub>2</sub>N<sub>9</sub>O<sub>4</sub>: C, 54.63; H, 5.47; N, 22.05. Found: C, 54.58; H, 5.37; N, 21.61. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 1.08 (1.5H, d, J=7 Hz), 1.24 (1.5H, d, J=7 Hz), 1.31–1.38 (6H, m), 3.50–4.06 (7H, m), 4.39 (0.5H, d, J=14 Hz), 4.59 (0.5H, d, J=14 Hz), 4.76 (0.5H, m), 4.93–5.08 (1.5H, m), 5.25–5.48 (1H, m), 6.73–6.83 (2H, m), 7.31–7.69 (5.5H, m), 7.79 (0.5H, s), 7.82 (0.5H, s), 8.24 (0.5H, s), 8.85 (0.5H, br), 8.94 (0.5H, s), 8.96 (0.5H, s), 9.16 (0.5H, s). IR (KBr): 3340, 3140, 2970, 1640, 1530, 1500, 1460, 1200, 1140 cm<sup>-1</sup>. [α]<sub>D</sub><sup>20</sup>: –106.9° (c=1.0, MeOH).

**1-[(1R,2R)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1H-1,2,4-triazol-1-yl)propyl]-3-[4-(1H-1-tetrazolyl)phenyl]-2-(1H,3H)-imidazolone (3b)** 2 N HCl (342 ml) was added to a solution of **9b** (39 g) in MeOH (342 ml). The mixture was stirred for 17 h at 50–55 °C and cooled. After having been neutralized with 2 N NaOH (342 ml), the whole was concentrated *in vacuo* and worked up (AcOEt; water, brine). The residue was crystallized from AcOEt-isoPr<sub>2</sub>O to give **3b** (28.6 g, 87%) as white powdery crystals. This product was identical to **3b** prepared in our preceding report<sup>1)</sup> upon direct comparison with the authentic sample.

**1-(2,2-Diethoxyethyl)-1-[(1R,2R)-2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1H-1,2,4-triazol-1-yl)propyl]-3-(4-nitrophenyl)urea (11)** A mixture of **8** (0.25 g), 4-NO<sub>2</sub>PhNCO (0.11 g) and DMF (2 ml) was stirred for 2 h. The reaction mixture was worked up (AcOEt; water, brine). The residue was purified by silica gel column chromatography (AcOEt-hexane, 1 : 3 → 1 : 2, v/v) to give **11** (0.29 g, 81%) as a pale yellow powder. *Anal.* Calcd for C<sub>25</sub>H<sub>30</sub>F<sub>2</sub>N<sub>6</sub>O<sub>6</sub>: C, 54.74; H, 5.51; N, 15.32. Found: C, 54.61; H, 5.29; N, 15.29. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 1.07 (1.5H, d, J=7 Hz), 1.23 (1.5H, d, J=7 Hz), 1.30–1.38 (6H, m), 3.50–4.10 (6.5H, m), 4.36 (0.5H, d, J=14 Hz), 4.53 (0.5H, d, J=14 Hz), 4.72–4.77 (0.5H, m), 4.91–5.01 (1H, m), 5.26–5.42 (2H, m), 6.70–6.84 (2H, m), 7.31–7.64 (3.5H, m), 7.78 (0.5H, s), 7.81 (0.5H, s), 8.16–8.23 (2.5H, m), 9.02 (0.5H, br), 9.37 (0.5H, s). IR (KBr): 3283, 2978, 1557, 1505, 1331, 1304, 1244, 1113 cm<sup>-1</sup>. [α]<sub>D</sub><sup>20</sup>: –120.9° (c=0.44, MeOH). SI-MS (m/z): 549 (MH<sup>+</sup>).

**1-[(1R,2R)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1H-1,2,4-triazol-1-yl)propyl]-3-(4-nitrophenyl)-2-(1H,3H)-imidazolone (12)** 1) 1 N HCl (2.8 ml) was added to a solution of **11** (0.15 g) in MeOH (3 ml). The mixture was stirred under reflux for 2 h and cooled. After having been neutralized by addition of NaHCO<sub>3</sub> (0.24 g) at 0 °C, the whole was worked up (AcOEt; water, brine) to give **12** (0.11 g, 86%) as a pale yellow powder. *Anal.* Calcd for C<sub>21</sub>H<sub>18</sub>F<sub>2</sub>N<sub>6</sub>O<sub>4</sub> · 1/2H<sub>2</sub>O: C, 54.19; H, 4.11; N, 18.06. Found: C, 54.53; H, 4.05; N, 18.13. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 1.20 (3H, d, J=7 Hz), 4.19 (1H, d, J=14 Hz), 5.02 (1H, q, J=7 Hz), 5.11 (1H, d, J=14 Hz), 5.41 (1H, br), 6.76–6.90 (4H, m), 7.41–7.53 (1H, m), 7.76 (1H, s), 7.82 (1H, s), 7.94 (2H, d, J=9.2 Hz), 8.34 (2H, d, J=9.2 Hz). IR (KBr): 1692, 1597, 1503, 1426, 1334, 1252, 855 cm<sup>-1</sup>. [α]<sub>D</sub><sup>20</sup>: –11.4° (c=1.0, MeOH). SI-MS (m/z): 457 (MH<sup>+</sup>).

2) A mixture of **8** (8.9 g), 4-NO<sub>2</sub>PhNCO (4.2 g) and dimethyl sulfoxide (DMSO, 30 ml) was stirred for 2 h, and the whole was worked up (AcOEt; water, brine). The residue was dissolved in MeOH (180 ml) and treated with 2 N HCl (115 ml) in a manner similar to that described above. The precipitate was filtered off, and the filtrate was neutralized by addition of NaHCO<sub>3</sub> (19.3 g). The whole was concentrated *in vacuo* and worked up (AcOEt; water, brine). The residue was purified by silica gel column chromatography (AcOEt-hexane, 2 : 1, v/v) to give **12** (8.6 g, 80% from **8**) as a pale yellow powder.

**(2R,3R)-2-(2,4-Difluorophenyl)-3-(2-hydroxyethyl)amino-1-(1H-1,2,4-triazol-1-yl)-2-butanol (14)** A mixture of **2** (30 g), Ti(O-isoPr)<sub>4</sub> (51 g), H<sub>2</sub>N(CH<sub>2</sub>)<sub>2</sub>OH (109.4 g) and PrOH (300 ml) was refluxed for 7.5 h under a nitrogen atmosphere. After having been cooled, the mixture was diluted with AcOEt (500 ml) and 2 N NaOH (98 ml) saturated with NaCl. The resulting mixture was stirred for 30 min, diluted with brine (195 ml) and stirred for a further 10 min. The supernatant was separated by decantation. AcOEt (400 ml) was added to the aqueous layer, and the mixture was stirred vigorously for 3 min. The supernatant was separated again by decantation. Addition of

AcOEt (300 ml each) followed by separation of the supernatant was repeated two more times. The supernatants were combined, washed with brine (250 ml) and extracted with 1 N HCl (225 ml $\times$ 2). The acidic aqueous extracts were combined and neutralized at 0 °C with 2 N NaOH (230 ml) saturated with NaCl. The whole was extracted with AcOEt and the extract was washed with brine. The organic layer was dried over MgSO<sub>4</sub> and concentrated *in vacuo* to give **14** (34.2 g, 89%) as a pale yellow oil. *Anal.* Calcd for C<sub>14</sub>H<sub>18</sub>F<sub>2</sub>N<sub>4</sub>O<sub>2</sub>·1/2H<sub>2</sub>O: C, 52.33; H, 5.96; N, 17.44. Found: C, 52.76; H, 6.06; N, 17.06. <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 0.92 (3H, d, *J*=6.6 Hz), 1.60–1.80 (2H, br), 2.61–2.73 (1H, m), 2.98–3.16 (2H, m), 3.65–3.71 (2H, m), 4.81 (1H, d, *J*=14 Hz), 4.96 (1H, d, *J*=14 Hz), 4.77–4.99 (1H, br), 6.70–6.80 (2H, m), 7.36–7.48 (1H, m), 7.78 (1H, s), 7.87 (1H, s). IR (neat): 3320, 1617, 1597, 1501, 1422, 1273, 1136, 1101 cm<sup>-1</sup>. [ $\alpha$ ]<sub>D</sub><sup>20</sup>: -66.4° (*c*=1.0, MeOH). SI-MS (*m/z*): 313 (MH<sup>+</sup>).

**1-[(1*R*,2*R*)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-1-(2-hydroxyethyl)-3-[4-(1*H*-1-tetrazolyl)phenyl]urea (**15**)** A mixture of **14** (34.2 g), **10b** (23.7 g), Et<sub>3</sub>N (17.6 ml) and AcOEt (600 ml) was stirred under reflux for 1.5 h. After having been cooled, the mixture was stirred for 2 h at 0 °C. The precipitate was collected by filtration, washed with AcOEt (50 ml) and dried at 40 °C *in vacuo* to give **15** (37.9 g, 86% based on **10b**) as white powdery crystals. mp 172–175 °C. *Anal.* Calcd for C<sub>22</sub>H<sub>23</sub>F<sub>2</sub>N<sub>9</sub>O<sub>3</sub>·1/4AcOEt: C, 52.97; H, 4.83; N, 24.17. Found: C, 53.01; H, 4.81; N, 24.19. <sup>1</sup>H-NMR (*d*<sub>6</sub>-DMSO)  $\delta$ : 0.93, 1.16 (3H, d, *J*=7.0 Hz), 3.47–4.09 (4.5H, m), 4.56 (1H, d, *J*=14 Hz), 4.91–5.03 (1H, m), 5.30 (0.5H, q, *J*=7 Hz), 5.79, 6.12, 6.21 (2H, br), 6.87–6.96 (1H, m), 7.13–7.45 (2H, m), 7.59–7.88 (5H, m), 8.21, 8.32 (1H, s), 9.41, 9.49 (1H, s), 10.02, 10.05 (1H, s). IR (KBr): 3240, 3136, 1649, 1524, 1473, 1338, 1207, 1138 cm<sup>-1</sup>. [ $\alpha$ ]<sub>D</sub><sup>20</sup>: -100.9° (*c*=0.94, MeOH). SI-MS (*m/z*): 500 (MH<sup>+</sup>).

**1-(2-Chloroethyl)-1-[(1*R*,2*R*)-2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-3-[4-(1*H*-1-tetrazolyl)phenyl]urea (**16**)** A solution of POCl<sub>3</sub> (0.51 ml) in AcOEt (6 ml) was added dropwise to a solution of **15** (2 g) in DMA–AcOEt (1 : 1, v/v, 20 ml) over a period of 1 h at around 22 °C. The mixture was stirred for 16 h and worked up (AcOEt; water). The residue was crystallized from diethyl ether (Et<sub>2</sub>O, 30 ml) to give **16** (1.44 g, 70%) as a white powder. *Anal.* Calcd for C<sub>22</sub>H<sub>22</sub>ClF<sub>2</sub>N<sub>9</sub>O<sub>2</sub>·H<sub>2</sub>O: C, 49.30; H, 4.51; N, 23.52. Found: C, 49.38; H, 4.50; N, 23.14. <sup>1</sup>H-NMR (*d*<sub>6</sub>-DMSO)  $\delta$ : 1.02, 1.16 (3H, d, *J*=7 Hz), 3.61–4.09 (4.5H, m), 4.43, 4.57 (1H, d, *J*=14 Hz), 4.87, 4.97 (1H, d, *J*=14 Hz), 5.20 (0.5H, q, *J*=7 Hz), 5.98 (1H, br), 6.89–6.98 (1H, m), 7.13–7.48 (2H, m), 7.64–7.85 (5H, m), 8.31 (1H, s), 9.02, 9.22 (1H, s), 10.02 (1H, s). IR (KBr): 3125, 1657, 1524, 1473, 1309, 1257, 1179 cm<sup>-1</sup>. SI-MS (*m/z*): 518 (MH<sup>+</sup>).

**1-[(1*R*,2*R*)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-1-(2-hydroxyethyl)-3-(4-nitrophenyl)urea (**17**)** A mixture of **14** (10 g), 4-NO<sub>2</sub>PhNCO (5.25 g) and DMSO (50 ml) was stirred for 1 h. The whole was worked up (AcOEt; water, brine). The residue was crystallized from AcOEt to give **17** (5.9 g, 39%) as yellow powdery crystals. mp 122–125 °C. *Anal.* Calcd for C<sub>21</sub>H<sub>22</sub>F<sub>2</sub>N<sub>6</sub>O<sub>5</sub>·1/2H<sub>2</sub>O: C, 51.96; H, 4.78; N, 17.31. Found: C, 51.72; H, 5.05; N, 17.14. <sup>1</sup>H-NMR (*d*<sub>6</sub>-DMSO)  $\delta$ : 0.94 (1.8H, d, *J*=7 Hz), 1.13 (1.2H, d, *J*=7 Hz), 3.40–4.05 (4.4H, m), 4.54, 4.58 (1H, d, *J*=14 Hz), 4.94, 4.97 (1H, d, *J*=14 Hz), 5.28 (0.6H, q, *J*=7 Hz), 6.08, 6.25 (2H, br), 6.87–6.96 (1H, m), 7.12–7.46 (2H, m), 7.59–7.72 (3.4H, m), 8.17–8.30 (3H, m), 9.90 (0.6H, br). IR (KBr): 1618, 1599, 1568, 1503, 1331, 1304, 1258 cm<sup>-1</sup>. SI-MS (*m/z*): 477 (MH<sup>+</sup>). [ $\alpha$ ]<sub>D</sub><sup>20</sup>: -111.1° (*c*=1.0, MeOH).

**1-[(1*R*,2*R*)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-3-(4-nitrophenyl)-2-imidazolidinone (**18**)** A solution of POCl<sub>3</sub> (0.88 ml) in AcOEt (10 ml) was added dropwise to a solution of **17** (3 g) in DMA–AcOEt (1 : 1, v/v, 30 ml) over a period of 20 min at around 21 °C. The mixture was stirred for 16 h, and then Et<sub>3</sub>N (8.8 ml) was added dropwise over a period of 20 min at -5 °C. The whole was stirred for 2 h and worked up (AcOEt; water, 5% H<sub>3</sub>PO<sub>4</sub>, aqueous NaHCO<sub>3</sub>, brine). The residue was purified by silica gel column chromatography (AcOEt–hexane, 6 : 1, v/v) to give **18** (2.3 g, 81%) as a pale yellow powder. *Anal.* Calcd for C<sub>27</sub>H<sub>26</sub>F<sub>2</sub>N<sub>8</sub>O<sub>2</sub>: C, 55.02; H, 4.40; N, 18.33. Found: C, 54.70; H, 4.44; N, 17.95. <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 1.07 (3H, d, *J*=7 Hz), 3.70–4.18 (4H, m), 4.48 (1H, d, *J*=14 Hz), 4.68–4.80 (1H, m), 5.11 (1H, d, *J*=14 Hz), 5.30 (1H, br), 6.73–6.85 (2H, m), 7.35–7.48 (1H, m), 7.75 (2H, d, *J*=9 Hz), 7.77 (1H, s), 7.84 (1H, s), 8.24 (2H, d, *J*=9 Hz). IR (KBr): 1701, 1597, 1501, 1426, 1329, 1267, 1113, 849 cm<sup>-1</sup>. SI-MS (*m/z*): 459 (MH<sup>+</sup>). [ $\alpha$ ]<sub>D</sub><sup>20</sup>: -69.0° (*c*=1.0, MeOH).

**1-(4-Aminophenyl)-3-[(1*R*,2*R*)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-2-imidazolidinone (**13**)** 1) A solution of **12** (1 g) in AcOH (15 ml) was hydrogenated over 10% Pd–C (1 g)

for 7 h at 40–45 °C. The catalyst was filtered off and washed with AcOH (30 ml). The filtrate and the washing were combined and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt–hexane, 9 : 1, v/v→AcOEt) and crystallized from AcOEt to give **13** (0.48 g, 52%) as white powdery crystals. mp 192–193 °C. *Anal.* Calcd for C<sub>21</sub>H<sub>22</sub>F<sub>2</sub>N<sub>6</sub>O<sub>2</sub>: C, 58.87; H, 5.18; N, 19.62. Found: C, 58.87; H, 5.08; N, 19.54. <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 1.06 (3H, d, *J*=7 Hz), 3.58–3.87 (6H, m), 4.46 (1H, br), 4.54 (1H, d, *J*=14 Hz), 5.07 (1H, d, *J*=14 Hz), 5.80 (1H, br), 6.69–6.81 (4H, m), 7.31 (2H, d, *J*=9 Hz), 7.35–7.52 (1H, m), 7.72 (1H, s), 7.92 (1H, s). IR (KBr): 3356, 1647, 1518, 1510, 1489, 1448, 1437, 1271 cm<sup>-1</sup>. [ $\alpha$ ]<sub>D</sub><sup>20</sup>: -62.5° (*c*=1.0, MeOH).

2) A mixture of **18** (1 g), AcOEt (18 ml) and water (2 ml) was hydrogenated over 10% Pd–C (0.5 g) for 3 h. The catalyst was filtered off and washed with AcOEt (50 ml). The filtrate and the washing were combined and concentrated *in vacuo*. The residue was crystallized from AcOEt to give **13** (0.35 g) as white powdery crystals. The mother liquor was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (AcOEt–acetone, 3 : 1, v/v) to give an additional amount of **13** (0.35 g). Yield: 0.7 g (75%).

**1-[(1*R*,2*R*)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-3-[4-(1*H*-1-tetrazolyl)phenyl]-2-imidazolidinone (**1b**; TAK-456)** 1) A solution of **3b** (27 g) in AcOH (500 ml) was hydrogenated over 10% Pd–C (27 g) for 48 h at 40 °C under a pressure of 50 kg/cm<sup>2</sup>. The catalyst was filtered off and washed with AcOH (100 ml). The filtrate and the washing were combined and concentrated *in vacuo*. The concentrate was worked up (AcOEt; brine), and the residue was crystallized from EtOH (1000 ml) to give **1b** (17.45 g, 64%) as colorless powdery crystals.

2) NaN<sub>3</sub> (0.18 g) and AcOH (2 ml) were added successively to a mixture of **13** (1 g) and CH(OEt)<sub>3</sub> (1.16 ml). The mixture was stirred for 20 min and for further 1.5 h at 80–85 °C under an argon atmosphere. After having been cooled, the mixture was diluted with water (2 ml) and 6 N HCl (0.85 ml). A 25% aqueous solution of sodium nitrite (25% NaNO<sub>2</sub>, 0.3 ml) was added, and the resulting mixture was cooled in an ice bath. The precipitate was collected by filtration, washed with water and recrystallized from EtOH to give **1b** (0.62 g, 55%) as colorless crystals.

3) A solution of **15** (0.2 g) in tetrahydrofuran (THF, 6 ml) was added dropwise over a period of 5 min to a mixture of Ph<sub>3</sub>P (0.145 g), a 40% solution of DEAD in toluene (0.24 g) and THF (6 ml) at -20 °C. The resulting mixture was stirred for 2 h. The solvent was distilled off *in vacuo*, and the residue was chromatographed on silica gel (hexane–AcOEt–acetone, 4 : 3 : 3, v/v→AcOEt). From the first eluate, **1b** was obtained as colorless powdery crystals (0.118 g, 67%). From the second eluate, the morpholine derivative **25** (0.021 g, 12%) was obtained as a white powder. Compound **25**: <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 1.19 (3H, d, *J*=6.8 Hz), 3.80–4.02 (2H, m), 4.50–4.58 (3H, m), 4.68 (1H, d, *J*=14.5 Hz), 5.04 (1H, d, *J*=14.5 Hz), 6.74–6.84 (2H, m), 7.32 (2H, d, *J*=8.8 Hz), 7.57 (2H, d, *J*=8.8 Hz), 7.50–7.88 (3H, m), 8.03 (1H, br), 8.93 (1H, s). SI-MS (*m/z*): 482 (MH<sup>+</sup>).

4) A solution of Et<sub>3</sub>N (0.96 ml) in AcOEt (2 ml) was added dropwise to a solution of **16** (1.2 g) in DMA–AcOEt (3 : 5, v/v, 16 ml) over a period of 15 min at an internal temperature of 0–2 °C. After having been stirred for 3 h, the mixture was diluted with ice-water (20 ml) and worked up (AcOEt; water, 5% H<sub>3</sub>PO<sub>4</sub>, aqueous NaHCO<sub>3</sub>, brine). The residue was crystallized from AcOEt to give a white powder, which was then recrystallized from EtOH to give **1b** (0.765 g, 71%) as colorless crystals.

5) A solution of POCl<sub>3</sub> (1.41 ml) in AcOEt (15 ml) was added dropwise to a solution of **15** (5 g) in DMA–AcOEt (1 : 1, v/v, 50 ml) over a period of 1.7 h at 22–25 °C. The mixture was stirred for 16 h, and then Et<sub>3</sub>N (12.8 ml) was added dropwise over a period of 20 min at around -4 °C. After having been stirred for 5 h, the whole was diluted with ice-water (20 ml) and worked up (AcOEt; water, 5% H<sub>3</sub>PO<sub>4</sub>, aqueous NaHCO<sub>3</sub>, brine). The AcOEt layer was treated with activated carbon and concentrated *in vacuo*. The residue was crystallized from AcOEt to give a white powder, which was then recrystallized from EtOH to give **1b** (3.4 g, 74%) as colorless crystals.

The product **1b** prepared by the methods described above was identical to the authentic sample<sup>11</sup> upon direct comparison.

**Phenyl 4-(1*H*-1,2,3-Triazol-1-yl)phenylcarbamate (**10a**)** A mixture of **19** (14.75 g), Cl<sub>2</sub>CHCH=NNHTs (10 g) and MeOH (400 ml) was stirred for 1 h at around -3 °C. After having been stirred overnight, the mixture was concentrated *in vacuo* to a volume of about 170 ml. The concentrate was allowed to stand in an ice-bath for 30 min. The precipitate was collected by filtration to give **20** (4.04 g) as pale yellow crystals. The mother liquor was concentrated *in vacuo*, and the residue was dissolved in AcOEt–THF (1 : 1, v/v, 400 ml). The solution was washed with 1 N HCl, 1 N NaOH, water and brine, successively. The organic layer was dried over MgSO<sub>4</sub> and evaporated

*in vacuo*. The residue was crystallized from Et<sub>2</sub>O to give an additional amount of **20** (0.47 g). Yield 4.51 g (67% based on the hydrazone).

A mixture of **20** (33.33 g), EtOH (200 ml) and THF (200 ml) was hydrogenated over 10% Pd-C (50% wet, 4 g) at 50 °C for 6 h. The catalyst was removed by filtration, and the filtrate was concentrated *in vacuo*. The residue was crystallized from isoPr<sub>2</sub>O to give **24a** (27.45 g, 94%) as pale yellow powdery crystals.

PhOCOCl (28.5 g) was added dropwise over a period of 15 min to an ice-cooled solution of **24a** (26.45 g) in acetone (550 ml) containing pyridine (14.4 g). After having been stirred for 1 h, the mixture was concentrated *in vacuo*. The concentrate was worked up (AcOEt-THF, 2:1, v/v; water, brine). The residue was crystallized from isoPr<sub>2</sub>O to give **10a** (39.68 g, 85%). Compounds **20**, **24a** and **10a** were identical to the authentic samples<sup>1)</sup> upon direct comparison.

**N,N-Dibenzyl-p-nitroaniline (22)** A mixture of **19** (10 g), PhCH<sub>2</sub>Br (49.5 g), K<sub>2</sub>CO<sub>3</sub> (22 g) and DMF (50 ml) was stirred for 32 h at 160 °C. After having been cooled, the mixture was worked up (AcOEt; water, brine). The residue was crystallized from isoPr<sub>2</sub>O-hexane to give **22** (19 g, 82%) as pale yellow crystals. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 4.76 (4H, s), 6.70 (2H, d, *J*=9.2 Hz), 7.81–7.41 (10H, m), 8.07 (2H, d, *J*=9.2 Hz).

**1-[4-(N,N-Dibenzylamino)phenyl]-1H-tetrazole (23)** A mixture of **22** (18 g), FeCl<sub>3</sub> (0.55 g), activated carbon (2.2 g) and MeOH (120 ml) was stirred under reflux for 30 min. N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O (27.4 ml) was added dropwise under reflux over a period of 1 h. The resulting mixture was stirred under reflux for 24 h and cooled. Activated carbon (2.2 g) and FeCl<sub>3</sub> (0.55 g) were added, and the mixture was refluxed for 10 min. N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O (27.4 ml) was added dropwise under reflux over a period of 1 h. The resulting mixture was stirred under reflux for further 24 h. After having been cooled, the whole was filtered to remove the activated carbon, and the filtrate was concentrated *in vacuo*. The concentrate was worked up (AcOEt; water, brine) and crystallized from hexane-isoPr<sub>2</sub>O to give *N,N*-dibenzyl-*p*-phenylenediamine (15 g, 92%) as purple crystals. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 3.29 (2H, br), 4.51 (4H, s), 6.57 (2H, d, *J*=8.4 Hz), 6.64 (2H, d, *J*=8.4 Hz), 7.18–7.34 (10H, m).

AcOH (40 ml) was added dropwise over a period of 10 min to a mixture of *N,N*-dibenzyl-*p*-phenylenediamine (15 g) prepared above, CH(OEt)<sub>3</sub> (25.5 ml) and NaN<sub>3</sub> (4 g). The mixture was stirred for 5 min and for further 4 h at 90 °C under a nitrogen atmosphere. After having been cooled, the mixture was diluted with water (50 ml) and 6*N* HCl (18 ml). A solution of NaNO<sub>2</sub> (2 g) in water (8.4 ml) was added, and the whole was cooled in an ice-bath. The precipitate was collected by filtration and recrystallized from EtOH-MeOH-THF to give **23** (12.4 g, 68%) as brown scaly crystals. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 4.74 (4H, s), 6.82 (2H, d, *J*=9.2 Hz), 7.23–7.43 (12H, m), 8.80 (1H, s).

**Phenyl 4-(1H-1-Tetrazolyl)phenylcarbamate (10b)** 1) Methyl orthoformate [CH(OMe)<sub>3</sub>, 2.74 ml] was added to a mixture of **19** (3 g) and AcOH (18 ml). The mixture was stirred for 30 min, and then NaN<sub>3</sub> (2.35 g) was added. The resulting mixture was stirred for 2 h at 83 °C. After having been cooled, the mixture was diluted with water (18 ml) and 6*N* HCl (5 ml). A solution of NaNO<sub>2</sub> (1.2 g) in water (3.6 ml) was added dropwise over a period of 5 min. The whole was stirred for 1 h in an ice-bath. The precipitate was collected by filtration and washed with water to obtain **21** as a wet solid. This solid was dissolved in THF (60 ml) at 60 °C. FeCl<sub>3</sub> (39.1 mg) and activated carbon (413 mg) were added to the solution, and then N<sub>2</sub>H<sub>3</sub>·H<sub>2</sub>O (3.79 ml) was added dropwise over a period of 30 min under reflux. The resulting mixture was stirred under reflux for 2.5 h and cooled. The activated carbon was removed by filtration and washed with AcOEt (30 ml). The filtrate and

the washing were combined and washed with brine. The organic layer containing **24b** was dried over MgSO<sub>4</sub>. The solution was cooled in an ice-bath, and pyridine (2.06 g) was added. PhOCOCl (4.08 g) was added dropwise to the solution over a period of 5 min. After having been stirred for 1 h, the whole was worked up (AcOEt; brine, water, brine). The residue was crystallized from isoPr<sub>2</sub>O to give **10b** (5.05 g, 83% over all yield from **19**).

2) A mixture of **23** (2 g), MeOH-THF (2:1, v/v, 30 ml) and 6*N* HCl (1 ml) was hydrogenated over 10% Pd-C (0.5 g) for 25 min. The catalyst was removed by filtration, and the filtrate was concentrated *in vacuo*. The residue was dissolved in a mixture of AcOEt-THF (2:1, v/v, 45 ml) and water (5 ml), and a solution of NaHCO<sub>3</sub> (1.3 g) in water (20 ml) was added dropwise under ice-cooling over a period of 5 min. PhOCOCl (0.89 g) was added dropwise to the solution over a period of 5 min. Water (10 ml) was added, and the resulting mixture was stirred for 30 min. The organic layer was worked up (brine). The residue was crystallized from isoPr<sub>2</sub>O to give **10b** (1.5 g, 91%).

The product **10b** obtained above was identical to the authentic sample<sup>1)</sup> upon direct comparison.

**Acknowledgements** We thank Dr. S. Kishimoto and Dr. A. Miyake for their encouragement throughout this work.

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# Novel Enantioselective Fluorinating Agents, (*R*)- and (*S*)-*N*-Fluoro-3-*tert*-butyl-7-nitro-3,4-dihydro-2*H*-benzo[*e*][1,2]thiazine 1,1-Dioxides

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**Enantioselective fluorinating agents, (*R*)- and (*S*)-*N*-fluoro-3-*tert*-butyl-7-nitro-3,4-dihydro-2*H*-benzo[*e*][1,2]-thiazine 1,1-dioxides (BNBT-F, **2**) are readily prepared in 3 steps from racemic 3-*tert*-butyl-7-nitro-3,4-dihydro-2*H*-benzo[*e*][1,2]thiazine 1,1-dioxides (**5**) via optical resolution and fluorination. These agents make accessible both enantiomers of optically active quaternary  $\alpha$ -fluoro carbonyl compounds in modest to high enantioselectivities. X-ray crystallographic analysis of chiral **2** reveals a unique structure wherein the nitrogen atom is highly pyramidalized and fluorine occupies an axial position.**

**Key words** asymmetric fluorination; *N*-fluorosultam;  $\alpha$ -fluoroketone

The extremely high reactivity of electrophilic fluorinating agents such as molecular fluorine,  $\text{FClO}_3$  and  $\text{CF}_3\text{OF}$  made selective fluorination reactions highly problematic. This limitation has been largely overcome by the more recent introduction of easy to handle fluorinating agents,<sup>1)</sup> of which *N*-fluoropyridinium salts,<sup>2)</sup> *N*-fluoroquinolizidinium salts<sup>3)</sup> and *N*-fluorosulfonamides<sup>4)</sup> are representative. Among current issues in this field, agent-controlled enantioselective fluorination remains a major challenge.<sup>5)</sup> Chiral *N*-fluorosulfonamide derivatives have been prepared in one approach to solving this problem.<sup>6)</sup> For example, we recently reported a novel chiral sultam derivative, *N*-fluoro-3-cyclohexyl-3-methyl-2,3-dihydro-benzo[1,2-*d*]isothiazole 1,1-dioxide (CMIT-F, **1**), as an enantioselective fluorinating agent.<sup>7)</sup> Unfortunately, the results to date with this class of agent have been less than satisfactory with respect to both chemical yield and enantioselectivity. In this paper, we report an additional enantioselective fluorinating agent, *N*-fluoro-3-*tert*-butyl-7-nitro-3,4-dihydro-2*H*-benzo[*e*][1,2]thiazine 1,1-dioxide (BNBT-F, **2**) which is effective in the enantioselective fluorination of cyclic ketones.

During our development of CMIT-F (**1**), we observed that the enantioselectivity of the fluorination reaction was dependent on the difference in steric bulkiness of the two substituents at the chiral center. To take advantage of this, we converted Oppolzer's sultam<sup>8)</sup> to the *N*-fluoro sultam **3**, which has both a *tert*-butyl group and hydrogen at the chiral center. We were disappointed to find that, under the conditions of the reaction with lithium enolates, **3** furnished the corresponding imine **4**. The easy loss of HF is clearly associated with the acidity of the proton at the chiral center. With these results in mind, we designed a new N-F type fluorinating agent, BNBT-F (**2**). The proton at the chiral center in **2** should be significantly less acidic than that in **3**, and the differential bulk of two substituents at the chiral center should

be efficient enough to induce high enantioselection. Based on a precedent in the literature,<sup>9)</sup> the electron withdrawing nitro group was introduced into the aromatic ring to increase the fluorinating ability of the agent.

**Preparation of BNBT-F** Chiral **2** was easily prepared from racemic 3-*tert*-butyl-7-nitro-3,4-dihydro-2*H*-benzo[*e*][1,2]thiazine 1,1-dioxide (**5**) in 3 steps, including optical resolution. The sultam **5**, prepared from 3-*tert*-butyl-3,4-dihydro-2*H*-benzo[*e*][1,2]thiazine 1,1-dioxide,<sup>4i)</sup> was derivatized with (+)-10-camphorsulfonyl chloride to furnish the diastereomeric mixture of **6** in moderate yield, accompanied by 45% of unreacted **5**. Diastereomer separation of **6** was effected by silica gel chromatography, and eluted with ethyl acetate/hexane to give the less polar isomer, (*3R*)-**6** (21%), and the more polar isomer, (*3S*)-**6** (31%). It is interesting to note that the mother liquid obtained by the recrystallization of recovered **5** (50% hexane in ethyl acetate) gave optical pure (*R*)-**5** in 20% yield. Removal of the chiral auxiliary of (*3R*)-**6** or (*3S*)-**6** was achieved with LiOH in aqueous THF to furnish (*R*)-**5** or (*S*)-**5** in 82% and 87% yield, respectively. Finally, fluorination of (*R*)-**5** with  $\text{FClO}_3$  in THF gave (*R*)-**2** in 66% yield. In the same way, (*S*)-**2** was obtained in 83% yield (Chart 1). X-ray crystallography was used to unambiguously determine the structure and absolute stereochemistry of (*S*)-**2**.

**Molecular Structure of (*S*)-BNBT-F** The molecular structure of (*S*)-BNBT-F (**2**) is shown in Figs. 2 and 3. The configuration of the nitrogen atom carrying fluorine in **2** is

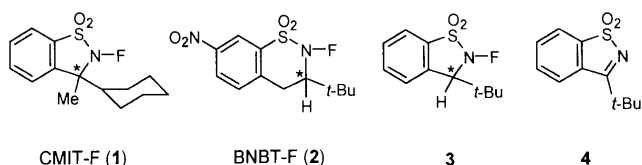
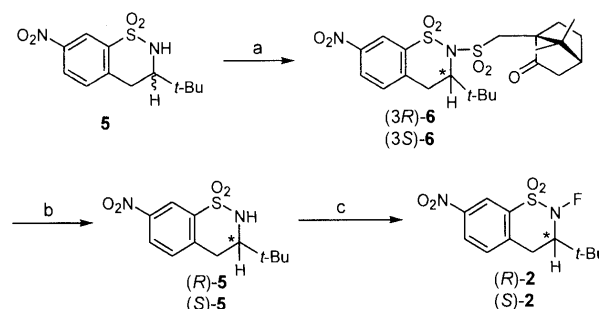


Fig. 1

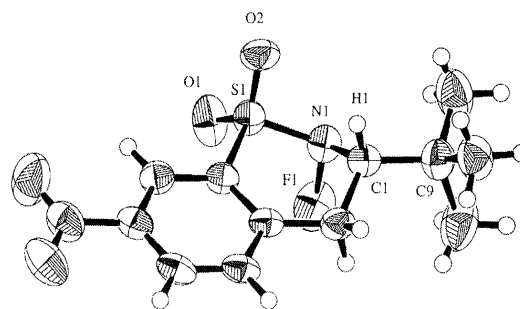
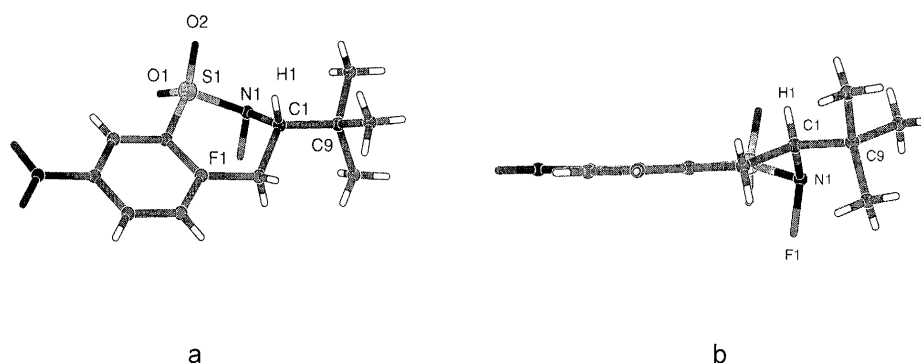


Key: (a) (+)-camphorsulfonyl chloride, NaH, THF, then separation; (b) 2M LiOH, aqueous THF; (c) NaH, THF;  $\text{FClO}_3$ .

Chart 1

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found to be pyramidal, with the sum of the bond angles around the nitrogen atom equaling  $324.6^\circ$ . The bond lengths of N(1)–S(1) and N(1)–C(1) are 1.685 (2) and 1.490 (3) Å, respectively. The length of the N(1)–F(1) bond is 1.425 (3) Å, slightly longer than that of the N–F bond of typical amines<sup>10)</sup> (1.406 (16) Å for F–NR<sup>1</sup>R<sup>2</sup>). To our slight surprise, fluorine is antiperiplanar to the hydrogen atom at the chiral center, in contrast to our previous observation.<sup>11)</sup> The dihedral angle F(1)–N(1)–C(1)–H(1) is  $160.8^\circ$ . The bulky *tert*-butyl moiety occupies the equatorial position, and the small hydrogen atom is axial. Fluorine, rather than the lone pair of nitrogens, takes an axial orientation, probably due to steric interactions with the bulky *tert*-butyl moiety.

Fig. 2. ORTEP Diagram of (*S*)-2Fig. 3. CHARON Diagram of (*S*)-2: (a) Front View; (b) Side ViewTable 1. Enantioselective Fluorination of Ketones **7** with **2**

| Ketone <b>7</b> | <b>2</b>              | Product <b>8</b> | ee (%)           | Yield (%) | Config. <sup>d)</sup> |
|-----------------|-----------------------|------------------|------------------|-----------|-----------------------|
|                 | <i>(R)</i> - <b>2</b> |                  | 62 <sup>a)</sup> | 79        | <i>R</i>              |
|                 | <i>(S)</i> - <b>2</b> |                  | 44 <sup>a)</sup> | 76        | <i>S</i>              |
|                 | <i>(R)</i> - <b>2</b> |                  | 60 <sup>a)</sup> | 59        | <i>R</i>              |
|                 | <i>(S)</i> - <b>2</b> |                  | 49 <sup>b)</sup> | 55        | <i>R</i>              |
|                 | <i>(S)</i> - <b>2</b> |                  | 43 <sup>a)</sup> | 73        | ND <sup>e)</sup>      |
|                 | <i>(S)</i> - <b>2</b> |                  | 52 <sup>a)</sup> | 74        | <i>S</i>              |
|                 | <i>(R)</i> - <b>2</b> |                  | 57 <sup>a)</sup> | 40        | <i>R</i>              |
|                 | <i>(R)</i> - <b>2</b> |                  | 69 <sup>b)</sup> | 70        | ND <sup>e)</sup>      |
|                 | <i>(S)</i> - <b>2</b> |                  | 60 <sup>c)</sup> | 56        | ND <sup>e)</sup>      |

a) Chiralcel OB column (10% *i*PrOH/hexane). b) Chiralcel OJ column (10% *i*PrOH/hexane). c) Chiralcel OJ column (EtOH). d) Ref. 7. e) ND: not determined.

**Enantioselective Fluorination of Prochiral Enolates by BNBT-F** With the target agent BNBT-F (**2**) in hand, we examined the ability of **2** to effect the enantioselective fluorination of enolates of a series of tetralones and indanones **7**. Fluorination of the preformed lithium enolate of 2-methyl-1-tetralone (**7a**) with (*R*)-**2** in THF at  $-50^{\circ}\text{C}$  furnished (*R*)-2-fluoro-2-methyl-1-tetralone (**8a**) in 79% yield with 62% ee. Other results of enantioselective fluorination using both (*R*)- and (*S*)-**2** are summarized in Table 1. Chiral agents **2** make accessible both enantiomers of optically active quaternary  $\alpha$ -fluoro carbonyl compounds in modest to high enantioselectivities. The absolute stereochemistry of **8** was determined by comparison of the specific rotation with that of the authentic samples reported.<sup>7)</sup>

On the bases of the X-ray crystallographic structure of **2** and the reaction mechanism of the fluorination by CMIT-F (**1**) reported in a previous paper,<sup>7)</sup> we propose a working model for the fluorination of **7** with (*S*)-**2**. At the asymmetric center of **2**, the bulky *tert*-butyl group occupies a pseudo-equatorial position, whereas the hydrogen atom is in an axial position. The fluorine atom occupies an axial position, and, as a consequence, the N–F bond is almost perpendicular to the benzene ring of **2**. The sulfonyl oxygen in the equatorial position can coordinate to the lithium ion of the enolate.<sup>7)</sup> Moreover, a stacking interaction between the benzene rings of **2** and **7** is expected to be energetically favorable. Therefore, **7** is predicted to approach **2** at the less hindered  $\alpha$ -face, as shown in Fig. 4.

In summary, we have synthesized BNBT-F (**2**) and have found it to be a new agent for enantioselective fluorination with moderate to high enantioselectivity. X-ray crystallographic analysis of **2** revealed that the N–F bond [1.425(3) Å] is slightly longer than that of Davis's (–)-*N*-fluoro-2,10-(3,3-dichlorocamphorsultam) 1.420(6) Å,<sup>6c)</sup> and slightly shorter than that of CMIT-F [1.435(3) Å].<sup>11)</sup> A relationship between N–F bond lengths and fluorination efficiency is now under investigation.

## Experimental

**General Methods** Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. IR spectra ( $\text{cm}^{-1}$ ) were recorded on a Perkin-Elmer 1600 spectrometer.  $^1\text{H}$ -NMR spectra were measured as solutions in  $\text{CDCl}_3$ , and chemical shifts are expressed in ppm relative to internal  $\text{Me}_4\text{Si}$  (0.00 ppm) and were recorded on a JEOL GX-270 (270 MHz) or a Varian Gemini 300 (300 MHz) spectrometer.  $^{19}\text{F}$ -NMR spectra were measured with  $\text{CFCl}_3$  as an internal standard and were taken with a JEOL GX-270 (254 MHz) spectrometer. Upfield shifts are quoted as negative  $\delta$  values. EI mass spectra were taken with a JEOL JMS-D300 spectrometer. Column chromatography and preparative TLC were performed on BW-200 (Fuji Silysia) and Kieselgel 60 (Merck, art. 7748), respectively. All reactions involving oxygen- or moisture-sensitive compounds were carried out under a  $\text{N}_2$  atmosphere. Unless otherwise noted, reagents were added by syringe. THF was distilled from sodium/benzophenone immediately prior to use.

**3-*tert*-Butyl-7-nitro-3,4-dihydro-2H-benzo[e][1,2]-thiazine 1,1-Dioxide (**5**)** A mixture of fuming  $\text{HNO}_3$  (1.76 ml, 42 mmol) and conc.  $\text{H}_2\text{SO}_4$  (6 ml) was dropped into a solution of 3-*tert*-butyl-3,4-dihydro-2H-benzo[e][1,2]-thiazine 1,1-dioxide<sup>4)</sup> (5.02 g, 21 mmol) at  $-20^{\circ}\text{C}$  and stirred for 1 h. The reaction mixture was poured onto crashed ice and extracted with AcOEt (150 ml $\times$ 3). The combined organic layer was washed with water, brine, and dried ( $\text{MgSO}_4$ ), then concentrated *in vacuo*. The residue was chromatographed (50%  $\text{CH}_2\text{Cl}_2$  in hexane, then 50% AcOEt in hexane) to give **5** (4.06 g, 68%) as a white powder. mp  $194^{\circ}\text{C}$  (AcOEt/hexane). IR (KBr)  $\text{cm}^{-1}$ : 3264, 3019, 2967, 1328, 1215, 758, 669.  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ )  $\delta$ : 1.06 (s, 9H, *tert*-Bu), 2.96 (dd,  $J=17.3$ , 11.7 Hz, 1H,  $\text{CHHAr}$ ), 3.08 (dd,  $J=17.3$ , 4.1 Hz, 1H,  $\text{CHHAr}$ ), 3.61 (ddd,  $J=12$ , 11, 4.1 Hz, 1H,  $\text{NCH<}$ ), 4.42 (d,

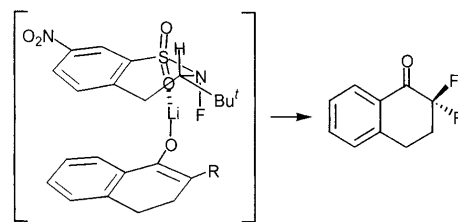


Fig. 4. Schematic Representation of the Transition State in the Fluorination of Enolates with (*S*)-**2**

$J=11.7$  Hz, 1H, NH), 7.45 (d,  $J=8.5$  Hz, 1H, ArH), 8.24 (dd,  $J=8.5$ , 2.4 Hz, 1H, ArH), 8.60 (d,  $J=2.4$  Hz, 1H, ArH). MS  $m/z$ : 284 ( $\text{M}^+$ ), 269 ( $\text{M}^+ - \text{Me}$ ), 227 ( $\text{M}^+ - \text{tert-Bu}$ ). HRMS Calcd for  $\text{C}_{12}\text{H}_{16}\text{N}_2\text{O}_4\text{S}$ : 284.0830. Found 284.0838. Anal. Calcd for  $\text{C}_{12}\text{H}_{16}\text{N}_2\text{O}_4\text{S}$ : C, 50.69; H, 5.67; N, 9.85. Found: C, 50.54; H, 5.60; N, 9.89.

***N*-Camphorsulfonyl-3-*tert*-butyl-7-nitro-3,4-dihydro-benzo[e][1,2]-thiazine 1,1-Dioxide (**6**)** A solution of **5** (1.04 g, 3.66 mmol) in THF (50 ml) was treated with NaH (60% oil dispersion, 292 mg, 7.72 mmol) at  $0^{\circ}\text{C}$  and stirred at room temperature for 1.5 h. (+)-10-Camphorsulfonyl chloride (1.38 g, 5.49 mmol) was added to the mixture, followed by stirring overnight. Cold saturated aqueous  $\text{NH}_4\text{Cl}$  was added, and the resulting mixture was extracted with AcOEt (50 ml $\times$ 3). The combined organic layer was washed with water, brine, and dried ( $\text{MgSO}_4$ ), then concentrated *in vacuo*. The residue was chromatographed (40%  $\text{CH}_2\text{Cl}_2$  in hexane, then 30% AcOEt in hexane) to give the less polar isomer (*R*)-**6** (0.38 g, 21%), the more polar isomer (*S*)-**6** (0.56 g, 31%) and 0.47 g of unreacted **5** as a white powder. The recovered **5** was recrystallized (50% AcOEt in hexane) to give racemic colorless crystals (0.26 g, 25%) and the enantiopure (*R*)-**5** (0.21 g, 20%) from the mother liquid.

**(3*R*)-*N*-Camphorsulfonyl-3-*tert*-butyl-7-nitro-3,4-dihydro-benzo[e][1,2]-thiazine 1,1-Dioxide (*R*)-**6**** Colorless crystals. mp  $152^{\circ}\text{C}$  (AcOEt/hexane).  $[\alpha]_D^{26} -14.1^{\circ}$  ( $c=0.88$ ,  $\text{CHCl}_3$ ). IR (KBr)  $\text{cm}^{-1}$ : 3020, 2966, 1747, 1534, 1216, 758, 669.  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ )  $\delta$ : 0.87 (s, 3H, gem Me), 1.07 (s, 3H, gem Me), 1.14 (s, 9H, *tert*-Bu), 1.39 (m, 1H), 1.61 (m, 1H), 2.03 (m, 1H), 2.35 (m, 2H), 3.22 (dd,  $J=14.9$ , 7.3 Hz, 1H,  $\text{CHHAr}$ ), 3.33 (dd,  $J=14.9$ , 11 Hz, 1H,  $\text{CHHAr}$ ), 3.83, 4.04 (ABq,  $J=14.9$  Hz, 2H,  $\text{CH}_2$ ), 4.55 (dd,  $J=11$ , 7.3 Hz, 1H,  $\text{NCH<}$ ), 7.56 (d,  $J=8.3$  Hz, 1H, ArH), 8.38 (dd,  $J=8.3$ , 2.2 Hz, 1H, ArH), 8.67 (d,  $J=2.2$  Hz, 1H, ArH). MS  $m/z$ : 499 ( $\text{M}^+ + 1$ ), 498 ( $\text{M}^+$ ), 483 ( $\text{M}^+ - \text{Me}$ ), 441 ( $\text{M}^+ - \text{tert-Bu}$ ). HRMS Calcd for  $\text{C}_{22}\text{H}_{30}\text{N}_2\text{O}_7\text{S}_2$ : 498.1495. Found 498.1463. Anal. Calcd for  $\text{C}_{22}\text{H}_{30}\text{N}_2\text{O}_7\text{S}_2$ : C, 52.99; H, 6.06; N, 5.62. Found: C, 52.76; H, 5.89; N, 5.44.

**(3*S*)-*N*-Camphorsulfonyl-3-*tert*-butyl-7-nitro-3,4-dihydro-benzo[e][1,2]-thiazine 1,1-Dioxide (*S*)-**6**** Colorless crystals. mp  $223^{\circ}\text{C}$  (AcOEt/hexane).  $[\alpha]_D^{26} +58.6^{\circ}$  ( $c=0.48$ ,  $\text{CHCl}_3$ ). IR (KBr)  $\text{cm}^{-1}$ : 3020, 2967, 1745, 1534, 1216, 758, 668.  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ )  $\delta$ : 0.90 (s, 3H, gem Me), 1.10 (s, 3H, gem Me), 1.14 (s, 9H, *tert*-Bu), 1.40 (m, 1H), 1.64 (m, 1H), 2.05 (m, 3H), 2.36 (m, 2H), 3.24 (dd,  $J=14.9$ , 7.3 Hz, 1H,  $\text{CHHAr}$ ), 3.35 (dd,  $J=14.9$ , 11.0 Hz, 1H,  $\text{CHHAr}$ ), 3.39, 4.38 (ABq,  $J=14.9$  Hz, 2H,  $\text{CH}_2$ ), 4.56 (dd,  $J=11.0$ , 7.6 Hz, 1H,  $\text{NCH<}$ ), 7.58 (d,  $J=8.3$  Hz, 1H, ArH), 8.40 (dd,  $J=8.3$ , 2.4 Hz, 1H, ArH), 8.71 (d,  $J=2.4$  Hz, 1H, ArH). MS  $m/z$ : 499 ( $\text{M}^+ + 1$ ), 498 ( $\text{M}^+$ ), 483 ( $\text{M}^+ - \text{Me}$ ), 441 ( $\text{M}^+ - \text{tert-Bu}$ ). HRMS Calcd for  $\text{C}_{22}\text{H}_{30}\text{N}_2\text{O}_7\text{S}_2$ : 498.1495. Found 498.1463. Anal. Calcd for  $\text{C}_{22}\text{H}_{30}\text{N}_2\text{O}_7\text{S}_2$ : C, 52.99; H, 6.06; N, 5.62. Found: C, 52.98; H, 6.05; N, 5.60.

**(3*R*)-3-*tert*-Butyl-7-nitro-3,4-dihydro-2H-benzo[e][1,2]-thiazine 1,1-Dioxide [(*R*)-**5**]** A solution of (*R*)-**6** (0.79 g, 1.58 mmol) in THF (15 ml) was treated with 2 M LiOH (7 ml) at room temperature and stirred for 1 h. The mixture was poured into 10% aqueous HCl solution and extracted with AcOEt (50 ml $\times$ 3). The combined organic layer was washed with water, brine, and dried ( $\text{MgSO}_4$ ), then concentrated *in vacuo*. The residue was chromatographed (20% AcOEt in hexane) to give (*R*)-**5** (0.37 g, 82%) as colorless crystals. mp  $223^{\circ}\text{C}$  (AcOEt/hexane).  $[\alpha]_D^{26} +52.4^{\circ}$  ( $c=0.96$ ,  $\text{CHCl}_3$ ). The optical pure (*R*)-**5** exhibited the same spectral properties (IR, MS and  $^1\text{H}$ -NMR) as the racemic **5**.

**(3*S*)-3-*tert*-Butyl-7-nitro-3,4-dihydro-2H-benzo[e][1,2]-thiazine 1,1-Dioxide [(*S*)-**5**]** In the same way, 0.6 g (1.2 mmol) of (*S*)-**6** was treated with 2 M LiOH (5 ml) to give (*S*)-**5** (0.30 g, 87%) as colorless crystals.  $[\alpha]_D^{26} -52.8^{\circ}$  ( $c=0.98$ ,  $\text{CHCl}_3$ ). The optical pure (*S*)-**5** exhibited the same spectral properties (IR, MS and  $^1\text{H}$ -NMR) as (*R*)-**5**.

**(3*R*)-*N*-Fluoro-3-*tert*-butyl-7-nitro-3,4-dihydro-2H-benzo[e][1,2]-thiazine 1,1-Dioxide [(*R*)-BNBT-F(**2**)]** A solution of (*R*)-**5** (1.83 g, 6.44 mmol) in THF (50 ml) was treated with NaH (60% dispersion in min-

eral oil, 386 mg, 9.66 mmol) under  $N_2$  at  $0^\circ C$ , and the mixture was stirred at room temperature for 1 h. To this mixture was introduced freshly prepared  $FCIO_3$  gas generated from  $KClO_4$  (4.46 g, 32.2 mmol) and  $FSO_3H$  (32.2 g, 322 mmol) for 3 h. The reaction was quenched by a saturated aqueous  $NH_4Cl$  solution and the resulting mixture was extracted with  $AcOEt$  (50 ml  $\times$  3). The combined organic layer was washed with water, brine, and dried ( $MgSO_4$ ), then concentrated *in vacuo*. The residue was chromatographed on silica gel (30%  $AcOEt$  in hexane) to give (R)-BNBTF(2) (1.28 g, 66%) as colorless crystals. mp  $143-145^\circ C$  ( $AcOEt$ /hexane).  $[\alpha]_D^{26} + 50.8^\circ$  ( $c=0.61$ ,  $CHCl_3$ ). IR (KBr)  $cm^{-1}$ : 3019, 2968, 1534, 1215, 758, 669.  $^{19}F$ -NMR ( $CDCl_3$ ,  $CFCl_3$ )  $\delta$ : -70.9 (brs, NF).  $^1H$ -NMR ( $CDCl_3$ )  $\delta$ : 1.18 (s, 9H, *tert*-Bu), 3.07 (m, 1H,  $CHHAr$ ), 3.37 (m, 1H,  $CHHAr$ ), 4.14 (brs, 1H,  $NCH<$ ), 7.60 (d,  $J=8.5$  Hz, 1H, ArH), 8.41 (dd,  $J=8.5$ , 2.2 Hz, 1H, ArH), 8.85 (d,  $J=2.2$  Hz, 1H, ArH). MS  $m/z$ : 302 ( $M^+$ ), 282 ( $M^+ - HF$ ), 244 ( $M^+ - tert$ -Bu). HRMS Calcd for  $C_{12}H_{15}FN_2O_4S$ : 302.0736. Found 302.0736. Anal. Calcd for  $C_{12}H_{15}FN_2O_4S$ : C, 47.67; H, 5.00; N, 9.27. Found: C, 47.83; H, 4.97; N, 9.39.

**(3S)-N-Fluoro-3-*tert*-butyl-7-nitro-3,4-dihydro-2H-benzo[e][1,2]-thiazine 1,1-Dioxide [(S)-BNBTF(2)]** In the same way, (S)-(2) (0.82 g, 83%) was obtained from (S)-5 (0.93 g, 3.26 mmol) as colorless crystals.  $[\alpha]_D^{26} - 47.3^\circ$  ( $c=0.69$ ,  $CHCl_3$ ). (S)-(2) exhibited the same spectral properties (IR, MS,  $^1H$ -NMR and  $^{19}F$ -NMR) as (R)-(2).

#### General Procedure for Asymmetric Fluorination of Ketones with 2:

**(R)-2-Fluoro-2-methyl-1-tetralone (8a)** To a stirred solution of 2-methyl-1-tetralone (16 mg, 0.10 mmol) in THF (1.0 ml) was added a 1.0 M solution of LHMDS in THF (0.15 ml, 0.15 mmol) under nitrogen at  $-78^\circ C$ . The mixture was stirred for 10 min at  $-78^\circ C$ , warmed to  $0^\circ C$ , stirred for an additional 1 h, and cooled to  $-50^\circ C$ . A solution of (R)-2 (36 mg, 0.12 mmol) in THF (1 ml) was added to the reaction mixture. After the reaction was complete, as indicated by TLC, the reaction mixture was quenched by the addition of saturated aqueous  $NH_4Cl$  (1 ml). The aqueous layer was extracted with  $AcOEt$  (5 ml  $\times$  3), the combined organic phase was washed with water (10 ml), brine (10 ml), and dried ( $MgSO_4$ ), then concentrated to give an oil which was purified by preparative TLC (10%  $AcOEt$  in hexane) to give **8a** (14.1 mg, 79%, 62% ee) as a colorless oil:  $[\alpha]_D^{26} + 21.7^\circ$  ( $c=0.63$ ,  $CHCl_3$ ); IR (neat)  $cm^{-1}$ : 2938, 1701.  $^{19}F$ -NMR  $\delta$ : -153.2 (m).  $^1H$ -NMR ( $CDCl_3$ )  $\delta$ : 1.60 (d,  $J=22.2$  Hz, 3H, Me), 2.21-2.36 (m, 1H,  $CHH$ ), 2.43-2.57 (m, 1H,  $CHH$ ), 3.01 (ddd,  $J=17.3$ , 9.3, 5.1 Hz, 1H,  $CHHAr$ ), 3.20 (dt,  $J=17.3$ , 5.3 Hz, 1H,  $CHHAr$ ), 7.26 (d,  $J=7.6$  Hz, 1H, ArH), 7.35 (t,  $J=7.3$ , 1.5 Hz, 1H, ArH), 7.52 (td,  $J=7.3$ , 1.5 Hz, 1H, ArH), 8.07 (dd,  $J=7.6$ , 1.5 Hz, 1H, ArH). MS  $m/z$  178 ( $M^+$ ). HRMS Calcd for  $C_{11}H_{11}FO$  178.0794. Found 178.0801.

**(S)-2-Ethyl-2-fluoro-1-tetralone (8b)** Fluorination of 2-ethyl-1-tetralone (15.4 mg, 0.1 mmol) with a 1 M solution of LHMDS (1.5 ml, 1.5 mmol) and (S)-2 (36 mg, 1.2 mmol) gave **8b** (14.6 mg, 79%, 44% ee) as a light yellow oil after purification by silica gel column chromatography (15%  $AcOEt$  in hexane).  $[\alpha]_D^{26} - 27.6^\circ$  ( $c=0.62$ ,  $CHCl_3$ ). IR (neat)  $cm^{-1}$ : 3019, 1700.  $^{19}F$ -NMR ( $CDCl_3$ ,  $CFCl_3$ )  $\delta$ : -163.2 (m);  $^1H$ -NMR ( $CDCl_3$ )  $\delta$ : 1.04 (t,  $J=7.3$  Hz, 3H, Me), 1.93 (m, 2H,  $CH_2$ ), 2.39 (m, 2H,  $CH_2$ ), 3.09 (m, 2H,  $CH_2Ar$ ), 7.25 (d,  $J=7.6$  Hz, 1H, ArH), 7.35 (t,  $J=7.6$  Hz, 1H, ArH), 7.52 (t,  $J=7.6$  Hz, 1H, ArH), 8.06 (d,  $J=7.8$  Hz, 1H, ArH). MS  $m/z$ : 192 ( $M^+$ ), 173 ( $M^+ - HF$ ), 163 ( $M^+ - Et$ ). HRMS Calcd for  $C_{12}H_{13}FO$ : 192.0950. Found 192.0967.

**(R)-5-Methoxy-2-ethyl-2-fluoro-1-tetralone (8c)** Fluorination of 5-methoxy-2-ethyl-1-tetralone (21 mg, 0.1 mmol) with a 1 M solution of LHMDS (1.5 ml, 1.5 mmol) and (R)-2 (36 mg, 1.2 mmol) gave **8c** (13.2 mg, 59%, 60% ee) as a light yellow oil after purification by silica gel column chromatography (15%  $AcOEt$  in hexane).  $[\alpha]_D^{26} + 16.8^\circ$  ( $c=0.43$ ,  $CHCl_3$ ). IR (neat)  $cm^{-1}$ : 3019, 1700.  $^{19}F$ -NMR ( $CDCl_3$ ,  $CFCl_3$ )  $\delta$ : -163.9 (m).  $^1H$ -NMR ( $CDCl_3$ )  $\delta$ : 1.02 (t,  $J=7.3$  Hz, 3H, Me), 1.89 (m, 2H,  $CH_2$ ), 2.34 (m, 2H,  $CH_2$ ), 2.78 (m, 1H,  $CHHAr$ ), 3.15 (m, 1H,  $CHHAr$ ), 3.87 (s, 3H, OMe), 7.04 (d,  $J=8.1$  Hz, 1H, ArH), 7.32 (t,  $J=7.8$  Hz, 1H, ArH), 7.65 (d,  $J=7.8$  Hz, 1H, ArH). MS  $m/z$ : 223 ( $M^+ + 1$ ), 222 ( $M^+$ ), 202 ( $M^+ - HF$ ), 202 ( $M^+ - Et$ ). HRMS Calcd for  $C_{13}H_{15}FO_2$ : 222.1056. Found 222.1065.

**(S)-2-Benzyl-2-fluoro-1-tetralone (8d)** Fluorination of 2-benzyl-1-tetralone (23.6 mg, 0.1 mmol) with a 1 M solution of LHMDS (1.5 ml, 1.5 mmol) and (S)-2 (36 mg, 1.2 mmol) gave **8d** (14 mg, 55%, 49% ee) as a light yellow oil after purification by silica gel column chromatography (15%  $AcOEt$  in hexane).  $[\alpha]_D^{26} - 28.6^\circ$  ( $c=0.54$ ,  $CHCl_3$ ). IR (neat)  $cm^{-1}$ : 3019, 1734.  $^{19}F$ -NMR ( $CDCl_3$ ,  $CFCl_3$ )  $\delta$ : -158.02 (m);  $^1H$ -NMR ( $CDCl_3$ )  $\delta$ : 1.98-2.25 (m, 2H,  $CH_2$ ), 2.89-3.07 (m, 3H,  $CH_2Ar$ ,  $CHHAr$ ), 3.26 (dd,  $J=17.6$ , 14.6 Hz, 1H,  $CHHAr$ ), 7.19-7.26 (m, 6H, ArH), 7.30 (t,  $J=7.6$  Hz, 1H, ArH), 7.47 (td, 1H,  $J=7.6$ , 1.5 Hz, ArH), 8.03 (dd, 1H,  $J=7.6$ , 1.5 Hz, ArH). MS  $m/z$ : 192 ( $M^+$ ), 173 ( $M^+ - HF$ ), 163 ( $M^+ - Et$ ). HRMS Calcd for

$C_{17}H_{15}FO$ : 254.1107. Found 254.1103.

**(-)-2-Carbomethoxy-2-fluoro-1-tetralone (8e)** Fluorination of 2-ethyl-1-tetralone (20.4 mg, 0.1 mmol) with a 1 M solution of LHMDS (1.5 ml, 1.5 mmol) and (S)-2 (36 mg, 1.2 mmol) gave **8e** (16.2 mg, 73%, 43% ee) as a light yellow oil after purification by silica gel column chromatography (30%  $AcOEt$  in hexane).  $[\alpha]_D^{26} - 6.7^\circ$  ( $c=0.71$ ,  $CHCl_3$ ). IR (neat)  $cm^{-1}$ : 3020, 1765, 1696.  $^{19}F$ -NMR ( $CDCl_3$ ,  $CFCl_3$ )  $\delta$ : -164.68 (dd,  $J=22.2$ , 11.1 Hz).  $^1H$ -NMR ( $CDCl_3$ )  $\delta$ : 2.51-2.85 (m, 2H,  $CH_2$ ), 3.02-3.28 (m, 2H,  $CH_2Ar$ ), 3.84 (s, 3H, OCH<sub>3</sub>), 7.28 (d,  $J=7.6$  Hz, 1H, ArH), 7.38 (t,  $J=7.6$  Hz, 1H, ArH), 7.56 (td,  $J=7.6$ , 1.5 Hz, 1H, ArH), 8.09 (dd,  $J=7.6$ , 1.5 Hz, 1H, ArH). MS  $m/z$ : 192 ( $M^+$ ), 173 ( $M^+ - HF$ ), 163 ( $M^+ - Et$ ). HRMS Calcd for  $C_{12}H_{11}FO_2$ : 222.2123. Found 222.2116.

**(S)-2-Methyl-2-fluoro-1-indanone (8f)** Fluorination of 2-methyl-1-indanone (14.6 mg, 0.1 mmol) with a 1 M solution of LHMDS (1.5 ml, 1.5 mmol) and (S)-2 (36 mg, 1.2 mmol) gave **8f** (12.1 mg, 74%, 52% ee) as a light yellow oil after purification by silica gel column chromatography (10%  $AcOEt$  in hexane).  $[\alpha]_D^{26} - 20.7^\circ$  ( $c=0.60$ ,  $CHCl_3$ ). IR (neat)  $cm^{-1}$ : 3023, 1730.  $^{19}F$ -NMR  $\delta$ : -152.8 (quintet d,  $J=22.5$ , 11 Hz).  $^1H$ -NMR (270 MHz)  $\delta$ : 1.63 (d,  $J=22.7$  Hz, 3H, Me), 3.30 (dd,  $J=17.6$ , 11.3 Hz, 1H,  $CHHAr$ ), 3.47 (dd,  $J=22.1$ , 17.6 Hz, 1H,  $CHHAr$ ), 7.43 (t,  $J=7.6$  Hz, 2H, ArH), 7.66 (t,  $J=7.6$  Hz, 1H, ArH), 7.82 (d,  $J=7.6$  Hz, 1H, ArH). MS  $m/z$  165 ( $M^+ + 1$ ), 164 ( $M^+$ ), 149 ( $M^+ - Me$ ). HRMS Calcd for  $C_{10}H_9FO$  164.0637. Found 164.0626.

**(R)-2-Benzyl-2-fluoro-1-indanone (8g)** Fluorination of 2-benzyl-1-indanone (22.2 mg, 0.1 mmol) with a 1 M solution of LHMDS (1.5 ml, 1.5 mmol) and (R)-2 (36 mg, 1.2 mmol) gave **8g** (9.6 mg, 40%, 57% ee) as a light yellow oil after purification by silica gel column chromatography (benzene):  $[\alpha]_D^{26} + 32.9^\circ$  ( $c=0.75$ ,  $CHCl_3$ ). IR (neat)  $cm^{-1}$ : 3023, 1726.  $^{19}F$ -NMR  $\delta$ : -154.72 (m).  $^1H$ -NMR (270 MHz)  $\delta$ : 2.95 (dd,  $J=30.3$ , 14.2 Hz, 1H,  $CHHAr$ ), 3.15 (dd,  $J=22.9$ , 17.8 Hz, 1H,  $CHHAr$ ), 3.39 (dd,  $J=17.8$ , 12.5 Hz, 1H,  $CHHAr$ ), 3.41 (t,  $J=14.1$  Hz, 1H,  $CHHAr$ ), 7.25-7.30 (m, 5H, ArH), 7.43 (m, 2H, ArH), 7.61 (td,  $J=7.6$ , 1.2 Hz, 1H, ArH), 7.81 (d,  $J=7.6$  Hz, 1H, ArH). MS  $m/z$  240 ( $M^+$ ), 221 ( $M^+ - HF$ ). HRMS Calcd for  $C_{16}H_{13}FO$ : 240.0950. Found 240.0953.

**(+)-2-*p*-Methoxybenzyl-2-fluoro-1-indanone (8h)** Fluorination of 2-*p*-methoxybenzyl-1-indanone (25.2 mg, 0.1 mmol) with a 1 M solution of LHMDS (1.5 ml, 1.5 mmol) and (R)-2 (36 mg, 1.2 mmol) gave **8h** (19 mg, 70%, 69% ee) as a light yellow oil after purification by silica gel column chromatography (50%  $CH_2Cl_2$  in hexane).  $[\alpha]_D^{26} + 46.2^\circ$  ( $c=0.56$ ,  $CHCl_3$ ). IR (neat)  $cm^{-1}$ : 3018, 1726.  $^{19}F$ -NMR ( $CDCl_3$ ,  $CFCl_3$ )  $\delta$ : -154.8 (m).  $^1H$ -NMR ( $CDCl_3$ )  $\delta$ : 2.92 (dd,  $J=29.7$ , 14.3 Hz, 1H,  $CHHAr$ ), 3.15 (dd,  $J=22.8$ , 17.3 Hz, 1H,  $CHHAr$ ), 3.35 (m, 2H,  $CH_2Ar$ ), 3.78 (s, 3H, OMe), 6.82 (d,  $J=8.6$  Hz, 2H, ArH), 7.18 (d,  $J=8.6$  Hz, 2H, ArH), 7.39 (dd,  $J=13.2$ , 7.6 Hz, 2H, ArH), 7.61 (t,  $J=7.6$  Hz, 1H, ArH), 7.80 (d,  $J=7.6$  Hz, 1H, ArH). MS  $m/z$ : 271 ( $M^+ + 1$ ), 270 ( $M^+$ ), 250 ( $M^+ - HF$ ). HRMS Calcd for  $C_{17}H_{15}FO_2$ : 270.1056. Found 270.1051.

**(-)-5,6-Dimethoxy-2-benzyl-2-fluoro-1-indanone (8i)** Fluorination of 5,6-dimethoxy-2-benzyl-1-indanone (28.2 mg, 0.1 mmol) with a 1 M solution of LHMDS (1.5 ml, 1.5 mmol) and (S)-2 (36 mg, 1.2 mmol) gave **8i** (16.8 mg, 56%, 60% ee) as a light yellow oil after purification by silica gel column chromatography (50%  $CH_2Cl_2$  in hexane).  $[\alpha]_D^{26} - 57.8^\circ$  ( $c=0.55$ ,  $CHCl_3$ ). IR (neat)  $cm^{-1}$ : 3021, 1710.  $^{19}F$ -NMR ( $CDCl_3$ ,  $CFCl_3$ )  $\delta$ : -153.5 (m);  $^1H$ -NMR ( $CDCl_3$ )  $\delta$ : 2.89 (dd,  $J=29.8$ , 14.1 Hz, 1H,  $CHHAr$ ), 2.98 (dd,  $J=22.9$ , 17.5 Hz, 1H,  $CHHAr$ ), 3.23 (dd,  $J=17.5$ , 12.0 Hz, 1H,  $CHHAr$ ), 3.33 (t,  $J=13.5$  Hz, 1H,  $CHHAr$ ), 3.84 (s, 1H, OMe), 3.87 (s, 1H, OMe), 6.68 (s, 1H, ArH), 7.13 (s, 1H, ArH), 7.20 (m, 5H, ArH). MS  $m/z$ : 301 ( $M^+ + 1$ ), 300 ( $M^+$ ), 280 ( $M^+ - HF$ ). HRMS Calcd for  $C_{18}H_{17}FO_3$ : 300.1162. Found 300.1174.

**Acknowledgment** This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan. N. S. wishes to thank the Uehara Memorial Foundation for support. We are also grateful to Dr. Bando Masahiko, Institute of Organic Chemistry, Otsuka Pharmaceutical Company, for his X-ray analysis of the absolute structure of compound (S)-2. We are much indebted to Dr. Kenneth L. Kirk (National Institutes of Health) for his constant advice and Ms. Hiroko Kakuda for providing insightful comments on the molecular structure of (S)-2.

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## Four New Stilbenoid C-Glucosides Isolated from the Stem Bark of *Shorea hemsleyana*

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**Four new C-glucopyranosides of resveratrol oligomers, hemsleyanosides A–D, were isolated from the bark of *Shorea hemsleyana*. The structures were established on the basis of spectroscopic evidence, including <sup>1</sup>H–<sup>1</sup>H and <sup>1</sup>H–<sup>13</sup>C long-range couplings and nuclear Overhauser effect experiments in the NMR spectrum.**

**Key words** *Shorea hemsleyana*; Dipterocarpaceae; stilbenoid; C-glucopyranoside; structure elucidation

In a previous paper,<sup>1)</sup> we reported the isolation and structural determination of four new stilbene derivatives, together with seven known resveratrol oligomers from an acetone extract of the bark of *Shorea hemsleyana*. Further investigation of more polar constituents of an acetone extract, four new C-glucosyl stilbene oligomers, were isolated. In nature, the occurrence of stilbene oligomer glycosides is very rare, especially as C-glycosides. Therefore, the structure of the compounds drew our attention in view of biological activities.<sup>2–5)</sup> In the present paper, we describe the isolation and structural determination of these compounds.

The fraction obtained by column chromatography of an acetone extract of the bark of *S. hemsleyana* [CHCl<sub>3</sub>–MeOH (5:1)] was further chromatographed on a reversed-phase (ODS) column eluted with a H<sub>2</sub>O–MeOH mixture (10% MeOH to 60% MeOH). The resulting fractions were further purified with a Sephadex LH 20 column and preparative TLC to give **1**–**4**.

Compound **1** (hemsleyanoside A), a dark brown amorphous powder, showed a positive reaction to the Gibbs

reagent. The negative ion FAB-MS showed an [M–H]<sup>–</sup> ion peak at *m/z* 777, indicating its molecular weight to be 778. The high-resolution (HR) negative FAB-MS at *m/z* 777.2409 corresponded to the molecular formula C<sub>40</sub>H<sub>42</sub>O<sub>16</sub>. The <sup>1</sup>H- and <sup>13</sup>C-NMR spectral data (Table 1) showed the presence of two C-glucopyranosyl moieties [ $\delta_{\text{H}}$  4.82, 4.93 (1H each, d, *J*=9.8 Hz, anomeric protons);  $\delta_{\text{C}}$  82.3, 79.7, 76.8, 74.5, 71.3, 62.1,  $\delta_{\text{C}}$  82.4, 79.8, 77.5, 73.6, 71.1, 62.4]. These signals were superimposed on those of 12-C-glucosylresveratrol. Considering the molecular composition, the aglycone moiety was presumed to be a resveratrol dimer. The <sup>1</sup>H-NMR and <sup>1</sup>H–<sup>1</sup>H shift correlation spectroscopy (COSY) spectrum (methanol-*d*<sub>4</sub>) showed the presence of two sets of *ortho*-coupled aromatic protons assignable to two 4-hydroxyphenyl groups [ $\delta$  7.02 (2H, d, *J*=8.5 Hz, H-2a, 6a), 6.67 (2H, d, *J*=8.5 Hz, H-3a, 5a);  $\delta$  7.07 (2H, d, *J*=8.8 Hz, H-2b, 6b), 6.58 (2H, d, *J*=8.8 Hz, H-3b, 5b)], two aromatic protons of a tetra-substituted benzene ring [ $\delta$  6.15 (2H, br s, H-10a, 14a)], an aromatic proton of a penta-substituted benzene ring ( $\delta$  6.78, s, H-14b), and a set of mutually coupled aliphatic pro-

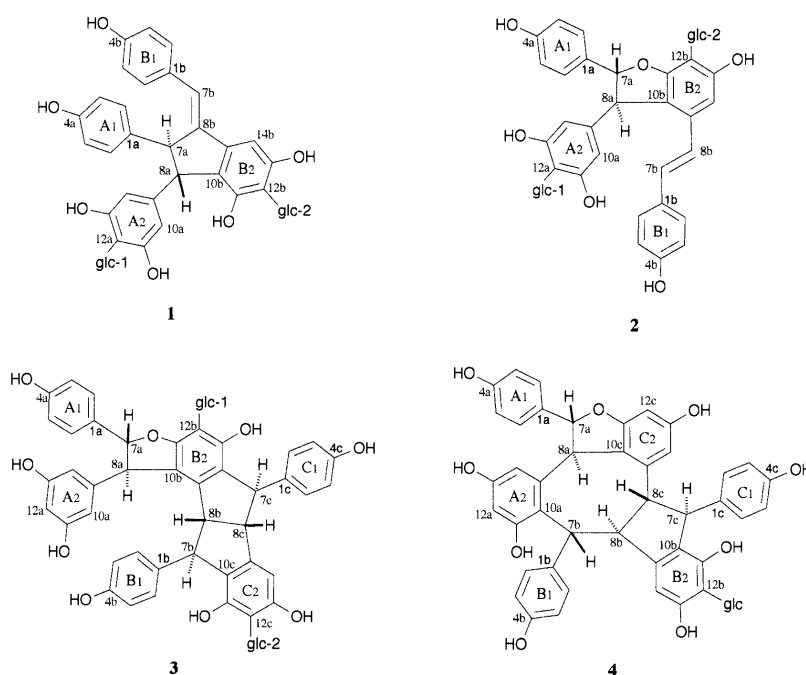


Fig. 1

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Table 1.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR Spectral Data of **1**–**4**

| No.            | <b>1</b> <sup>a)</sup> |                     | <b>2</b> <sup>a)</sup> |                     | <b>3</b> <sup>a)</sup> |                     | <b>4</b> <sup>b)</sup> |                     |
|----------------|------------------------|---------------------|------------------------|---------------------|------------------------|---------------------|------------------------|---------------------|
|                | $\delta_{\text{H}}$    | $\delta_{\text{C}}$ | $\delta_{\text{H}}$    | $\delta_{\text{C}}$ | $\delta_{\text{H}}$    | $\delta_{\text{C}}$ | $\delta_{\text{H}}$    | $\delta_{\text{C}}$ |
| 1a             |                        | 137.8               |                        | 134.3               |                        | 132.9               |                        | 134.6               |
| 2a, 6a         | 7.02 (d, 8.5)          | 129.0               | 7.24 (d, 8.5)          | 127.9               | 7.22 (d, 8.5)          | 129.1               | 6.88 (d, 8.8)          | 127.2               |
| 3a, 5a         | 6.67 (d, 8.5)          | 116.4               | 6.75 (d, 8.5)          | 116.2               | 6.81 (d, 8.5)          | 115.4               | 6.75 (d, 8.8)          | 116.0               |
| 4a             |                        | 156.7               |                        | 158.2               |                        | 158.4               |                        | 153.2 <sup>c)</sup> |
| 7a             | 4.17 (brs)             | 59.9                | 5.42 (d, 5.4)          | 94.3                | 5.27 (d, 8.5)          | 95.5                | 5.85 (d, 4.1)          | 88.1                |
| 8a             | 4.09 (brs)             | 59.1                | 4.31 (d, 5.4)          | 58.2                | 4.75 (d, 8.5)          | 57.9                | 5.09 (d, 4.1)          | 52.7                |
| 9a             |                        | 149.1               |                        | 147.1               |                        | 144.8               |                        | 141.1               |
| 10a            | 6.15 (brs)             | 107.7               | 6.28 (brs)             | 108.2               | 6.19 (d, 2.4)          | 108.1               |                        | 123.5               |
| 11a            |                        | 157.9 <sup>c)</sup> |                        | 158.7               |                        | 159.9               |                        | 156.4               |
| 12a            |                        | 110.1               |                        | 111.1               | 6.25 (t, 2.4)          | 102.4               | 6.33 (d, 2.4)          | 101.4               |
| 13a            |                        | 157.9 <sup>c)</sup> |                        | 158.7               |                        | 159.9               |                        | 158.1               |
| 14a            | 6.15 (brs)             | 107.7               | 6.28 (brs)             | 108.2               | 6.19 (d, 2.4)          | 108.1               | 6.26 (d, 2.4)          | 105.1               |
| 1b             |                        | 130.1               |                        | 130.2               |                        | 136.7               |                        | 136.7               |
| 2b, 6b         | 7.07 (d, 8.8)          | 131.2               | 7.08 (d, 8.8)          | 128.9               | 6.30 (d, 8.3)          | 129.3               | 7.29 (d, 8.5)          | 129.9               |
| 3b, 5b         | 6.58 (d, 8.8)          | 116.0               | 6.67 (d, 8.8)          | 116.5               | 6.42 (d, 8.3)          | 116.2               | 6.70 (d, 8.5)          | 115.9               |
| 4b             |                        | 157.5               |                        | 158.5               |                        | 156.2               |                        | 156.0 <sup>c)</sup> |
| 7b             | 6.97 (brs)             | 123.3               | 6.83 (d, 14.9)         | 130.6               | 4.43 (s)               | 50.5                | 5.36 (d, 10.0)         | 47.1                |
| 8b             |                        | 147.0               | 6.56 (d, 14.9)         | 123.3               | 3.74 (d, 6.0)          | 60.5                | 4.32 (dd, 10.0, 7.8)   | 53.6                |
| 9b             |                        | 143.2               |                        | 136.6               |                        | 149.7 <sup>d)</sup> |                        | 149.8 <sup>f)</sup> |
| 10b            |                        | 125.6               |                        | 120.1               |                        | 111.7               |                        | 124.8               |
| 11b            |                        | 154.7               |                        | 161.4               |                        | 161.0               |                        | 156.1 <sup>c)</sup> |
| 12b            |                        | 112.9               |                        | 107.7               |                        | 107.7               |                        | 111.1               |
| 13b            |                        | 157.9 <sup>c)</sup> |                        | 158.3               |                        | 153.8               |                        | 156.6               |
| 14b            | 6.78 (s)               | 99.5                | 6.68 (s)               | 105.2               |                        | 127.4               | 6.10 (s)               | 104.4               |
| 1c             |                        |                     |                        |                     |                        | 137.7               |                        | 139.7               |
| 2c, 6c         |                        |                     |                        |                     | 7.02 (d, 8.5)          | 129.3               | 7.17 (d, 8.3)          | 130.0               |
| 3c, 5c         |                        |                     |                        |                     | 6.71 (d, 8.5)          | 116.0               | 6.65 (d, 8.3)          | 115.8               |
| 4c             |                        |                     |                        |                     |                        | 155.7               |                        | 158.8 <sup>c)</sup> |
| 7c             |                        |                     |                        |                     | 4.58 (s)               | 53.7                | 4.74 (d, 6.3)          | 51.9                |
| 8c             |                        |                     |                        |                     | 3.66 (d, 6.0)          | 59.9                | 3.41 (dd, 7.8, 6.3)    | 56.5                |
| 9c             |                        |                     |                        |                     |                        | 144.4 <sup>d)</sup> |                        | 144.1 <sup>f)</sup> |
| 10c            |                        |                     |                        |                     |                        | 124.9               |                        | 120.4               |
| 11c            |                        |                     |                        |                     |                        | 153.6               |                        | 160.5               |
| 12c            |                        |                     |                        |                     |                        | 111.3               | 6.24 (brs)             | 95.8                |
| 13c            |                        |                     |                        |                     |                        | 157.2               |                        | 158.8               |
| 14c            |                        |                     |                        |                     | 6.61 (s)               | 104.7               | 6.80 (brs)             | 106.6               |
| glucose-1 (-1) | 4.82 (d, 9.8)          | 76.8                | 4.79 (d, 9.8)          | 76.6                | 4.71 (d, 9.8)          | 77.0                | 4.84 (d, 9.8)          | 77.3                |
| (-2)           | 3.63 (m)               | 74.5                | 4.15 (m)               | 73.1                | 3.87 (m)               | 73.5                | 3.61 (m)               | 74.8                |
| (-3)           | 3.43 (m)               | 79.7                | 3.48 (m)               | 80.0                | 3.45 (m)               | 79.6                | 3.51 (m)               | 79.4                |
| (-4)           | 3.48 (m)               | 71.3                | 3.39–3.48 (m)          | 71.3                | 3.50 (m)               | 70.9                | 3.58 (m)               | 70.6                |
| (-5)           | 3.28 (m)               | 82.3                | 3.39–3.48 (m)          | 82.3                | 3.36 (m)               | 82.1                | 3.40 (m)               | 82.2                |
| (-6)           | 3.72, 3.78 (m)         | 62.1                | 3.93–3.72 (m)          | 62.3                | 3.71, 3.81 (m)         | 62.4                | 3.75 (m)               | 61.6                |
| glucose-2 (-1) | 4.93 (d, 9.8)          | 77.5                | 4.91 (d, 9.8)          | 76.7                | 4.88 (d, 9.8)          | 77.4                |                        |                     |
| (-2)           | 3.85 (m)               | 73.6                | 3.93 (m)               | 73.6                | 3.69 (m)               | 74.3                |                        |                     |
| (-3)           | 3.44 (m)               | 79.8                | 3.45 (m)               | 80.0                | 3.48 (m)               | 79.5                |                        |                     |
| (-4)           | 3.42 (m)               | 71.1                | 3.39–3.48 (m)          | 71.8                | 3.43 (m)               | 71.3                |                        |                     |
| (-5)           | 3.38 (m)               | 82.4                | 3.39–3.48 (m)          | 82.4                | 3.35 (m)               | 82.1                |                        |                     |
| (-6)           | 3.67, 3.76 (m)         | 62.4                | 3.93–3.72 (m)          | 63.0                | 3.74, 3.85 (m)         | 61.9                |                        |                     |

a) Measured in methanol- $d_4$ . b) Measured in acetone- $d_6$ . c) Overlapping. d–f) Interchangeable. Assignment of glucose units are interchangeable between glucose-1 and glucose-2. All protons and carbons were assigned by  $^1\text{H}$ – $^1\text{H}$ ,  $^1\text{H}$ – $^{13}\text{C}$  long range,  $^{13}\text{C}$ – $^1\text{H}$  COSY and COLOC spectrum.

tons [ $\delta$  4.17 (1H, br s, H-7a), 4.09 (1H, br s, H-8a)], in addition to a signal attributed to an olefinic proton [ $\delta$  6.97 (1H, br s)]. The  $^1\text{H}$ -NMR spectral data in acetone- $d_6$  (see Experimental) showed the presence of six phenolic hydroxyl groups [ $\delta$  7.91, 8.12, 8.33 (2H each, br s)]. All protonated carbon signals were assigned by the  $^{13}\text{C}$ – $^1\text{H}$  shift correlation spectroscopy (CH-COSY) spectrum, and the results are shown in Table 1. In the  $^1\text{H}$ – $^1\text{H}$  long range COSY (H–H long-range COSY) spectrum, H-7a had a correlation with H-2a (6a), and H-8a with H-10a (14a), respectively. In the correlation spectroscopy involving long range coupling (COLOC) spectra ( $J=8$  Hz) (Fig. 2), significant correlations were observed be-

tween the aromatic carbons on the penta-substituted benzene ring (ring B<sub>2</sub>) and the methine protons as follows: C-9b/H-8a, C-10b/H-7a and C-9b/H-7a. In addition, long-range correlations were observed for C-8b/H-7a, C-8b/H-8a and C-7b/H-7a. These results indicate that the resveratrol moiety (ring A<sub>1</sub>–C-7a–C-8a–ring A<sub>2</sub>) formed a five-membered ring with ring B<sub>2</sub> and C-8b. The  $^3J$  long-range correlation between H-7b and C-2b (6a) indicated that the 4-hydroxyphenyl group (B<sub>1</sub>) was substituted at C-7b. The *E* configuration of a double bond was determined by an nuclear Overhauser effect (NOE) between H-7b/H-14b. NOEs observed for H-7a/H-10a(14a) and H-7b/H-14b suggested that rings A<sub>1</sub> and A<sub>2</sub>

were *trans*. Therefore the structure of **1**, including the relative stereochemistry of the aglycon, was determined to be as shown in Fig. 1. The aglycone is then ampelopsin D isolated from *Ampelopsis brevipedunculata* var. *hancei*.<sup>6)</sup> The positions of two glucose moieties were confirmed at C-12a and C-12b in the aglycone by analysis of the COLOC spectrum, as shown in Fig. 2.

Compound **2** (hemsleyanoside B), a dark brown amorphous powder, showed a positive reaction to the Gibbs reagent. The compound gave an  $[M-H]^-$  ion peak at  $m/z$  777 in negative ion FAB-MS, corresponding to the molecular formula  $C_{40}H_{42}O_{16}$ . The  $^1H$ - and  $^{13}C$ -NMR spectral data (Table 1) suggested the presence of two *C*-glucosyl moieties. The  $^1H$ -NMR spectrum showed the presence of two 4-hydroxyphenyl groups ( $A_1$ ,  $B_1$ ), *trans* olefines [ $\delta$  protons assignable 6.56, 6.83 (1H each, d,  $J=14.9$  Hz)] and mutually coupled protons [ $\delta$  4.31, 5.42 (1H each, d,  $J=5.4$  Hz)] on a dihydrofuran ring. These spectral data were similar to those

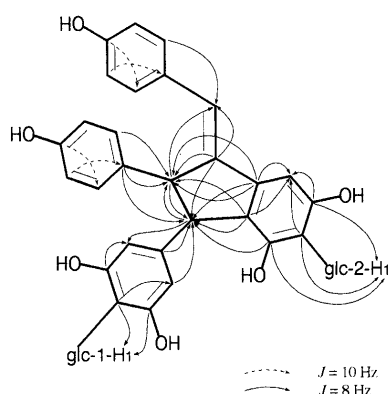


Fig. 2. CH Long Range Correlations in a COLOC Spectrum ( $J=8, 10$  Hz) of **1**

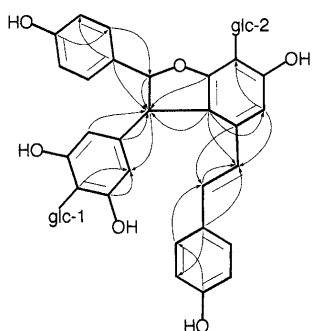


Fig. 3. COLOC Spectrum ( $J=10$  Hz) of **2**

of  $\epsilon$ -viniferin, except for the disappearance of two aromatic hydrogens on the 1,3-dioxygenated benzene rings caused by the introduction of two glucosyl moieties, indicating that **2** was a *C*-glucosyl derivative of  $\epsilon$ -viniferin.<sup>7)</sup> The positions of two glucosyl moieties were determined at C-12a and C-12b by the COLOC spectrum (Fig. 3) and by comparison of NMR spectral data with those of  $\epsilon$ -viniferin. Consequently, the structure of hemsleyanoside B was determined to be 12a,12b-di-*C*-glucopyranosyl- $\epsilon$ -viniferin.

Compound **3** (hemsleyanoside C), a dark brown amorphous powder, showed a positive reaction to the Gibbs reagent. The compound gave an  $[M-H]^-$  ion peak at  $m/z$  1003 in negative ion FAB-MS corresponding to the molecular formula  $C_{54}H_{52}O_{19}$ . The  $^1H$ -NMR spectrum (Table 1) showed the presence of three sets of *ortho*-coupled aromatic protons assignable to three 4-hydroxyphenyl groups [ $\delta$  7.22 (2H, d,  $J=8.5$  Hz, H-2a, 6a), 6.81 (2H, d,  $J=8.5$  Hz, H-3a, 5a);  $\delta$  6.30 (2H, d,  $J=8.3$  Hz, H-2b, 6b), 6.42 (2H, d,  $J=8.3$  Hz, H-3b, 5b);  $\delta$  7.02 (2H, d,  $J=8.5$  Hz, H-2c, 6c), 6.71 (2H, d,  $J=8.5$  Hz, H-3c, 5c)], a 3,5-dihydroxyphenyl group [ $\delta$  6.19 (2H, d,  $J=2.4$  Hz, H-10a, 14a), 6.25 (1H, t,  $J=2.4$  Hz, H-12a)], an aromatic proton on a penta-substituted benzene ring in singlet [ $\delta$  6.61 (1H, s, H-14c)], a set of mutually coupled aliphatic protons due to a dihydrofuran ring [ $\delta$  5.27 (1H, d,  $J=8.5$  Hz, H-7a), 4.75 (1H, d,  $J=8.5$  Hz, H-8a)], and a sequence of four aliphatic methine protons [ $\delta$  4.43 (1H, s, H-7b), 3.74 (1H, d,  $J=6.0$  Hz, H-8b), 4.58 (1H, s, H-7c), 3.66 (1H, d,  $J=6.0$  Hz, H-8c)] in addition to two glucosyl anomeric protons [ $\delta$  4.71, 4.88 (1H each, d,  $J=9.8$  Hz)], as shown in Table 1. All protonated carbons were assigned by CH-COSY spectrum. The above results indicated that **3** is a stilbene trimer having two *C*-glucosyl moieties. In the HH long-range COSY spectrum, an oxymethine proton (H-7a) had a correlation with H-2a(6a). A significant long-range correlation was observed between C-2b(6b)/H-7a and C-10a(14a)/H-8a in the COLOC spectrum (Fig. 4). These results showed that rings  $A_1$  and  $B_1$  were connected at C-7a and C-7b, respectively. Therefore, a plausible partial structure (**3A**) could be drawn as shown in Fig. 4. On the other hand,  $^3J$  long-range correlations were observed between C-2a(6a)/H-7c, C-1b/H-8c, C-1c/H-8c, C-8c/H-7b, C-8b/H-7c and C-2c(6c)/H-7c in the COLOC spectrum, suggesting that ring  $B_1$ , C7b, C8b, C8c, C7c and ring  $C_1$  were connected in this order. Other significant long-range correlations were observed between the quaternary carbons on ring  $C_2$  and the aliphatic methine protons as follows: C-9c/H-7c, C-9c/H-8b, C-10c/H-8b, C-11c/H-7b. In addition, correlations were ob-

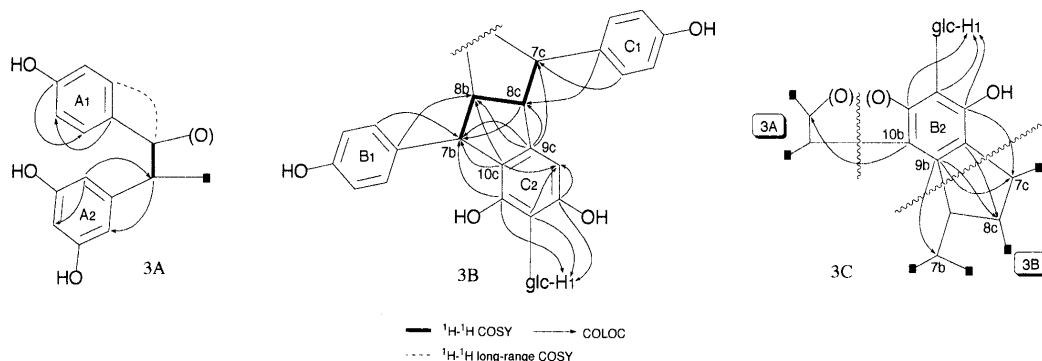
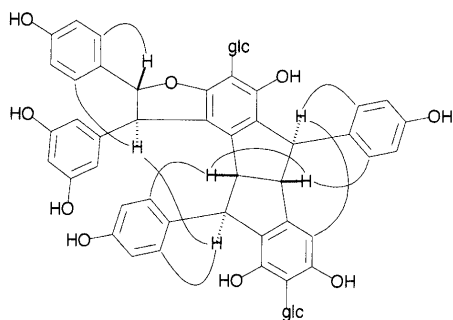
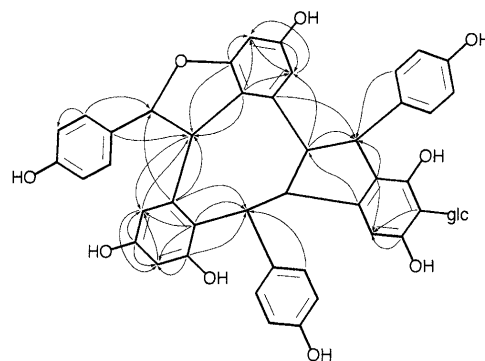
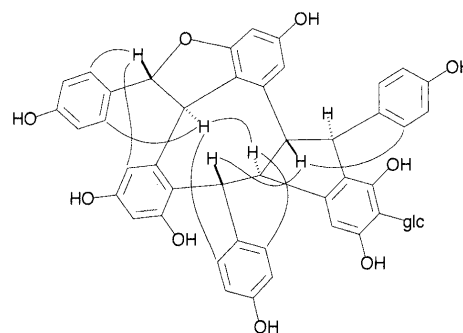


Fig. 4. Possible Partial Structures (**3A**, **3B**, **3C**) of **3**

Fig. 5. NOE Interactions in the Differential (DIF) Spectrum of **3**

served between the aromatic carbons on ring C<sub>2</sub> ( $\delta$  157.2, 153.6, 111.3) and a glucosidic anomeric proton ( $\delta$  4.88). Therefore, the glucosyl moiety was attached at the middle of two hydroxyl groups. These results indicated that **3** had a 12-C-glucopyranosyl resveratrol unit (ring C<sub>1</sub>–C7c–C8c–ring C<sub>2</sub>) which forms a benzocyclopentane ring with the methines C-7b and C-8b. The other partial structure (**3B**) could then be depicted as in Fig. 4. The remaining partial structure (**3C**) which consists of another glucopyranosyl residue and ring B<sub>2</sub> was determined as follows. Considering the chemical shifts of carbons with an oxygen function ( $\delta$  153.8, 161.0), ring B<sub>2</sub> was regarded as a 1,3-dioxygenated benzene. In the COLOC spectrum, the glucosyl anomeric proton ( $\delta$  4.71) was correlated to the above carbons, one of which ( $\delta$  153.8) was further correlated with H-7c on the partial structure of **3B**. The <sup>3</sup>J long-range correlations between the aromatic carbon on ring B<sub>2</sub> (C-9b) and two methine protons (H-7b, H-8c) supported the partial structure **3C**. The aromatic carbon due to C-10b on ring B<sub>2</sub> was correlated to H-8a, which indicated that the resveratrol moiety (ring A<sub>1</sub>–C7a–C8a–ring A<sub>2</sub>) formed a dihydrobenzofuran ring with ring B<sub>2</sub>. Thus, the total and planer structure of hemsleyanoside C (**3**) was composed by connecting the above three partial structures. The relative stereochemistry was substantiated by means of NOE spectrum (Fig. 5). An NOE between H-8a/H-2a (6a) showed that the two methine protons (H-7a, 8a) on the dihydrofuran ring were *trans*. NOEs between H-8b/H-2b (6b) and H-8c/H-2c (6c) indicated that both 4-hydroxyphenyl groups and the methine protons (ring B<sub>1</sub> and H-8b; ring C<sub>1</sub> and H-8c) were *cis*, respectively. In addition, an NOE observed between H-8b/H-8c suggested that the configuration of both methine protons were  $\beta$ . The  $\alpha$ -configuration of H-8a was also deduced by NOE results between H-8a/H-7b. Consequently, the relative structure of hemsleyanoside C was characterized as **3**.

Compound **4** (hemsleyanoside D), a brown amorphous powder, showed positive to the Gibbs reagent and had the molecular formula C<sub>48</sub>H<sub>42</sub>O<sub>14</sub>, supported by the HR-FAB-MS (*m/z* 841.2499) in negative ion mode. The <sup>1</sup>H- and <sup>13</sup>C-NMR spectrum (Table 1) showed a presence of a C-glucopyranosyl unit [ $\delta$ <sub>H</sub> 4.84 (1H, d, *J*=9.8 Hz, anomeric proton),  $\delta$ <sub>C</sub> 82.2, 79.4, 77.3, 74.8, 70.6, 61.6]. These results indicated that **4** was a mono-C-glucopyranosyl resveratrol trimer. The <sup>1</sup>H-NMR spectrum showed the presence of three sets of *ortho*-coupled aromatic protons assignable to three 4-hydroxyphenyl groups [ $\delta$  6.88 (2H, d, *J*=8.8 Hz, H-2a, 6a), 6.75 (2H, d, *J*=8.8 Hz, H-3a, 5a);  $\delta$  7.29 (2H, d, *J*=8.5 Hz, H-2b, 6b), 6.70 (2H, d, *J*=8.5 Hz, H-3b, 5b);  $\delta$  7.17 (2H, d, *J*=8.3

Fig. 6. COLOC Spectrum (*J*=8 Hz) of **4**Fig. 7. NOEs Observed in the DIFNOE Spectrum of **4**

Hz, H-2c, 6c), 6.65 (2H, d, *J*=8.3 Hz, H-3c, 5c)], two sets of *meta*-coupled aromatic protons of a 1,2,3,5-tetra-substituted benzene ring [ $\delta$  6.33 (1H, d, *J*=2.4 Hz, H-12a), 6.26 (1H, d, *J*=2.4 Hz, H-14a);  $\delta$  6.24 (1H, brs, H-12c), 6.80 (1H, brs, H-14c)], an aromatic proton on a penta-substituted benzene ring [ $\delta$  6.10 (H-14b)] in singlet, and a set of mutually coupled aliphatic protons [ $\delta$  5.85 (1H, d, *J*=4.1 Hz, H-7a), 5.09 (1H, d, *J*=4.1 Hz, H-8a)] in addition to a sequence of four aliphatic methine protons connected successively in this order [ $\delta$  5.36 (1H, d *J*=10.0 Hz, H-7b), 4.32 (1H, dd, *J*=10.0, 7.8 Hz, H-8b), 3.41 (1H, dd, *J*=7.8, 6.3 Hz, H-8c), 4.74 (1H, d *J*=6.3 Hz, H-7c)] in the HH COSY. The CH COSY spectrum supplied the complete assignment of all protonated carbons, as shown in Table 1. In the COLOC spectrum (Fig. 6), the correlations were observed between C-2a (6a)/H-7a, C-14a/H-8a, C-10c/H-8a and C-11c/H-8a, respectively. These results indicated that the resveratrol (ring A<sub>1</sub>–C7a–C8a–ring A<sub>2</sub>) formed a dihydrobenzofuran ring with ring C<sub>2</sub>. On the other hand, the HH long-range COSY correlations observed between H-7b/H-2b (6b) and H-8b/H-14b indicated a connection between ring B<sub>1</sub>/C-7b and B<sub>2</sub>/C-8b, respectively. The connectivities were further confirmed by the COLOC correlations. In the COLOC spectrum, long range correlations were observed between C-2c (6c)/H-7c, C-8c/H-14c, C-9b/H-8c and C-10b/H-7c. The planar structure of the aglycone could then be depicted as in Fig. 7. The position of a C-glucosyl residue was determined at C-12b as follows. A quaternary carbon ( $\delta$  111.1) on which the C-glucosyl residue was substituted caused a long-range correlation with H-14b in the COLOC spectrum. The relative stereochemistry of **4** was characterized by the results of NOE experiments (Fig. 7). The NOE interactions between H-2a (6a)/H-8a, H-8a/H-8b, H-8b/H-2b (6b) and H-8a/H-2b (6b)

indicated that ring A<sub>1</sub>, H-8a, H-8b and ring B<sub>1</sub> are in the same orientation. On the basis of NOE results [H-2c (6c)/H-8c, H-7b/H-8c], the relative configuration of **4** was determined to be as shown in Fig. 7. The aglycone, except the absolute structure, was the same as stenophyllol B isolated from the roots of *Sophora stenophylla*.<sup>8)</sup> The absolute stereochemistry of all compounds has not been determined yet. To the best of our knowledge, this occurrence of C-glycosylated stilbene oligomers is the first instance from nature.

#### Experimental

<sup>1</sup>H- and <sup>13</sup>C-NMR spectra were recorded on JNM EX-400 (JEOL) spectrometers. Chemical shifts are shown as  $\delta$ -values, with tetramethylsilane (TMS) as the internal reference. Peak multiplicities are quoted in Hz. Negative ion FAB-MS was measured on a JMS-DX-300 spectrometer equipped with a JMA 3500 data analysis system (JEOL). UV spectra were recorded on a UV-2200 spectrometer (Shimadzu), optical rotations on a P-1020 (JASCO) polarimeter. Silica gel 60 (70–230 mesh, Merck), Chromatorex (100–200 mesh, Fuji) and Sephadex LH-20 (Pharmacia) were used for column chromatography; Silica gel 60H (Merck) was used for vacuum liquid chromatography (VLC); Kiesel-gel 60F<sub>254</sub> (Merck) was used for analytical and preparative TLC.

**Extraction and Isolation of Compounds (1–4)** The bark of *Shorea hemsleyana* (1 kg) collected in Indonesia in Oct. 1997 was air-dried, powdered and extracted successively with acetone (31×3), MeOH (31×3) and 70% MeOH (31×3) at room temperature. A part (40 g) of the acetone extract (47 g) was chromatographed on Si gel (800 g) and eluted with CHCl<sub>3</sub>–MeOH mixtures to divide it into 34 fractions (fr. 1–34). Fraction 31–33 [CHCl<sub>3</sub>–MeOH (5:1) fraction] were combined and chromatographed by a reversed-phase ODS column (Chromatorex) under a vacuum condition using H<sub>2</sub>O–MeOH mixtures (10% MeOH–60% MeOH) to give 7 fractions. Compounds **1** (1.2 g), **2** (20 mg) and **3** (117 mg) were obtained in a pure form from the third (30% MeOH) fraction after purification by a Sephadex LH 20 column [Acetone–H<sub>2</sub>O (20:1) and MeOH] and preparative TLC (EtOAc: CHCl<sub>3</sub>: MeOH: H<sub>2</sub>O=20:10:11:5). The fourth (40% MeOH) fraction afforded **4** (25 mg) after purification by a Sephadex LH 20 column (MeOH) and preparative TLC (EtOAc: CHCl<sub>3</sub>: MeOH: H<sub>2</sub>O=15:8:4:1).

**Compound 1 (Hemsleyanoside A)** A dark brown amorphous solid. Negative ion HR-FAB-MS: ([M–H]<sup>–</sup>) *m/z* 777.2381 (Calcd 777.2394 for C<sub>40</sub>H<sub>41</sub>O<sub>16</sub>); Negative ion FAB-MS: *m/z* 777 ([M–H]<sup>–</sup>). UV  $\lambda_{\max}$  (MeOH)

nm: 224, 290 sh, 320 sh, 329, 340 sh. [ $\alpha$ ]<sub>D</sub><sup>24</sup> +2° (*c*=0.05, MeOH). The <sup>1</sup>H- and <sup>13</sup>C-NMR spectral data are listed in Table 1. <sup>1</sup>H-NMR (400 MHz, acetone-*d*<sub>6</sub>)  $\delta$ : 3.40–4.30 (10H, m, glucose), 4.10 (1H, brs, H-8a), 4.29 (1H, brs, H-7a), 4.86 (1H, d, *J*=9.8 Hz, glucose H-1 at C-12a), 4.93 (1H, d, *J*=9.3 Hz, glucose H-1 at C-12b), 6.16 (2H, s, H-10a, 14a), 6.68 (1H, d, *J*=8.5 Hz, H-3b, 5b), 6.76 (1H, d, *J*=8.5 Hz, H-3a, 5a), 6.84 (1H, s, H-14b), 7.08 (1H, s, H-7b), 7.11 (2H, d, *J*=8.5 Hz, H-2a, 6a), 7.20 (2H, d, *J*=8.5 Hz, H-2b, 6b), 7.91 (2H, brs, OH×2), 8.12 (2H, brs, OH×2), 8.33 (2H, brs, OH×2).

**Compound 2 (Hemsleyanoside B)** A dark brown amorphous solid. Negative ion HR-FAB-MS ([M–H]<sup>–</sup>) *m/z* 777.2409 (Calcd 777.2394 for C<sub>40</sub>H<sub>41</sub>O<sub>16</sub>); Negative ion FAB-MS: *m/z* 777 ([M–H]<sup>–</sup>). UV  $\lambda_{\max}$  (MeOH) nm: 211, 222 sh, 326. [ $\alpha$ ]<sub>D</sub><sup>24</sup> –25° (*c*=0.10, MeOH). The <sup>1</sup>H- and <sup>13</sup>C-NMR spectral data are listed in Table 1.

**Compound 3 (Hemsleyanoside C)** A dark brown amorphous solid. Negative ion HR-FAB-MS ([M–H]<sup>–</sup>) *m/z* 1003.3022 (Calcd 1003.3024 for C<sub>54</sub>H<sub>51</sub>O<sub>19</sub>); Negative ion FAB-MS: *m/z* 1003 ([M–H]<sup>–</sup>). UV  $\lambda_{\max}$  (MeOH) nm: 220, 271. [ $\alpha$ ]<sub>D</sub><sup>24</sup> –11° (*c*=0.10, MeOH). The <sup>1</sup>H- and <sup>13</sup>C-NMR spectral data are listed in Table 1.

**Compound 4 (Hemsleyanoside D)** A dark brown amorphous solid. Negative ion HR-FAB-MS ([M–H]<sup>–</sup>) *m/z* 841.2499 (Calcd 841.2496 for C<sub>48</sub>H<sub>41</sub>O<sub>14</sub>); Negative ion FAB-MS: *m/z* 841 ([M–H]<sup>–</sup>). UV  $\lambda_{\max}$  (MeOH) nm: 230, 285. [ $\alpha$ ]<sub>D</sub><sup>24</sup> –2.3° (*c*=0.10, MeOH). The <sup>1</sup>H- and <sup>13</sup>C-NMR spectral data are listed in Table 1.

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## Development of Potent and Selective Plasmin and Plasma Kallikrein Inhibitors and Studies on the Structure–Activity Relationship<sup>1)</sup>

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Received July 17, 2000; accepted September 16, 2000

Based on structure–activity relationship studies, we designed and synthesized plasmin (PL) and plasma kallikrein (PK) inhibitors. *Trans*-(4-aminomethylcyclohexanecarbonyl)-Tyr(*O*-Pic)-octylamide inhibited PL, PK, urokinase (UK) and thrombin (TH) with IC<sub>50</sub> values of 0.53, 30, 5.3 and >400 μM, respectively. *Trans*-(4-aminomethylcyclohexanecarbonyl)-Tyr(*O*-2-Pyrim)-4-carboxyanilide inhibited PL, PK, UK and TH with IC<sub>50</sub> values of 36, 0.56, 440 and >1000 μM, respectively.

**Key words** plasmin inhibitor; plasma kallikrein inhibitor; selectivity; structure–activity relationship

Proteinases and their natural inhibitors regulate biological functions cooperatively to maintain homeostasis, while an imbalance between them may cause serious disorders.<sup>2,3)</sup> With regard to plasmin (PL), α<sub>2</sub>-macroglobulin (α<sub>2</sub>-M)<sup>4)</sup> and α<sub>2</sub>-plasmin inhibitor (α<sub>2</sub>-PI)<sup>5)</sup> are known as endogenous inhibitors. Data has shown that α<sub>2</sub>-PI consists of two active regions: one binds to the active site (catalytic site) and another to the lysine binding site (LBS) of PL. An imbalance between PL and its natural inhibitors causes a serious syndrome, for instance, over activation of PL may induce hyperfibrinolysis.<sup>6–8)</sup> It was reported that PL selective inhibitor inhibited the increase of tumor cells in sarcoma 180 bearing mice.<sup>9)</sup> Plasma prekallikrein circulates in the blood as the zymogen of plasma kallikrein (PK) and is activated by factor XIIa to form plasma PK.<sup>10)</sup> PK releases bradykinin from high molecular weight kininogen.<sup>11)</sup> It is well known that the PK–kinin system is associated with various diseases, such as disseminated intravascular coagulation (DIC),<sup>12)</sup> allergy<sup>13)</sup> and ARDS (adult respiratory distress syndrome).<sup>14)</sup> Furthermore, it has been reported that PK activates factor XII,<sup>15)</sup> prourokinase,<sup>16)</sup> and plasminogen.<sup>17)</sup> PK is also known to cause neutrophil aggregation<sup>18)</sup> and elastase release.<sup>19)</sup> These observations suggest that although PK has many functions, its precise role remains to be determined.

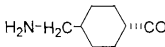
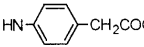
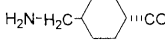
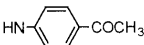
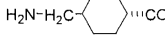
Keeping these circumstances in mind, our studies were directed toward the development of selective PL and PK in-

hibitors. Previously, we reported active center-directed inhibitors of PL<sup>20–22)</sup> and studies on their structure–activity relationship.<sup>23)</sup> Our inhibitors consist of three parts, P<sub>1</sub>, P<sub>1</sub>', and P<sub>2</sub>',<sup>24)</sup> and their structure–activity relationships are summarized in Table 1.

As shown in Table 1, compound I inhibits PK specifically. Compound II inhibits both PL and PK, and compound III specifically inhibits PL. The phenyl ring of Phe in compound I may interact with some functional domain of PK, while it does not appear to react with PL, thereby producing the difference in the inhibitory activity between against PK and PL. The benzene moiety of 2-Br-Z of compound II and III, however, may interact with PK and PL. The octyl amide moiety at the position P<sub>2</sub>' of compound III interacts with PL more suitably than PK. These results showed that we could design enzyme-selective inhibitors by combining various substituents at positions P<sub>1</sub>, P<sub>1</sub>', and P<sub>2</sub>'. This report deals with design and synthesis of selective PL and PK inhibitors and their structure–activity relationship.

First of all, 2-Br-Z moiety at position P<sub>1</sub>' of the compound III was modified in order to increase aqueous solubility. As shown in Table 2, the compound **1** exhibited similar inhibitory profile to that of compound III. Compound **2** exhibited an interesting inhibitory profile because it inhibited UK with an IC<sub>50</sub> value of 5.3 μM. On the other hand, compounds **1** and **3** did not inhibit UK. Interestingly, the compound **5** in-

Table 1. IC<sub>50</sub> Values (μM) of Compounds I–III for PL and PK

| No. | P <sub>1</sub>                                                                                                             | P <sub>1</sub> '                                                                                                                | P <sub>2</sub> '                                                                                          | PL     | PK     |
|-----|----------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|--------|--------|
|     |                                                                                                                            |                                                                                                                                 |                                                                                                           | S-2251 | S-2302 |
| I   | H <sub>2</sub> N–H <sub>2</sub> C–  –CO | Phe                                                                                                                             |  –CH <sub>2</sub> COOH | 630    | 1.3    |
| II  | H <sub>2</sub> N–H <sub>2</sub> C–  –CO | Tyr (O–CO <sub>2</sub> –CH <sub>2</sub> –  ) |  –COCH <sub>3</sub>    | 0.23   | 0.37   |
| III | H <sub>2</sub> N–H <sub>2</sub> C–  –CO | Tyr (O–CO <sub>2</sub> –CH <sub>2</sub> –  ) | HN–(CH <sub>2</sub> ) <sub>7</sub> –CH <sub>3</sub>                                                       | 0.80   | 16     |

S-2251: D-Val-Leu-Lys-pNA, S-2302: D-Pro-Phe-Arg-pNA

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Table 2.  $IC_{50}$  Values ( $\mu M$ ) of Compounds **1**–**11** for Various Enzymes

| No.       | $P_1$                    | $P_{1'}$                                                                                                                       | $P_2$                                                                                                                       | PL     |       | PK     | UK     | TH     |       |
|-----------|--------------------------|--------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------|--------|-------|--------|--------|--------|-------|
|           |                          |                                                                                                                                |                                                                                                                             | S-2251 | Fn    | S-2302 | S-2444 | S-2238 | Fg    |
| III       | $H_2N-H_2C-C_6H_{11}-CO$ | Tyr (O-CO <sub>2</sub> -CH <sub>2</sub> -  )  | NH-(CH <sub>2</sub> ) <sub>7</sub> -CH <sub>3</sub>                                                                         | 0.80   | 0.23  | 16     | >50    | >50    | >25   |
| <b>1</b>  | $H_2N-H_2C-C_6H_{11}-CO$ | Tyr (O-CH <sub>2</sub> -  )                   | NH-(CH <sub>2</sub> ) <sub>7</sub> -CH <sub>3</sub>                                                                         | 0.65   | 0.29  | 38     | >50    | >40    | >20   |
| <b>2</b>  | $H_2N-H_2C-C_6H_{11}-CO$ | Tyr (O-CH <sub>2</sub> -  )                   | NH-(CH <sub>2</sub> ) <sub>7</sub> -CH <sub>3</sub>                                                                         | 0.53   | 0.36  | 30     | 5.3    | >400   | >100  |
| <b>3</b>  | $H_2N-H_2C-C_6H_{11}-CO$ | Tyr (O-CO <sub>2</sub> -CH <sub>2</sub> -  )  | NH-(CH <sub>2</sub> ) <sub>7</sub> -CH <sub>3</sub>                                                                         | 1.0    | 0.85  | 12     | 81     | >200   | >200  |
| <b>4</b>  | $H_2N-H_2C-C_6H_{11}-CO$ | Tyr (O-CO <sub>2</sub> -CH <sub>2</sub> -  )  | NH-(CH <sub>2</sub> ) <sub>2</sub> -CH(  ) | 0.46   | 0.056 | 2.1    | 260    | 70     | >100  |
| <b>5</b>  | $H_2N-H_2C-C_6H_{11}-CO$ | Tyr (O-CH <sub>2</sub> -  )                   | NH-(CH <sub>2</sub> ) <sub>2</sub> -CH(  ) | 1.9    | 1.7   | 28     | 5.9    | >1000  | >1000 |
| <b>6</b>  | $H_2N-H_2C-C_6H_{11}-CO$ | Tyr (O-CO <sub>2</sub> -CH <sub>2</sub> -  )  | NH-(CH <sub>2</sub> ) <sub>2</sub> -CH(  ) | 2.2    | 2.0   | 7.0    | 75     | >1000  | >1000 |
| <b>7</b>  | $H_2N-H_2C-C_6H_{11}-CO$ | Tyr (O-CH <sub>2</sub> -  )                   | NH-CH <sub>2</sub> -C <sub>6H<sub>11</sub></sub> -CO <sub>2</sub> -(CH <sub>2</sub> ) <sub>7</sub> -CH <sub>3</sub>         | 1.2    | 0.6   | 30     | 25     | >100   | >10   |
| <b>8</b>  | $H_2N-(CH_2)_5-CO$       | Tyr (O-CO <sub>2</sub> -CH <sub>2</sub> -  )  | NH-CH <sub>2</sub> -C <sub>6H<sub>11</sub></sub> -CO <sub>2</sub> -(CH <sub>2</sub> ) <sub>7</sub> -CH <sub>3</sub>         | >100   | 5.0   | >400   | >100   | >100   | >10   |
| <b>9</b>  | $H_2N-(CH_2)_5-CO$       | Tyr (O-CH <sub>2</sub> -  )                   | NH-CH <sub>2</sub> -C <sub>6H<sub>11</sub></sub> -CO <sub>2</sub> -(CH <sub>2</sub> ) <sub>7</sub> -CH <sub>3</sub>         | 23     | 9.5   | >1000  | >100   | >100   | >20   |
| <b>10</b> | $H_2N-(CH_2)_5-CO$       | Tyr (O-CO <sub>2</sub> -CH <sub>2</sub> -  ) | NH-(CH <sub>2</sub> ) <sub>7</sub> -CH <sub>3</sub>                                                                         | 7.0    | 1.8   | 75     | >250   | >50    | >25   |
| <b>11</b> | $H_2N-(CH_2)_5-CO$       | Tyr (O-CH <sub>2</sub> -  )                 | NH-(CH <sub>2</sub> ) <sub>7</sub> -CH <sub>3</sub>                                                                         | 14     | 6.6   | >400   | 120    | >400   | >200  |

S-2251: D-Val-Leu-Lys-pNA, S-2302: D-Pro-Phe-Arg-pNA, S-2444: Glp-Gly-Arg-pNA, S-2238: D-Phe-Pip-Arg-pNA

Table 3.  $IC_{50}$  Values ( $\mu M$ ) of Compounds **2**, **12** and **13** for Various Enzymes

| No.       | R                                                                                                                                          | PL     |      | PK     | UK     | TH     |       |
|-----------|--------------------------------------------------------------------------------------------------------------------------------------------|--------|------|--------|--------|--------|-------|
|           |                                                                                                                                            | S-2251 | Fn   | S-2302 | S-2444 | S-2238 | Fg    |
| <b>2</b>  | -NH-CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>3</sub> | 0.53   | 0.36 | 30     | 5.3    | >400   | >100  |
| <b>12</b> | -NH-CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> -COOH            | 5.5    | 4.6  | 32     | 29     | >500   | >500  |
| <b>13</b> | -NH-CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> -NH <sub>2</sub> | 3.8    | 2.4  | 330    | 31     | >1000  | >1000 |

S-2251: D-Val-Leu-Lys-pNA, S-2302: D-Pro-Phe-Arg-pNA, S-2444: Glp-Gly-Arg-pNA, S-2238: D-Phe-Pip-Arg-pNA

hibited UK with an  $IC_{50}$  value of 5.9  $\mu M$ , while the compounds **4**<sup>23</sup> and **6**, which have bulkier moiety at the position  $P_{1'}$ , than that in compounds **2** and **5**, only inhibited PL. The combination of the picolyl moiety at position  $P_{1'}$  along with alkylamide at position  $P_2$  produced UK inhibitors. Compounds **8**,<sup>23</sup> **9**, **10**<sup>23</sup> and **11** exhibited similar inhibitory profiles to each other, although their inhibitory activity was weak. *Trans*-4-aminomethylcyclohexanecarbonyl (Tra) moiety at the position  $P_1$  is more suitable for interaction with active center of PL and PK than  $\epsilon$ -aminocaproyl moiety at the position  $P_1$ .

As summarized in Table 3, the compound **12**, which has carboxy group at position  $P_2$ , reduced inhibitory activity against PL and UK compared with the compound **2**, while **12** exhibited similar inhibitory activity against PK relative to **2**.

Similar phenomena were reported previously.<sup>25,26</sup> Compound **13**, which contains an amino group at position  $P_2$ , had a similar inhibitory profile against PL and UK in respect to compound **12**, with decreased inhibitory activity toward PK compared with compound **12**. The effect of carboxyl or amino function at position  $P_2$  on enzymatic activity implicated new strategies for the development of enzyme inhibitors.

As shown in Table 1, compound I is a selective PK inhibitor, compound II inhibited both PL and PK and compound III specifically inhibited PL. Figure 1 schematically shows the mode of interaction between the compound II and PK and PL. Although compound II inhibited PL and PK with  $IC_{50}$  values of 0.23 and 0.37  $\mu M$ , respectively, Tra-Phe-ACA (4-acetylanilide) inhibited PL and PK with  $IC_{50}$  values of 36 and 0.85  $\mu M$ , respectively,<sup>22</sup> indicating that Tra-Phe-ACA is

a more PK specific inhibitor. While the benzene moiety of Phe residue interacts with some part in PK, such part appears to be excluded from PL. On the other hand, the benzene moiety of 2-Br-Z at position P<sub>1'</sub> of compound II interacts with both PL and PK. Based on the above results, we further designed and synthesized PK specific inhibitors and summarized in Table 4. At position P<sub>1'</sub>, Tyr(*O*-2-Pyrim) or D,L-*m*-Tyr(*O*-2-Pyrim) was employed, in which methylene or methyloxycarbonyl group between *O* of Tyr residue and second aromatic moiety was deleted and at position P<sub>2'</sub>, carboxyl function was introduced. Compound **14** inhibited PK, PL, UK and TH with IC<sub>50</sub> values of 0.56, 36, 440 and >1000  $\mu$ M, respectively. The compound **15** inhibited the same enzymes with IC<sub>50</sub> values of 0.71, 32, 650 and >1000  $\mu$ M, respectively. Both compounds inhibited PK preferentially rather than PL as expected. As shown in Table 4, compounds **16** and **17** exhibited weaker inhibitory activity against PL and PK compared to compound **14** and **15**, although the difference in inhibitory activity against PL and PK is more than those of compounds **14** and **15**.

Compounds **2** and **15** will be candidates for development of novel drugs, such as anti-tumor medicine and anti-allergy medicine, by inhibiting PL and PK, respectively.

#### Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus without correction. Optical rotations were measured with an auto-

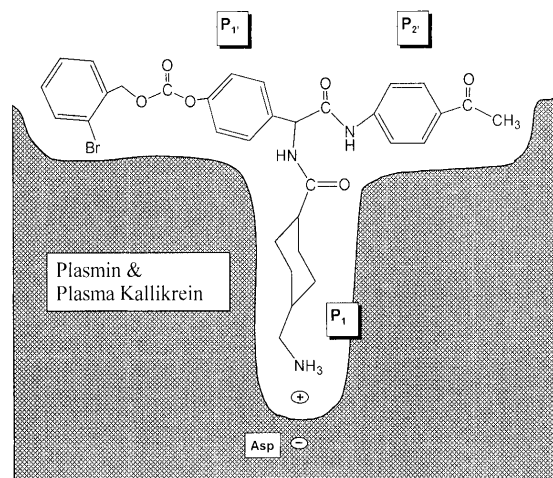


Fig. 1. Schematic Representation of Interaction of Tra-Tyr(*O*-BrZ)-ACA (II) with PL and PK

matic polarimeter, model DIP-360 (Japan Spectroscopic Co.). Matrix assisted laser desorption ionization time-of-flight mass spectra (MALDI-TOF-MAS) were obtained on a Kratos MALDI II mass spectrometer (Kratos Analysis). Waters model 600E was used for analytical or preparative HPLC. If necessary, peptides were purified by reverse phase HPLC on a C18 semi-preparative column (20×250 mm, Nakalai). The column was eluted in 40 min using a linear gradient from 10 to 50% acetonitrile in water, containing 0.05% TFA at flow rate of 10 ml/min; Detection was at 220 nm. 2-Chloropyrimidine, 4-picoly chloride and D,L-*m*-tyrosine (D,L-*m*-Tyr) were purchased from Tokyo Kasei (Japan). On TLC (Kieselgel G, Merck), *R*<sub>f</sub><sup>1</sup>, *R*<sub>f</sub><sup>2</sup>, *R*<sub>f</sub><sup>3</sup>, *R*<sub>f</sub><sup>4</sup>, *R*<sub>f</sub><sup>5</sup>, *R*<sub>f</sub><sup>6</sup>, and *R*<sub>f</sub><sup>7</sup> values refer to the systems of CHCl<sub>3</sub>, MeOH and AcOH (90:8:2); CHCl<sub>3</sub>, MeOH and H<sub>2</sub>O (89:10:1); CHCl<sub>3</sub>, MeOH and H<sub>2</sub>O (8:3:1, lower phase); *n*-BuOH, AcOH and H<sub>2</sub>O (4:1:5, upper phase); *n*-BuOH, AcOH, pyridine and H<sub>2</sub>O (4:1:1:2); *n*-BuOH, AcOH, pyridine and H<sub>2</sub>O (1:1:1:1) and CHCl<sub>3</sub> and ether (1:1), respectively.

***p*-Hydroxymethylbenzoic Acid** To a solution of *p*-formylbenzoic acid (0.50 g, 3.3 mmol) in dry MeOH (30 ml) was added sodium borohydride (0.37 g, 10 mmol) at 0°C. After 5 min in an ice-bath, the reaction mixture was stirred at room temperature for 2 h. After neutralization with AcOH, the solvent was removed under reduced pressure. The residue was extracted with AcOEt and the extract was washed with 10% AcOH, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated down. Petroleum ether was added to the residue to give a white precipitate, which was collected by filtration, yield 0.42 g (84%), mp 177–180°C, *R*<sub>f</sub><sup>1</sup> 0.38. Anal. Calcd for C<sub>8</sub>H<sub>8</sub>O<sub>3</sub>: C, 63.2; H, 5.30. Found: C, 62.9; H, 5.30.

**Methyl *p*-Hydroxymethylbenzoate** To dry methanol (30 ml), thionyl chloride (0.70 ml, 8.0 mmol) was added dropwise at –5 to –10°C. *p*-Hydroxymethylbenzoic acid (1.0 g, 8.0 mmol) was added to the above solution. The reaction mixture was stirred at 50°C for 3 h. After removal of the solvent, petroleum ether was added to the residue to yield a white precipitate, which was collected by filtration, yield 0.90 g (83%), mp 43.5–44°C, *R*<sub>f</sub><sup>1</sup> 0.61. Anal. Calcd for C<sub>9</sub>H<sub>10</sub>O<sub>3</sub>·1/10H<sub>2</sub>O: C, 64.4; H, 6.06. Found: C, 64.5; H, 5.93.

***p*-Methyloxycarbonylbenzyl Chloride** Methyl *p*-hydroxymethylbenzoate (0.32 g, 2.0 mmol) was added to a cooled thionyl chloride (0.34 ml, 4.0 mmol). After all the methyl *p*-hydroxymethylbenzoate had been added, the reaction mixture was stirred at room temperature for 15 h. The reaction mixture was poured into the cracked ice with stirring. The aqueous solution was extracted with ether, and the extract was dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated down to give oily material, which was dried over P<sub>2</sub>O<sub>5</sub> *in vacuo*, yield 0.29 g (78%), *R*<sub>f</sub><sup>1</sup> 0.77.

**Boc-Tyr(*O*-CH<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>-COOCH<sub>3</sub>)-octylamide** To a solution of Boc-Tyr-octylamide (1.3 g, 4.0 mmol) [prepared from Boc-Tyr(2-Br-Z)-octylamide<sup>23</sup>] by catalytic hydrogenation as usual in DMF (30 ml) was added 50% sodium hydride (0.21 g, 5.2 mmol) at 0°C. After the evolution of hydrogen gas ceased, *p*-methyloxycarbonylbenzyl chloride (1.9 g, 11 mmol) in DMF (10 ml) and sodium iodide (0.69 g, 4.0 mmol) were added to the solution. The reaction mixture was stirred at 40°C for 6 h. After neutralization with AcOH, the solvent was removed and the residue extracted with AcOEt. The extract was washed with water, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated down. Petroleum ether was added to the residue to give a precipitate, which was collected by filtration. The crude product dissolved in CHCl<sub>3</sub> (2 ml) was applied to a column of silica gel (3.0×30 cm), equilibrated and eluted with CHCl<sub>3</sub>. The solvent of the effluent (300–600 ml) was removed by evaporation. Pe-

Table 4. IC<sub>50</sub> Values ( $\mu$ M) of Compounds **14**–**17** for Various Enzymes

| No.       | P <sub>1</sub>                                                      | P <sub>1'</sub>                                                                                              | P <sub>2'</sub>                         | PL     |      | PK     |        | UK     |       | TH |  |
|-----------|---------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------|-----------------------------------------|--------|------|--------|--------|--------|-------|----|--|
|           |                                                                     |                                                                                                              |                                         | S-2251 | Fn   | S-2302 | S-2444 | S-2238 | Fg    |    |  |
| <b>14</b> | H <sub>2</sub> N-H <sub>2</sub> C-C <sub>6</sub> H <sub>4</sub> -CO | Tyr (O-  )                | -NH-C <sub>6</sub> H <sub>4</sub> -COOH | 36     | 22   | 0.56   | 440    | >1000  | >1000 |    |  |
| <b>15</b> | H <sub>2</sub> N-H <sub>2</sub> C-C <sub>6</sub> H <sub>4</sub> -CO | Tyr (O-  )                | -NH-C <sub>6</sub> H <sub>4</sub> -COOH | 32     | 29   | 0.71   | 650    | >1000  | >1000 |    |  |
| <b>16</b> | H <sub>2</sub> N-H <sub>2</sub> C-C <sub>6</sub> H <sub>4</sub> -CO | D,L- <i>m</i> -Tyr (O-  ) | -NH-C <sub>6</sub> H <sub>4</sub> -COOH | >400   | >400 | 3.6    | >400   | >400   | >400  |    |  |
| <b>17</b> | H <sub>2</sub> N-H <sub>2</sub> C-C <sub>6</sub> H <sub>4</sub> -CO | D,L- <i>m</i> -Tyr (O-  ) | -NH-C <sub>6</sub> H <sub>4</sub> -COOH | >500   | 410  | 6.8    | >500   | >500   | >500  |    |  |

S-2251: D-Val-Leu-Lys-*p*NA, S-2302: D-Pro-Phe-Arg-*p*NA, S-2444: Glp-Gly-Arg-*p*NA, S-2238: D-Phe-Pip-Arg-*p*NA

toluene ether was added to the residue to yield a precipitate, which was collected by filtration, yield 1.16 g (57%), mp 134–137 °C,  $[\alpha]_D^{25} +5.1^\circ$  ( $c=1.0$ , MeOH),  $R_f^1$  0.85. *Anal.* Calcd for  $C_{31}H_{44}N_2O_6$ : C, 67.7; H, 8.25; N, 5.09. Found: C, 68.1; H, 8.02; N, 4.98.

**Boc-Tyr(O-iNoc)-OH** To a solution of Boc-Tyr-OH (2.8 g, 10 mmol) in DMF (50 ml) was added 50% sodium hydride (1.1 g, 22 mmol) at 0 °C. After the evolution of hydrogen gas ceased, isonicotinyl *p*-nitrophenyl carbonate (3.0 g, 11 mmol) was added to the solution. The reaction mixture was stirred at 25–30 °C for 5 h to give a clear solution. After neutralization with AcOH, the solvent was removed *in vacuo*. The solution of the residue in water (30 ml) was washed with ether. The pH of the aqueous phase was adjusted to 6 with AcOH under cooling to give a precipitate, which was collected by filtration and recrystallized from EtOH, yield 1.8 g (43%), mp 161–162 °C,  $[\alpha]_D^{25} +9.0^\circ$  ( $c=0.9$ , MeOH),  $R_f^1$  0.60,  $R_f^2$  0.12. *Anal.* Calcd for  $C_{29}H_{41}N_3O_7 \cdot 1/4 H_2O$ : C, 59.9; H, 5.86; N, 6.65. Found: C, 59.9; H, 5.86; N, 6.74.

**Boc-Tyr(O-iNoc)-R [R: NH(CH<sub>2</sub>)<sub>7</sub>CH<sub>3</sub>, NH(CH<sub>2</sub>)<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>]** A mixed anhydride [prepared from Boc-Tyr(O-iNoc)-OH (1.2 g, 2.8 mmol) and isobutyl chloroformate (0.36 ml, 2.6 mmol) as usual] in THF (60 ml) was added to an ice-cooled solution of the corresponding amine (3.0 mmol) in THF (10 ml). The reaction mixture was stirred at 0 °C for 1 h and at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 5% NaHCO<sub>3</sub>, 10% citric acid and water, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated down. Ether was added to the residue to give a white precipitate, which was collected by filtration. R: NH(CH<sub>2</sub>)<sub>7</sub>CH<sub>3</sub>, yield 81%, mp 118–122 °C,  $[\alpha]_D^{25} +5.5^\circ$  ( $c=0.9$ , MeOH),  $R_f^1$  0.60,  $R_f^3$  0.12. *Anal.* Calcd for  $C_{29}H_{41}N_3O_6 \cdot 1/4 H_2O$ : C, 65.5; H, 7.83; N, 7.89. Found: C, 65.7; H, 8.04; N, 7.74. R: NH(CH<sub>2</sub>)<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>, yield 79%, mp 149–151 °C,  $[\alpha]_D^{25} +4.1^\circ$  ( $c=1.0$ , MeOH),  $R_f^1$  0.67,  $R_f^3$  0.67. *Anal.* Calcd for  $C_{26}H_{35}N_3O_6$ : C, 64.3; H, 7.27; N, 8.65. Found: C, 64.1; H, 7.39; N, 8.58.

**Boc-Tyr-NH(CH<sub>2</sub>)<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>** Boc-Tyr(2-Br-Z)-NH(CH<sub>2</sub>)<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub><sup>23</sup> (3.1 g, 5.5 mmol) in MeOH (30 ml) was hydrogenated over a Pd catalyst. After removal of Pd and the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid and water, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated down. Petroleum ether was added to the residue to give a precipitate, which was collected by filtration, yield 1.7 g (88%), mp 147–150 °C,  $[\alpha]_D^{25} +6.1^\circ$  ( $c=0.3$ , MeOH),  $R_f^1$  0.70,  $R_f^2$  0.60. *Anal.* Calcd for  $C_{19}H_{30}N_2O_4 \cdot 1/4 H_2O$ : C, 64.3; H, 8.66; N, 7.89. Found: C, 64.6; H, 8.60; N, 7.87.

**Boc-Tyr(O-Pic)-NH(CH<sub>2</sub>)<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>** To a solution of Boc-Tyr-NH(CH<sub>2</sub>)<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub> (1.7 g, 4.8 mmol) in DMF (50 ml) was added 60% sodium hydride (0.20 g, 5.0 mmol) at 0 °C. After the evolution of hydrogen gas ceased, 4-picolyl chloride [prepared from 4-picolyl chloride hydrochloride (0.82 g, 5.0 mmol)] in DMF (10 ml) was added to the solution. The reaction mixture was stirred at room temperature overnight. After neutralization with AcOH, the solvent was removed *in vacuo*. The residue was extracted with AcOEt. The extract was washed with 5% NaHCO<sub>3</sub>, 10% citric acid and water, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated down. Petroleum ether was added to the residue to give a precipitate, which was collected by filtration. The crude product in CHCl<sub>3</sub> (2 ml) was applied to a column of silica gel (3.0×25 cm), equilibrated and eluted with CHCl<sub>3</sub>. The solvent of the effluent (1800–3000 ml) was removed by evaporation. Petroleum ether was added to the

residue to form a precipitate, which was collected by filtration, yield 1.0 g (47%), mp 131–132 °C,  $[\alpha]_D^{25} +5.8^\circ$  ( $c=0.9$ , MeOH),  $R_f^2$  0.53. *Anal.* Calcd for  $C_{25}H_{35}N_3O_4$ : C, 68.2; H, 8.33; N, 9.65. Found: C, 68.2; H, 8.10; N, 9.62.

**General Procedure for Preparation of Boc-Tra-Tyr(X)-R [R: NH-(CH<sub>2</sub>)<sub>7</sub>-CH<sub>3</sub>, NH(CH<sub>2</sub>)<sub>2</sub>-CH(CH<sub>3</sub>)<sub>2</sub>, X: iNoc, Pic, CH<sub>2</sub>--COOMe]**

Boc-Tra-OH (0.31 g, 1.2 mmol) was added to the corresponding amine component [prepared from the corresponding N<sup>α</sup>-protected Tyr derivative (1.2 mmol) and TFA (4.6 ml, 60 mmol) in the presence of anisole (0.13 ml, 1.2 mmol) as usual] in acetonitrile (30 ml) containing DIPEA (0.41 ml, 2.4 mmol) and BOP reagent (0.53 g, 1.2 mmol). The reaction mixture was stirred at room temperature for 15 h. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% NaHCO<sub>3</sub> and water, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated down. Ether was added to the residue to give a white precipitate, which was collected by filtration and recrystallized from AcOEt. Yield, mp,  $[\alpha]_D^{25}$  value, elemental analysis and  $R_f$  values are summarized in Table 5.

**General Procedure for Preparation of H-Tra-Tyr(X)-R·HCl [R: NH-(CH<sub>2</sub>)<sub>7</sub>-CH<sub>3</sub>, NH(CH<sub>2</sub>)<sub>2</sub>-CH(CH<sub>3</sub>)<sub>2</sub>, X: iNoc, Pic, CH<sub>2</sub>--COOMe]**

Boc-Tra-Tyr(X)-R (0.30 mmol) was dissolved in TFA (1.28 ml, 17 mmol) containing anisole (0.04 ml, 0.34 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 60 min. Ether was added to the solution to yield a white precipitate, which was collected by filtration and dried over KOH pellets *in vacuo*. Each product was lyophilized from 0.1 M HCl (10 ml) to afford an amorphous powder. Yield, mp,  $[\alpha]_D^{25}$  value, elemental analysis and  $R_f$  values are summarized in Table 6.

**Boc-Tyr(O-Pic)-Tra-Octyl Ester** To a solution of Boc-Tyr-Tra-octyl ester (1.75 g, 3.3 mmol) [prepared from Boc-Tyr(2-Br-Z)-Tra-octyl ester by catalytic hydrogenation as usual] in DMF (100 ml) was added 60% sodium hydride (0.33 g, 8.2 mmol) at 0 °C. After the evolution of hydrogen gas ceased, 4-picolyl chloride [prepared from 4-picolyl chloride hydrochloride (0.60 g, 0.82 mmol)] in DMF (30 ml) was added to the solution. The reaction mixture was stirred at room temperature overnight. After neutralization with AcOH, the solvent was removed *in vacuo*. The residue was extracted with AcOEt. The extract was washed with 5% NaHCO<sub>3</sub>, 10% citric acid and water, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated down. Petroleum ether was added to the residue to give an oily product. The crude product in CHCl<sub>3</sub> (2 ml) was applied to a column of silica gel (2.0×40 cm), equilibrated and eluted with CHCl<sub>3</sub>. The solvent of the effluent (500–1300 ml) was removed by evaporation. Petroleum ether was added to the residue to give purified oily material, yield 1.25 g (61%),  $R_f^1$  0.76.

**Boc-Tra-Tyr(O-Pic)-Tra-Octyl Ester** A mixed anhydride [prepared from Boc-Tra-OH (205 mg, 0.80 mmol), isobutyl chloroformate (0.11 ml, 0.80 mmol) and Et<sub>3</sub>N (0.11 ml, 0.78 mmol) as usual] in THF (30 ml) was added to a solution of H-Tyr(O-Pic)-Tra-octyl ester [prepared from Boc-Tyr(O-Pic)-Tra-octyl ester (500 mg, 0.80 mmol), TFA (0.6 ml, 8.0 mmol) and anisole (0.26 ml, 2.4 mmol) as usual] in DMF (40 ml) containing Et<sub>3</sub>N (0.11 ml, 0.78 mmol) under cooling with ice-water. The reaction mixture was stirred at room temperature overnight. After removal of the solvents, the residue was extracted with AcOEt. The extract was washed with 5% Na<sub>2</sub>CO<sub>3</sub>, 10% citric acid and water, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated down. Petroleum ether was added to the residue to afford crystals, which were col-

Table 5. Yield, Melting Point, Optical Rotation, Elemental Analysis and  $R_f$  Values of Boc-Tra-Tyr(X)-R

| X:                                                                                                                        | R:                                                                    | Yield (%) | mp (°C)   | $[\alpha]_D^{25}$ (DMF) | Formula                             | Elemental analysis<br>Calcd (Found) |                |                | $R_f^1$ |
|---------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------|-----------|-----------|-------------------------|-------------------------------------|-------------------------------------|----------------|----------------|---------|
|                                                                                                                           |                                                                       |           |           |                         |                                     | C                                   | H              | N              |         |
| iNoc                                                                                                                      | NH-(CH <sub>2</sub> ) <sub>7</sub>                                    | 52.0      | 169–179   | −10.2<br>( $c=1.0$ )    | $C_{29}H_{54}N_4O_7 \cdot 1/4 H_2O$ | 65.8<br>(66.0)                      | 8.20<br>(8.10) | 8.28<br>(8.30) | 0.55    |
| iNoc                                                                                                                      | NH-(CH <sub>2</sub> ) <sub>2</sub> -CH(CH <sub>3</sub> ) <sub>2</sub> | 75.0      | 196–197   | −10.2<br>( $c=1.0$ )    | $C_{34}H_{48}N_4O_7 \cdot 1/2 H_2O$ | 64.4<br>(64.5)                      | 7.79<br>(7.82) | 7.79<br>(7.82) | 0.58    |
| Pic                                                                                                                       | NH-(CH <sub>2</sub> ) <sub>7</sub>                                    | 72.0      | 188–191   | −11.0<br>( $c=1.0$ )    | $C_{36}H_{54}N_4O_5$                | 69.4<br>(69.1)                      | 8.74<br>(8.86) | 9.00<br>(8.90) | 0.62    |
| Pic                                                                                                                       | NH-(CH <sub>2</sub> ) <sub>2</sub> -CH(CH <sub>3</sub> ) <sub>2</sub> | 75.0      | 143–145   | −11.0<br>( $c=1.0$ )    | $C_{33}H_{48}N_4O_5 \cdot 1/2 H_2O$ | 65.2<br>(65.3)                      | 8.45<br>(8.08) | 9.21<br>(9.42) | 0.58    |
| CH <sub>2</sub> -  -COOCH <sub>3</sub> | NH-(CH <sub>2</sub> ) <sub>7</sub>                                    | 74.0      | 198.5–201 | −9.1<br>( $c=0.9$ )     | $C_{39}H_{57}N_3O_7$                | 68.9<br>(68.7)                      | 8.45<br>(8.57) | 6.18<br>(6.15) | 0.63    |

Table 6. Yield, Melting Point, Optical Rotation, Elemental Analysis and *R<sub>f</sub>* Values of H-Tra-Tyr(X)-R·HCl

| X:                                                                                                                      | R:                                                                    | Yield (%) | mp (°C)   | [α] <sub>D</sub> <sup>25</sup><br>(MeOH) | Formula                                                                                        | Elemental analysis<br>Calcd (Found) |                |                | TLC<br><i>R<sub>f</sub></i> <sup>3</sup> |
|-------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------|-----------|-----------|------------------------------------------|------------------------------------------------------------------------------------------------|-------------------------------------|----------------|----------------|------------------------------------------|
|                                                                                                                         |                                                                       |           |           |                                          |                                                                                                | C                                   | H              | N              |                                          |
| iNoc                                                                                                                    | NH-(CH <sub>2</sub> ) <sub>7</sub>                                    | 78.0      | Amorphous | +1.9<br>( <i>c</i> =0.4)                 | C <sub>37</sub> H <sub>46</sub> N <sub>4</sub> O <sub>5</sub> ·<br>HCl·TFA                     | 54.5<br>(54.9)                      | 6.45<br>(6.79) | 7.47<br>(7.76) | 0.34                                     |
| iNoc                                                                                                                    | NH-(CH <sub>2</sub> ) <sub>2</sub> -CH(CH <sub>3</sub> ) <sub>2</sub> | 83.0      | Amorphous | -2.8<br>( <i>c</i> =0.9)                 | C <sub>29</sub> H <sub>40</sub> N <sub>4</sub> O <sub>5</sub> ·<br>2HCl·2H <sub>2</sub> O      | 55.0<br>(54.9)                      | 7.31<br>(7.18) | 8.84<br>(8.78) | 0.29                                     |
| Pic                                                                                                                     | NH-(CH <sub>2</sub> ) <sub>7</sub>                                    | 72.0      | Amorphous | +2.5<br>( <i>c</i> =1.0)                 | C <sub>31</sub> H <sub>46</sub> N <sub>4</sub> O <sub>3</sub> ·<br>2HCl·5/2H <sub>2</sub> O    | 68.7<br>(68.4)                      | 8.33<br>(8.80) | 8.74<br>(8.66) | 0.42                                     |
| Pic                                                                                                                     | NH-(CH <sub>2</sub> ) <sub>2</sub> -CH(CH <sub>3</sub> ) <sub>2</sub> | 75.0      | Amorphous | +1.0<br>( <i>c</i> =0.5)                 | C <sub>28</sub> H <sub>40</sub> N <sub>4</sub> O <sub>3</sub> ·<br>HCl·TFA·5/2H <sub>2</sub> O | 53.3<br>(53.1)                      | 7.00<br>(6.77) | 8.28<br>(8.53) | 0.29                                     |
| CH <sub>2</sub> -  -COOCH <sub>3</sub> | NH-(CH <sub>2</sub> ) <sub>7</sub>                                    | 74.0      | Amorphous | +4.4<br>( <i>c</i> =0.3)                 | C <sub>34</sub> H <sub>49</sub> N <sub>3</sub> O <sub>5</sub> ·<br>HCl·3/2H <sub>2</sub> O     | 63.5<br>(63.6)                      | 8.30<br>(8.00) | 6.53<br>(6.38) | 0.44                                     |

lected by filtration, yield 427 mg (69.8%), mp 153–155 °C, [α]<sub>D</sub><sup>25</sup> -2.65° (*c*=1.0, CHCl<sub>3</sub>), *R<sub>f</sub>*<sup>1</sup> 0.79. *Anal.* Calcd for C<sub>44</sub>H<sub>66</sub>N<sub>4</sub>O<sub>7</sub>·1/2H<sub>2</sub>O: C, 68.4; H, 8.76; N, 7.25. Found: C, 68.6; H, 8.83; N, 7.20.

**H-Tra-Tyr(O-Pic)-Tra-Octyl Ester·2HCl** Boc-Tra-Tyr(O-Pic)-Tra-octyl ester (150 mg, 0.2 mmol) was dissolved in 7.2 N HCl/dioxane (0.14 ml, 1 mmol) containing anisole (0.7 ml, 0.6 mmol). After dilution of the above reaction mixture with dioxane (0.14 ml), the solution was stored at room temperature for 2 h. Dry ether was added to the solution to afford a precipitate, which was collected by filtration, yield 135 mg (93.8%), mp 214–216 °C, [α]<sub>D</sub><sup>25</sup> -3.4° (*c*=1.0, MeOH), *R<sub>f</sub>*<sup>3</sup> 0.58. *Anal.* Calcd for C<sub>39</sub>H<sub>58</sub>N<sub>4</sub>O<sub>5</sub>·2HCl·3/2H<sub>2</sub>O: C, 61.4; H, 8.32; N, 7.31. Found: C, 61.8; H, 8.09; N, 7.00.

**Boc-EACA-Tyr(O-Pic)-Tra-Octyl Ester** A mixed anhydride [prepared from Boc-EACA-OH (185 mg, 0.80 mmol), isobutyl chloroformate (0.11 ml, 0.80 mmol) and Et<sub>3</sub>N (0.11 ml, 0.78 mmol) as usual] in THF (30 ml) was added to a solution of H-Tyr(O-Pic)-Tra-octyl ester [prepared from Boc-Tyr(O-Pic)-Tra-octyl ester (500 mg, 0.80 mmol), TFA (0.6 ml, 8.0 mmol) and anisole (0.26 ml, 2.4 mmol) as usual] in DMF (40 ml) containing Et<sub>3</sub>N (0.11 ml, 0.78 mmol) under cooling with ice-water. The reaction mixture was stirred at room temperature overnight. After removal of the solvents, the residue was extracted with AcOEt. The extract was washed with 5% Na<sub>2</sub>CO<sub>3</sub>, 10% citric acid and water, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration, yield 430 mg (73.1%), mp, 85–88 °C, [α]<sub>D</sub><sup>25</sup> -3.3° (*c*=1.0, CHCl<sub>3</sub>), *R<sub>f</sub>*<sup>1</sup> 0.70. *Anal.* Calcd for C<sub>42</sub>H<sub>64</sub>N<sub>4</sub>O<sub>7</sub>: C, 68.4; H, 8.77; N, 7.60. Found: C, 68.3; H, 8.94; N, 7.36.

**H-EACA-Tyr(O-Pic)-Tra-Octyl Ester·2HCl** Boc-EACA-Tyr(O-Pic)-Tra-octyl ester (150 mg, 0.20 mmol) was dissolved in 7.2 N HCl/dioxane (0.14 ml, 1 mmol) containing anisole (0.7 ml, 0.6 mmol). After dilution of the above reaction mixture with dioxane (0.14 ml), the solution was stored at room temperature for 2 h. Dry ether was added to the solution to afford a precipitate, which was collected by filtration, yield 140 mg (99%), amorphous, [α]<sub>D</sub><sup>25</sup> +1.5° (*c*=1.0, MeOH), *R<sub>f</sub>*<sup>3</sup> 0.61. *Anal.* Calcd for C<sub>37</sub>H<sub>56</sub>N<sub>4</sub>O<sub>5</sub>·2HCl·2H<sub>2</sub>O: C, 59.6; H, 8.37; N, 7.51. Found: C, 60.0; H, 8.01; N, 7.36.

**Boc-EACA-Tyr(O-Pic)-NH-(CH<sub>2</sub>)<sub>7</sub>-CH<sub>3</sub>** A mixed anhydride [prepared from Boc-EACA-OH (185 mg, 0.80 mmol), isobutyl chloroformate (0.11 ml, 0.80 mmol) and Et<sub>3</sub>N (0.11 ml, 0.78 mmol) as usual] in THF (30 ml) was added to a solution of H-Tyr(O-Pic)-octylamide [prepared from Boc-Tyr(O-Pic)-octylamide (500 mg, 0.80 mmol), TFA (0.6 ml, 8.0 mmol) and anisole (0.26 ml, 2.4 mmol) as usual] in DMF (40 ml) containing Et<sub>3</sub>N (0.11 ml, 0.78 mmol) under cooling with ice-water. The reaction mixture was stirred at room temperature overnight. After removal of the solvents, the residue was extracted with AcOEt. The extract was washed with 5% Na<sub>2</sub>CO<sub>3</sub>, 10% citric acid and water, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated down. Petroleum ether was added to the residue to give crystals, which were collected by filtration and washed with ether, yield 420 mg (87.9%), mp, 92–94 °C, [α]<sub>D</sub><sup>25</sup> -8.7° (*c*=1.0, DMF), *R<sub>f</sub>*<sup>1</sup> 0.56. *Anal.* Calcd for C<sub>34</sub>H<sub>52</sub>N<sub>4</sub>O<sub>5</sub>: C, 68.4; H, 8.78; N, 9.39. Found: C, 68.2; H, 8.91; N, 9.29.

**H-EACA-Tyr(O-Pic)-NH-(CH<sub>2</sub>)<sub>7</sub>-CH<sub>3</sub>·2HCl** Boc-EACA-Tyr(O-Pic)-octylamide (150 mg, 0.25 mmol) was dissolved in 7.2 N HCl/dioxane (0.14 ml, 1 mmol) containing anisole (0.7 ml, 0.6 mmol). After dilution of the above reaction mixture with dioxane (0.14 ml), the solution was stored at room temperature for 2 h. Dry ether was added to the solution to afford a

precipitate, which was collected by filtration, yield 140 mg (90%), amorphous, [α]<sub>D</sub><sup>25</sup> +10.6° (*c*=1.0, MeOH), *R<sub>f</sub>*<sup>3</sup> 0.27. *Anal.* Calcd for C<sub>29</sub>H<sub>44</sub>N<sub>4</sub>O<sub>3</sub>·2HCl·2H<sub>2</sub>O: C, 57.5; H, 8.32; N, 9.25. Found: C, 57.9; H, 8.09; N, 9.27.

**Boc-NH(CH<sub>2</sub>)<sub>7</sub>-COOH** (Boc)<sub>2</sub>O (8.1 g, 37.2 mmol) in dioxane (30 ml) was added to a solution of 8-aminooctanoic acid (5.0 g, 31 mmol) in water (30 ml) containing Et<sub>3</sub>N (6.5 ml, 46.5 mmol). The reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was dissolved in water. The pH of the solution was adjusted to 4 with citric acid and a precipitate was extracted with AcOEt. The extract was washed with water, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration, yield 6.87 g (85.5%), mp 56–56.5 °C, *R<sub>f</sub>*<sup>1</sup> 0.37, *R<sub>f</sub>*<sup>2</sup> 0.59. *Anal.* Calcd for C<sub>13</sub>H<sub>25</sub>NO<sub>4</sub>: C, 60.2; H, 9.72; N, 5.40. Found: 60.2; H, 9.82; 5.37.

**Boc-NH(CH<sub>2</sub>)<sub>7</sub>-COOPac** Boc-NH(CH<sub>2</sub>)<sub>7</sub>-COOH (2.59 g, 10 mmol) was dissolved in MeOH (20 ml) containing Cs<sub>2</sub>CO<sub>3</sub> (1.62 g, 5.0 mmol) in H<sub>2</sub>O (8 ml). Phenacyl bromide (1.99 g, 10 mmol) was added to the above solution and the reaction mixture was stirred at room temperature for 4 d. After removal of the white precipitate (CsBr), the solvents were removed by evaporation. The residue was extracted with AcOEt. The extract was washed with 5% Na<sub>2</sub>CO<sub>3</sub> and water, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration, yield 3.8 g (100%), mp 68–69 °C, *R<sub>f</sub>*<sup>1</sup> 0.76. *Anal.* Calcd for C<sub>21</sub>H<sub>31</sub>NO<sub>5</sub>: C, 66.8; H, 8.2%; N, 3.71. Found: C, 66.7; H, 8.18; N, 3.64.

**Boc-Tyr(O-Pic)-NH(CH<sub>2</sub>)<sub>7</sub>-COOPac** To a solution of Boc-Tyr(O-Pic)-OH (0.93 g, 2.5 mmol), H<sub>2</sub>N-(CH<sub>2</sub>)<sub>7</sub>-COOPac·TFA [prepared from corresponding Boc derivative (1.0 g, 2.4 mmol), TFA (4.6 ml, 60 mmol) and anisole (0.5 ml, 4.6 mmol) as usual] in DMF (10 ml) containing Et<sub>3</sub>N (0.84 ml, 6 mmol), BOP reagent (1.54 g, 3.5 mmol) was added. The reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with water, 5% Na<sub>2</sub>CO<sub>3</sub> and water, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from EtOH, yield 1.03 g (65.3%), mp 131–134 °C, [α]<sub>D</sub><sup>25</sup> -5.4° (*c*=1.0, DMF), *R<sub>f</sub>*<sup>1</sup> 0.70. *Anal.* Calcd for C<sub>36</sub>H<sub>45</sub>N<sub>3</sub>O<sub>7</sub>·H<sub>2</sub>O: C, 66.5; H, 7.29; N, 6.46. Found: C, 66.7; H, 7.10; N, 6.44.

**Boc-Tra-Tyr(O-Pic)-NH(CH<sub>2</sub>)<sub>7</sub>-COOPac** To a solution of H-Tyr(O-Pic)-NH(CH<sub>2</sub>)<sub>7</sub>-COOPac·TFA [prepared from corresponding Boc-derivative (720 mg, 1.2 mmol), TFA (1.83 ml, 24 mmol) and anisole (0.18 ml, 1.6 mmol) as usual] and Boc-Tra-OH (308 mg, 1.2 mmol) in DMF (10 ml) containing Et<sub>3</sub>N (0.37 ml, 2.6 mmol), BOP reagent was added. The reaction mixture was stirred at room temperature overnight. After removal of the solvent the residue was extracted with AcOEt. The extract was washed with 5% Na<sub>2</sub>CO<sub>3</sub> and water, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated down. Ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from EtOH, yield 410 mg (44%), mp 180–181 °C, [α]<sub>D</sub><sup>25</sup> -10.7° (*c*=1.0, DMF), *R<sub>f</sub>*<sup>1</sup> 0.48. *Anal.* Calcd for C<sub>44</sub>H<sub>58</sub>N<sub>4</sub>O<sub>8</sub>: C, 68.5; H, 7.58; N, 7.27. Found: C, 68.3; H, 7.56; N, 7.14.

**H-Tra-Tyr(O-Pic)-NH-(CH<sub>2</sub>)<sub>7</sub>-COOH·2TFA** Boc-Tra-Tyr(O-Pic)-NH(CH<sub>2</sub>)<sub>7</sub>-COOPac (200 mg, 0.26 mmol) was dissolved in 90% AcOH (10 ml) and DMF (8 ml). To the solution, Zn powder 1.70 g (26 mmol) was added. The reaction mixture was stirred at 40 °C overnight. After removal of

Zn powder and the solvents, the residue was extracted with AcOEt. The extract was washed with 10% citric acid and water, dried over  $\text{Na}_2\text{SO}_4$  and evaporated down. Ether was added to the residue to produce crystals, which were collected by filtration, yield 160 mg (92.6%), mp 209—211 °C,  $R_f^1$  0.46. Boc-Tra-Tyr(O-Pic)-NH(CH<sub>2</sub>)<sub>7</sub>-COOH (70 mg, 0.11 mmol) was dissolved in TFA (0.5 ml, 6.5 mmol) containing anisole (50  $\mu\text{l}$ ). The solution was stored at room temperature for 1 h. Ether was added to the solution to give a precipitate, which was collected by centrifugation and lyophilized from water, yield 76 mg. The crude material was purified with preparative HPLC to give 45 mg of purified material (31.9%),  $R_f^1$  0.05. *Anal.* Calcd for  $\text{C}_{31}\text{H}_{44}\text{N}_4\text{O}_5 \cdot 2\text{TFA} \cdot 2\text{H}_2\text{O}$ : C, 52.6; H, 6.06; N, 7.01. Found: 52.3; H, 5.78; N, 7.08.

**Fmoc-Tyr(O-Pic)-OH** To a suspension of H-Tyr(O-Pic)-OH  $\cdot$  TFA [prepared from Boc-Tyr(O-Pic)-OH (7.87 g, 0.02 mol), TFA (30.6 ml, 0.4 mol) and anisole (3.0 ml, 0.02 mol) as usual] in 10%  $\text{Na}_2\text{CO}_3$  (60 ml), Fmoc-OSu (13.13 g, 0.039 mol) in dimethoxyethane (100 ml) was added dropwise under cooling with ice. The reaction mixture was stirred at room temperature overnight. After removal of insoluble material by filtration, the pH of the filtrate was adjusted to 7 with AcOH and the solvent was removed by evaporation. The residue was extracted with AcOEt. The extract was washed with 0.1 M HCl and water. The AcOEt layer was concentrated to afford crystals, which were collected by filtration and washed with water, yield 9.5 g (92.4%), mp 208—211 °C,  $[\alpha]_D^{25}$  -17.7° ( $c=1.0$ , DMF). *Anal.* Calcd for  $\text{C}_{30}\text{H}_{26}\text{N}_2\text{O}_5$ : C, 72.9; H, 5.30; N, 5.67. Found: C, 73.0; H, 5.23; N, 5.49.

**Boc-NH-(CH<sub>2</sub>)<sub>7</sub>-NH<sub>2</sub><sup>27</sup>** (Boc)<sub>2</sub>O (5 g, 23 mmol) in 50 ml dioxane was added over 3 h to 1,7-diaminoheptane (11.9 g, 92 mmol) in dioxane (100 ml). The mixture was stirred at room temperature for 2 h. After removal of the solvents, the residue was extracted with AcOEt. The extract was washed with 5%  $\text{Na}_2\text{CO}_3$  (7  $\times$  200 ml) and water, dried over  $\text{Na}_2\text{SO}_4$  and evaporated down to give an oily product of 4.2 g (86.8%). The crude product (1.49 g) in  $\text{CHCl}_3$  (2 ml) was applied to a column of silica gel (1.7  $\times$  30 cm), equilibrated and eluted with  $\text{CHCl}_3$ . The solvent of the effluent (1200 ml—3900 ml) was removed by evaporation, which was collected as an oily material, yield 1.5 g (79.2%),  $R_f^c=0.36$ , MALDI-TOF-MAS:  $m/z=231.50$  ( $M+1$ )<sup>+</sup>.

**Fmoc-Tyr(O-Pic)-NH(CH<sub>2</sub>)<sub>7</sub>-NH-Boc** A solution of Boc-NH(CH<sub>2</sub>)<sub>7</sub>-NH<sub>2</sub> (2 g, 8.7 mmol) in DMF (100 ml) was added to Fmoc-Tyr(O-Pic)-OH in DMF (100 ml) in presence of PyBOP (5.41 g, 10.4 mmol), HOBT  $\cdot$  H<sub>2</sub>O (1.78 g, 10.4 mmol) and DIPEA (1.8 ml, 10.4 mmol) at 0 °C and the reaction mixture was stirred at the same temperature for 5 min and was further stirred at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5%  $\text{Na}_2\text{CO}_3$  and water, dried over  $\text{Na}_2\text{SO}_4$  and evaporated down. Ether was added to the residue to give a white precipitate which was collected by filtration and recrystallized from EtOH, yield 3.68 g (63.2%), mp 134—135.5 °C,  $[\alpha]_D^{25}$  -12.6° ( $c=1.0$ ,  $\text{CHCl}_3$ ),  $R_f^1$  0.66. *Anal.* Calcd For  $\text{C}_{42}\text{H}_{50}\text{N}_4\text{O}_6$ : C, 71.4; H, 7.13; N, 7.93. Found: C, 71.3; H, 7.24; N, 8.00.

**Boc-Tra-Tyr(O-Pic)-NH(CH<sub>2</sub>)<sub>7</sub>-NH-Boc** Piperidine (8 ml) in DMF (40 ml) was added to the solution of Fmoc-Tyr(O-Pic)-NH(CH<sub>2</sub>)<sub>7</sub>-NH-Boc (1 g, 1.39 mmol) in DMF (50 ml) and stirred at room temperature for 1.5 h. After removal of the solvent, ether was added to the residue to afford crystals [H-Tyr(O-Pic)-NH(CH<sub>2</sub>)<sub>7</sub>-NH-Boc], which were collected by filtration, yield 0.42 g, (78.7%). A solution of H-Tyr(O-Pic)-NH(CH<sub>2</sub>)<sub>7</sub>-NH-Boc in DMF (20 ml) was added to Boc-Tra-OH (0.36 g, 1.4 mmol) in DMF (20 ml) in the presence of PyBOP (0.73 g, 1.4 mmol), HOBT  $\cdot$  H<sub>2</sub>O (0.24 g, 1.6 mmol) and DIPEA (0.24 ml, 1.4 mmol) at 0 °C. The reaction mixture was stirred at room temperature overnight. After removal of the solvents, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5%  $\text{Na}_2\text{CO}_3$  and water, dried over  $\text{Na}_2\text{SO}_4$  and evaporated to dryness. Ether was added to the residue to give crystals, which were collected by filtration and recrystallized from EtOH, yield 0.45 g (56.5%), mp 175—176 °C,  $[\alpha]_D^{25}$  -3.8° ( $c=1.0$ ,  $\text{CHCl}_3$ ),  $R_f^1=0.63$ . *Anal.* Calcd For  $\text{C}_{42}\text{H}_{61}\text{N}_5\text{O}_5$ : C, 65.6; H, 8.53; N, 9.56. Found: C, 65.6; H, 8.45; N, 9.60.

**H-Tra-Tyr(O-Pic)-NH(CH<sub>2</sub>)<sub>7</sub>-NH<sub>2</sub>  $\cdot$  3TFA** Boc-Tra-Tyr(O-Pic)-NH-(CH<sub>2</sub>)<sub>7</sub>-NH-Boc (0.25 g, 0.35 mmol) was dissolved in TFA (0.79 ml, 6.9 mmol) containing anisole (0.79 ml, 6.9 mmol) at 0° and the reaction mixture was stirred at 0 °C for 10 min and was further stirred at room temperature for 1 h. After removal of the solvent, dry ether was added to the residue to give a precipitate, which was collected by filtration, yield 0.17 g (94.9%), mp 134—136 °C  $[\alpha]_D^{25}$  -9.5° ( $c=1.0$ ,  $\text{CHCl}_3$ ),  $R_f^6$  0.7. *Anal.* Calcd For  $\text{C}_{30}\text{H}_{45}\text{N}_5\text{O}_5 \cdot 3\text{TFA} \cdot 2\text{H}_2\text{O}$ : C, 48.0; H, 5.59; N, 7.77. Found: C, 47.7; H, 5.42; N, 7.95. MALDI-TOF-MAS:  $m/z=524.75$  ( $M+1$ )<sup>+</sup>.

**N-Boc-*p*- or -*m*-Aminobenzoic Acid** (Boc)<sub>2</sub>O (4.8 g, 22 mmol) in dioxane (100 ml) was added to a solution of *p*- or *m*-aminobenzoic acid (4.3 g, 20 mmol) in water (50 ml) containing Et<sub>3</sub>N (4.2 ml, 30 mmol). The reaction

mixture was stirred at room temperature overnight. After removal of the solvent, the residue was dissolved in water. The pH of the solution was adjusted to 4 with citric acid to give an oily precipitate. The precipitate was extracted with AcOEt. The extract was washed with water, dried over  $\text{Na}_2\text{SO}_4$  and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from EtOH. Boc-*p*-amino benzoic acid: yield 2.0 g (41.2%), mp 193—195 °C,  $R_f^1$  0.59,  $R_f^2$  0.77. *Anal.* Calcd for  $\text{C}_{12}\text{H}_{13}\text{NO}_4$ : C, 60.8; H, 6.37; N, 5.90. Found: C, 60.9; H, 6.39; N, 5.75. Boc-*m*-aminobenzoic acid: yield 1.5 g (32.6%), mp 198—199 °C,  $R_f^1$  0.50. *Anal.* Calcd for  $\text{C}_{12}\text{H}_{13}\text{NO}_4$ : C, 60.8; H, 6.37; N, 5.90. Found: C, 60.8; H, 6.48; N, 5.94.

**Benzyl N-Boc-*p*-Aminobenzoate** N-Boc-*p*-aminobenzoic acid (2.4 g, 10 mmol) was dissolved in MeOH (40 ml) and water (5 ml) and the pH of the solution was adjusted to 8 by adding 5%  $\text{Cs}_2\text{CO}_3$  in water (5 ml). After removal of the solvents, the residue was dissolved in DMF (30 ml) and the DMF was removed. To a solution of the residue in DMF (25 ml), benzyl bromide (1.34 ml, 11 mmol) was added under cooling with ice. After 4 h, the solvent was removed and water was added to the residue to yield a white powder, which was collected by filtration. The crude material was dissolved in AcOEt. The AcOEt layer was washed with water, dried over  $\text{Na}_2\text{SO}_4$  and evaporated down. Petroleum ether was added to the residue to afford white powder, which was collected by filtration, yield 2.7 g (82.5%), mp 148—150 °C,  $R_f^2$  0.83. *Anal.* Calcd for  $\text{C}_{19}\text{H}_{21}\text{NO}_4$ : C, 69.7; H, 6.47; N, 4.28. Found: C, 69.6; H, 6.37; N, 4.10.

**Benzyl N-Boc-*m*-Aminobenzoate** N-Boc-*m*-aminobenzoic acid (4.7 g, 19.4 mmol) was dissolved in MeOH (80 ml) and water (10 ml) and the pH of the solution was adjusted to 8 by adding 20%  $\text{Cs}_2\text{CO}_3$  in water (10 ml). After removal of the solvents, the residue was dissolved in DMF (50 ml) and the DMF was removed. To a solution of the residue in DMF (40 ml), benzyl bromide (2.4 ml, 19.6 mmol) was added under cooling with ice. After stirring at 30 °C for 10 h, the solid material (CsBr) and the solvent were removed. The residue was extracted with AcOEt. The AcOEt layer was washed with 5%  $\text{Na}_2\text{CO}_3$  and water, dried over  $\text{Na}_2\text{SO}_4$  and evaporated down. Ether was added to the residue to afford white powder, which was collected by filtration, yield 3.0 g (47.2%), mp 143—145 °C,  $R_f^1$  0.78. *Anal.* Calcd for  $\text{C}_{19}\text{H}_{21}\text{NO}_4$ : C, 69.7; H, 6.47; N, 4.28. Found: C, 69.5; H, 6.30; N, 4.20.

**Boc-Tyr(O-2-Pyrim)-OH** To a solution of Boc-Tyr-OH (2.81 g, 10 mmol) in DMF (50 ml) was added 60% sodium hydride (0.88 g, 22 mmol) at 0 °C. After the evolution of hydrogen gas ceased, 2-pyrimidyl chloride (1.37 g, 12 mmol) was added. The reaction mixture was stirred at 33 °C for 4 h. After removal of the solvent, the residue was extracted with AcOEt and water. The pH of the water layer was adjusted to 4 with AcOH to afford a white precipitate, which was extracted with AcOEt. The extract was washed with water, dried over  $\text{Na}_2\text{SO}_4$  and evaporated down. Petroleum ether was added to the residue to afford crude product. The crude product in  $\text{CHCl}_3$  (2 ml) was applied to a column of silica gel (3.0  $\times$  25 cm), equilibrated and eluted with  $\text{CHCl}_3$  (1300 ml) followed by 1% MeOH in  $\text{CHCl}_3$  (500 ml). The solvent of the effluent (800—1700 ml) was removed by evaporation. Petroleum ether and ether were added to the residue to afford a precipitate, which was collected by filtration, yield 1.36 g (37%), mp 145—147 °C,  $[\alpha]_D^{25}$  -18.0° ( $c=1.0$ , DMF),  $R_f^1$  0.56,  $R_f^2$  0.39. *Anal.* Calcd For  $\text{C}_{18}\text{H}_{21}\text{N}_3\text{O}_5 \cdot 1/4\text{H}_2\text{O}$ : C, 59.4; H, 5.95; N, 11.6. Found: C, 59.6; H, 5.95; N, 11.2.

**N-Boc-D,L-Tyr-(O-2-Pyrim)-OH** To a solution of H-D,L-Tyr-OH (4.0 g, 22 mmol) in H<sub>2</sub>O (30 ml) containing Et<sub>3</sub>N (4.6 ml, 33 mmol), (Boc)<sub>2</sub>O (5.8 g, 26 mmol) in dioxane (30 ml) was added. The reaction mixture was stirred at room temperature for 4 h. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid and water, dried over  $\text{Na}_2\text{SO}_4$  and evaporated down. Petroleum ether was added to the residue to give crystals, which were collected by filtration, yield 5.78 g (93.4%), mp 133—136 °C,  $R_f^1$  0.26,  $R_f^2$  0.13. To a solution of Boc-D,L-Tyr-OH (5.0 g, 18 mmol) in DMF (100 ml) was added 60% sodium hydride (1.4 g, 40 mmol) at 0 °C. After the evolution of hydrogen gas ceased, 2-pyrimidyl chloride (2.5 g, 22 mmol) was added. The reaction mixture was stirred at 40 °C for 4 h. After removal of the solvent, the residue was extracted with 5%  $\text{Na}_2\text{CO}_3$  and AcOEt. The pH of the water layer was adjusted to 4 with AcOH to afford an oily material, which was extracted with AcOEt. The extract was washed with water, dried over  $\text{Na}_2\text{SO}_4$  and evaporated down. Petroleum ether was added to the residue to afford crude product, which was collected by filtration and recrystallized from petroleum ether and ether, yield 2.21 g (34.1%), mp 160—162 °C,  $[\alpha]_D^{25}$  -2.2° ( $c=1.0$ , DMF),  $R_f^1$  0.55,  $R_f^2$  0.10. *Anal.* Calcd For  $\text{C}_{18}\text{H}_{21}\text{N}_3\text{O}_5$ : C, 60.2; H, 5.89; N, 11.7. Found: C, 59.9; H, 5.89; N, 11.4.

**Benzyl N-Boc-Tyr(O-2-Pyrim)-*p*-Aminobenzoate** A mixed anhydride

[prepared from Boc-Tyr(*O*-2-Pyrim)-OH (0.72 g, 2.0 mmol), isobutyl chloroformate (0.26 ml, 2.0 mmol) and Et<sub>3</sub>N (0.28 ml, 2.0 mmol) as usual] in THF (20 ml) was added to a solution of benzyl *p*-aminobenzoate [prepared from corresponding Boc derivative (0.65 g, 2.0 mmol), TFA (4.6 ml, 60 mmol) and anisole (0.50 ml, 4.6 mmol) as usual] in THF (20 ml) containing Et<sub>3</sub>N (0.42 ml, 3 mmol) under cooling with ice. The reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 5% Na<sub>2</sub>CO<sub>3</sub> and water, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated down to afford an oily material. The crude material in CHCl<sub>3</sub> (2 ml) was applied to a silica gel column (3.0×21.0 cm), equilibrated and eluted with CHCl<sub>3</sub>. The solvent of the effluent (700–1200 ml) was removed by evaporation. Petroleum ether was added to the residue to afford amorphous powder, which was collected by filtration, yield 0.57 g (25.2%), [ $\alpha$ ]<sub>D</sub><sup>25</sup> +47.6° (*c*=0.2, DMF), *R*<sub>f</sub><sup>7</sup> 0.15. *Anal.* Calcd for C<sub>32</sub>H<sub>32</sub>N<sub>4</sub>O<sub>6</sub>: C, 67.6; H, 5.67; N, 9.85. Found: C, 67.8; H, 5.96; N, 9.47.

**Benzyl *N*-Boc-Tyr(*O*-2-Pyrim)-*m*-Aminobenzoate** A mixed anhydride [prepared from Boc-Tyr(*O*-2-Pyrim)-OH (1.57 g, 4.37 mmol), pivaloyl chloride (0.54 ml, 4.5 mmol) and Et<sub>3</sub>N (0.63 ml, 4.5 mmol) as usual] in THF (20 ml) was added to a solution of benzyl *m*-aminobenzoate [prepared from the corresponding Boc derivative (0.98 g, 3 mmol), TFA (4.6 ml, 86 mmol) and anisole (0.46 ml, 3 mmol) as usual and converted to HCl salt by 7.1 N HCl/dioxane (0.81 ml, 6 mmol)] in DMF (20 ml) containing Et<sub>3</sub>N (0.42 ml, 3 mmol) under cooling with ice. The reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 5% Na<sub>2</sub>CO<sub>3</sub> and water, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated down to afford an oily material. The crude material in CHCl<sub>3</sub> (2 ml) was applied to a silica gel column (3.0×21.0 cm), equilibrated and eluted with CHCl<sub>3</sub>. The solvent of the effluent (400–800 ml) was removed by evaporation. Petroleum ether was added to the residue to afford amorphous powder, which was collected by filtration, yield 0.51 g (24.0%), mp 74–78°C, [ $\alpha$ ]<sub>D</sub><sup>25</sup> +16.8° (*c*=1.0, DMF), *R*<sub>f</sub><sup>1</sup> 0.59, *R*<sub>f</sub><sup>7</sup> 0.14. *Anal.* Calcd for C<sub>32</sub>H<sub>32</sub>N<sub>4</sub>O<sub>6</sub>: C, 67.6; H, 5.67; N, 9.85. Found: C, 67.4; H, 5.81; N, 9.47.

**Benzyl *N*-Boc-D,L-Tyr(*O*-2-Pyrim)-*p*-Aminobenzoate** A mixed anhydride [prepared from Boc-D,L-Tyr(*O*-2-Pyrim)-OH (0.72 g, 2.0 mmol), pivaloyl chloride (0.24 ml, 2.0 mmol) and Et<sub>3</sub>N (0.84 ml, 6.0 mmol) as usual] in THF (20 ml) was added to a solution of benzyl *p*-aminobenzoate [prepared from the corresponding Boc derivative (0.65 g, 2.0 mmol), TFA (3.1 ml, 40 mmol) and anisole (0.30 ml, 3.0 mmol) as usual] in THF (20 ml) containing Et<sub>3</sub>N (0.42 ml, 3 mmol) under cooling with ice. The reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 5% Na<sub>2</sub>CO<sub>3</sub> and water, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated down to afford an oily material. The crude material in CHCl<sub>3</sub> (2 ml) was applied to a silica gel column (3.0×19.0 cm), equilibrated and eluted with CHCl<sub>3</sub> (1300 ml), followed by 1% MeOH in CHCl<sub>3</sub> (600 ml). The solvent of the effluent (500–1700 ml) was removed by evaporation. Petroleum ether was added to the residue to give a powder, which was collected by filtration, yield 0.25 g (22.0%), mp 112–113°C, [ $\alpha$ ]<sub>D</sub><sup>25</sup> –2.3° (*c*=1.0, DMF), *R*<sub>f</sub><sup>1</sup> 0.55, *R*<sub>f</sub><sup>7</sup> 0.11. *Anal.* Calcd for C<sub>32</sub>H<sub>32</sub>N<sub>4</sub>O<sub>6</sub>: C, 67.6; H, 5.67; N, 9.85. Found: C, 67.3; H, 5.72; N, 9.81.

**Benzyl *N*-Boc-D,L-Tyr(*O*-2-Pyrim)-*m*-Aminobenzoate** A mixed anhydride [prepared from Boc-D,L-Tyr(*O*-2-Pyrim)-OH (0.72 g, 2.0 mmol), pivaloyl chloride (0.24 ml, 2.0 mmol) and Et<sub>3</sub>N (0.28 ml, 2.0 mmol) as usual] in THF (20 ml) was added to a solution of benzyl *m*-aminobenzoate [prepared from the corresponding Boc derivative (0.65 g, 2.0 mmol), TFA (3.1 ml, 40 mmol) and anisole (0.30 ml, 3.0 mmol) as usual] in THF (20 ml) containing Et<sub>3</sub>N (0.42 ml, 3 mmol) under cooling with ice. The reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 5% Na<sub>2</sub>CO<sub>3</sub> and water, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated down. Petroleum ether was added to the residue to give a powder. The crude material in CHCl<sub>3</sub> (2 ml) was applied to a silica gel column (3.0×19.0 cm), equilibrated and eluted with CHCl<sub>3</sub> (1300 ml). The solvent of the effluent (400–900 ml) was removed by evaporation. Petroleum ether was added to the residue to afford a powder, which was collected by filtration, yield 0.70 g (61.6%), mp 70–79°C, [ $\alpha$ ]<sub>D</sub><sup>25</sup> –3.3° (*c*=1.0, DMF), *R*<sub>f</sub><sup>1</sup> 0.64, *R*<sub>f</sub><sup>7</sup> 0.20. *Anal.* Calcd for C<sub>32</sub>H<sub>32</sub>N<sub>4</sub>O<sub>6</sub>: C, 67.6; H, 5.67; N, 9.85. Found: C, 67.4; H, 5.65; N, 9.72.

**Benzyl *N*-Boc-Tra-Tyr(*O*-2-Pyrim)-*p*-Aminobenzoate** A mixed anhydride [prepared from Boc-Tra-OH (60 mg, 0.21 mmol), isobutyl chloroformate (27  $\mu$ l, 0.21 mmol) and Et<sub>3</sub>N (29  $\mu$ l, 0.21 mmol) as usual] in THF (20 ml) was added to a solution of H-Tyr(*O*-2-Pyrim)-*p*-aminobenzoic acid benzyl ester trifluoroacetate [prepared from the corresponding Boc derivative (120 mg, 0.21 mmol), TFA (0.48 ml, 6.3 mmol) and anisole (0.05 ml, 0.46 mmol) as usual] in DMF (20 ml) containing Et<sub>3</sub>N (29  $\mu$ l, 0.21 mmol). The

reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na<sub>2</sub>CO<sub>3</sub> and water, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from EtOH, yield 120.0 mg (80.8%), mp 182.5–184°C, [ $\alpha$ ]<sub>D</sub><sup>25</sup> +37.1° (*c*=0.4, DMF), *R*<sub>f</sub><sup>1</sup> 0.66, *R*<sub>f</sub><sup>2</sup> 0.84. *Anal.* Calcd for C<sub>40</sub>H<sub>45</sub>N<sub>5</sub>O<sub>7</sub>: C, 67.8; H, 6.41; N, 9.90. Found: C, 67.8; H, 6.45; N, 9.74.

**Benzyl *N*-Boc-Tra-Tyr(*O*-2-Pyrim)-*m*-Aminobenzoate** A mixed anhydride [prepared from Boc-Tra-OH (210 mg, 0.81 mmol), isobutyl chloroformate (0.10 ml, 0.81 mmol) and Et<sub>3</sub>N (0.11 ml, 0.81 mmol) as usual] in THF (20 ml) was added to a solution of H-Tyr(*O*-2-Pyrim)-*m*-aminobenzoic acid benzyl ester trifluoroacetate [prepared from the corresponding Boc derivative (350 mg, 0.6 mmol), TFA (0.94 ml, 12 mmol) and anisole (0.1 ml, 0.9 mmol) as usual] in THF (20 ml) containing Et<sub>3</sub>N (0.10 ml, 0.71 mmol). The reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 5% Na<sub>2</sub>CO<sub>3</sub> and water, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from AcOEt, yield 170 mg (40.0%), mp 180–182°C, [ $\alpha$ ]<sub>D</sub><sup>25</sup> +20.5° (*c*=1.0, DMF), *R*<sub>f</sub><sup>1</sup> 0.56, *R*<sub>f</sub><sup>2</sup> 0.79. *Anal.* Calcd for C<sub>40</sub>H<sub>45</sub>N<sub>5</sub>O<sub>7</sub>·1/4H<sub>2</sub>O: C, 67.4; H, 6.43; N, 9.83. Found: C, 67.6; H, 6.46; N, 9.79.

**Benzyl *N*-Boc-Tra-D,L-Tyr(*O*-2-Pyrim)-*p*-Aminobenzoate** A mixed anhydride [prepared from Boc-Tra-OH (110 mg, 0.42 mmol), isobutyl chloroformate (50  $\mu$ l, 0.42 mmol) and Et<sub>3</sub>N (60  $\mu$ l, 0.42 mmol) as usual] in THF (20 ml) was added to a solution of H-D,L-Tyr(*O*-2-Pyrim)-*p*-aminobenzoic acid benzyl ester trifluoroacetate [prepared from the corresponding Boc derivative (240 mg, 0.42 mmol), TFA (0.7 ml, 8.4 mmol) and anisole (70  $\mu$ l, 0.65 mmol) as usual] in DMF (20 ml) containing Et<sub>3</sub>N (60  $\mu$ l, 0.42 mmol). The reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na<sub>2</sub>CO<sub>3</sub> and water, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration. A crude material in CHCl<sub>3</sub> (2 ml) was applied to a WAKO gel 300 column (2.5×21 cm), equilibrated and eluted with CHCl<sub>3</sub> (255 ml), followed by 1% MeOH in CHCl<sub>3</sub> (600 ml). The solvent of an effluent (435–800 ml) was removed by evaporation. Petroleum ether was added to the residue to afford a powder, which was collected by filtration, yield 80 mg (26.9%), mp 152–153°C, [ $\alpha$ ]<sub>D</sub><sup>25</sup> +0.49° (*c*=1.0, MeOH), *R*<sub>f</sub><sup>1</sup> 0.58, *R*<sub>f</sub><sup>7</sup> 0.16. *Anal.* Calcd for C<sub>40</sub>H<sub>45</sub>N<sub>5</sub>O<sub>7</sub>·1/4H<sub>2</sub>O: C, 67.4; H, 6.43; N, 9.83. Found: C, 67.7; H, 6.38; N, 9.87.

**Benzyl *N*-Boc-Tra-D,L-Tyr(*O*-2-Pyrim)-*m*-Aminobenzoate** A mixed anhydride [prepared from Boc-Tra-OH (140 mg, 0.53 mmol), isobutyl chloroformate (70  $\mu$ l, 0.53 mmol) and Et<sub>3</sub>N (70  $\mu$ l, 0.53 mmol) as usual] in THF (20 ml) was added to a solution of H-D,L-Tyr(*O*-2-Pyrim)-*m*-aminobenzoic acid benzyl ester trifluoroacetate [prepared from the corresponding Boc derivative (300 mg, 0.53 mmol), TFA (1.0 ml, 13 mmol) and anisole (0.1 ml, 0.9 mmol) as usual] in THF (20 ml) containing Et<sub>3</sub>N (0.10 ml, 0.71 mmol). The reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na<sub>2</sub>CO<sub>3</sub> and water, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration. A crude material in AcOEt (2 ml) was applied to a silica gel column (3×21 cm), equilibrated and eluted with AcOEt and *n*-hexane (1:1, 390 ml), followed by AcOEt and *n*-hexane (2:1, 800 ml). The solvent of the effluent (120–1100 ml) was removed by evaporation. Petroleum ether was added to the residue to afford a powder, which was collected by filtration, yield 110 mg (29.3%), mp 178–181°C, [ $\alpha$ ]<sub>D</sub><sup>25</sup> –3.2° (*c*=1.0, DMF), *R*<sub>f</sub><sup>1</sup> 0.60, *R*<sub>f</sub><sup>7</sup> 0.15. *Anal.* Calcd for C<sub>40</sub>H<sub>45</sub>N<sub>5</sub>O<sub>7</sub>·1/4H<sub>2</sub>O: C, 67.4; H, 6.43; N, 9.83. Found: C, 67.5; H, 6.46; N, 9.90.

**H-Tra-Tyr(*O*-2-Pyrim)-*p*-aminobenzoic Acid·TFA** Benzyl *N*-Boc-Tra-Tyr(*O*-2-Pyrim)-*p*-aminobenzoate (150 mg, 0.21 mmol) in DMF (2 ml) and MeOH (20 ml) was hydrogenated for 2 h over a Pd catalyst. After removal of Pd and the solvent, ether was added to the residue to yield crystals, which were collected by filtration. The crude material in CHCl<sub>3</sub> and MeOH (1 ml+1 ml) was applied to a silica gel column (3×13 cm), equilibrated with CHCl<sub>3</sub> and eluted stepwise with CHCl<sub>3</sub> (300 ml), 1% MeOH in CHCl<sub>3</sub> (200 ml), 3% MeOH in CHCl<sub>3</sub> (400 ml), 5% MeOH in CHCl<sub>3</sub> (200 ml) and 8% MeOH in CHCl<sub>3</sub> (800 ml). The solvent of the effluent (5% and 8% MeOH in CHCl<sub>3</sub>) was removed by evaporation to yield solid material, which was collected by filtration and recrystallized from AcOEt and ether, yield 117 mg (88%), mp 241–244°C, *R*<sub>f</sub><sup>1</sup> 0.56, *R*<sub>f</sub><sup>2</sup> 0.42. Boc-Tra-Tyr(*O*-2-Pyrim)-*p*-aminobenzoic acid (80 mg, 0.13 mmol) was dissolved in TFA (0.5

ml, 6.5 mmol) containing anisole (0.05 ml, 0.46 mmol) and the solution was stored at room temperature for 1 h. Ether was added to the solution to give a precipitate, which was collected by centrifugation and washed with ether. The crude material in 3% AcOH (2 ml) was applied to a Sephadex G-15 column (2.3×64 cm), equilibrated and eluted with 5% AcOH. The solvent of the effluent (180–300 ml) was completely removed by evaporation. EtOH was added to the residue to afford a solid material, yield 75.0 mg (67.1%), mp 165–175 °C,  $[\alpha]_D^{25} + 37.4^\circ$  ( $c=0.7$ , MeOH),  $R_f^4$  0.24,  $R_f^5$  0.67. *Anal.* Calcd for  $C_{28}H_{31}N_5O_5 \cdot 0.5TFA$ : C, 60.6; H, 5.52; N, 12.2. Found: C, 60.7; H, 6.01; N, 12.0.

**H-Tra-Tyr(O-2-Pyrim)-m-Aminobenzoic Acid·TFA** Benzyl *N*-Boc-Tra-Tyr(O-2-Pyrim)-*m*-aminobenzoate (180 mg, 0.25 mmol) in DMF (2 ml) and MeOH (20 ml) was hydrogenated for 2 h over a Pd catalyst. After removal of a Pd and the solvent, ether was added to the residue to afford crystals, which were collected by filtration, yield 140 mg (88%), mp 224–229 °C. A solution of *N*-Boc-Tra-Tyr(O-2-Pyrim)-*m*-aminobenzoic acid (80 mg, 0.13 mmol) in TFA (0.70 ml, 5.0 mmol) containing anisole (50  $\mu$ l) was stored at room temperature for 90 min. Ether was added to the solution to afford a precipitate, which was collected by centrifugation, yield 68 mg (85.0%), mp 241–244 °C,  $[\alpha]_D^{25} + 16.6^\circ$  ( $c=0.56$ , 10% AcOH),  $R_f^2$  0.22. *Anal.* Calcd for  $C_{28}H_{31}N_5O_5 \cdot 1.5TFA \cdot H_2O$ : C, 52.7; H, 4.92; N, 9.91. Found: C, 52.8; H, 4.83; N, 9.92.

**H-Tra-D,L-m-Tyr(O-2-Pyrim)-p-Aminobenzoic Acid·TFA** Benzyl *N*-Boc-Tra-D,L-m-Tyr(O-2-Pyrim)-*p*-aminobenzoic acid (100 mg, 0.14 mmol) in DMF (2 ml) and MeOH (20 ml) was hydrogenated for 2 h over a Pd catalyst. After removal of Pd and the solvent, ether was added to the residue to afford crystals, which were collected by filtration, yield 80 mg (81%),  $R_f^1$  0.58,  $R_f^2$  0.33. A solution of *N*-Boc-Tra-D,L-m-Tyr(O-2-Pyrim)-*p*-aminobenzoic acid (80 mg, 0.13 mmol) in TFA (0.70 ml, 5.0 mmol) containing anisole (50  $\mu$ l) was stored at room temperature for 90 min. Ether was added to the solution to afford a precipitate, which was collected by centrifugation, purified with HPLC, yield 68 mg (85.0%), amorphous,  $[\alpha]_D^{25} -1.5^\circ$  ( $c=0.70$ , 10% AcOH),  $R_f^3$  0.40,  $R_f^4$  0.40,  $R_f^5$  0.47. *Anal.* Calcd for  $C_{28}H_{31}N_5O_5 \cdot 1.5TFA$ : C, 54.1; H, 4.75; N, 10.2. Found: C, 54.2; H, 5.02; N, 10.6.

**H-Tra-D,L-m-Tyr(O-2-Pyrim)-m-Aminobenzoic Acid·TFA** Benzyl *N*-Boc-Tra-D,L-m-Tyr(O-2-Pyrim)-*m*-aminobenzoic acid (100 mg, 0.14 mmol) in DMF (2 ml) and MeOH (20 ml) was hydrogenated for 2 h over a Pd catalyst. After removal of Pd and the solvent, ether was added to the residue to afford crystals, which were collected by filtration, yield 80 mg (81%),  $R_f^1$  0.58,  $R_f^2$  0.33. A solution of *N*-Boc-Tra-D,L-m-Tyr(O-2-Pyrim)-*m*-aminobenzoic acid (80 mg, 0.13 mmol) in TFA (0.70 ml, 5.0 mmol) containing anisole (50  $\mu$ l) was stored at room temperature for 90 min. Ether was added to the solution to afford a precipitate, which was collected by centrifugation, purified with HPLC, yield 60 mg (75.0%), amorphous,  $[\alpha]_D^{25} -1.6^\circ$  ( $c=0.45$ , 10% AcOH),  $R_f^3$  0.39,  $R_f^4$  0.40,  $R_f^5$  0.47. *Anal.* Calcd for  $C_{28}H_{31}N_5O_5 \cdot 1.5TFA$ : C, 54.1; H, 4.75; N, 10.2. Found: C, 54.2; H, 5.02; N, 10.6.

**Assay Procedure** The enzymes used were as follows: human PL and PK (KABI Co.), bovine TH (Mochida Seiyaku Co.), porcine glandular kallikrein (GK) (Sigma Chemical Co.) and human UK (Green Cross). Enzymatic activities of PL, PK, TH, GK, and UK were determined by the method described previously,<sup>28)</sup> using D-Val-Leu-Lys-pNA (S-2251), D-Pro-Phe-Arg-pNA (S-2302), D-Phe-Pip-Arg-pNA (S-2238), D-Val-Leu-Arg-pNA (S-2266), and <Glu-Gly-Arg-pNA (S-2444), respectively. Fn and Fg were used as substrates for PL and TH, respectively.  $IC_{50}$  values were determined as follows: 1) Antiamidolytic assay<sup>22)</sup>, the  $IC_{50}$  value was taken as the concentration of inhibitor which reduced the absorbance at 405 nm by 50% compared with the absorbance measured under the same conditions without inhibitor. 2) Antifibrinolytic assay<sup>20)</sup>, the  $IC_{50}$  value was taken as the concentration of inhibitor which prolonged the complete lysis time two-fold compared with that without inhibitor. 3) Antifibrinogenolytic assay: to a borate saline buffer (pH 7.4) was added solutions containing various concentrations of the inhibitor to be tested (0.5 ml), 0.2% bovine Fg in the above buffer (0.4 ml), and bovine TH 4 U/ml (0.1 ml). The assay was carried out at 37 °C and the clotting time was measured. The  $IC_{50}$  value was taken as the concentration of inhibitor which prolonged the clotting time two-fold compared with that without inhibitor.

**Acknowledgements** The authors are grateful for the assistance of Drs. L. H. Lazarus and S. D. Bryant of the National Institute of Environmental Health Sciences (NIEHS) for their kind help during the preparation of this manuscript.

## References and Notes

- 1) The customary L-configuration for amino acid residues is omitted. Abbreviations used in this report for amino acids, peptides and their derivatives are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature: *Biochemistry*, **5**, 2485–2489 (1966); **6**, 362–364 (1967); **11**, 1726–1732 (1972). The following additional abbreviations are used: AcOEt, ethyl acetate; DMF, *N,N*-dimethylformamide; TFA, trifluoroacetic acid; DIPEA, *N,N*-diisopropylethylamine; Boc, *tert*-butoxycarbonyl; TEA, triethylamine; (Boc)<sub>2</sub>O, di-*tert*-butyldicarbonate; 2-Br-Z, 2-bromobenzyloxycarbonyl; OPac, phenacyl ester; Pic, 4-picolyl; 2-Pyrim, 2-pyrimidyl; EACA, 6-amino-hexanoyl; TH, thrombin; UK, urokinase; Fg, fibrinogen; Fn, fibrin; THF, tetrahydrofuran; (DIEA=DIPEA) BOP, benzotriazole-1-yl-oxy-tris(dimethylamino)-phosphoniumhexafluorophosphate; Pac, phenacyl; iNoc, isonicotinylloxycarbonyl; pNA, *p*-nitroanilide; Fmoc, 9-fluorenylmethoxycarbonyl; HOBt, *N*-hydroxybenzotriazole; Py BOP, benzotriazol-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate.
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# Hydrophobicity Parameters Determined by Reversed-Phase Liquid Chromatography. XIV.<sup>1)</sup> Application of a New Hydrogen-accepting Scale of Monosubstituted Pyrazines to Analysis of the Relationship between Octanol–Water Partition Coefficients and Retention Factors Measured in Different Mobile Phases

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Received July 31, 2000; accepted October 10, 2000

We recently proposed a new H-accepting scale,  $S_{\text{HA}}$ , for monosubstituted pyrazines, and demonstrated that this parameter works effectively in expressing the relationship between  $\log P$  ( $P$ : 1-octanol/water partition coefficient) and  $\log k'$  ( $k'$ : retention factor derived from reversed phase liquid chromatography) with aqueous methanol solutions as the mobile phase, according to the equation:  $\log k' = a \log P + \rho \sigma_1 + s S_{\text{HA}} + \text{const.}$ , where  $\sigma_1$  represents the electronic substituent constant. In this work, we have extended the same treatment to analysis of  $\log k'$  measured in mobile phases containing different organic modifiers such as 1-propanol, acetonitrile, and dioxane, and found that the above equation is still useful. By comparing the correlations obtained, it was confirmed that the parameter  $S_{\text{HA}}$  could be universally utilized for representing the difference in H-bonding effects involved in different partitioning systems.

**Key words** H-bonding; H-accepting scale; hydrophobicity; partition coefficient; retention factor; reversed phase liquid chromatography

The hydrophobicity of molecules plays an important role in structure–activity relationship studies for various bioactive compounds, along with other physicochemical properties such as electronic and steric (including three-dimensional structure) factors.<sup>2–4)</sup> In connection with recently developed high throughput screening techniques, the necessity has been increasing that such prospective parameters should be measured or predicted, not only accurately but also rapidly. To express hydrophobicity, the logarithm of the octanol/water partition coefficient,  $\log P$ , has been used<sup>2,3)</sup> and large compilations<sup>5)</sup> are available. As an alternative approach, the retention factor,  $\log k'$ , obtained by reversed phase liquid chromatography, RPLC, has been used as a convenient proxy for  $\log P$ . Extensive studies for predicting  $\log P$  from  $\log k'$  have been reported.<sup>6–9)</sup> One of the difficulties in utilizing this RPLC method lies in interference from H-bonding between the solutes and the environmental medium that affects linearity between  $\log P$  and  $\log k'$ .<sup>6–9)</sup>

We have been systematically studying the relationship between  $\log P$  and  $\log k'$  for various series of heteroaromatic compounds under different RPLC conditions.<sup>1,10–13)</sup> In our earlier work,<sup>11)</sup> we analyzed the relationship between  $\log P$  and  $\log k'$  determined for monosubstituted diazines (pyrazines and pyrimidines) by using various combinations of stationary and mobile phases. These studies demonstrated that  $\log k'$  can be described by Eq. 1.

$$\log k' = a \log P + \rho \sigma_1 + h_{\text{CO}} HB_{\text{CO}} + h_{\text{A}} HB_{\text{A}} + h_{\text{AM}} HB_{\text{AM}} + \text{const.} \quad (1)$$

In Eq. 1,  $\sigma_1$  represents the inductive-electronic substituent constant<sup>14)</sup> expressing the electronic effect of substituents on the hydrogen bonding capacity of the ring  $N$ -atom(s). The  $HB$  terms serve to correct for variation in H-bonding associated with substituents.<sup>15)</sup> The parameters  $HB_{\text{CO}}$  and  $HB_{\text{A}}$  are indicator variables which take either the value 1 for strong H-accepting substituents with two sites (e.g.  $\text{CO}_2\text{R}$  and  $\text{CONMe}_2$ ) and one site (e.g.  $\text{CN}$  and  $\text{Ac}$ ) respectively, or 0

for non-H-bonders (e.g. H, alkyls, halogens, OR, SMe, and  $\text{NMe}_2$ ); the parameter  $HB_{\text{AM}}$  is that for amphiprotic substituents.

Although Eq. 1 works well for each series, classification of substituents into an appropriate category has to be done arbitrarily; it is clearly desirable to seek an alternative, universal H-bonding parameter. The new H-acceptor scale,  $S_{\text{HA}}$ , which we have recently proposed,<sup>1)</sup> appears to satisfy this need. The value of  $S_{\text{HA}}$  for each member of the (di)azine series is derived from heats of formation calculated by the COSMO method.<sup>1)</sup> When this parameter is applied to analyses of the data used with Eq. 1, omitting the data for amphiprotic substituents, the results show that when the organic modifier in the eluent is methanol, the two  $HB$  terms,  $HB_{\text{CO}}$  and  $HB_{\text{A}}$ , can be replaced by the single term  $s S_{\text{HA}}$  (Eq. 2).

$$\log k' = a \log P + \rho \sigma_1 + s S_{\text{HA}} + \text{const.} \quad (2)$$

Accordingly, we were interested in examining the possibility that Eq. 2 might be applied to other mobile phases in order to evaluate the validity of using the  $S_{\text{HA}}$  parameter generally as an H-acceptor scale. In this study, we have re-analyzed, by means of Eq. 2, the values of  $\log k'$  that had been obtained for eluents containing a wide range of organic modifiers and previously analyzed by Eq. 1.

## Materials and Methods

**Compounds** Compounds used in this work are the same as those used previously but excluding those containing amphiprotic substituents (see Table 2).<sup>11)</sup>

**$\log P$  and  $\log k'$  Values** The 1-octanol/water  $\log P$  (Table 1) and  $\log k'$  obtained with eluents other than aqueous methanol solutions are taken from our previous work unless otherwise noted.<sup>11)</sup> The RPLC conditions used for obtaining  $\log k'$  values were as follows:

Columns: C18 (CAPCELL PAK C18-AG or SG type, Shiseido), Ph (CAPCELL PAK Ph, Shiseido); the size of each column is  $4.6 \times 150$  mm; packing materials of C18 and Ph columns are silicone-polymer coated silica gels chemically modified with octadecyl and phenyl groups, respectively.

Eluents: Organic modifiers used in this work are acetonitrile (A), dioxane

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Table 1. Physicochemical Parameters Used for Analyses by Eq. 2.

| Substituent        | $\log P^{a)}$ | $S_{HA}^{b)}$      | $\sigma_1^{c)}$ |
|--------------------|---------------|--------------------|-----------------|
| H                  | -0.26         | 1.00               | 0.00            |
| F                  | 0.29          | 0.99               | 0.54            |
| Cl                 | 0.70          | 0.97               | 0.47            |
| Me                 | 0.21          | 0.96               | -0.01           |
| Et                 | 0.69          | 0.91               | -0.01           |
| OMe                | 0.73          | 1.02               | 0.30            |
| OEt                | 1.28          | 1.00               | 0.28            |
| OPr                | 1.84          | 0.99               | 0.28            |
| SMe                | 1.17          | 0.96               | 0.30            |
| NMe <sub>2</sub>   | 0.93          | 1.09               | 0.17            |
| CN                 | -0.01         | 1.21               | 0.57            |
| Ac                 | 0.20          | 1.31               | 0.30            |
| COOMe              | -0.23         | 1.62               | 0.32            |
| COOEt              | 0.28          | 1.60               | 0.30            |
| CONMe <sub>2</sub> | -0.80         | 1.78 <sup>c)</sup> | 0.28            |

a) Taken from ref. 10. b) Taken from ref. 1. c) Taken from ref. 14.

Table 2.  $\log k'$  Values for Monosubstituted Pyrazines.<sup>a)</sup>

| Substituent        | M5 <sup>b)</sup> | M15   | M30    | M50    | M70    |
|--------------------|------------------|-------|--------|--------|--------|
| H                  | 0.443            | 0.102 | -0.263 | -0.590 | -0.786 |
| F                  | 0.583            | 0.296 | 0.000  | -0.317 | -0.570 |
| Cl                 | 0.934            | 0.637 | 0.336  | -0.068 | -0.369 |
| Me                 | 0.949            | 0.474 | 0.031  | -0.383 | -0.671 |
| Et                 | 1.417            | 0.897 | 0.401  | -0.084 | -0.463 |
| OMe                | 1.202            | 0.834 | 0.430  | 0.000  | -0.341 |
| OEt                | 1.730            | 1.318 | 0.850  | 0.329  | -0.112 |
| OPr                | — <sup>c)</sup>  | 1.846 | 1.317  | 0.685  | 0.140  |
| SMe                | 1.547            | 1.153 | 0.694  | 0.205  | -0.193 |
| NMe <sub>2</sub>   | 1.653            | 1.846 | 0.551  | -0.021 | -0.409 |
| CN                 | 0.493            | 0.177 | -0.154 | -0.481 | -0.766 |
| Ac                 | 0.940            | 0.526 | 0.097  | -0.296 | -0.600 |
| CO <sub>2</sub> Me | 0.921            | 0.441 | -0.065 | -0.514 | -0.822 |
| CO <sub>2</sub> Et | 1.456            | 0.917 | 0.352  | -0.179 | -0.574 |
| CONMe <sub>2</sub> | 0.684            | 0.140 | -0.386 | -0.834 | -1.096 |

a) Remeasured in this work. b) In 0.01 M phosphate buffer (pH 7.4). The figures after M represent the % volume of MeOH. c) The retention time was too long to be measured.

(D), 1-propanol (P) and trimethylene glycol (TMG). An appropriate composition of eluent was prepared by volume using a phosphate buffer solution (0.01 M, pH 7.4) as the aqueous component. As the retention times of the solutes eluted by the mobile phases mentioned above were shorter than those eluted by aqueous methanol solutions with the same percentage of water, the data could be obtained only for highly water-rich eluents. To compare the analytical results with those from aqueous methanol eluents under equivalent conditions, we remeasured  $\log k'$  values with methanol–buffer (pH 7.4) mixtures containing 5, 15, 30, 50 and 70% MeOH (M5, M15, M30, M50 and M70, respectively), designated as the M-series. The newly obtained  $\log k'$  values are given in Table 2.

**Hydrogen Accepting Scale,  $S_{HA}$**  Values for the H-accepting parameter,  $S_{HA}$ , are taken from our previous work.<sup>1)</sup> The heat of formation,  $\Delta H_f$ , of the minimum energy conformation of a given compound (Ar-X) was calculated for the gaseous phase and also for several dielectric ( $\epsilon$ ) environments with the AM1 Hamiltonian<sup>16)</sup> and by using the eps command to perform the COSMO procedure<sup>17)</sup> incorporated in the MOPAC 93 program package.<sup>18)</sup> By plotting  $\Delta H_f$  values for Ar-X, calculated at these  $\epsilon$  values, against the corresponding  $\Delta H_f$  values for the unsubstituted compound (Ar-H), a straight line was obtained; the slope of this line was defined as  $S_{HA}$  for the X substituent,  $S_{HA} = \delta\Delta H_f(\text{Ar-X})/\delta\Delta H_f(\text{Ar-H})$ .

**Analyses for the Relationship between  $\log P$  and  $\log k'$**  Regression analyses were performed as previously described by means of Eq. 2 using the parameters given in Table 1. The level of significance for the correlation and for each term was estimated by the  $F$  and  $t$  tests, respectively.

## Results and Discussion

Correlations between  $\log P$  and  $\log k'$  under various RPLC conditions are summarized in Tables 3–5. For each set, two results are reported: (a) first, those from fitting the data to the equation  $\log k' = a \log P + \text{const.}$ ; and (b) from fitting the data to Eq. 2. When inclusion of neither  $\sigma_1$  nor  $S_{HA}$  parameters improved the correlation, only the result in the case of (a) is given. Confidence intervals (95%) for the parameters are shown in parentheses for the statistically most significant correlation, designated “best correlation” in subsequent discussion. The results (Table 3) obtained for  $\log k'$  from the M-series shown in Table 2 were essentially the same as those previously reported.<sup>1)</sup> It can be clearly seen that the contributions of  $\sigma_1$  and  $S_{HA}$  terms increase with decreased methanol concentration:  $\rho$  becomes more negative and  $s$  more positive, confirming that H-bonding effects are more significant in more water-rich solvents.

Correlations for other organic modifiers measured on C18 and Ph columns are in Tables 4 and 5, respectively. Inspection of these tables indicates that the  $\log k'$  values are also well expressed by Eq. 2 for each set regardless of the properties of the organic solvents. Here again, the contributions of the correction terms become more significant as the modifier concentration approaches zero, reaching their highest values at organic modifier concentrations of 5%. The excellent predictive ability of Eq. 2 is shown in Fig. 1, for 5% modifier-containing eluents with the Ph column, in comparison with that of the uncorrected equation.

By examining the coefficients of each term in the correlations thus obtained, we can evaluate the contributions provided by the components of Eq. 2. The most significant is the coefficient,  $a$ , of the  $\log P$  term. In Fig. 2, the value of  $a$  at each mobile phase composition is plotted as a function of the volume fraction,  $f$ , of methanol (M-series) and acetonitrile (A-series). In each case, whereas values of  $a$  from the uncorrected equation provide no linear correlation against  $f$  (dotted line), those from the best correlations show good linear correlations (solid line), yielding Eqs. 3–6. It is particularly noteworthy that good linear correlation covers the full range of methanol concentrations in the M-series.

### M-series

$$\text{C18: } a = -0.783f + 1.006 \quad n=5, r=1.000, s=0.007 \quad (3)$$

$$\text{Ph: } a = -0.823f + 0.898 \quad n=5, r=0.999, s=0.009 \quad (4)$$

### A-series

$$\text{C18: } a = -1.334f + 0.998 \quad n=3, r=1.000, s=0.003 \quad (5)$$

$$\text{Ph: } a = -1.249f + 0.767 \quad n=3, r=0.997, s=0.017 \quad (6)$$

We have previously shown that  $\log k'$  for pyrazines increases non-linearly with decreases in  $f$ . It follows that the good linearities indicated for Eqs. 3–6 must arise from relative changes in pure hydrophobicity ascribable to substituent effects. This demonstrates the validity of analyzing the chromatographic hydrophobicity ( $\log k'$ ) in terms of an intrinsic factor reflecting  $\log P$  and separable specific substituent-modified H-bonding effects by Eq. 2. The intercepts for Ph column are smaller than those for C18, consistent with the fact that the stationary phase bonded with phenyl groups is more polar than that with octadecyl hydrocarbon groups.

The dependence of  $a$  on  $f$  is greater for acetonitrile-containing eluents (Eqs. 5 and 6) than for methanolic eluents

Table 3. Analyses of log  $k'$  for Pyrazines Determined with Methanol as Organic Modifier by Using Eq. 2

| Set        | Mobile phase <sup>a)</sup> | Coefficient           |                       |                       | Const.  | $n^{b)}$ | $r^{c)}$ | $s^{d)}$ | $F^{e)}$ |
|------------|----------------------------|-----------------------|-----------------------|-----------------------|---------|----------|----------|----------|----------|
|            |                            | $\log P$              | $\sigma_1$            | $S_{HA}$              |         |          |          |          |          |
| C18 column |                            |                       |                       |                       |         |          |          |          |          |
| 1          | M5                         | 0.588                 |                       |                       | 0.851   | 14       | 0.790    | 0.279    | 19.9     |
|            |                            | 0.958                 | −0.888                | 1.135                 | −0.372  | 14       | 0.992    | 0.062    | 215.1    |
|            |                            | (0.088) <sup>f)</sup> | (0.202)               | (0.181)               | (0.232) |          |          |          |          |
| 2          | M15                        | 0.673                 |                       |                       | 0.410   | 15       | 0.925    | 0.195    | 77.5     |
|            |                            | 0.893                 | −0.543                | 0.832                 | −0.509  | 15       | 0.998    | 0.033    | 1033.0   |
|            |                            | (0.038)               | (0.108)               | (0.092)               | (0.117) |          |          |          |          |
| 3          | M30                        | 0.651                 |                       |                       | −0.025  | 15       | 0.971    | 0.112    | 218.0    |
|            |                            | 0.778                 | −0.226                | 0.481                 | −0.580  | 15       | 0.997    | 0.037    | 710.8    |
|            |                            | (0.042)               | (0.120) <sup>g)</sup> | (0.102)               | (0.130) |          |          |          |          |
| 4          | M50                        | 0.564                 |                       |                       | −0.434  | 15       | 0.989    | 0.061    | 562.2    |
|            |                            | 0.614                 |                       | 0.187                 | −0.675  | 15       | 0.994    | 0.045    | 516.2    |
|            |                            | (0.050)               |                       | (0.120) <sup>h)</sup> | (0.157) |          |          |          |          |
| 5          | M70                        | 0.454                 |                       |                       | −0.721  | 15       | 0.990    | 0.047    | 608.5    |
|            |                            | (0.040)               |                       |                       | (0.032) |          |          |          |          |
| Ph column  |                            |                       |                       |                       |         |          |          |          |          |
| 6          | M5                         | 0.578                 |                       |                       | 0.534   | 15       | 0.850    | 0.253    | 33.8     |
|            |                            | 0.849                 | −0.790                | 1.024                 | −0.563  | 15       | 0.991    | 0.069    | 204.9    |
|            |                            | (0.079)               | (0.225)               | (0.192)               | (0.245) |          |          |          |          |
| 7          | M15                        | 0.562                 |                       |                       | 0.227   | 15       | 0.898    | 0.194    | 54.3     |
|            |                            | 0.774                 | −0.558                | 0.803                 | −0.650  | 15       | 0.994    | 0.054    | 291.3    |
|            |                            | (0.061)               | (0.174)               | (0.149)               | (0.189) |          |          |          |          |
| 8          | M30                        | 0.518                 |                       |                       | −0.085  | 15       | 0.943    | 0.129    | 105.1    |
|            |                            | 0.664                 | −0.282                | 0.550                 | −0.714  | 15       | 0.996    | 0.040    | 411.1    |
|            |                            | (0.045)               | (0.129)               | (0.110)               | (0.140) |          |          |          |          |
| 9          | M50                        | 0.413                 |                       |                       | −0.463  | 15       | 0.976    | 0.065    | 259.1    |
|            |                            | 0.484                 |                       | 0.266                 | −0.805  | 15       | 0.996    | 0.027    | 818.8    |
|            |                            | (0.030)               |                       | (0.071)               | (0.093) |          |          |          |          |
| 10         | M70                        | 0.295                 |                       |                       | −0.750  | 15       | 0.980    | 0.038    | 287.9    |
|            |                            | 0.318                 |                       | 0.123                 | −0.900  | 15       | 0.990    | 0.028    | 261.1    |
|            |                            | (0.033)               |                       | (0.084) <sup>i)</sup> | (0.105) |          |          |          |          |

a) In 0.01 M phosphate buffer (pH 7.4). The figures after M represent the % volume of MeOH. b) Number of compounds used for correlations. c) Correlation coefficients. d) Standard deviations. e) Values of  $F$ -ratio between regression and residual variances. f) Figures in parentheses are 95% confidence intervals. The terms except the constant term are justified >99.9% unless otherwise noted. g) Justified at the 99.8% by  $t$ -test. h) Justified at the 99.4% by  $t$ -test. i) Justified at the 99.1% by  $t$ -test.

Table 4. Analyses of log  $k'$  for Pyrazines with Various Mobile Phases on the C18 Column by Using Eq. 2

| Set        | Mobile phase <sup>a)</sup> | Coefficient           |                       |                 | Const.  | $n^{b)}$ | $r^{c)}$ | $s^{d)}$ | $F^{e)}$ |
|------------|----------------------------|-----------------------|-----------------------|-----------------|---------|----------|----------|----------|----------|
|            |                            | $\log P$              | $\sigma_1$            | $S_{\text{HA}}$ |         |          |          |          |          |
| C18 column |                            |                       |                       |                 |         |          |          |          |          |
| 11         | A5                         | 0.658                 |                       |                 | 0.495   | 15       | 0.895    | 0.232    | 52.1     |
|            |                            | 0.933                 | −0.313                | 1.035           | −0.749  | 15       | 0.996    | 0.051    | 450.8    |
|            |                            | (0.058) <sup>f)</sup> | (0.165) <sup>g)</sup> | (0.141)         | (0.179) |          |          |          |          |
| 12         | A15                        | 0.661                 |                       |                 | −0.053  | 15       | 0.957    | 0.142    | 139.9    |
|            |                            | 0.796                 | 0.244                 | 0.502           | −0.766  | 15       | 0.995    | 0.055    | 341.7    |
|            |                            | (0.062)               | (0.177) <sup>h)</sup> | (0.152)         | (0.193) |          |          |          |          |
| 13         | A30                        | 0.597                 |                       |                 | −0.438  | 15       | 0.952    | 0.135    | 126.3    |
|            |                            | 0.599                 | 0.612                 |                 | −0.608  | 15       | 0.989    | 0.067    | 274.0    |
|            |                            | (0.058)               | (0.210)               |                 | (0.075) |          |          |          |          |
| 14         | D5                         | 0.750                 |                       |                 | 0.179   | 15       | 0.945    | 0.183    | 109.4    |
|            |                            | 0.960                 |                       | 0.786           | −0.831  | 15       | 0.997    | 0.043    | 1092.0   |
|            |                            | (0.048)               |                       | (0.115)         | (0.151) |          |          |          |          |
| 15         | D10                        | 0.768                 |                       |                 | −0.070  | 15       | 0.969    | 0.137    | 202.6    |
|            |                            | 0.921                 |                       | 0.573           | −0.807  | 15       | 0.997    | 0.047    | 919.5    |
|            |                            | (0.052)               |                       | (0.125)         | (0.164) |          |          |          |          |
| 16         | P5                         | 0.758                 |                       |                 | 0.072   | 15       | 0.953    | 0.170    | 128.5    |
|            |                            | 0.867                 | −0.811                | 0.421           | −0.244  | 15       | 0.996    | 0.053    | 474.9    |
|            |                            | (0.061)               | (0.174)               | (0.149)         | (0.189) |          |          |          |          |
| 17         | P15                        | 0.836                 |                       |                 | −0.502  | 15       | 0.976    | 0.133    | 257.2    |
|            |                            | 0.834                 | −0.452                |                 | −0.376  | 15       | 0.986    | 0.103    | 215.3    |
|            |                            | (0.089)               | (0.323) <sup>i)</sup> |                 | (0.115) |          |          |          |          |

a) In 0.01 M phosphate buffer (pH 7.4). The figures after the symbol of organic modifier (A: acetonitrile, D: dioxane, P: 1-propanol) represent the % volume of the modifier. b—e) See the footnotes in Table 3. f) Figures in parentheses are 95% confidence intervals. The terms except the constant term are justified >99.9% unless otherwise noted. g) Justified at the 99.8% by  $t$ -test. h) Justified at the 98.8% by  $t$ -test. i) Justified at the 98.9% by  $t$ -test.

Table 5. Analyses of  $\log k'$  for Pyrazines with Various Mobile Phases on the Ph Column by Using Eq. 2

| Set       | Mobile phase <sup>a)</sup> | Coefficients          |                       |                       | Const.  | $n^{b)}$ | $r^{c)}$ | $s^{d)}$ | $F^{e)}$ |
|-----------|----------------------------|-----------------------|-----------------------|-----------------------|---------|----------|----------|----------|----------|
|           |                            | $\log P$              | $\sigma_1$            | $S_{\text{HA}}$       |         |          |          |          |          |
| Ph column |                            |                       |                       |                       |         |          |          |          |          |
| 18        | A5                         | 0.501                 |                       |                       | 0.119   | 15       | 0.879    | 0.192    | 44.1     |
|           |                            | 0.713                 | −0.497                | 0.802                 | −0.775  | 15       | 0.991    | 0.060    | 191.2    |
|           |                            | (0.068) <sup>f)</sup> | (0.195)               | (0.167)               | (0.212) |          |          |          |          |
| 19        | A15                        | 0.452                 |                       |                       | −0.245  | 15       | 0.950    | 0.106    | 118.9    |
|           |                            | 0.566                 |                       | 0.427                 | −0.794  | 15       | 0.992    | 0.044    | 366.8    |
|           |                            | (0.049)               |                       | (0.119)               | (0.155) |          |          |          |          |
| 20        | A30                        | 0.396                 |                       |                       | −0.577  | 15       | 0.962    | 0.079    | 163.1    |
|           |                            | 0.398                 | 0.338                 |                       | −0.671  | 15       | 0.988    | 0.046    | 254.1    |
|           |                            | (0.039)               | (0.143)               |                       | (0.051) |          |          |          |          |
| 21        | D5                         | 0.549                 |                       |                       | −0.017  | 15       | 0.911    | 0.176    |          |
|           |                            | 0.743                 | −0.446                | 0.734                 | −0.837  | 15       | 0.993    | 0.056    | 247.2    |
|           |                            | (0.064)               | (0.182)               | (0.155)               | (0.198) |          |          |          |          |
| 22        | D10                        | 0.536                 |                       |                       | −0.206  | 15       | 0.947    | 0.128    |          |
|           |                            | 0.681                 | −0.243                | 0.547                 | −0.842  | 15       | 0.995    | 0.044    | 357.6    |
|           |                            | (0.050)               | (0.142) <sup>g)</sup> | (0.122)               | (0.155) |          |          |          |          |
| 23        | D15                        | 0.524                 |                       |                       | −0.346  | 15       | 0.968    | 0.095    | 195.6    |
|           |                            | 0.626                 |                       | 0.384                 | −0.840  | 15       | 0.995    | 0.041    | 562.3    |
|           |                            | (0.045)               |                       | (0.109)               | (0.143) |          |          |          |          |
| 24        | P5                         | 0.555                 |                       |                       | −0.061  | 15       | 0.911    | 0.178    | 63.3     |
|           |                            | 0.702                 | −0.746                | 0.562                 | −0.576  | 15       | 0.991    | 0.063    | 201.7    |
|           |                            | (0.071)               | (0.203)               | (0.174)               | (0.221) |          |          |          |          |
| 25        | P15                        | 0.586                 |                       |                       | −0.427  | 15       | 0.976    | 0.092    | 264.6    |
|           |                            | 0.635                 | −0.434                | 0.190                 | −0.551  | 15       | 0.997    | 0.038    | 531.2    |
|           |                            | (0.043)               | (0.124)               | (0.106) <sup>h)</sup> | (0.135) |          |          |          |          |
| 26        | TMG5                       | 0.524                 |                       |                       | 0.196   | 15       | 0.903    | 0.176    | 57.7     |
|           |                            | 0.650                 | −0.771                | 0.480                 | −0.206  | 15       | 0.986    | 0.074    | 129.8    |
|           |                            | (0.084)               | (0.240)               | (0.205)               | (0.261) |          |          |          |          |
| 27        | TMG10                      | 0.527                 |                       |                       | 0.051   | 15       | 0.934    | 0.143    | 88.2     |
|           |                            | 0.630                 | −0.623                | 0.395                 | −0.283  | 15       | 0.990    | 0.060    | 187.8    |
|           |                            | (0.068)               | (0.195)               | (0.167)               | (0.212) |          |          |          |          |

a) The figures after the symbol of organic modifier (A: acetonitrile, D: dioxane, P: 1-propanol, TMG: trimethylene glycol) represent the % volume of the modifier. b—e) See the footnotes in Table 3. f) Figures in parentheses are 95% confidence intervals. The terms except the constant term are justified >99.9% unless otherwise noted. g) Justified at the 99.6% by *t*-test. h) Justified at the 98.7% by *t*-test.

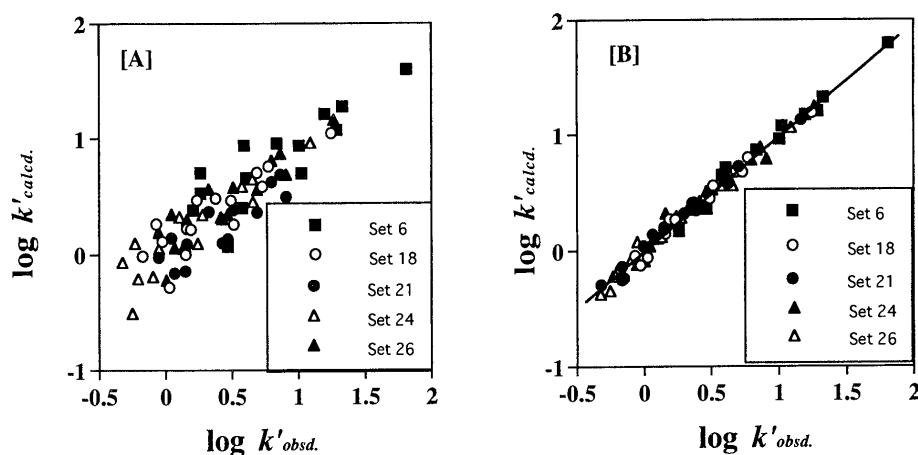


Fig. 1. The Predictive Power of Eq. 2 for  $\log k'$  Values of Monosubstituted Pyrazines with Eluents Containing 5% Organic Modifiers on the Ph Column  
[A]  $\log k'$  values calculated by the uncorrected equation:  $\log k' = a \log P + \text{const.}$  [B]  $\log k'$  values calculated by Eq. 2.

(Eqs. 3 and 4). Addition of aprotic acetonitrile to water by changing the volume fraction will exert a more drastic effect on properties of the resulting eluent compared to addition of protic methanol to water. As pyrazines have polar aza functions in the parent nucleus, their  $\log k'$  should be more dependent on the characteristics of the mobile phase and hence vary more widely in aqueous acetonitrile than in aqueous methanol. This concept seems to be justified by finding that

preliminary study of a series of less polar monosubstituted benzenes showed much smaller and closer dependence of  $a$  on  $f$  with gradients of  $-0.60$  and  $-0.66$  for the M- and A-eluent series, respectively.<sup>19)</sup>

In the preceding discussion, we have shown that our  $S_{HA}$  parameter can effectively work as an H-acceptor scale for substituents so as to separate the overall  $\log k'$  value into hydrophobic and H-bonding components. Recently, we have

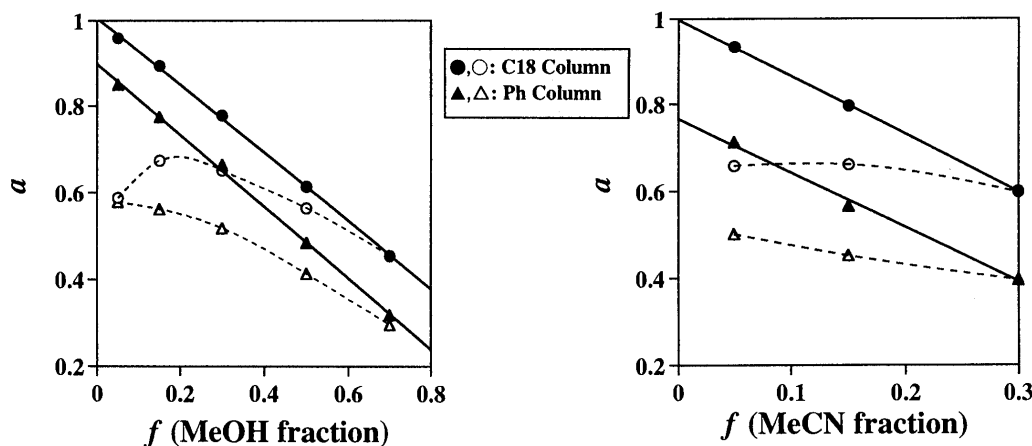


Fig. 2. Plots of  $a$  Values against Volume Fraction,  $f$ , of Methanol and Acetonitrile in Eluents

The solid symbols represent  $a$  values for the best correlations. The hollow symbols represent  $a$  values derived from the equation;  $\log k = a \log P + \text{const.}$

also succeeded in utilizing  $S_{\text{HA}}$  to correlate  $\log P_{\text{oct}}$  ( $P_{\text{oct}}$ : octanol/water partition coefficient) and  $\log P_{\text{CL}}$  ( $P_{\text{CL}}$ : chloroform/water partition coefficient) for monosubstituted (di)azines and presented the rationale as an H-bond acceptor scale.<sup>20</sup> For each series of pyridines, pyrazines and pyrimidines, the values for  $\log P_{\text{CL}}$  are formulated with high precision by Eq. 7.<sup>20</sup>

$$\log P_{\text{CL}} = a \log P_{\text{oct}} + sS_{\text{HA}} + \text{const.} \quad (7)$$

In an earlier study, we had analyzed the same data set by using the indicator variable  $HB$  according to Eq. 8.<sup>21</sup>

$$\log P_{\text{CL}} = a \log P_{\text{oct}} + hHB + \text{const.} \quad (8)$$

where  $HB=0, 1$  or  $2$  depending on the H-bonding ability of the substituent. Although analysis according to Eq. 8 yielded very good statistical correlations, and the physical interpretation of the discrete H-bond parameter,  $HB$ , could be explained rationally,<sup>21</sup> classification of compounds into their appropriate groups could still be achieved only on the basis of trial and error. In contrast, use of the  $S_{\text{HA}}$  parameter removes the necessity to employ this intellectually unsatisfactory and arbitrary procedure.

The present results, demonstrating that the use of the  $S_{\text{HA}}$  parameter can be applied to a variety of solvent systems, may provide confirmation that  $S_{\text{HA}}$  can be universally utilized to represent the difference in H-bonding effects involved in various combinations of two different partitioning systems. The solutes so far studied were limited to within a series of congeners of similar structure. A study of  $S_{\text{HA}}$  for a wide range of aromatic H-accepting solutes is now under way.

**Acknowledgment** This work was partly supported by Kobe Pharmaceutical University Collaboration Fund.

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## New Strong Fibrates with Piperidine Moiety

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Received August 14, 2000; accepted September 8, 2000

**New fibrates containing piperidine, 4-hydroxypiperidine, piperidin-3-ene, and piperazine moieties in the structures were synthesized and evaluated. Among the synthesized compounds, 2-[3-[1-(4-fluorobenzoyl)-piperidin-4-yl]phenoxy]-2-methylpropanoic acid (9aA: AHL-157) showed very superior activities in decreasing triglyceride, cholesterol, and blood sugar compared to bezafibrate in mice and rats.**

**Key words** fibrate; piperidine; hypolipidemic; hypoglycemic

2-Methyl-2-phenoxypropanoic acid (fibric acid) derivatives are called fibrates<sup>1–3)</sup> and clinically useful in hyperlipidemic patients who show from middle to high levels of triglyceridemia. Although clofibrate,<sup>4)</sup> one of the first generation fibrates, has been frequently used until quite recently, the second generation fibrates such as bezafibrate,<sup>5–8)</sup> fenofibrate,<sup>9–16)</sup> and gemfibrozil<sup>4,17)</sup> have been developed and are now clinically used for the treatment of hyperlipidemia. Gemfibrozil having a 5-phenoxy-pentanoic acid moiety instead of the fibric acid moiety, is also called an exceptionally new fibrates because of having almost similar pharmacological activity as the other fibrates possessing fibric acid moieties.

Although these fibrates are clinically used, drugs significantly reducing cholesterol and triglyceride in the blood and possessing a more powerful hypoglycemic effect are desired.

By chance, we found that 2-[3-[1-[4-chloro(phenylsulfonyl)]-4-hydroxypiperidin-4-yl]-phenoxy]acetic acid (**4a**), which was synthesized during the development of thromboxane-receptor antagonists, has total cholesterol- and  $\beta$ -lipoprotein-lowering effects in mice. Since **4a** shows almost equipotent total cholesterol- and  $\beta$ -lipoprotein-lowering activities to bezafibrate used as a reference compound and has a similar structure to bezafibrate, we started to modify the chemical structure of **4a** in order to find new fibrates.

As shown in Chart 1, our strategy to synthesize and develop stronger fibrates consists of modifying four parts (A—D) of **4a**. First, we modified the phenoxyacetic acid moiety (A part) of **4a**, which was converted to the 2-methyl-2-phenoxypropanoic acid moiety (fibrate moiety). Since the product, 2-[3-[1-(4-chlorophenyl)sulfonyl]-4-hydroxypiperidin-4-yl]phenoxy]-2-methylpropanoic acid (**4b**), has almost equipotent total cholesterol- and  $\beta$ -lipoprotein-lowering activities to **4a**, the *N*-phenylsulfonyl moiety (part C) was changed to the *N*-benzoyl moiety. Although the *N*-benzoyl derivative (**4c**) also has almost equipotent activities to **4a**, the derivatives when the 4-chloro group of part D was changed to the 4-fluoro group 2-[3-[1-(4-fluorobenzoyl)-4-hydroxypiperidin-4-yl]phenoxy]-2-methylpropanoic acid (**4d**) had about three times higher activity than **4a**. Finally, the derivatives with the modified 4-hydroxypiperidine moiety of **4d** into piperidine, piperidin-3-ene, and piperazine moieties were synthesized and evaluated. In this paper, we describe the synthesis and hypolipidemic activities of the derivatives.

**Chemistry** The 4-hydroxypiperidine derivatives (**4a—d**)

were synthesized as shown in Chart 2. The reaction of the Grignard reagent prepared from 3-benzyloxybromobenzene with 1-benzyl-4-piperidone gave the 4-aryl-4-hydroxypiperidine derivative (**1**), which was transformed into the *O,N*-debenzylated 4-hydroxypiperidine derivative (**2**) by the palladium-catalyzed hydrogenolysis. Compound **2** was allowed to react with 4-substituted benzenesulfonyl or benzoyl chlorides to afford the corresponding *N*-arylsulfonyl or *N*-benzoyl derivatives (**3a—c**). The reaction of **3a—c** with ethyl 2-bromoacetate and 2-bromo-2-methylpropanoate followed by alkaline hydrolysis gave the expected 2-phenoxy-2-methylpropanoic acids (**4a—d**).

The piperidine derivatives (**9**) were synthesized from 1-benzyl-4-piperidinone according to the scheme shown in Chart 3. The piperidin-3-ene derivatives (**5a—c**) were obtained from the reaction of 1-benzyl-4-piperidone with the Grignard reagents derived from the methoxybromobenzenes followed by dehydration using hydrochloric acid. The palladium-catalyzed hydrogenation and debenzylation of **5a—c** gave the piperidine derivatives (**6a—c**) which were demethylated with aqueous hydrobromic acid to the phenols (**7a—c**). The reaction of **7a—c** with benzoyl chlorides gave *N*-benzoylpiperidines (**8**), which were allowed to react with ethyl 2-bromo-2-methylpropanoate followed by hydrolysis to give the 2-phenoxy-2-methylpropanoic acids (**9**).

The piperidin-3-ene derivative (**12**) was synthesized from **3c** according to the scheme shown in Chart 4. Namely, the reaction of the phenol (**3c**) with ethyl 2-bromo-2-methylpropanoate gave 2-phenoxy-2-methylpropanoate (**10**), which was dehydrated with *p*-toluenesulfonic acid (TsOH) followed by alkaline hydrolysis to give the piperidin-3-ene derivative (**12**).

The piperazine derivative (**15**) was synthesized from the

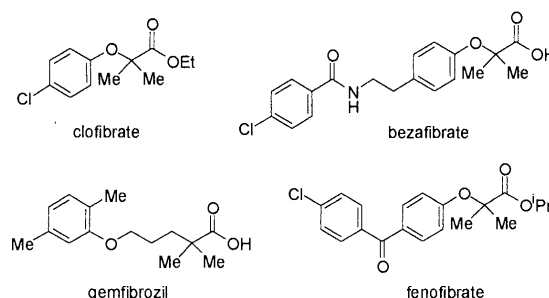


Fig. 1

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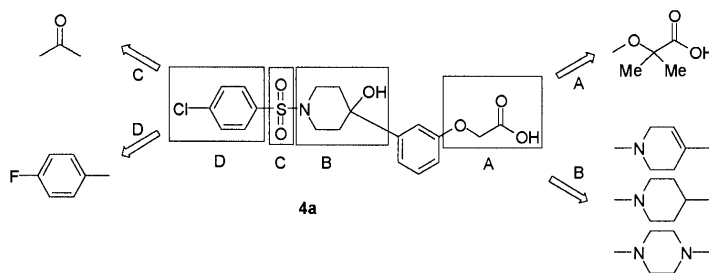


Chart 1

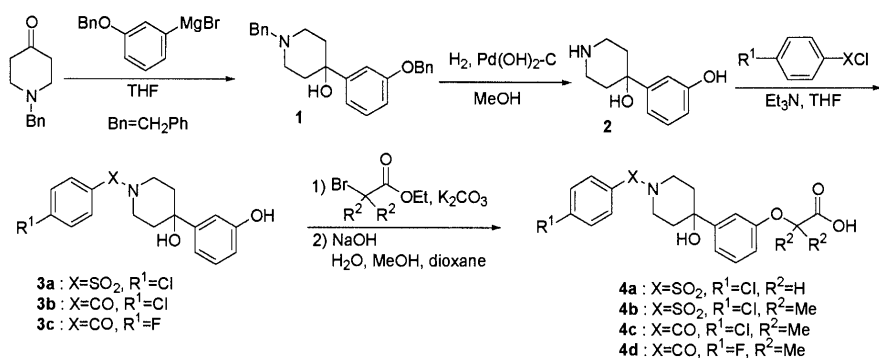


Chart 2

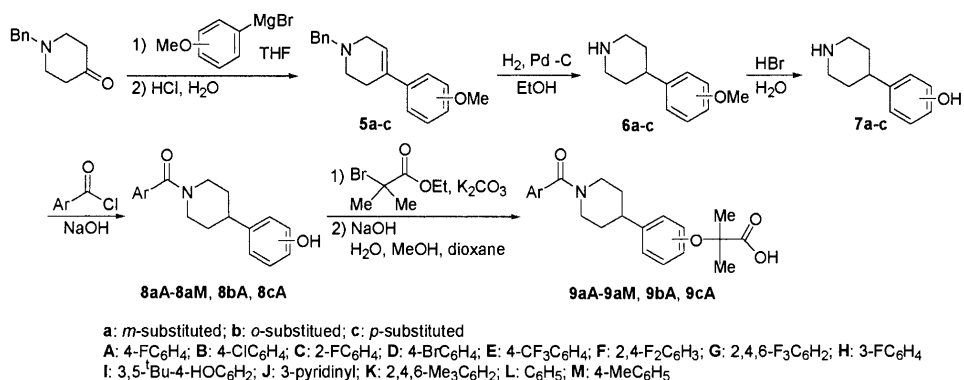


Chart 3

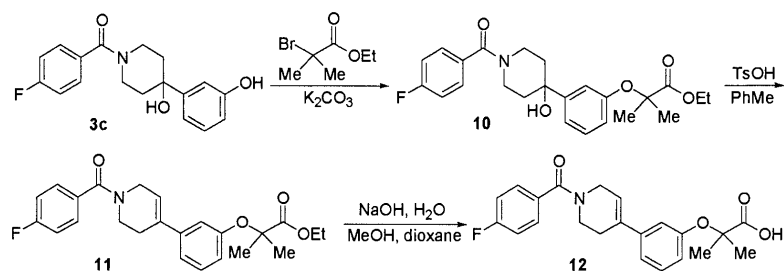


Chart 4

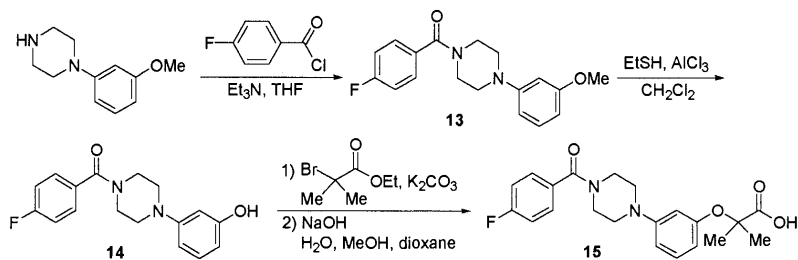


Chart 5

Table 1. Hypolipidemic Activities of **4**, **9**, **12**, **15**, and Bezafibrate in High-Cholesterol Diet-Fed Mice

| Compound    | T-CHOL (MED: mg/kg) | $\beta$ -LP (MED: mg/kg) |
|-------------|---------------------|--------------------------|
| Bezafibrate | 100                 | 100                      |
| <b>9aA</b>  | 0.3                 | 0.3                      |
| <b>9aB</b>  | 1                   | 1                        |
| <b>9aC</b>  | 1                   | 1                        |
| <b>9aD</b>  | 3                   | 3                        |
| <b>9aE</b>  | 3                   | 3                        |
| <b>9aF</b>  | 3                   | 3                        |
| <b>9aG</b>  | 3                   | 3                        |
| <b>9aH</b>  | 3                   | 3                        |
| <b>9aI</b>  | 30                  | 30                       |
| <b>9aJ</b>  | 30                  | 30                       |
| <b>9aK</b>  | 30                  | 30                       |
| <b>9aL</b>  | NT                  | NT                       |
| <b>9aM</b>  | NT                  | NT                       |
| <b>9bA</b>  | 10                  | 10                       |
| <b>9cA</b>  | 100                 | 100                      |
| <b>4a</b>   | 100                 | 100                      |
| <b>4b</b>   | 100                 | 100                      |
| <b>4c</b>   | 100                 | 100                      |
| <b>4d</b>   | 30                  | 30                       |
| <b>12</b>   | 10                  | 10                       |
| <b>15</b>   | 100                 | 100                      |

Mice ( $n=6$ ) were given high-cholesterol diet (1% cholesterol and 0.5% cholic acid) for 7 d. Drugs were orally administered on days 6 and 7. Each value represents the MED (minimum effective dose, reducing 15% in T-CHOL and 20% in  $\beta$ -LP, respectively). NT: Not tested.

commercially available 1-(3-methoxyphenyl)piperazine as shown in Chart 5. The *N*-benzoylpiperazine derivative (**13**) prepared by the acylation of 1-(3-methoxyphenyl)piperazine with 4-fluorobenzoyl chloride was demethylated using ethanethiol and aluminum chloride to give the phenol (**14**) which was reacted with ethyl 2-bromo-2-methylpropanoate followed by alkaline hydrolysis to yield the 2-phenoxypropanoic acid (**15**).

**Pharmacological Activity** The pharmacological activity of **4b** having the phenoxyacetic acid moiety of **4a** replaced with the 2-phenoxy-2-methylpropanoic acid moiety, which is common to fibrate-type compounds, was evaluated. The potency of reducing cholesterol and  $\beta$ -lipoprotein ( $\beta$ -LP) in serum did not change when compared with **4a**. Compound **4c**, having the sulfonyl group replaced with the benzoyl group, showed almost equipotent activity to **4b**. Compound **4d**, having the chlorine group of the phenyl ring of **4c** replaced with the fluorine group, showed a stronger total cholesterol (T-CHOL)- and  $\beta$ -LP-lowering effect than **4a**—**c** or bezafibrate.

Among the compounds (**4d**, **9aA**, **12**, **15**) having a 4-hydroxypiperidine, piperidine, piperidin-3-ene, or piperazine moiety, **9aA** showed the strongest activity. When the substitution position of the 2-methylpropanoic acid moiety on the phenyl group was changed from *meta* to *ortho* or *para*, the *meta*-substituted compound (**9aA**) showed the strongest activity among the derivatives.

Although several compounds (**9aB**—**9aM**) had the 4-fluorobenzoyl group of **9aA** replaced with other substituted benzoyl or 3-pyridinecarbonyl groups, **9aA** showed the most potent activity in reducing the T-CHOL and  $\beta$ -LP in serum. As shown in Table 1, compounds **9aL** and **9aM** were not tested in the high-cholesterol diet-fed mice, but they were estimated

Table 2. T-CHOL,  $\beta$ -LP, and TG Reducing Effects of **9aD**, **9aE**, **9aJ**, **9aL**, and **9aM** on Serum Lipids of Normal Mice

| Treatment  | T-CHOL (mg/dl)      | $\beta$ -LP (mg/dl) | TG (mg/dl)          |
|------------|---------------------|---------------------|---------------------|
| Control    | 198 $\pm$ 8         | 294 $\pm$ 23        | 227 $\pm$ 23        |
| <b>9aD</b> | 137 $\pm$ 4** (69)  | 58 $\pm$ 7** (20)   | 66 $\pm$ 3** (29)   |
| <b>9aE</b> | 136 $\pm$ 16** (69) | 78 $\pm$ 11** (27)  | 87 $\pm$ 7** (38)   |
| <b>9aJ</b> | 178 $\pm$ 15 (90)   | 253 $\pm$ 28 (86)   | 214 $\pm$ 22 (94)   |
| <b>9aL</b> | 181 $\pm$ 14 (91)   | 116 $\pm$ 14** (39) | 102 $\pm$ 8** (45)  |
| <b>9aM</b> | 130 $\pm$ 12** (66) | 110 $\pm$ 17** (37) | 103 $\pm$ 14** (45) |

Drugs were orally administrated 10 mg/kg for 3 d. Blood was taken about 24 h after the final administration. Each value represents the mean $\pm$ S.E. ( $n=5$ ). Percent of control is indicated in parentheses. \* and \*\*: Significantly different from the control at  $p<0.05$  and  $p<0.01$  (2-side).

Table 3. T-CHOL and TG Reducing Effect of **9aA** and Bezafibrate in High-Cholesterol Diet-Fed Rats

| Drugs       | (%)   | T-CHOL (mg/dl)                  | TG (mg/dl)        |
|-------------|-------|---------------------------------|-------------------|
| Normal      |       | 62 $\pm$ 2                      | 141 $\pm$ 4       |
| Control     |       | 174 $\pm$ 9 <sup>##</sup> (281) | 130 $\pm$ 11 (92) |
| <b>9aA</b>  | 0.001 | 125 $\pm$ 7** (72)              | 120 $\pm$ 11 (92) |
|             | 0.003 | 110 $\pm$ 4** (63)              | 86 $\pm$ 7* (66)  |
|             | 0.01  | 133 $\pm$ 7** (76)              | 78 $\pm$ 10* (60) |
| Bezafibrate | 0.01  | 149 $\pm$ 6 (86)                | 127 $\pm$ 17 (98) |
|             | 0.03  | 123 $\pm$ 13* (71)              | 72 $\pm$ 9* (55)  |
|             | 0.1   | 138 $\pm$ 14 (79)               | 78 $\pm$ 12 (60)  |

Rats were given a high-cholesterol diet (1.5% cholesterol, 0.5% cholic acid) for 2 weeks. Each value represents the mean $\pm$ S.E. ( $n=5$ ). Percent of control is indicated in parentheses. #: Significantly different from the normal group ( $p<0.01$ , 2-side). \*, \*\*: Significantly different from the control group ( $p<0.05$ ,  $p<0.01$ , 2-side).

using normal mice and the results are shown in Table 2. They showed the reducing potencies between those of **9aD** (or **9aE**) and **9aJ** on  $\beta$ -LP and triglyceride (TG) in serum.

The pharmacological profiles of **9aA** were studied in rats and mice. First, the reducing effects of **9aA** on the serum TG and T-CHOL levels were estimated in high-cholesterol diet-fed rats in comparison with bezafibrate (Table 3). Second, the reducing effects of **9aA** on the serum TG were estimated in fructose-induced hypertriglyceremic rats and compared with bezafibrate, fenofibrate and gemfibrozil (Table 4). The reducing effects of **9aA** on the serum glucose and TG were then estimated in KK-A<sup>y</sup> mice and compared with bezafibrate (Table 5). As a result, the reducing activity of **9aA** on the TG and T-CHOL levels was greater than 10 times more potent than that of bezafibrate. The hypoglycemic effects of **9aA** in KK-A<sup>y</sup> mice were also greater than 10 times more potent than those of bezafibrate.

Now, Patients with NIDDM (non-insulin-dependent diabetes mellitus) commonly have dyslipidaemia (especially hypertriglyceridaemia and low HDL (high density lipoprotein) cholesterol levels) and are at high risk of coronary heart disease.<sup>8)</sup> In this paper, **9aA** improved the lipid profile in several animal models, additionally this compound showed hypoglycemic effect in a spontaneous NIDDM mouse model. These results indicate that **9aA** may be a useful therapeutic candidate for NIDDM patients with hyperlipidaemia.

As mentioned above, after evaluation of several pharmacological studies, it was clarified that **9aA** possesses an additional hypoglycemic effect along with the strong reducing effect on serum cholesterol and TG. Detailed studies of the mechanism and action of **9aA** will be reported separately.

Since 2-[3-[1-(4-fluorobenzoyl)piperidin-4-yl]phenoxy]-2-methylpropanoic acid (**9aA**: AHL-157) was selected as a novel synthesized fibrate-type compound as described above,

Table 4. TG Reducing Effects of **9aA**, Bezafibrate, Fenofibrate, and Gemfibrozil in Fructose-Induced Hypertriglyceridemia in Rat

| Drugs          | Dose (mg/kg/d) | TG (mg/dl)              | Inhibition (%) |
|----------------|----------------|-------------------------|----------------|
| Normal Control |                | 100.9±12.0              |                |
| Control        |                | 186.4±25.0 <sup>#</sup> |                |
| <b>9aA</b>     | 0.03           | 182.9±37.6              | (1.9%)         |
|                | 0.1            | 191.5±47                | (-2.7%)        |
|                | 0.3            | 121.7±18                | (34.7%)        |
|                | 1.0            | 80.1±11**               | (57.0%)        |
| Bezafibrate    | 0.3            | 161.6±14                | (13.3%)        |
|                | 1.0            | 179.5±30.5              | (3.7%)         |
|                | 3.0            | 156.3±12                | (16.1%)        |
|                | 10.0           | 98.1±14*                | (47.4%)        |
| Fenofibrate    | 0.3            | 189.3±20                | (-1.6%)        |
|                | 1.0            | 221.1±26.6              | (-18.6%)       |
|                | 3.0            | 129.6±18                | (30.5%)        |
|                | 10.0           | 110.2±15                | (40.9%)        |
| Gemfibrozil    | 0.3            | 222.3±26.5              | (-19.3%)       |
|                | 1.0            | 188.1±23.0              | (-0.9%)        |
|                | 3.0            | 126.2±16                | (32.3%)        |
|                | 10.0           | 95.9±9.1*               | (48.6%)        |

Rats (*n*=8) were given 25% fructose solution for 21 d, and drugs were orally administered once a day for the latter 7 d of the experimental period at each dose. Blood samples were drawn from inferior vena cava 24 h after the last administration. Each data represents the mean±S.E. #: Significantly different from Normal control by Aspin-Welch *t*-test (*p*<0.05, 2-side). \*, \*\*: Significantly different from the control by Dunnett's test (*p*<0.05, *p*<0.01, 2-side). The % TG lowering action relative to control are shown in parentheses.

we examined the procedure for the large scale synthesis of **9aA**. The transformation of **8a** to **9aA** was effectively achieved under the reaction conditions<sup>18)</sup> using acetone, chloroform, and sodium hydroxide as shown in Chart 6.

## Conclusion

Novel fibrates with 4-hydroxypiperidine, piperidine, piperidin-3-ene, and piperazine moieties in the center of the structure were synthesized. It was found that 1) **9aA** with the piperidine moiety showed a strong total cholesterol- and  $\beta$ -LP-lowering effect in mice, 2) the reducing activity of **9aA** on TG in rats and mice is greater than 10 times more potent than that of bezafibrate, and 3) the hypoglycemic effect of **9aA** in KK-A<sup>y</sup> mice is also greater than 10 times more potent than that of bezafibrate. **9aA** was finally selected as the most

Table 5. Glucose and TG Reducing Effects of **9aA** and Bezafibrate in KK-A<sup>y</sup> Mice

|             | Glucose (mg/dl)           | TG (mg/dl)          |
|-------------|---------------------------|---------------------|
| Control     | 551.6±15.3                | 868.9±65.1          |
| <b>9aA</b>  | 0.003% 469.7±34.9 (85.2)  | 708.3±72.2 (81.5)   |
|             | 0.01% 398.4±23.8** (72.2) | 536.0±29.1** (61.7) |
|             | 0.03% 414.7±31.6** (75.2) | 594.0±52.7** (68.4) |
|             | 0.03% 496.0±39.5 (89.9)   | 746.9±83.2 (86.0)   |
| Bezafibrate | 0.1% 440.7±22.7* (79.9)   | 661.0±24.8 (76.1)   |
|             | 0.3% 258.1±18.6** (46.8)  | 451.4±18.4** (52.0) |

Mice were given drug containing diet (0.003% to 0.3%) for 14 d. Each value represents the mean±S.E. of 8 mice. The values in parentheses represent percent of control. \*, \*\*: Significantly different from the control by Dunnett's test (*p*<0.05, *p*<0.01, 2-side).

Table 6. Yields, Melting Points, Mass Spectral and Analytical Data for **4**, **9**, **12**, and **15**

| Compd. No. | Yield (%)    | mp (°C) | Formula                                                                              | FAB-MS ( <i>m/z</i> )                                                                | Anal. Calcd (Found) |             |             |
|------------|--------------|---------|--------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|---------------------|-------------|-------------|
|            |              |         |                                                                                      |                                                                                      | C                   | H           | N           |
| <b>4a</b>  | 80           | 143—144 | C <sub>19</sub> H <sub>20</sub> ClNO <sub>6</sub> S                                  | 426 (M <sup>+</sup> +1, <sup>35</sup> Cl), 428 (M <sup>+</sup> +3, <sup>37</sup> Cl) | 53.58 (53.77)       | 4.73 (4.76) | 3.29 (3.46) |
| <b>4b</b>  | 45           | 181—182 | C <sub>21</sub> H <sub>24</sub> ClNO <sub>6</sub> S                                  | 454 (M <sup>+</sup> +1, <sup>35</sup> Cl), 456 (M <sup>+</sup> +3, <sup>37</sup> Cl) | 55.56 (55.41)       | 5.33 (5.28) | 3.09 (2.95) |
| <b>4c</b>  | 67           | 158—159 | C <sub>22</sub> H <sub>24</sub> ClNO <sub>5</sub> ·0.2H <sub>2</sub> O               | 418 (M <sup>+</sup> +1, <sup>35</sup> Cl), 420 (M <sup>+</sup> +3, <sup>37</sup> Cl) | 62.70 (62.75)       | 5.83 (5.87) | 3.32 (3.32) |
| <b>4d</b>  | 67           | 77—79   | C <sub>22</sub> H <sub>24</sub> FNO <sub>5</sub> ·0.25H <sub>2</sub> O               | 402 (M <sup>+</sup> +1)                                                              | 65.10 (64.96)       | 6.08 (6.16) | 3.45 (3.47) |
| <b>9aA</b> | 60           | 138—139 | C <sub>22</sub> H <sub>24</sub> FNO <sub>4</sub>                                     | 386 (M <sup>+</sup> +1)                                                              | 68.56 (68.56)       | 6.28 (6.45) | 3.63 (3.40) |
| <b>9aB</b> | 73           | 140—141 | C <sub>22</sub> H <sub>24</sub> ClNO <sub>4</sub>                                    | 402 (M <sup>+</sup> +1, <sup>35</sup> Cl), 404 (M <sup>+</sup> +3, <sup>37</sup> Cl) | 65.75 (65.71)       | 6.02 (5.92) | 3.49 (3.40) |
| <b>9aC</b> | 71           | 121—122 | C <sub>22</sub> H <sub>24</sub> FNO <sub>4</sub>                                     | 386 (M <sup>+</sup> +1)                                                              | 68.56 (68.60)       | 6.28 (6.07) | 3.63 (3.72) |
| <b>9aD</b> | 81           | 153—154 | C <sub>22</sub> H <sub>24</sub> BrNO <sub>4</sub>                                    | 446 (M <sup>+</sup> +1, <sup>79</sup> Br), 448 (M <sup>+</sup> +3, <sup>81</sup> Br) | 59.20 (59.01)       | 5.42 (5.66) | 3.14 (3.05) |
| <b>9aE</b> | 61           | 148—149 | C <sub>23</sub> H <sub>24</sub> F <sub>3</sub> NO <sub>4</sub> ·0.25H <sub>2</sub> O | 436 (M <sup>+</sup> +1)                                                              | 62.79 (62.93)       | 5.61 (5.71) | 3.18 (3.13) |
| <b>9aF</b> | 78           | 110—112 | C <sub>22</sub> H <sub>23</sub> F <sub>2</sub> NO <sub>4</sub>                       | 404 (M <sup>+</sup> +1)                                                              | 65.50 (65.41)       | 5.75 (5.77) | 3.47 (3.36) |
| <b>9aG</b> | 55           | 115—116 | C <sub>22</sub> H <sub>22</sub> F <sub>3</sub> NO <sub>4</sub>                       | 422 (M <sup>+</sup> +1)                                                              | 62.70 (62.45)       | 5.26 (5.27) | 3.32 (3.34) |
| <b>9aH</b> | 58           | 122—123 | C <sub>22</sub> H <sub>24</sub> FNO <sub>4</sub>                                     | 386 (M <sup>+</sup> +1)                                                              | 68.56 (68.37)       | 6.28 (6.28) | 3.63 (3.55) |
| <b>9aI</b> | 80           | 148—150 | C <sub>30</sub> H <sub>41</sub> NO <sub>5</sub> ·0.25H <sub>2</sub> O                | 496 (M <sup>+</sup> +1)                                                              | 72.04 (71.85)       | 8.36 (8.46) | 2.80 (2.81) |
| <b>9aJ</b> | 45           | 149—150 | C <sub>21</sub> H <sub>24</sub> N <sub>2</sub> O <sub>4</sub>                        | 369 (M <sup>+</sup> +1)                                                              | 68.46 (68.55)       | 6.57 (6.52) | 7.60 (7.74) |
| <b>9aK</b> | 72           | 168—169 | C <sub>25</sub> H <sub>31</sub> NO <sub>4</sub> ·0.25H <sub>2</sub> O                | 410 (M <sup>+</sup> +1)                                                              | 72.53 (72.72)       | 7.67 (7.67) | 3.38 (3.33) |
| <b>9aL</b> | 84           | 122—123 | C <sub>22</sub> H <sub>25</sub> NO <sub>4</sub>                                      | 368 (M <sup>+</sup> +1)                                                              | 71.91 (72.03)       | 6.86 (7.01) | 3.81 (3.91) |
| <b>9aM</b> | 85           | 124—125 | C <sub>23</sub> H <sub>27</sub> NO <sub>4</sub> ·0.2H <sub>2</sub> O                 | 382 (M <sup>+</sup> +1)                                                              | 71.74 (71.98)       | 7.17 (7.38) | 3.64 (3.64) |
| <b>9bA</b> | 56           | 185—186 | C <sub>22</sub> H <sub>24</sub> FNO <sub>4</sub>                                     | 386 (M <sup>+</sup> +1)                                                              | 68.56 (68.69)       | 6.28 (6.29) | 3.63 (3.52) |
| <b>9cA</b> | 63           | 174—175 | C <sub>22</sub> H <sub>24</sub> FNO <sub>4</sub>                                     | 386 (M <sup>+</sup> +1)                                                              | 68.56 (68.44)       | 6.28 (6.32) | 3.63 (3.52) |
| <b>12</b>  | 75 (3 steps) | 129—130 | C <sub>22</sub> H <sub>22</sub> FNO <sub>4</sub>                                     | 384 (M <sup>+</sup> +1)                                                              | 68.92 (68.85)       | 5.78 (5.94) | 3.65 (3.66) |
| <b>15</b>  | 48           | 143—144 | C <sub>21</sub> H <sub>23</sub> FN <sub>2</sub> O <sub>4</sub>                       | 387 (M <sup>+</sup> +1)                                                              | 65.27 (65.29)       | 6.00 (6.00) | 7.25 (7.25) |

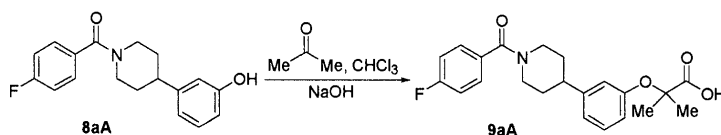


Chart 6

Table 7. <sup>1</sup>H-NMR and IR Spectral Data for **4**, **9**, **12**, and **15**

| Compd.     | <sup>1</sup> H-NMR (CHCl <sub>3</sub> ) δ                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | IR (KBr) cm <sup>-1</sup> |
|------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------|
| <b>4a</b>  | 1.57—1.68 (2H, m), 1.90—2.04 (2H, m), 2.60—2.72 (2H, m), 3.51—3.60 (2H, m), 4.64 (2H, s), 4.94 (1H, s), 6.75 (1H, dd, <i>J</i> =7.8, 2.4 Hz), 6.99 (1H, s), 7.00 (1H, d, <i>J</i> =7.8 Hz), 7.21 (1H, t, <i>J</i> =7.8 Hz), 7.24 (2H, d, <i>J</i> =8.6 Hz), 7.81 (2H, d, <i>J</i> =8.6 Hz), 12.80—14.30 (1H, br) [DMSO- <i>d</i> <sub>6</sub> ]                                                                                                                                                                                                                                                                                                         | 3500<br>1725              |
| <b>4b</b>  | 1.49 (6H, s), 1.58—1.70 (2H, m), 1.84—1.97 (2H, m), 2.60—2.72 (2H, m), 3.50—3.62 (2H, m), 4.93 (1H, s), 6.66 (1H, dd, <i>J</i> =8.0, 2.0 Hz), 6.92 (1H, t, <i>J</i> =2.0 Hz), 7.01 (1H, d, <i>J</i> =8.0 Hz), 7.19 (1H, t, <i>J</i> =8.0 Hz), 7.74 (2H, d, <i>J</i> =8.8 Hz), 7.80 (2H, d, <i>J</i> =8.8 Hz), 13.30—14.20 (1H, br) [DMSO- <i>d</i> <sub>6</sub> ]                                                                                                                                                                                                                                                                                       | 3448<br>1712              |
| <b>4c</b>  | 1.55—2.20 (4H, m), 1.60 (6H, s), 3.15—4.00 (4H, m), 4.45—4.72 (1H, br), 6.81 (1H, dd, <i>J</i> =8.1, 2.0 Hz), 7.07 (1H, t, <i>J</i> =2.0 Hz), 7.11 (1H, d, <i>J</i> =8.1 Hz), 7.24 (1H, t, <i>J</i> =8.1 Hz), 7.36 (2H, d, <i>J</i> =8.8 Hz), 7.39 (2H, d, <i>J</i> =8.8 Hz)                                                                                                                                                                                                                                                                                                                                                                            | 3400<br>1722<br>1595      |
| <b>4d</b>  | 1.50—2.16 (4H, m), 1.58 (6H, s), 3.10—3.40 (1H, m), 3.40—3.80 (2H, m), 4.37—4.62 (1H, m), 4.62—5.17 (2H, br), 6.78 (1H, dd, <i>J</i> =7.8, 2.0 Hz), 7.02—7.12 (4H, m), 7.20 (1H, t, <i>J</i> =7.8 Hz), 7.36—7.46 (2H, m)                                                                                                                                                                                                                                                                                                                                                                                                                                | 3422<br>1734<br>1605      |
| <b>9aA</b> | 1.50 (6H, s), 1.50—1.65 (2H, m), 1.70—1.90 (2H, m), 2.72—2.83 (1H, m), 2.90—3.10 (2H, m), 3.70—4.50 (2H, br), 6.69 (1H, dd, <i>J</i> =7.8, 2.0 Hz), 6.77 (1H, t, <i>J</i> =2.0 Hz), 6.89 (1H, d, <i>J</i> =7.8 Hz), 7.18 (1H, t, <i>J</i> =7.8 Hz), 7.20—7.30 (2H, m), 7.45—7.55 (2H, m), 11.5—13.5 (1H, br) [DMSO- <i>d</i> <sub>6</sub> ]                                                                                                                                                                                                                                                                                                             | 1734<br>1605              |
| <b>9aB</b> | 1.45—1.65 (2H, m), 1.50 (6H, s), 1.65—1.98 (2H, m), 2.68—2.80 (1H, m), 2.80—3.25 (2H, m), 3.45—3.80 (1H, m), 4.40—4.80 (1H, m), 6.65 (1H, dd, <i>J</i> =8.0, 2.0 Hz), 6.75 (1H, t, <i>J</i> =2.0 Hz), 6.88 (1H, d, <i>J</i> =8.0 Hz), 7.18 (1H, t, <i>J</i> =8.0 Hz), 7.45 (2H, d, <i>J</i> =8.3 Hz), 7.50 (2H, d, <i>J</i> =8.3 Hz), 12.75—13.00 (1H, br) [DMSO- <i>d</i> <sub>6</sub> ]                                                                                                                                                                                                                                                               | 1734<br>1597              |
| <b>9aC</b> | 1.47—1.90 (3H, m), 1.59 (6H, s), 1.90—2.00 (1H, m), 2.65—2.80 (1H, m), 2.80—2.95 (1H, m), 3.02—3.18 (1H, m), 3.61—3.77 (1H, br), 4.83—5.00 (1H, m), 5.00—6.00 (1H, br), 6.76 (1H, dd, <i>J</i> =8.4, 2.2 Hz), 6.80 (1H, s), 6.90 (1H, d, <i>J</i> =8.4 Hz), 7.10 (1H, t, <i>J</i> =8.4 Hz), 7.17—7.26 (2H, m), 7.34—7.50 (2H, m)                                                                                                                                                                                                                                                                                                                        | 1724<br>1599              |
| <b>9aD</b> | 1.48—2.00 (4H, m), 1.59 (6H, s), 2.66—2.78 (1H, m), 2.78—3.00 (1H, m), 3.00—3.24 (1H, m), 3.63—3.97 (1H, m), 4.68—4.96 (1H, m), 6.74 (1H, dd, <i>J</i> =7.8, 2.0 Hz), 6.80 (1H, t, <i>J</i> =2.0 Hz), 6.88 (1H, d, <i>J</i> =7.8 Hz), 7.19 (1H, t, <i>J</i> =7.8 Hz), 7.31 (2H, d, <i>J</i> =8.3 Hz), 7.54 (2H, d, <i>J</i> =8.3 Hz)                                                                                                                                                                                                                                                                                                                    | 1736<br>1587              |
| <b>9aE</b> | 1.48 (6H, s), 1.50—1.65 (2H, m), 1.65—1.95 (2H, m), 2.70—2.80 (1H, m), 2.80—3.00 (1H, m), 3.30—3.75 (2H, m), 4.45—4.75 (1H, m), 6.66 (1H, dd, <i>J</i> =7.8, 2.0 Hz), 6.75 (1H, s), 6.86 (1H, d, <i>J</i> =7.8 Hz), 7.16 (1H, t, <i>J</i> =7.8 Hz), 7.65 (2H, d, <i>J</i> =8.1 Hz), 7.81 (2H, d, <i>J</i> =8.1 Hz) [DMSO- <i>d</i> <sub>6</sub> ]                                                                                                                                                                                                                                                                                                       | 1721<br>1595              |
| <b>9aF</b> | 1.50—1.90 (3H, m), 1.60 (6H, s), 1.90—2.04 (1H, m), 2.65—2.80 (1H, m), 2.80—2.95 (1H, m), 3.05—3.30 (1H, m), 3.60—3.70 (1H, m), 4.83—4.95 (1H, m), 6.77 (1H, dd, <i>J</i> =8.0, 2.0 Hz), 6.80 (1H, t, <i>J</i> =2.0 Hz), 6.83—6.88 (1H, m), 6.92 (1H, d, <i>J</i> =8.0 Hz), 6.93—6.98 (1H, m), 7.21 (1H, t, <i>J</i> =8.0 Hz), 7.26—7.44 (1H, m)                                                                                                                                                                                                                                                                                                        | 1740<br>1602              |
| <b>9aG</b> | 1.60 (6H, s), 1.60—1.80 (2H, m), 1.80—1.90 (1H, m), 1.90—2.02 (1H, m), 2.68—2.80 (1H, m), 2.80—2.95 (1H, m), 3.15—3.23 (1H, m), 3.58—3.70 (1H, m), 4.86—4.96 (1H, m), 6.67—6.83 (4H, m), 6.91 (1H, d, <i>J</i> =7.8 Hz), 7.22 (1H, t, <i>J</i> =7.8 Hz)                                                                                                                                                                                                                                                                                                                                                                                                 | 1754<br>1614              |
| <b>9aH</b> | 1.50—2.05 (4H, m), 1.60 (6H, s), 2.70—2.80 (1H, m), 2.80—3.00 (1H, m), 3.00—3.23 (1H, m), 3.85—3.96 (1H, m), 4.71—4.98 (1H, m), 6.67 (1H, dd, <i>J</i> =8.0, 2.0 Hz), 6.81 (1H, t, <i>J</i> =2.0 Hz), 6.92 (1H, d, <i>J</i> =8.0 Hz), 7.06—7.18 (2H, m), 7.18—7.24 (2H, m), 7.34—7.46 (1H, m)                                                                                                                                                                                                                                                                                                                                                           | 1724<br>1597              |
| <b>9aI</b> | 1.39 (18H, s), 1.42 (6H, s), 1.43—1.62 (2H, m), 1.73—1.86 (1H, m), 2.63—2.78 (1H, m), 2.83—3.05 (1H, m), 3.05—3.50 (2H, m), 3.92—4.50 (1H, br), 6.69 (1H, d, <i>J</i> =7.8 Hz), 6.72 (1H, s), 6.75 (1H, d, <i>J</i> =7.8 Hz), 7.08 (1H, t, <i>J</i> =7.8 Hz), 7.17 (2H, s), 7.17—7.33 (1H, br) [DMSO- <i>d</i> <sub>6</sub> ]                                                                                                                                                                                                                                                                                                                           | 3448<br>1602              |
| <b>9aJ</b> | 1.52—2.04 (4H, m), 1.62 (6H, s), 2.68—2.80 (1H, m), 2.80—2.97 (1H, m), 3.08—3.28 (1H, m), 3.66—3.87 (1H, m), 4.74—4.94 (1H, m), 6.78 (1H, d, <i>J</i> =7.8 Hz), 6.81 (1H, s), 6.84 (1H, d, <i>J</i> =7.8 Hz), 7.18 (1H, t, <i>J</i> =7.8 Hz), 7.44 (1H, dd, <i>J</i> =7.7, 4.8 Hz), 7.87 (1H, d, <i>J</i> =7.7 Hz), 8.00—9.20 (1H, br), 8.68 (1H, d, <i>J</i> =4.8 Hz), 8.73 (1H, s)                                                                                                                                                                                                                                                                    | 1718<br>1631              |
| <b>9aK</b> | 1.45—1.59 (1H, m), 1.60 (6H, s), 1.60—1.75 (1H, m), 1.75—1.83 (1H, m), 1.95—2.05 (1H, m), 2.18 (3H, s), 2.28 (3H, s), 2.30 (3H, s), 2.65—2.77 (1H, m), 2.80—2.90 (1H, m), 3.00—3.13 (1H, m), 3.45—3.57 (1H, m), 4.90—5.00 (1H, m), 6.74 (1H, dd, <i>J</i> =7.8, 2.1 Hz), 6.77 (1H, t, <i>J</i> =2.1 Hz), 6.83 (1H, d, <i>J</i> =7.8 Hz), 6.85 (1H, s), 6.88 (1H, s), 7.18 (1H, t, <i>J</i> =7.8 Hz), 1.40—2.15 (4H, m), 1.59 (6H, s), 2.60—3.40 (3H, m), 3.68—4.05 (1H, m), 4.20—5.60 (2H, br), 6.76 (1H, dd, <i>J</i> =7.8, 2.0 Hz), 6.81 (1H, t, <i>J</i> =2.0 Hz), 6.90 (1H, d, <i>J</i> =7.8 Hz), 7.20 (1H, t, <i>J</i> =7.8 Hz), 7.35—7.55 (5H, m) | 1722<br>1574              |
| <b>9aL</b> | 1.40—2.15 (4H, m), 1.59 (6H, s), 2.60—3.40 (3H, m), 3.68—4.05 (1H, m), 4.20—5.60 (2H, br), 6.76 (1H, dd, <i>J</i> =7.8, 2.0 Hz), 6.81 (1H, t, <i>J</i> =2.0 Hz), 6.90 (1H, d, <i>J</i> =7.8 Hz), 7.20 (1H, t, <i>J</i> =7.8 Hz), 7.35—7.55 (5H, m)                                                                                                                                                                                                                                                                                                                                                                                                      | 1741<br>1594              |
| <b>9aM</b> | 1.48—1.75 (2H, m), 1.59 (6H, s), 1.75—2.03 (2H, m), 2.38 (3H, s), 2.65—2.80 (1H, m), 2.80—3.25 (2H, m), 3.58—4.24 (1H, m), 4.24—5.37 (2H, br), 6.76 (1H, dd, <i>J</i> =7.8, 2.0 Hz), 6.81 (1H, t, <i>J</i> =2.0 Hz), 6.89 (1H, d, <i>J</i> =7.8 Hz), 7.19 (1H, t, <i>J</i> =7.8 Hz), 7.21 (2H, d, <i>J</i> =8.3 Hz), 7.33 (2H, d, <i>J</i> =8.3 Hz)                                                                                                                                                                                                                                                                                                     | 1718<br>1578              |
| <b>9bA</b> | 1.53—2.05 (4H, m), 1.63 (6H, s), 2.76—3.00 (1H, m), 3.00—3.32 (2H, m), 3.78—4.00 (1H, m), 4.70—5.00 (1H, m), 6.75 (1H, d, <i>J</i> =7.3 Hz), 6.98 (1H, t, <i>J</i> =7.3 Hz), 7.05—7.15 (3H, m), 7.17 (1H, dd, <i>J</i> =7.3, 1.5 Hz), 7.38—7.50 (2H, m)                                                                                                                                                                                                                                                                                                                                                                                                 | 1708<br>1600              |
| <b>9cA</b> | 1.58 (6H, s), 1.50—2.05 (4H, m), 2.68—2.80 (1H, m), 2.80—3.00 (1H, m), 3.00—3.24 (1H, m), 3.77—3.97 (1H, m), 4.77—4.95 (1H, m), 6.86 (2H, d, <i>J</i> =8.8 Hz), 7.05—7.17 (4H, m), 7.40—7.50 (2H, m)                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 1715<br>1569              |
| <b>12</b>  | 1.60 (6H, s), 2.45—2.66 (2H, m), 3.50—3.70 (1H, m), 3.85—4.05 (1H, m), 4.05—4.20 (1H, m), 4.25—4.45 (1H, m), 5.82—6.20 (1H, m), 6.84 (1H, dd, <i>J</i> =8.3, 2.0 Hz), 6.96 (1H, s), 7.02—7.18 (3H, m), 7.24 (1H, t, <i>J</i> =8.3 Hz), 7.40—7.50 (2H, m)                                                                                                                                                                                                                                                                                                                                                                                                | 1732<br>1603              |
| <b>15</b>  | 1.59 (6H, s), 3.00—3.37 (4H, m), 3.43—4.10 (4H, m), 4.80—6.00 (1H, br), 6.45 (1H, dd, <i>J</i> =8.1, 2.2 Hz), 6.52 (1H, t, <i>J</i> =2.2 Hz), 6.63 (1H, dd, <i>J</i> =8.1, 2.2 Hz), 7.05—7.20 (3H, m), 7.40—7.50 (2H, m)                                                                                                                                                                                                                                                                                                                                                                                                                                | 1724<br>1603              |

promising candidate of our new fibrate, and is now in pre-clinical stage.

#### Experimental

Melting points were determined on a Yanaco micro melting point apparatus without correction. IR spectra were measured with a Perkin-Elmer 1600 FT-IR spectrometer. <sup>1</sup>H-NMR spectra were recorded on a JEOL JNM-

EX400 FT-NMR spectrometer in CDCl<sub>3</sub> or dimethyl sulfoxide (DMSO)-*d*<sub>6</sub> using tetramethylsilane as the internal reference. The following abbreviations were used: s=singlet, d=doublet, dd=double doublet, dt=double triplet, t=triplet, q=quartet, m=multiplet and br=broad. FAB-MS, electron ionization mass spectrometry (EI-MS) or high-resolution mass spectrometry (HR-MS) were obtained using JEOL JMS-DX303 or JEOL JMS-AX500 mass spectrometer. TLC was performed by using Silica gel 60F<sub>254</sub> (Merck). Column chromatography was performed with Silica gel 60 (70—230 mesh)

(Merck). Sodium sulfate was employed as the drying agent. Palladium hydroxide [20 wt.%, Pd (dry basis) on carbon, wet] was obtained from Aldrich Chemical Company, Inc. The yields, physical and spectral data for **4a**–**d**, **9aA**–**9aM**, **9bA**, **9cA**, **12** and **15** are shown in Tables 6 and 7.

**1-Benzyl-4-[3-(benzyloxy)phenyl]-4-hydroxypiperidine (1)** A solution of 3-benzyloxy bromobenzene (13.0 g, 49.4 mmol) in tetrahydrofuran (THF) (50 ml) was dropwise added to a stirred mixture of Mg (1.21 g, 49.8 mmol) and I<sub>2</sub> (catalytic amount) in THF (30 ml) at 40 °C over 30 min, and the mixture was stirred at reflux for 2 h. After cooling, a solution of 1-benzyl-4-piperidone (7.20 g, 37.7 mmol) in THF (50 ml) was added dropwise to the stirred mixture at room temperature over a 30 min period, and then stirred at reflux for 2 h. After cooling, aq. sat. NH<sub>4</sub>Cl (50 ml) was added dropwise, and the reaction mixture was extracted with Et<sub>2</sub>O. The organic layer was dried, concentrated *in vacuo* to give a pale brown oil which was purified by column chromatography on silica gel, eluting with 50% AcOEt in *n*-hexane to give **1** (10.3 g, 73%) as a pale yellow viscous liquid. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 1.50–3.00 (1H, br), 1.67–1.80 (2H, m), 2.08–2.23 (2H, m), 2.42–2.55 (2H, m), 2.73–2.85 (2H, m), 3.58 (2H, s), 5.05 (2H, s), 6.86 (1H, dd, *J*=7.8, 2.0 Hz), 7.09 (1H, dd, *J*=7.8, 2.0 Hz), 7.18 (1H, t, *J*=2.0 Hz), 7.22–7.50 (11H, m). EI-MS *m/z*: 373 (M<sup>+</sup>). HR-MS *m/z*: 373.2026 (Calcd for C<sub>25</sub>H<sub>27</sub>NO<sub>2</sub>: 373.2042). IR ν (neat) cm<sup>-1</sup>: 3380.

**4-Hydroxy-4-(3-hydroxyphenyl)piperidine (2)** A solution of **1** (10.0 g, 26.8 mmol) in MeOH (100 ml) was hydrogenated in the presence of Pd(OH)<sub>2</sub> (3.0 g) under a H<sub>2</sub> atmosphere (4 atm) at room temperature for 6 h. The catalyst was removed by filtration and the filtrate was concentrated *in vacuo* to give **2** (4.00 g, 77%) as a white solid. mp 188–189 °C. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ: 1.42–1.52 (2H, m), 1.66–1.80 (2H, m), 2.62–2.76 (2H, m), 2.84–2.97 (2H, m), 4.40–4.80 (1H, br), 6.54–6.60 (1H, m), 6.85 (1H, dd, *J*=7.8, 2.0 Hz), 6.89 (1H, t, *J*=2.0 Hz), 7.07 (1H, t, *J*=7.8 Hz), 8.00–10.00 (1H, br). EI-MS *m/z*: 193 (M<sup>+</sup>). HR-MS *m/z*: 193.1097 (Calcd for C<sub>11</sub>H<sub>15</sub>NO<sub>2</sub>: 193.1102). IR ν (KBr) cm<sup>-1</sup>: 3273.

**1-[4-Halobenzoyl or (4-Chlorophenyl)sulfonyl]-4-hydroxy-4-(3-hydroxyphenyl)piperidine (3) (General Procedure A)** A solution of a benzoyl or phenylsulfonyl chloride (44 mmol) in THF (20 ml) was added dropwise to a stirred solution of **2** (20 mmol) and Et<sub>3</sub>N (43 mmol) in THF (80 ml) at 0 °C over a 15 min period, and then was stirred at room temperature for 2 h. The reaction mixture was concentrated *in vacuo*, and the residue was dissolved in CHCl<sub>3</sub>. The CHCl<sub>3</sub> layer was washed with H<sub>2</sub>O, and concentrated *in vacuo*. The residue was dissolved in a mixture of 1 N NaOH (50 ml), MeOH (50 ml) and 1,4-dioxane (50 ml), then stirred at room temperature for 30 min. The mixture was acidified with dil. HCl to pH 3 at 0 °C. The mixture was extracted with CHCl<sub>3</sub>, washed with sat. aq. NaHCO<sub>3</sub> and H<sub>2</sub>O, dried, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel, eluting with 5% MeOH in CHCl<sub>3</sub> to give **3** as a white solid.

**1-[(4-Chlorophenyl)sulfonyl]-4-hydroxy-4-(3-hydroxyphenyl)piperidine (3a)**: mp 195–196 °C. Yield 75%. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ: 1.52–1.70 (2H, m), 1.82–2.00 (2H, m), 2.57–2.76 (2H, m), 3.48–3.68 (2H, m), 4.84 (1H, s), 6.80 (1H, d, *J*=7.8 Hz), 6.86 (1H, t, *J*=2.0 Hz), 7.08 (1H, t, *J*=7.8 Hz), 7.24 (2H, d, *J*=8.8 Hz), 7.80 (2H, d, *J*=8.8 Hz), 9.19 (1H, s). EI-MS *m/z*: 367 (M<sup>+</sup>). HR-MS *m/z*: 367.0627 (Calcd for C<sub>17</sub>H<sub>18</sub>ClNO<sub>4</sub>S: 367.0645). IR ν (KBr) cm<sup>-1</sup>: 3444, 3340.

**1-(4-Chlorobenzoyl)-4-hydroxy-4-(3-hydroxyphenyl)piperidine (3b)**: mp 168–170 °C. Yield 80%. <sup>1</sup>H-NMR (CHCl<sub>3</sub>) δ: 1.53–1.70 (1H, m), 1.70–1.93 (2H, m), 1.93–2.15 (1H, m), 3.16–3.43 (2H, m), 3.43–3.63 (2H, m), 4.40–4.58 (1H, m), 6.66 (1H, dd, *J*=7.9, 2.0 Hz), 6.84 (1H, d, *J*=7.9 Hz), 6.88 (1H, t, *J*=2.0 Hz), 7.11 (1H, t, *J*=7.9 Hz), 7.30 (2H, d, *J*=8.3 Hz), 7.33 (2H, d, *J*=8.3 Hz). EI-MS *m/z*: 331 (M<sup>+</sup>). HR-MS *m/z*: 331.0987 (Calcd for C<sub>18</sub>H<sub>18</sub>ClNO<sub>3</sub>: 331.0975). IR ν (KBr) cm<sup>-1</sup>: 3227, 1614.

**1-(4-Fluorobenzoyl)-4-hydroxy-4-(3-hydroxyphenyl)piperidine (3c)**: mp 195–196 °C. Yield 87%. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 1.60–2.00 (3H, m), 2.00–2.20 (1H, m), 3.22–3.45 (1H, m), 3.45–3.78 (2H, m), 4.45–4.70 (1H, m), 6.74 (1H, dd, *J*=8.0, 2.0 Hz), 6.92 (1H, d, *J*=8.0 Hz), 6.96 (1H, t, *J*=2.0 Hz), 7.07–7.16 (2H, m), 7.20 (1H, t, *J*=8.0 Hz), 7.40–7.48 (2H, m). EI-MS *m/z*: 315 (M<sup>+</sup>). HR-MS *m/z*: 315.1268 (Calcd for C<sub>17</sub>H<sub>18</sub>FNO<sub>3</sub>: 315.1271). IR ν (KBr) cm<sup>-1</sup>: 3241, 1615.

**2-[3-[1-[4-Halobenzoyl or (4-Chlorophenyl)sulfonyl]-4-hydroxypiperidin-4-yl]phen-oxyl]acetic or -2-Methylpropanoic Acid (4) (General Procedure B)** A mixture of **3** (10 mmol), ethyl 2-bromo-2-methylpropanoate (0.1 mol) and K<sub>2</sub>CO<sub>3</sub> (30 mmol) was stirred at 100 °C for 8 h. The mixture was poured into H<sub>2</sub>O and extracted with CHCl<sub>3</sub>, and the CHCl<sub>3</sub> layer was concentrated *in vacuo*. The residue was dissolved in a mixture of 1 N NaOH (50 ml), MeOH (50 ml), and 1,4-dioxane (50 ml), and then stirred for 1 h at room temperature. The mixture was poured into H<sub>2</sub>O, acidified with dil. HCl to pH 3, and extracted with CHCl<sub>3</sub>. The CHCl<sub>3</sub> layer was washed with H<sub>2</sub>O,

dried, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel, eluting with 5% MeOH in CHCl<sub>3</sub> to give an oil which was crystallized from Et<sub>2</sub>O to give **4** as a white solid.

**1-Benzyl-4-(methoxyphenyl)-1,2,3,6-tetrahydropyridine · HCl (5) (General Procedure C)** A solution of methoxybromobenzene (1.10 mol) in THF (350 ml) was added dropwise to a stirred mixture of Mg (1.10 mol) and I<sub>2</sub> (catalytic amount) in THF (100 ml) at 40 °C over a 1 h period, then stirred at reflux for 1 h. After cooling, a solution of 1-benzyl-4-piperidinone (1.0 mol) in THF (250 ml) was added dropwise to the stirred mixture at room temperature over a 1 h period, and then stirred at reflux for 1 h. After cooling, sat. aq. NH<sub>4</sub>Cl (400 ml) and H<sub>2</sub>O (200 ml) were added to the mixture, then allowed to stand for a short time. The mixture was partitioned between THF and H<sub>2</sub>O, and the organic layer was washed with brine, then concentrated *in vacuo*. The residue was dissolved in a mixture of 1,4-dioxane (500 ml) and 6 N HCl (1000 ml), then refluxed for 3 h. After cooling, the mixture was concentrated *in vacuo*, and the residue was triturated with Et<sub>2</sub>O to give **5** as a white solid.

**1-Benzyl-4-(3-methoxyphenyl)-1,2,3,6-tetrahydropyridine · HCl (5a)**: mp 193–195 °C. Yield 62%. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ: 2.64–2.83 (1H, m), 2.83–3.02 (1H, m), 3.10–3.30 (1H, m), 3.47–3.62 (1H, m), 3.62–3.80 (2H, m), 3.77 (3H, s), 4.30–4.50 (2H, m), 6.13–6.18 (1H, br), 6.89 (1H, dd, *J*=8.1, 2.0 Hz), 6.98 (1H, t, *J*=2.0 Hz), 7.03 (1H, d, *J*=8.1 Hz), 7.29 (1H, t, *J*=8.1 Hz), 7.42–7.52 (3H, m), 7.66–7.76 (2H, m), 11.20–11.60 (1H, br). EI-MS *m/z*: 279 (M<sup>+</sup>). HR-MS *m/z*: 279.1606 (Calcd for C<sub>19</sub>H<sub>21</sub>NO: 279.1623). IR ν (KBr) cm<sup>-1</sup>: 2515.

**1-Benzyl-4-(2-methoxyphenyl)-1,2,3,6-tetrahydropyridine · HCl (5b)**: mp 163–165 °C. Yield 86%. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ: 2.52–2.65 (1H, m), 2.75–3.00 (1H, m), 3.10–3.30 (1H, m), 3.30–3.50 (1H, m), 3.60–3.80 (2H, m), 3.78 (3H, s), 4.35–4.50 (2H, m), 5.75–5.80 (1H, m), 6.94 (1H, t, *J*=7.8 Hz), 7.02 (1H, d, *J*=7.8 Hz), 7.15 (1H, dd, *J*=7.8, 1.5 Hz), 7.29 (1H, dt, *J*=7.8, 1.5 Hz), 7.36–7.54 (3H, m), 7.60–7.75 (2H, m), 10.90–11.20 (1H, br). EI-MS *m/z*: 279 (M<sup>+</sup>). HR-MS *m/z*: 279.1635 (Calcd for C<sub>19</sub>H<sub>21</sub>NO: 279.1623). IR ν (KBr) cm<sup>-1</sup>: 2498.

**1-Benzyl-4-(4-methoxyphenyl)-1,2,3,6-tetrahydropyridine · HCl (5c)**: mp 228–229 °C. Yield 69%. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ: 2.65–2.80 (1H, m), 2.80–2.95 (1H, m), 3.10–3.30 (1H, m), 3.50–3.62 (1H, m), 3.62–3.78 (2H, m), 3.76 (3H, s), 4.35–4.48 (2H, m), 6.01–6.06 (1H, m), 6.93 (2H, d, *J*=8.8 Hz), 7.40 (2H, d, *J*=8.8 Hz), 7.43–7.50 (3H, m), 7.65–7.72 (2H, m), 11.01–11.30 (1H, br). EI-MS *m/z*: 279 (M<sup>+</sup>). HR-MS *m/z*: 279.1609 (Calcd for C<sub>19</sub>H<sub>21</sub>NO: 279.1623). IR ν (KBr) cm<sup>-1</sup>: 2484.

**4-(Methoxyphenyl)piperidine · HCl (6) (General Procedure D)** A solution of **5** (0.5 mol) in a mixture of MeOH (440 ml) and H<sub>2</sub>O (360 ml) was hydrogenated in the presence of 5% Pd-C (32.0 g) under a H<sub>2</sub> atmosphere (1 atm) at room temperature for 16 h. The catalyst was filtered off, and the filtrate was concentrated *in vacuo* to give **6** as a white solid.

**4-(3-Methoxyphenyl)piperidine · HCl (6a)**: mp 171–172 °C. Yield ca. 100%. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ: 1.81–2.00 (4H, m), 2.71–2.86 (1H, m), 2.86–3.06 (2H, m), 3.23–3.40 (2H, m), 3.74 (3H, s), 6.74–6.86 (3H, m), 7.25 (1H, t, *J*=7.8 Hz), 9.00–9.46 (2H, br). EI-MS *m/z*: 191 (M<sup>+</sup>). HR-MS *m/z*: 191.1306 (Calcd for C<sub>12</sub>H<sub>17</sub>NO: 191.1310). IR ν (KBr) cm<sup>-1</sup>: 2490.

**4-(2-Methoxyphenyl)piperidine · HCl (6b)**: mp 228–230 °C. Yield 74%. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ: 1.78–2.00 (4H, m), 2.90–3.08 (2H, m), 3.09–3.22 (1H, m), 3.24–3.38 (2H, m), 3.80 (3H, s), 6.94 (1H, t, *J*=7.7 Hz), 6.99 (1H, d, *J*=7.7 Hz), 7.14 (1H, dd, *J*=7.7, 1.5 Hz), 7.21 (1H, dt, *J*=7.7, 1.5 Hz), 9.00–9.30 (2H, br). EI-MS *m/z*: 191 (M<sup>+</sup>). HR-MS *m/z*: 191.1324 (Calcd for C<sub>12</sub>H<sub>17</sub>NO: 191.1310). IR ν (KBr) cm<sup>-1</sup>: 2506.

**4-(4-Methoxyphenyl)piperidine · HCl (6c)**: mp 205–206 °C. Yield 81%. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ: 1.80–1.95 (4H, m), 2.75–2.85 (1H, m), 2.85–3.00 (2H, m), 3.20–3.30 (2H, m), 3.73 (3H, s), 6.89 (2H, d, *J*=8.8 Hz), 7.14 (2H, d, *J*=8.8 Hz), 9.08–9.34 (2H, m). EI-MS *m/z*: 191 (M<sup>+</sup>). HR-MS *m/z*: 191.1320 (Calcd for C<sub>12</sub>H<sub>17</sub>NO: 191.1310). IR ν (KBr) cm<sup>-1</sup>: 2502.

**4-(Hydroxyphenyl)piperidine (7) (General Procedure E)** A mixture of **6** (500 mmol) and 47% hydrobromic acid (300 g, 1.74 mol) was refluxed with stirring for 2 h. The mixture was concentrated *in vacuo* and the residue was dissolved in H<sub>2</sub>O, then made alkaline to pH 9.5 with aq. NaOH. The resulting precipitate (**7**) was collected by filtration as a white solid.

**4-(3-Hydroxyphenyl)piperidine (7a)**: mp 227–228 °C. Yield 89%. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ: 1.34–1.55 (2H, m), 1.55–1.75 (2H, m), 2.36–2.62 (3H, m), 2.62–3.80 (1H, br), 2.90–3.07 (2H, m), 6.52–6.66 (3H, m), 7.05 (1H, t, *J*=7.8 Hz), 8.40–10.20 (1H, br). EI-MS *m/z*: 177 (M<sup>+</sup>). HR-MS *m/z*: 177.1146 (Calcd for C<sub>11</sub>H<sub>15</sub>NO: 177.1154). IR ν (KBr) cm<sup>-1</sup>: 3265.

**4-(2-Hydroxyphenyl)piperidine (7b)**: mp 172–173 °C. Yield 68%. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 1.70–1.93 (4H, m), 2.70–2.90 (2H, m), 2.95–3.10 (1H, m), 3.10–3.32 (2H, m), 4.00–5.20 (2H, br), 6.70 (1H, dd, *J*=7.7, 1.4 Hz),

6.84 (1H, dt,  $J=7.7, 1.4$  Hz), 7.04 (1H, dt,  $J=7.7, 1.4$  Hz), 7.14 (1H, dd,  $J=7.7, 1.4$  Hz). EI-MS  $m/z$ : 177 ( $M^+$ ). HR-MS  $m/z$ : 177.1155 (Calcd for  $C_{11}H_{15}NO$ : 177.1154). IR  $\nu$  (KBr)  $cm^{-1}$ : 3312.

4-(4-Hydroxyphenyl)piperidine (**7c**): mp 218–220 °C. Yield 67%.  $^1H$ -NMR (DMSO- $d_6$ )  $\delta$ : 1.30–1.50 (2H, m), 1.50–1.70 (2H, m), 2.30–2.65 (3H, m), 2.90–3.10 (2H, m), 6.70 (2H, d,  $J=8.3$  Hz), 6.99 (2H, d,  $J=8.3$  Hz). EI-MS  $m/z$ : 177 ( $M^+$ ). HR-MS  $m/z$ : 177.1138 (Calcd for  $C_{11}H_{15}NO$ : 177.1154). IR  $\nu$  (KBr)  $cm^{-1}$ : 3300.

**(1-Aroyl-4-hydroxyphenyl)piperidine (8) (General Procedure F)** An aroyl chloride (0.5 mol) was dropwise added to a stirred solution of **7** (0.45 mol), NaOH (21.6 g, 0.54 mol), isopropanol (240 ml), and  $H_2O$  (240 ml) at 40 °C over a 15 min period, then the mixture was stirred at 40 °C for 1 h. A solution of MeOH (400 ml) and aq. NaOH (20% w/w) (59.7 g, 0.498 mol) was next added to the mixture, then stirred at 50 °C for 1 h. After cooling, the mixture was acidified with aq. HCl to pH 4, and the resulting precipitate (**8**) was collected by filtration as a white solid.

Compounds **8** except **8aI** and **8aK** were synthesized according to general procedure F. Compound **8aI** was synthesized from **7a** according to the procedure for the synthesis of **13** and the compound **8aK** was synthesized from **7a** according to general procedure A.

1-(4-Fluorobenzoyl)-4-(3-hydroxyphenyl)piperidine (**8aA**): mp 163–164 °C. Yield ca. 100%.  $^1H$ -NMR ( $CHCl_3$ )  $\delta$ : 1.40–2.15 (4H, m), 2.65–3.32 (3H, m), 3.70–4.10 (1H, m), 4.70–5.05 (1H, m), 5.82–6.36 (1H, br), 6.65–6.73 (2H, m), 6.75 (1H, d,  $J=8.0$  Hz), 7.06–7.13 (2H, m), 7.16 (1H, t,  $J=8.0$  Hz), 7.40–7.50 (2H, m). EI-MS  $m/z$ : 299 ( $M^+$ ). HR-MS  $m/z$ : 299.1327 (Calcd for  $C_{18}H_{18}FNO_2$ : 299.1321). IR  $\nu$  (KBr)  $cm^{-1}$ : 3449, 1602.

1-(4-Chlorobenzoyl)-4-(3-hydroxyphenyl)piperidine (**8aB**): mp 142–143 °C. Yield ca. 100%.  $^1H$ -NMR ( $CHCl_3$ )  $\delta$ : 1.40–2.15 (4H, m), 2.60–2.80 (1H, m), 2.80–3.00 (1H, m), 3.00–3.30 (1H, m), 3.68–4.03 (1H, m), 4.65–5.08 (1H, m), 6.42–6.60 (1H, br), 6.60–6.80 (3H, m), 7.15 (1H, t,  $J=8.2$  Hz), 7.32–7.43 (4H, m). EI-MS  $m/z$ : 315 ( $M^+$ ). HR-MS  $m/z$ : 315.1040 (Calcd for  $C_{18}H_{18}ClNO_2$ : 315.1026). IR  $\nu$  (KBr)  $cm^{-1}$ : 3226, 1610.

1-(2-Fluorobenzoyl)-4-(3-hydroxyphenyl)piperidine (**8aC**): mp 149–150 °C. Yield 96%.  $^1H$ -NMR ( $CHCl_3$ )  $\delta$ : 1.62–2.10 (4H, m), 2.63–2.80 (1H, m), 2.80–2.97 (1H, m), 3.00–3.36 (1H, br), 3.60–3.75 (1H, m), 4.85–5.00 (1H, m), 6.63–6.80 (3H, m), 6.95–7.27 (4H, m), 7.33–7.50 (2H, m). EI-MS  $m/z$ : 299 ( $M^+$ ). HR-MS  $m/z$ : 299.1322 (Calcd for  $C_{18}H_{18}FNO_2$ : 299.1321). IR  $\nu$  (KBr)  $cm^{-1}$ : 3270, 1620.

1-(4-Bromobenzoyl)-4-(3-hydroxyphenyl)piperidine (**8aD**): mp 163–164 °C. Yield 93%.  $^1H$ -NMR ( $CHCl_3$ )  $\delta$ : 1.43–2.15 (4H, m), 2.63–2.80 (1H, m), 2.80–3.00 (1H, m), 3.00–3.30 (1H, m), 3.70–4.00 (1H, m), 4.73–5.00 (1H, m), 6.15 (1H, s), 6.64–6.72 (2H, m), 6.74 (1H, d,  $J=7.8$  Hz), 7.16 (1H, t,  $J=7.8$  Hz), 7.31 (2H, d,  $J=8.3$  Hz), 7.55 (2H, d,  $J=8.3$  Hz). EI-MS  $m/z$ : 359 ( $M^+$ ). HR-MS  $m/z$ : 359.0501 (Calcd for  $C_{18}H_{18}BrNO_2$ : 359.0520). IR  $\nu$  (KBr)  $cm^{-1}$ : 3208, 1614.

4-(3-Hydroxyphenyl)-1-[4-(trifluoromethyl)benzoyl]piperidine (**8aE**): mp 156–157 °C. Yield 76%.  $^1H$ -NMR ( $CHCl_3$ )  $\delta$ : 1.47–1.68 (1H, m), 1.68–1.90 (2H, m), 1.90–2.10 (1H, m), 2.66–2.70 (1H, m), 2.70–3.00 (1H, m), 3.05–3.27 (1H, m), 3.67–3.86 (1H, m), 4.78–5.04 (1H, m), 5.80–7.10 (1H, m), 6.64–6.72 (2H, m), 6.74 (1H, d,  $J=7.8$  Hz), 7.15 (1H, t,  $J=7.8$  Hz), 7.54 (2H, d,  $J=8.1$  Hz), 7.68 (2H, d,  $J=8.1$  Hz). EI-MS  $m/z$ : 349 ( $M^+$ ). HR-MS  $m/z$ : 349.1295 (Calcd for  $C_{19}H_{18}F_3NO_2$ : 349.1290). IR  $\nu$  (KBr)  $cm^{-1}$ : 3260, 1620.

1-(2,4-Difluorobenzoyl)-4-(3-hydroxyphenyl)piperidine (**8aF**): mp 147–148 °C. Yield ca. 100%.  $^1H$ -NMR ( $CHCl_3$ )  $\delta$ : 1.40–1.90 (3H, m), 1.90–2.05 (1H, m), 2.63–2.80 (1H, m), 2.80–2.95 (1H, m), 3.00–3.35 (1H, m), 3.58–3.62 (1H, m), 4.82–5.00 (1H, m), 5.80–6.30 (1H, br), 6.63–6.71 (2H, m), 6.74 (1H, d,  $J=7.8$  Hz), 6.79–6.90 (1H, m), 6.90–6.99 (1H, m), 7.16 (1H, t,  $J=7.8$  Hz), 7.35–7.48 (1H, m). EI-MS  $m/z$ : 317 ( $M^+$ ). HR-MS  $m/z$ : 317.1241 (Calcd for  $C_{18}H_{17}F_2NO_2$ : 317.1228). IR  $\nu$  (KBr)  $cm^{-1}$ : 3194, 1614.

4-(3-Hydroxyphenyl)-1-(2,4,6-trifluorobenzoyl)piperidine (**8aG**): mp 168–169 °C. Yield ca. 100%.  $^1H$ -NMR ( $CHCl_3$ )  $\delta$ : 1.55–1.80 (2H, m), 1.80–1.92 (1H, m), 1.92–2.05 (1H, m), 2.67–2.80 (1H, m), 2.82–2.96 (1H, m), 3.15–3.28 (1H, m), 3.57–3.68 (1H, m), 4.87–4.97 (1H, m), 6.15–6.45 (1H, br), 6.63–6.78 (5H, m), 7.15 (1H, t,  $J=8.1$  Hz). EI-MS  $m/z$ : 335 ( $M^+$ ). HR-MS  $m/z$ : 335.1149 (Calcd for  $C_{18}H_{16}F_3NO_2$ : 335.1133). IR  $\nu$  (KBr)  $cm^{-1}$ : 3245, 1616.

1-(3-Fluorobenzoyl)-4-(3-hydroxyphenyl)piperidine (**8aH**): mp 155–156 °C. Yield ca. 100%.  $^1H$ -NMR ( $CHCl_3$ )  $\delta$ : 1.40–2.10 (4H, m), 2.65–2.80 (1H, m), 2.80–3.00 (1H, m), 3.00–3.25 (1H, m), 3.70–3.95 (1H, m), 4.72–5.00 (1H, m), 5.60–6.10 (1H, br), 6.65–6.73 (2H, m), 6.76 (1H, d,  $J=7.8$  Hz), 7.08–7.24 (3H, m), 7.35–7.44 (2H, m). EI-MS  $m/z$ : 299 ( $M^+$ ).

HR-MS  $m/z$ : 299.1335 (Calcd for  $C_{18}H_{18}FNO_2$ : 299.1322). IR  $\nu$  (KBr)  $cm^{-1}$ : 3187, 1598.

1-[3,5-Di(*tert*-butyl)-4-hydroxybenzoyl]-4-(3-hydroxyphenyl)piperidine (**8aI**): mp 263–265 °C. Yield 24%.  $^1H$ -NMR ( $CHCl_3$ )  $\delta$ : 1.43 (18H, s), 1.56–2.12 (4H, m), 2.67–2.80 (1H, m), 2.80–3.25 (2H, m), 3.84–4.30 (1H, m), 4.60–5.05 (1H, m), 5.40 (1H, s), 6.68 (1H, dd,  $J=7.8, 2.0$  Hz), 6.73 (1H, s), 6.77 (1H, d,  $J=7.8$  Hz), 7.17 (1H, t,  $J=7.8$  Hz), 7.27 (2H, s). EI-MS  $m/z$ : 409 ( $M^+$ ). HR-MS  $m/z$ : 409.2597 (Calcd for  $C_{26}H_{35}NO_3$ : 409.2616). IR  $\nu$  (KBr)  $cm^{-1}$ : 3568, 3169, 1598.

4-(3-Hydroxyphenyl)-1-(3-pyridinecarbonyl)piperidine (**8aJ**): mp 188–189 °C. Yield 48%.  $^1H$ -NMR ( $CHCl_3$ )  $\delta$ : 1.46–2.10 (4H, m), 2.66–2.80 (1H, m), 2.80–3.00 (1H, m), 3.05–3.32 (1H, m), 3.65–3.92 (1H, m), 4.70–4.92 (1H, m), 6.65–6.76 (5H, m), 7.15 (1H, t,  $J=7.8$  Hz), 7.42 (1H, dd,  $J=8.1, 4.9$  Hz), 7.82 (1H, dt,  $J=8.1, 1.6$  Hz). EI-MS  $m/z$ : 282 ( $M^+$ ). HR-MS  $m/z$ : 282.1369 (Calcd for  $C_{17}H_{18}N_2O_2$ : 282.1368). IR  $\nu$  (KBr)  $cm^{-1}$ : 3200, 1631.

4-(3-Hydroxyphenyl)-1-(2,4,6-trimethylbenzoyl)piperidine (**8aK**): mp 194–195 °C. Yield 72%.  $^1H$ -NMR ( $CHCl_3$ )  $\delta$ : 1.43–1.58 (1H, m), 1.62–1.85 (2H, m), 1.92–2.05 (1H, m), 2.19 (3H, s), 2.27 (3H, s), 2.29 (3H, s), 2.60–2.74 (1H, m), 2.76–2.92 (1H, m), 2.98–3.12 (1H, m), 3.46–3.56 (1H, m), 4.96–5.06 (1H, m), 6.60–6.74 (1H, m), 6.84 (1H, s), 6.85 (1H, s), 6.91 (1H, s), 7.12 (1H, t,  $J=8.1$  Hz). EI-MS  $m/z$ : 323 ( $M^+$ ). HR-MS  $m/z$ : 323.1895 (Calcd for  $C_{21}H_{25}NO_2$ : 323.1885). IR  $\nu$  (KBr)  $cm^{-1}$ : 3198, 1607.

1-Benzoyl-4-(3-hydroxyphenyl)piperidine (**8aL**): mp 189–190 °C. Yield ca. 100%.  $^1H$ -NMR ( $CHCl_3$ )  $\delta$ : 1.30–2.15 (4H, m), 2.62–2.80 (1H, m), 2.80–3.00 (1H, m), 3.00–3.25 (1H, m), 3.76–4.05 (1H, m), 4.72–5.05 (1H, m), 5.55–6.25 (1H, br), 6.65–6.73 (2H, m), 6.76 (1H, d,  $J=7.8$  Hz), 7.16 (1H, t,  $J=7.8$  Hz), 7.36–7.49 (5H, m). EI-MS  $m/z$ : 281 ( $M^+$ ). HR-MS  $m/z$ : 281.1409 (Calcd for  $C_{18}H_{19}NO_2$ : 281.1416). IR  $\nu$  (KBr)  $cm^{-1}$ : 3154, 1614.

4-(3-Hydroxyphenyl)-1-(4-methylbenzoyl)piperidine (**8aM**): mp 137–138 °C. Yield 93%.  $^1H$ -NMR ( $CHCl_3$ )  $\delta$ : 1.48–2.12 (4H, m), 2.37 (3H, s), 2.65–2.80 (1H, m), 2.80–3.00 (1H, m), 3.00–3.28 (1H, m), 3.79–4.08 (1H, m), 4.73–5.02 (1H, m), 6.56–6.66 (1H, br), 6.66–6.78 (3H, m), 7.14 (1H, t,  $J=7.8$  Hz), 7.20 (2H, d,  $J=7.8$  Hz), 7.34 (2H, d,  $J=7.8$  Hz). EI-MS  $m/z$ : 295 ( $M^+$ ). HR-MS  $m/z$ : 295.1575 (Calcd for  $C_{19}H_{21}NO_2$ : 295.1572). IR  $\nu$  (KBr)  $cm^{-1}$ : 3184, 1593.

1-(4-Fluorobenzoyl)-4-(2-hydroxyphenyl)piperidine (**8bA**): mp 211–212 °C. Yield 70%.  $^1H$ -NMR ( $CHCl_3$ )  $\delta$ : 1.43–2.08 (5H, m), 2.76–3.02 (1H, m), 3.05–3.28 (1H, m), 3.75–4.00 (1H, m), 4.74–4.98 (1H, m), 5.70–6.20 (1H, m), 6.74 (1H, dd,  $J=7.6, 1.0$  Hz), 6.89 (1H, dt,  $J=7.6, 1.0$  Hz), 7.01–7.17 (4H, m), 7.40–7.50 (2H, m). EI-MS  $m/z$ : 299 ( $M^+$ ). HR-MS  $m/z$ : 299.1334 (Calcd for  $C_{18}H_{18}FNO_2$ : 299.1321). IR  $\nu$  (KBr)  $cm^{-1}$ : 3133, 1601.

1-(4-Fluorobenzoyl)-4-(4-hydroxyphenyl)piperidine (**8aC**): mp 133–185 °C. Yield 79%.  $^1H$ -NMR (DMSO- $d_6$ )  $\delta$ : 1.40–1.60 (2H, m), 1.60–1.90 (2H, m), 2.60–2.75 (1H, m), 2.75–3.30 (2H, m), 3.50–4.00 (1H, m), 4.50–4.80 (1H, m), 6.68 (2H, d,  $J=8.5$  Hz), 7.05 (2H, d,  $J=8.5$  Hz), 7.22–7.30 (2H, m), 7.42–7.56 (2H, m), 8.95–9.20 (1H, br). EI-MS  $m/z$ : 299 ( $M^+$ ). HR-MS  $m/z$ : 299.1325 (Calcd for  $C_{18}H_{18}FNO_2$ : 299.1321). IR  $\nu$  (KBr)  $cm^{-1}$ : 3126, 1607.

**(1-Aroylpiperidin-4-yl)phenoxy-2-methylpropanoic Acid (9)** Compounds **9** were synthesized from **8** according to general procedure B.

**Modified Synthetic Procedure of 9aA** NaOH (60.0 g, 2.50 mol) was added in small portions to a stirred solution of **8aA** (30.0 g, 0.1 mol) in acetone (420 g, 7.23 mol) at room temperature over a 15 min period, and then stirred at 35 °C for 30 min.  $CHCl_3$  (53.9 g, 0.451 mol) was added dropwise to the stirred mixture in such a way as to maintain the reaction temperature at 35 °C for 1 h. The entire mixture was stirred at 35 °C for 1 h, and then concentrated *in vacuo*. The residue was dissolved in  $H_2O$ , and the mixture was washed with  $CHCl_3$  and acidified with dilute HCl to pH 3. The resulting precipitate was collected by filtration and dissolved in  $CHCl_3$ . The  $CHCl_3$  solution was extracted with aq.  $Na_2CO_3$  and the aqueous layer was acidified with dilute HCl to pH 3 again, and the resulting precipitate was collected by filtration and washed with  $H_2O$ . The solid was recrystallized from toluene to give **9aA** (29.4 g, 76%) as a white solid.

**Ethyl 2-[3-[1-(4-Fluorobenzoyl)-4-hydroxypiperidin-4-yl]phenoxy]-2-methylpropanoate (10)** A mixture of **3c** (4.44 g, 14.1 mmol), ethyl 2-bromo-2-methylpropanoate (12.5 g, 64.1 mmol) and  $K_2CO_3$  (4.50 g, 32.6 mmol) was stirred at 100 °C for 14 h. The mixture was poured into  $H_2O$  and extracted with  $CHCl_3$ . The  $CHCl_3$  layer was washed with  $H_2O$ , dried, and concentrated *in vacuo* to give **10** (6.05 g, ca. 100%) as a white solid. mp 103–104 °C.  $^1H$ -NMR ( $CHCl_3$ )  $\delta$ : 1.25 (3H, t,  $J=7.0$  Hz), 1.45–2.25 (4H, m), 1.60 (6H, s), 3.20–3.86 (3H, m), 4.24 (2H, q,  $J=7.0$  Hz), 4.50–4.77

(1H, m), 6.71 (1H, dd,  $J=8.1$ , 2.0 Hz), 7.03 (1H, t,  $J=2.0$  Hz), 7.05—7.15 (3H, m), 7.23 (1H, t,  $J=8.1$  Hz), 7.40—7.49 (2H, m). EI-MS  $m/z$ : 429 ( $M^+$ ). HR-MS  $m/z$ : 429.1936 (Calcd for  $C_{24}H_{28}FNO_5$ : 429.1952). IR  $\nu$  (KBr)  $cm^{-1}$ : 3403, 1732, 1607.

**Ethyl 2-[3-[1-(4-Fluorobenzoyl)-1,2,3,6-tetrahydropyridin-4-yl]phenoxy]-2-methylpropanoate (11)** A mixture of **10** (1.38 g, 3.22 mmol), TsOH acid monohydrate (700 mg, 3.68 mmol), and toluene (50 ml) was refluxed with stirring using a condenser attached to a water separator for 3 h. After cooling, the reaction mixture was washed with sat. aq.  $NaHCO_3$  and  $H_2O$ , dried, and concentrated *in vacuo* to give **11** (1.12 g, 85%) as a pale yellow viscous liquid.  $^1H$ -NMR ( $CHCl_3$ )  $\delta$ : 1.25 (3H, t,  $J=7.0$  Hz), 1.60 (6H, s), 2.42—2.68 (2H, m), 3.45—3.72 (1H, m), 3.83—4.00 (1H, m), 4.00—4.20 (1H, m), 4.24 (2H, q,  $J=7.0$  Hz), 4.25—4.50 (1H, m), 5.76—6.24 (1H, m), 6.73 (1H, dd,  $J=8.1$ , 2.0 Hz), 6.90 (1H, s), 7.01 (1H, d,  $J=8.1$  Hz), 7.06—7.17 (2H, m), 7.20 (1H, t,  $J=8.1$  Hz), 7.42—7.51 (2H, m). EI-MS  $m/z$ : 411 ( $M^+$ ). HR-MS  $m/z$ : 411.1842 (Calcd for  $C_{24}H_{26}FNO_4$ : 411.1845). IR  $\nu$  (KBr)  $cm^{-1}$ : 1731, 1633.

**2-[3-[1-(4-Fluorobenzoyl)-1,2,3,6-tetrahydropyridin-4-yl]phenoxy]-2-methylpropanoic Acid (12)** A solution of **11** (1.00 g, 2.43 mmol) in a mixture of 1 N NaOH (10 ml), MeOH (10 ml), and 1,4-dioxane (10 ml) was stirred at room temperature for 1 h. The mixture was acidified with dilute HCl to pH 3 and extracted with  $CHCl_3$ . The  $CHCl_3$  layer was washed with  $H_2O$ , dried, and concentrated *in vacuo* to give an oil which was crystallized from  $Et_2O$  to give **12** (820 mg, 88%) as a white solid.

**1-(4-Fluorobenzoyl)-4-(3-methoxyphenyl)piperazine (13)** A solution of 4-fluorobenzoyl chloride (1.80 g, 11.4 mmol) in THF (20 ml) was dropwise added to a stirred solution of 1-(3-methoxyphenyl)piperazine (2.0 g, 10.2 mmol) and  $Et_3N$  (3.0 ml, 21.6 mmol) in THF (40 ml) at 0°C over a 15 min period, and the mixture was stirred at room temperature for 1 h. The mixture was concentrated *in vacuo*, and the residue was dissolved in  $CHCl_3$ . The  $CHCl_3$  layer was washed with  $H_2O$ , dried, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel, eluting with 3% MeOH in  $CHCl_3$  to give **13** (3.10 g, 97%) as a pale yellow viscous liquid.  $^1H$ -NMR ( $CHCl_3$ )  $\delta$ : 3.00—3.36 (4H, m), 3.43—4.12 (4H, m), 3.79 (3H, s), 6.43—6.50 (2H, m), 6.54 (1H, dd,  $J=8.2$ , 2.0 Hz), 7.07—7.15 (2H, m), 7.19 (1H, t,  $J=8.2$  Hz), 7.40—7.50 (2H, m). EI-MS  $m/z$ : 314 ( $M^+$ ). HR-MS  $m/z$ : 314.1439 (Calcd for  $C_{18}H_{19}FN_2O_2$ : 314.1431). IR  $\nu$  (KBr)  $cm^{-1}$ : 1632.

**1-(4-Fluorobenzoyl)-4-(3-hydroxyphenyl)piperazine (14)**  $AlCl_3$  (4.0 g, 29.9 mmol) was added in small portions to a stirred solution of **13** (3.14 g, 10.0 mmol) and ethanethiol (4.0 ml, 53.5 mmol) in  $CH_2Cl_2$  (120 ml) at 0°C over a 5 min period, and the mixture was stirred at room temperature for 8 h and concentrated *in vacuo*. The residue was dissolved in  $H_2O$ , and the aqueous mixture was extracted with  $CHCl_3$ -MeOH (15:1). The organic layer was washed with  $H_2O$ , dried, and concentrated *in vacuo* to give an oil which was purified by column chromatography on silica gel, eluting with 3% MeOH in  $CHCl_3$  to give **14** (2.10 g, 70%) as a white solid, mp 134—135°C.  $^1H$ -NMR ( $DMSO-d_6$ )  $\delta$ : 3.00—3.20 (4H, m), 3.40—3.85 (4H, m), 6.25 (1H, dd,  $J=8.1$ , 2.0 Hz), 6.33 (1H, t,  $J=2.0$  Hz), 6.38 (1H, dd,  $J=8.1$ , 2.0 Hz), 6.99 (1H, t,  $J=8.1$  Hz), 7.23—7.35 (2H, m), 7.45—7.55 (2H, m), 9.09 (1H, s). EI-MS  $m/z$ : 300 ( $M^+$ ). HR-MS  $m/z$ : 300.1270 (Calcd for  $C_{17}H_{17}FN_2O_2$ : 300.1274). IR  $\nu$  (KBr)  $cm^{-1}$ : 3092, 1576.

**2-[3-[4-(4-Fluorobenzoyl)piperadino]phenoxy]-2-methylpropanoic Acid (15)** Compound **15** was synthesized from **14** according to general procedure B.

**Animals** Male ICR mice and male SD rats were obtained from Charles River Japan, Inc. (Tokyo, Japan). Male KK-A $^y$  mice were also obtained from CLEA Japan Inc. (Tokyo, Japan).

**Chemicals** Bezafibrate, fenofibrate, and gemfibrozil were purchased from Sigma (St. Louis, U.S.A.). For oral administration, the compounds were suspended in a vehicle (0.5% carboxymethylcellulose sodium solution containing 0.05% Tween 80). For the admixture in diet, normal-chow diet (MF; Oriental Yeast Co., Ltd., Tokyo, Japan), high-cholesterol diet (MF supplemented with 1.5% cholesterol and 0.5% cholic acid) and high-cholesterol diets containing 0.001, 0.003, and 0.01% **9aA**, and 0.01, 0.03, and 0.1% bezafibrate were prepared (Oriental Yeast Co., Ltd., Tokyo, Japan). Serum T-CHOL, TG,  $\beta$ -LP, and glucose levels were measured with commercially available kits (Wako Chemicals Japan).

#### Hypolipidemic Activities in Normal or Hypercholesterolemic Mice

In the case of normal mice, drugs were orally administered to male ICR mice (7-weeks old,  $n=5$ ) for 3 d. In the case of hypercholesterolemic mice, the high-cholesterol diet (1% cholesterol and 0.5% cholic acid) was given to male ICR mice (7-weeks old,  $n=6$ ) for 7 d, and drugs were orally administered on days 6 and 7. The T-CHOL,  $\beta$ -LP, and TG levels in the serum were measured.

**Hypolipidemic Effect in Hypercholesterolemic Rats** Male SD rats (7 weeks old,  $n=5$ ) were given the high-cholesterol diet (1.5% cholesterol and 0.5% cholic acid) or high-cholesterol diet containing drugs for 2 weeks. The T-CHOL and TG levels in serum were measured.

**Hypolipidemic Effect in Fructose-Induced Hypertriglyceridemic Rats** Male SD rats (6 weeks old) were given 25% fructose in drinking water during the experimental period. After 12 d, rats were divided into the control and treatment groups ( $n=8$ ), and were orally administered with **9aA**, bezafibrate, fenofibrate, and gemfibrozil for 7 d. Twenty-four hours after the final administration, the serum TG levels were measured.

**Hypoglycemic Effect in KK-A $^y$  mice** Male KK-A $^y$  mice (13 weeks old,  $n=8$ ) were administered **9aA** (0.003%, 0.01%, and 0.03%) and bezafibrate (0.03%, 0.1%, and 0.3%) admixture in their diets for 2 weeks. The serum glucose and TG levels were measured.

**Statistical Analysis** Data were expressed as mean  $\pm$  S.E. A statistical analysis of difference between the groups was performed with Dunnett's multiple comparison test followed by Bartlett's test. A Student's  $t$ -test or Aspin-Welch  $t$ -test were also used for comparison between the two groups. A  $p$ -value level less than 0.05 (2-side) was considered as statistically significant.

**Acknowledgements** We thank Messrs. M. Ogawa and M. Ikeda of SSP Co., Ltd. for their support of synthesis of the compounds. We are also grateful to Dr. T. Koda of SSP Co., Ltd. Central Research Labs. for his helpful discussions and the biological experiments.

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## Complex Formation of Cyclomaltononaose $\delta$ -Cyclodextrin ( $\delta$ -CD) with Macrocyclic Compounds<sup>1)</sup>

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The complex forming ability of  $\delta$ -cyclodextrin  $\delta$ -CD with 7 kinds of macrocyclic compounds (MCCs) with 8—15 carbon atoms in the ring as models of large guest molecules was studied in aqueous solution and compared with the complexation properties of  $\alpha$ -,  $\beta$ - and  $\gamma$ -CD. Both  $\alpha$ - and  $\beta$ -CD formed relatively stable complexes with small MCCs, while  $\gamma$ - and  $\delta$ -CD were more efficient in binding larger MCCs. The solid MCC/ $\delta$ -CD complexes were precipitated with the larger MCCs with 11—15 carbon atoms in the ring, while no such precipitates were obtained with smaller MCCs with only 8—10 carbon atoms in the ring. The formation of the solid complexes was confirmed by powder X-ray diffractometry and differential scanning calorimetry. The cell dimensions of cycloundecanone (11 carbon atoms in the ring)/ $\delta$ -CD complex were determined by X-ray crystallography. The preliminary crystal data were: Monoclinic,  $P2_1$ ,  $a=32.50$  (2) Å,  $b=19.02$  (3) Å,  $c=16.60$  (1) Å,  $\beta=98.37$  (5)°,  $V=10148$  (16) Å<sup>3</sup>.

**Key words**  $\delta$ -cyclodextrin; complex forming ability; macrocyclic compound; cycloundecanone

Cyclodextrins (CDs) are cyclic oligosaccharides composed of  $\alpha$ -1,4-linked glucose units, and  $\alpha$ -,  $\beta$ - and  $\gamma$ -CD, which are composed of 6, 7 and 8 glucose units, have been well studied and used in various fields because of their inclusion complex formation ability.<sup>2-4)</sup> However, their cavities are too small to form inclusion complexes with large guest molecules with molecular weights higher than several hundred. Large-ring CDs (LR-CDs) composed of more than nine glucose units do exist in nature and have large cavities.<sup>5,6)</sup> Purification of LR-CDs and portions of their X-ray crystal structure have been reported.<sup>7-12)</sup> The macrocyclic rings of  $\alpha$ -,  $\beta$ - and  $\gamma$ -CD are round in shape, but  $\delta$ -CD has a considerably warped macrocyclic ring.<sup>6)</sup> Recently, we studied the properties and complex formation abilities of these LR-CDs with some small compounds, but the relationship between complex forming ability and the guest molecule structure has not yet been elucidated in detail.<sup>7,13)</sup> The object of this study was to identify the structural relationship between LR-CDs and guest molecules. We selected a cyclomaltononaose ( $\delta$ -

CD) composed of nine glucose units as a LR-CD, as well as 7 kinds of macrocyclic compounds (MCCs) with 8—15 carbon atoms in the ring, as models of large guest molecules (Fig. 1). The complex forming ability of  $\delta$ -CD was studied in aqueous solution and compared with the complexation properties of  $\alpha$ -,  $\beta$ - and  $\gamma$ -CD. Preliminary structural analysis of cycloundecanone (11 carbon atoms in the ring)/ $\delta$ -CD complex was also performed using X-ray crystallography.

### Experimental

**Materials**  $\delta$ -CD with a purity of more than 95% was prepared by the method described previously.<sup>7,14)</sup> All other chemicals and solvents were of analytical reagent grade. Milli-Q water was used in all of the experiments.

**Simple Precipitation Test** Various amounts of MCC (the final mole ratio of MCC : CD was always 1 : 1) were added to 2 ml of  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -CD aqueous solution (15 mg/ml) in microtubes, which were then sealed and incubated at 25 °C. To achieve equilibrium, the sample solution was occasionally ultrasonicated (power: 80 W). After reaching equilibration (3 d), the precipitates were separated from the solution by centrifugation (3000 rpm for 30 min). The precipitates were dried under a vacuum at 60 °C. The MCC/pullulan systems were also examined as controls because pullulan does not

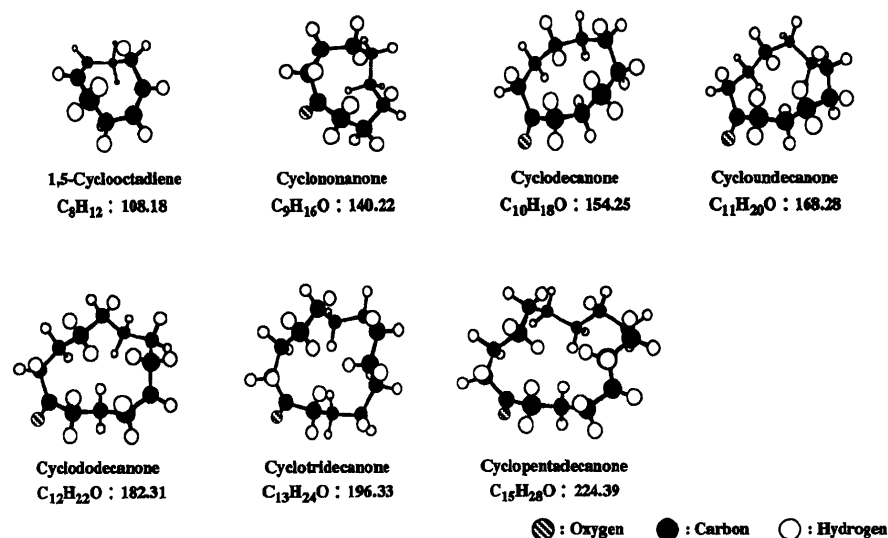


Fig. 1. Structures of the Macrocyclic Compounds Used

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form complexes with MCCs.

**Powder X-Ray Diffraction** Powder X-ray diffractometry was carried out using a RINT 1400 X-ray diffractometer (Rigaku Corp., Tokyo, Japan). X-ray measurements were carried out at 4°/min from 3° to 35° of the 2 $\theta$  range. Measurements were performed at 50 kV and 100 mA.

**Differential Scanning Calorimetry (DSC)** DSC was performed using a DSC8240D under the TAS 200 thermal analysis system (Rigaku Corp.) at a heating rate of 3 °C/min from 0 °C to 250 °C. Alumina was used as a reference.

**Preparation of Single Crystals of the Cycloundecanone/ $\delta$ -CD Complex** Aliquots of 4  $\mu$ l of cycloundecanone were added to 2 ml of an aqueous solution containing 45 mg of  $\delta$ -CD. Crystal growth was controlled by a temperature gradient provided by the PCC-700 programmable circulator (Tokyo Rikakikai Co., Ltd., Tokyo, Japan) for crystallization. The solution was shaken at 60 °C for several minutes, cooled to 45 °C over 6 h and then cooled to 10 °C over 24 h. Finally, it was kept at 10 °C for 48 h, and colorless crystals were obtained.

**X-Ray Analysis of the Cycloundecanone/ $\delta$ -CD Complex** X-ray intensity data were obtained with a Rigaku automatic four circle diffractometer (AFC-7R, CuK $\alpha$ ,  $\lambda$ =1.5418 Å, 60 kV, 200 mA, at 20 °C). Lattice constants were determined using 25 diffractions with the 2 $\theta$  range from 18.9° to 21.7°.

## Results and Discussion

**Simple Precipitation Test<sup>15)</sup>** To evaluate the complex forming ability of  $\delta$ -CD with MCC, a simple precipitation test was carried out, and the results were compared with those for conventional  $\alpha$ -,  $\beta$ - and  $\gamma$ -CD. Table 1 summarizes the yields of precipitation of CDs with MCC expressed as the percentage of the weight of each precipitate by the total weight of each system excluding the solvent.  $\gamma$ -CD and  $\beta$ -CD formed precipitates with almost all the MCCs tested, which had from eight to fifteen carbon atoms in the ring. In contrast,  $\alpha$ -CD produced precipitates with molecules such as 1,5-cyclooctadiene, cyclononanone and cyclodecanone, with eight, nine and ten carbon atoms in the ring, respectively.  $\beta$ -CD produced small amounts of precipitate (yield: 4.4%) with cyclopentadecanone with fifteen carbon atoms in the ring. The diameters of the  $\alpha$ - and  $\beta$ -CD cavity were too small to produce complete inclusion complexes with MCC. The precipitation reaction of  $\alpha$ - and  $\beta$ -CD with some MCC may be interpreted in the following manner. The structures of CD complexes differ significantly in solid state and in solution. In solution, the guest molecule resides in the cavity, and the whole complex is surrounded by a solvate shell of water molecules. In the solid state, the guest molecules can be intermolecular cavities formed by a crystal lattice, or sandwich-like between two complex molecules. However, there is still no clear understanding of the structures of precipitates of

CDs with MCC. It is beyond the scope of this paper to delve into the detailed structures of precipitates of  $\alpha$ - and  $\beta$ -CD with MCC.  $\delta$ -CD clearly produced precipitates with those MCC with more than eleven carbon atoms in the ring. If the  $\delta$ -CD cavity was too wide, it would not be able to hold small guest molecules such as MCC with less than ten carbon atoms in the ring tightly enough to prevent their slipping out. These results suggested that the yield of a complex precipitate of MCC for  $\alpha$ -,  $\beta$ -,  $\gamma$ - or  $\delta$ -CD was dependent on the cavity diameter of CDs. Precipitation of the complex alone does not imply the stability of these complexes, but these comparative experiments suggested that  $\delta$ -CD may be a good host molecule for relatively large guest molecules.

**Evidence of Inclusion Complexation** To confirm whether the precipitates consisted of inclusion complexes or simple coprecipitating mixtures of CDs and MCC, all precipitates were subjected to powder X-ray diffraction and DSC analyses. Figure 2 shows the powder X-ray diffraction patterns of cyclododecanone/ $\delta$ -CD precipitate in comparison with those of the physical mixture at the same mole ratio (guest :  $\delta$ -CD=1 : 1). The diffraction patterns of the physical mixtures were found to be simple superpositions of those of the components, while those of the precipitates were different, corresponding to a new solid phase. With the exception of cyclododecanone, cyclotridecanone and cyclopentadecanone, the MCCs used were liquid at room temperature, but there were no differences in diffraction patterns of CDs in physical mixtures in comparison with intact CDs.

Figure 3 shows the DSC curves of cyclododecanone/ $\delta$ -CD. The exothermic peaks at around 169 °C and 176 °C, which were observed for intact cyclododecanone and the physical mixture of cyclododecanone with  $\delta$ -CD, respec-

Table 1. Precipitation of CDs by the Formation of Insoluble Inclusion Complexes with MCCs (25 °C)

|                    | $\alpha$ -CD | $\beta$ -CD | $\gamma$ -CD | $\delta$ -CD | Pullulan <sup>a)</sup> |
|--------------------|--------------|-------------|--------------|--------------|------------------------|
| 1,5-Cyclooctadiene | 38%          | 72%         | 53%          | —            | —                      |
| Cyclononanone      | 63%          | 75%         | 55%          | —            | —                      |
| Cyclodecanone      | 33%          | 96%         | 66%          | —            | —                      |
| Cycloundecanone    | —            | 84%         | 87%          | 35%          | —                      |
| Cyclododecanone    | —            | 33%         | 36%          | 42%          | —                      |
| Cyclotridecanone   | —            | 73%         | 89%          | 56%          | —                      |
| Cyclopentadecanone | —            | 4.4%        | 22%          | 53%          | —                      |

a) Pullulan was used as an inactive additive. —: Not detected.

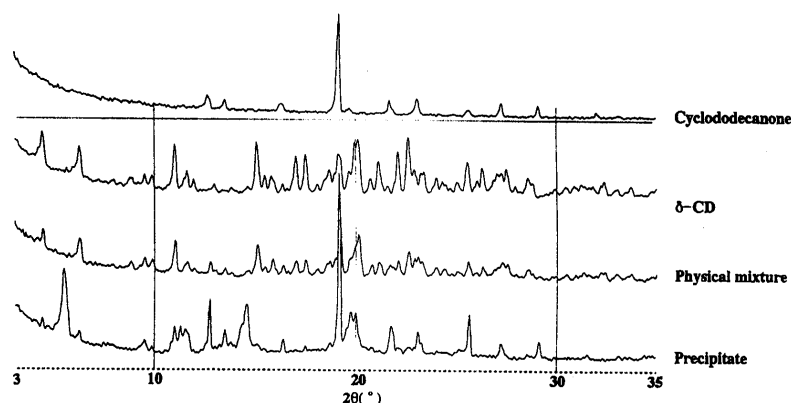
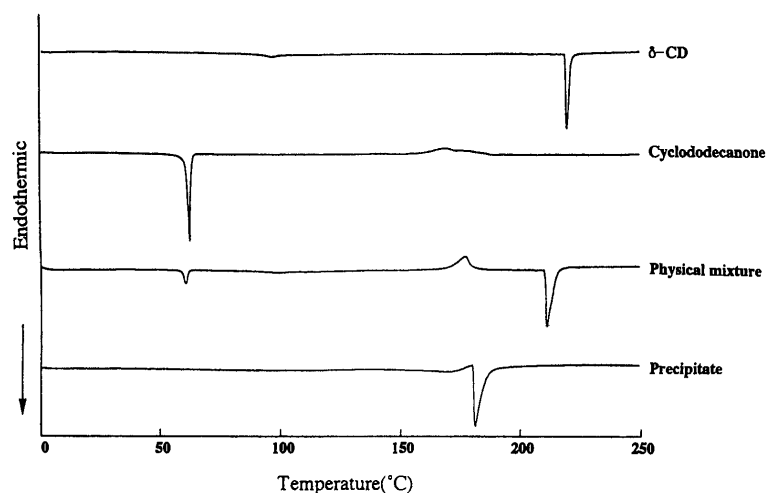
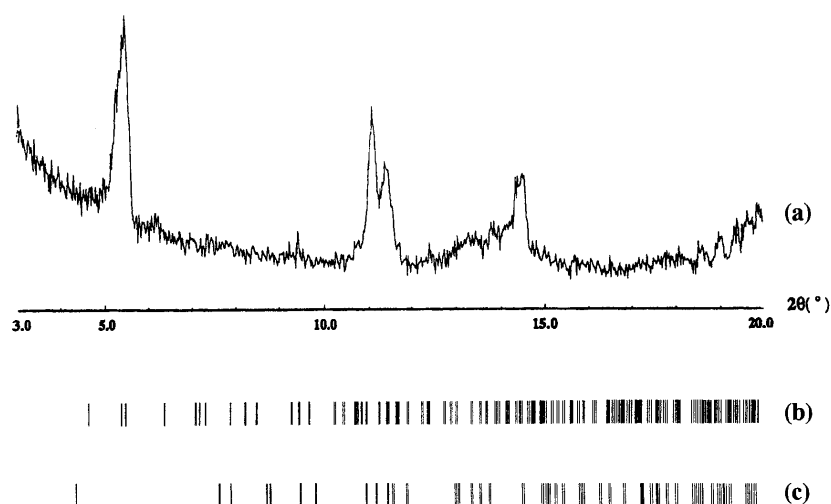


Fig. 2. Powder X-Ray Diffraction Patterns of the Cyclododecanone/ $\delta$ -CD System

Fig. 3. DSC Thermograms of the Cyclododecanone/ $\delta$ -CD SystemFig. 4. X-Ray Diffraction Pattern of the Cycloundecanone/ $\delta$ -CD Precipitate (a), Diffraction Angles of Cycloundecanone/ $\delta$ -CD Crystals (b) and Diffraction Angles of  $\delta$ -CD Crystals (c)

tively, disappeared in the cyclododecanone/ $\delta$ -CD precipitate. The endothermic peak at around 60 °C was shown to be due to the melting of cyclododecanone. This peak was observed for intact cyclododecanone and the physical mixture of cyclododecanone with  $\delta$ -CD, but not for the cyclododecanone/ $\delta$ -CD precipitate. These results corresponded to the changes observed in the X-ray diffraction pattern and indicated that the precipitates were inclusion complexes comprised of CDs and MCC.

**X-Ray Analysis of the Cycloundecanone/ $\delta$ -CD Complex** Table 2 summarizes the lattice constants of cycloundecanone/ $\delta$ -CD crystals and those of pure  $\delta$ -CD crystals reported by Fujiwara *et al.*<sup>6)</sup> The lattice constants of cycloundecanone/ $\delta$ -CD crystals differed from those of  $\delta$ -CD crystals. The volume of the direct-lattice unit cell of cycloundecanone/ $\delta$ -CD crystals was about three-fold larger than that of  $\delta$ -CD crystals. These results suggest that cycloundecanone/ $\delta$ -CD crystals were not crystals of  $\delta$ -CD itself. The following three possible structures were considered: a polymorphic form of  $\delta$ -CD, single crystals of cycloundecanone or an inclusion complex of  $\delta$ -CD and cycloundecanone. The possibility of a  $\delta$ -CD polymorphic form was very low because the  $\delta$ -CD concentration in the preparation of single crystals in

Table 2. Lattice Constants of Cycloundecanone/ $\delta$ -CD Crystals

|                     | Cycloundecanone/ $\delta$ -CD | $\delta$ -CD |
|---------------------|-------------------------------|--------------|
| Crystal system      | Monoclinic                    | Monoclinic   |
| Space group         | $P2_1$                        | $P2_1$       |
| Z                   | —                             | 2            |
| a (Å)               | 32.50 (2)                     | 20.881 (3)   |
| b (Å)               | 19.02 (3)                     | 11.538 (1)   |
| c (Å)               | 16.60 (1)                     | 16.222 (2)   |
| $\beta$ (°)         | 98.37 (5)                     | 104.33 (1)   |
| V (Å <sup>3</sup> ) | 10148 (16)                    | 3786.5 (8)   |
| $D_x$               | —                             | 1.497        |
| $D_m$               | —                             | 1.474        |

the cycloundecanone/ $\delta$ -CD system was one-quarter of the  $\delta$ -CD solubility, so it would have been difficult to precipitate pure  $\delta$ -CD itself. The next possibility, *i.e.* single crystals of cycloundecanone, was also very unlikely because the melting point of cycloundecanone was 16 °C, so it was difficult to measure at room temperature (20 °C). Therefore, the most likely structure was an inclusion complex of  $\delta$ -CD and cycloundecanone.

To obtain more detailed information about the relationship

between the single cycloundecanone/ $\delta$ -CD crystals and the cycloundecanone/ $\delta$ -CD precipitate, the diffraction angle of the cycloundecanone/ $\delta$ -CD crystals was calculated using the lattice constants by RIETAN.<sup>16)</sup> Figure 4 shows the observed diffraction pattern of cycloundecanone/ $\delta$ -CD precipitate and the calculated diffraction angles of the cycloundecanone/ $\delta$ -CD crystals and  $\delta$ -CD crystals. The observed diffraction pattern had three main peaks around 5–6°, 11–12° and 14–15° on the  $2\theta$  scale. The calculated and observed values were in good agreement for the 5–6° and 14–15° peaks. The small differences were probably due to the breadth of the lattice constant value, the use of imprecise X-ray crystal data and the unstable crystal structure. In addition, the diffraction angle of  $\delta$ -CD crystals was calculated in the same way. Figure 4 also shows a clear difference between the diffraction angles of  $\delta$ -CD crystals and those of cycloundecanone/ $\delta$ -CD crystals. Based on these results, it was previously suggested that the cycloundecanone/ $\delta$ -CD precipitates described were microcrystalline aggregates of cycloundecanone/ $\delta$ -CD crystals. The density of cycloundecanone/ $\delta$ -CD crystals was calculated from the lattice constants to estimate the compositional ratio. Highly accurate results could not be obtained in this experiment. However, it was presumed that the molar ratio of cycloundecanone:  $\delta$ -CD was 1 : 2 if the unit cell had two inclusion complex molecules and 1 : 1 or 2 : 1 if the unit cell had four inclusion complex molecules. This is the first report of the interaction between  $\delta$ -CD and guest molecules with preliminary crystal data. If more detailed structural data of a drug/LR-CD complex could be obtained, this kind of complex formation study of LR-CDs might provide valuable insight into drug delivery systems for use with large unstable drug molecules.

**Acknowledgments** The authors would like to thank Ms. H. Kato, Ms. M. Kaneko, Ms. N. Kamura, Ms. Y. Kobayashi, Ms. A. Fukushima, Mr. A. Hyodou, Mr. S. Matsumoto and Mr. M. Takahashi for their assistance with the experimental work.

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## Two New Alkaloids from Cigarette Smoke Condensate

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Received May 16, 2000; accepted September 2, 2000

**Chemical constituents of MeOH extracts of cigarette smoke were studied. Two new alkaloids, named cigatin A (1), 2-(Pyridine-3-yloxy)-benzene-1',4'-diol and B (2), 2-(4-Methyl-pyridin-3-yloxy)-benzene-1',4'-diol, were isolated from a mainstream condensate of cigarette together with seven known alkaloids. Their structures were determined on the basis of spectral data and chemical methods.**

**Key words** cigarette; mainstream; alkaloid; cigatin A; cigatin B

Nicotine was isolated as the major pharmacologically active ingredient in tobacco in 1828, and its chemical structure was determined to be 3-(1-methyl-pyrrolidin-2-yl)pyridine in 1895.<sup>1)</sup> Several investigators have studied the chemical composition of the basic portion of tobacco smoke condensate, and a great number of compounds (at least 3,796 different compounds<sup>2)</sup>) have been identified by using GC/FID or GC/MS.<sup>3–5)</sup> However, the reliability of their structures is questionable, because their structures were only deduced from their MS fragments in most cases. We have examined the smoke of cigarette and report here the isolation and characterization of two novel alkaloids.

Cigatin A (**1**) was obtained as yellowish plates whose FAB mass spectroscopy showed a molecular ion peak at  $m/z$  204 ( $M+H$ )<sup>+</sup>. HR-FAB-MS measurement gave a formula of  $C_{11}H_{10}NO_3$  ( $[M+H]^+$ ;  $m/z$  204.0652). The <sup>1</sup>H-NMR spectrum of **1** exhibited 4 proton signals at  $\delta$  8.76 (s), 8.22 (d), 8.06 (dd) and 8.49 (d), suggesting a pyridine skeleton in which one proton is substituted. Furthermore, the coupling constants of aromatic proton signals at  $\delta$  7.11 and 6.98 (each 1H, d,  $J=8.7$ ) are attributed to *ortho*-coupled aromatic protons. (Table 1)

In the <sup>13</sup>C NMR spectrum, four quaternary carbons signals at  $\delta$  159.6, 151.7, 143.7, and 131.4 indicated the presence of an oxygen bearing function. Weak correlation between H-2 ( $\delta$  8.76) and C-1' ( $\delta$  131.4) in the heteronuclear multi-bond correlation (HMBC) spectrum was also observed. On the other hand, in order to confirm the presence of hydroxyl groups, **1** was acetylated with pyridine and acetic anhydride. LC-ACPI-MS of the acetylated **1** showed a molecular ion peak at  $m/z$  288 [ $M+H$ ]<sup>+</sup>. The acetylated **1** exhibited two acetyl proton signals at  $\delta$  2.17 (s, 3H $\times$ 2) in the <sup>1</sup>H-NMR spectrum. The HMBC and HMQC (heteronuclear

multi quantum correlation) spectra were used to assign <sup>1</sup>H- and <sup>13</sup>C- signals of **1**. Based on these results, the structure of **1** was formulated as shown in Fig. 1.

Cigatin B (**2**) gave a molecular ion at  $m/z$  218 ( $M+H$ )<sup>+</sup> in positive ion FAB mass spectrometry, and the molecular formula  $C_{12}H_{12}NO_3$ , which was elucidated by HR-FABMS. By comparison of the NMR data with those of **1**, it was considered that the structure of **2** was similar to **1**, having a methyl group. Since correlations were shown, respectively, between a methyl proton signal at  $\delta$  2.52 and C-3 ( $\delta$  157.6), C-4 ( $\delta$  147.3), and C-5 ( $\delta$  128.8) in the HMBC spectrum, it was suggested that **2** has a methyl group at C-4. <sup>1</sup>H- and <sup>13</sup>C-NMR data are shown in Table 1.

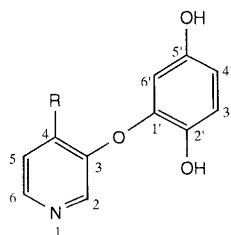
In addition to **1** and **2**, seven compounds were also obtained and were identified as harman, norharman, 3-methyl- $\beta$ -carboline, 1-ethyl- $\beta$ -carbolin, 3-pyridinol, 6-methyl-3-pyridinol and *N*-methylnicotinamide, respectively, by <sup>1</sup>H- and <sup>13</sup>C-NMR, and GC/MS spectroscopy. Among these compounds, 3-methyl- $\beta$ -carbolin and 1-ethyl- $\beta$ -carbolin are reported for the first time from cigarette mainstream smoke.

### Experimental

**General Procedures** FAB-MS and HR-FAB-MS were obtained on a JEOL JMS-SX 102 spectrometer. UV and IR spectra were recorded on a Hitachi U-2000 spectrophotometer and a JASCO IR-5300 spectrophotometer, respectively. NMR spectra were measured on a JEOL JNM-alpha (500 MHz) with acetone-*d*<sub>6</sub> and methanol-*d*<sub>4</sub> as the solvents. Spectra were referenced internally to TMS in <sup>1</sup>H-NMR and to the solvent in <sup>13</sup>C-NMR. GC/MS was performed with a Shimadzu GC/MS-5050A using the following conditions:

Table 1. <sup>1</sup>H- and <sup>13</sup>C-NMR Assignments of Cigatin A (**1**) and B (**2**) in Acetone-*d*<sub>6</sub>

| Position | <b>1</b>                                     |                          | <b>2</b>                                     |                          |
|----------|----------------------------------------------|--------------------------|----------------------------------------------|--------------------------|
|          | $\delta$ <sup>1</sup> H (int, mul, $J$ (Hz)) | $\delta$ <sup>13</sup> C | $\delta$ <sup>1</sup> H (int, mul, $J$ (Hz)) | $\delta$ <sup>13</sup> C |
| 2        | 8.76(1H, s)                                  | 135.5                    | 9.04 (1H, d, 1.3)                            | 132.8                    |
| 3        |                                              | 159.6                    |                                              | 157.6                    |
| 4        | 8.22(1H, d, 7.9)                             | 133.6                    |                                              | 147.3                    |
| 5        | 8.06 (1H, dd, 7.9, 5.4)                      | 128.8                    | 7.95 (1H, d, 5.9)                            | 128.8                    |
| 6        | 8.49 (1H, d, 5.4)                            | 137.0                    | 8.40 (1H, dd, 1.3, 5.9)                      | 136.6                    |
| 1'       |                                              | 131.4                    |                                              | 131.4                    |
| 2'       |                                              | 143.7                    |                                              | 143.7                    |
| 3'       | 7.11 (1H, d, 8.7)                            | 119.3                    | 7.11 (1H, d, 8.8)                            | 119.5                    |
| 4'       | 6.98 (1H, d, 8.7)                            | 120.2                    | 6.97 (1H, dd, 2.5, 8.8)                      | 120.0                    |
| 5'       |                                              | 151.7                    |                                              | 151.8                    |
| 6'       | 7.12 (1H, s)                                 | 113.2                    | 7.12 (1H, d, 2.5)                            | 113.3                    |
| 4-Me     |                                              |                          | 2.52 (3H, s)                                 | 16.6                     |



(1) 2-(Pyridin-3-yloxy)-benzene-1,4'-diol, R=H  
(2) 2-(4-Methyl-Pyridin-3-yloxy)-benzene-1,4'-diol, R=CH<sub>3</sub>

Fig. 1

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column, CBP-20 (0.25 mm i.d.  $\times$  25 m, Shimadzu); inj. temp., 240 °C, column temp., 50 °C  $\rightarrow$  2.0 °C/min  $\rightarrow$  240 °C; carrier gas, He 1.4 ml/min; detect. temp., 250 °C; ionization voltage, 70 eV; acceleration voltage, 1.0 kV. Column chromatography was performed using silica gel 60 (Art. 7734; Merck) and ODS (Fuji Silysia Chemical Ltd.). TLC was conducted on pre-coated silica gel 60 F<sub>254</sub> plates (Art. 5715; Merck). Prep. HPLC was performed with a JASCO HPLC system using the following conditions: column, Inertsil ODS (20 mm i.d.  $\times$  250 mm); mobile phase, CH<sub>3</sub>CN/H<sub>2</sub>O (15:85); flow rate, 6 ml/min; detection, UV at 254 nm.

**Extraction and Isolation** Cigarette smoke, which was generated under standard conditions (puff volume, 35 ml; puff duration, 2 s; puff frequency, once/min) by an inhalation apparatus, "Hamburg II," was passed through glass filters, and the collected materials were extracted with MeOH (3 l) to give an extract (308.0 g). The MeOH extract was partitioned between CHCl<sub>3</sub> and H<sub>2</sub>O. The CHCl<sub>3</sub> extract was fractionated by silica gel column chromatography using a CHCl<sub>3</sub>-MeOH gradient solvent system, and the CHCl<sub>3</sub>-MeOH (8:2) eluate was further partitioned with 1 M HCl and CHCl<sub>3</sub>. The CHCl<sub>3</sub> part was chromatographed on ODS MPLC with 35% CH<sub>3</sub>CN and on ODS HPLC (15% CH<sub>3</sub>CN), affording cigatin A (**1**, 5.3 mg) and B (**2**, 2.1 mg).

Cigatin A (**1**): yellowish plates. IR  $\nu_{\max}$  (KBr) cm<sup>-1</sup>: 3190 (OH), 1670, and 1580 (C=C). UV  $\lambda_{\max}$  (in MeOH): 333 ( $\epsilon$  5,530), 216 ( $\epsilon$  25673), Positive FAB-MS  $m/z$ : 204 (M+H)<sup>+</sup>, HR-FAB-MS  $m/z$  Calcd. for C<sub>11</sub>H<sub>10</sub>NO<sub>3</sub>: 204.0661, Found: 204.0652. <sup>1</sup>H- and <sup>13</sup>C-NMR: Table 1.

Acetylation of **1** in pyridine (1 ml) and Ac<sub>2</sub>O (1 ml) was carried out at

room temperature for 10 h.

Diacetate of **1**: yellowish plates. <sup>1</sup>H-NMR (in CD<sub>3</sub>OD)  $\delta$ : 7.64 (H-6, dd,  $J$ =5.5, 1.5 Hz), 7.60 (H-2, dd,  $J$ =1.5, 2.8 Hz), 7.50 (H-5, dd,  $J$ =5.5, 8.8 Hz), 7.40 (H-4, dd,  $J$ =2.8, 8.8 Hz), 7.22 (H-6', d,  $J$ =2.8 Hz), 7.11 (H-4', dd,  $J$ =2.8, 9.0 Hz), 6.99 (H-3', d,  $J$ =9.0 Hz), 2.16 (6H, s, 2 $\times$ COCH<sub>3</sub>). Positive ACPI-MS  $m/z$ : 288 (M+H)<sup>+</sup>.

Cigatin B (**2**): yellowish plates. IR  $\nu_{\max}$  (KBr) cm<sup>-1</sup>: 3190 (OH), 1640, and 1580 (C=C). UV  $\lambda_{\max}$  (in MeOH): 328 ( $\epsilon$  2,782), 220 ( $\epsilon$  10,695), Positive FAB-MS  $m/z$ : 218 (M+H)<sup>+</sup>, HR-FAB-MS  $m/z$  Calcd. for C<sub>12</sub>H<sub>12</sub>NO<sub>3</sub>: 218.0817, Found: 218.0824. <sup>1</sup>H- and <sup>13</sup>C-NMR: Table 1.

**Acknowledgements** This work was supported in part by a Grant-in-Aid for young scientists from the Japan Foundation for Aging and Health.

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## A Novel Anti-HBeAg Homolignan, Taiwanschirin D from *Kadsura matsudai*

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Received May 18, 2000; accepted August 7, 2000

**A novel C<sub>19</sub> homolignan, taiwanschirin D (1), possessing a 3,4-{1-[(Z)-2-methoxy-2-oxoethylidene]}pentano (2,3-dihydrobenzo[*b*]furan)-3(2-oxoacetate) skeleton, was isolated from the stem of *Kadsura matsudai* HAYATA. Its structure was determined from physical and spectral data including 2D NMR spectra. The Anti-HBeAg test revealed that taiwanschirin D (1) had moderate activity at a concentration of 94.3  $\mu$ M (50  $\mu$ g/ml).**

**Key words** *Kadsura matsudai* HAYATA; Anti-HBeAg; C<sub>19</sub> homolignans; taiwanschirin; 3,4-{1-[(Z)-2-methoxy-2-oxoethylidene]} pentano (2,3-dihydrobenzo[*b*]furan)-3(2-oxoacetate)

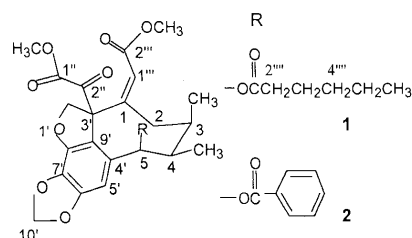
Several C<sub>18</sub> dibenzocyclooctadiene type lignans have been isolated from plants of Schizandraceae.<sup>1–7)</sup> These lignans showed some pharmacological effects such as antioxidant, antihepatitis, antihepatotoxic and antilipid peroxidative effects.<sup>1–5)</sup> We have reported the isolation of two types of novel cytotoxic C<sub>19</sub> homolignans, 3,4-{1-[(Z)-2-methoxy-2-oxoethylidene]}pentano(2,3-dihydrobenzo[*b*]furan)-3(2-oxoacetate) skeleton [taiwanschirins A, B, C] and 5,4-butan-2,4-cyclohexadienone-6-spiro-3-(2,3-dihydrobenzo[*b*]furan) skeleton [schiarisanrins A—D] from *Schizandra arisanensis*.<sup>8,9)</sup> Recently, we obtained a new anti-HBeAg C<sub>18</sub> dibenzocyclooctadiene lignan, kadsumarin A from another Taiwanese Schizandraceae plant, *Kadsura matsudai* HAYATA.<sup>10)</sup> Further investigation of the *K. matsudai* led to the isolation and characterization of a novel analog of taiwanschirins, namely taiwanschirin D (1), along with a known cytotoxic taiwanschirin C (2) and two C<sub>18</sub> dibenzocyclooctadiene lignans, kadsurarin (3) and kadsulignan N (4). We report herein the structural elucidation of compound 1 determined by employing two dimensional (2D) NMR techniques including <sup>1</sup>H–<sup>1</sup>H correlation spectroscopy (COSY), <sup>1</sup>H–<sup>13</sup>C heteronuclear multiple quantum coherence (HMQC), <sup>1</sup>H–<sup>13</sup>C heteronuclear multiple bonds coherence (HMBC), and (NOESY) spectra.

Taiwanschirin D (1) was obtained as colorless, cubic crystals. The IR spectrum indicated the presence of ester carbonyl (1745, 1735 cm<sup>–1</sup>), conjugated ester carbonyl (1718 cm<sup>–1</sup>), vinyl (1655 cm<sup>–1</sup>) and aromatic (1620 cm<sup>–1</sup>) groups. The <sup>13</sup>C-NMR and DEPT of 1 indicated the signals of an aromatic group at  $\delta_C$  132.9 (s), 102.3 (d), 130.4 (s), 150.8 (s), 145.5 (s) and 118.1 (s) (C-4', -5', -6', -7', -8' and -9', respectively). HMBC correlations from oxygenated methylene protons (H-2') to C-8', -9' and from a methylenedioxy proton (H-10') to C-6' and -7', indicated the presence of an oxygenated benzene moiety. The correlations between H-2' and a quaternary carbon (C-3') and C-9' indicated the connectivity of a dihydrofuran linkage an oxygenated benzene, which suggested a 2,3-dihydrobenzo[*b*]furan with a methylenedioxy moiety. A butyl chain (C<sub>2</sub>–C<sub>3</sub>–C<sub>4</sub>–C<sub>5</sub>) was predicted due to the prominent cross peaks of H-3, 2, 4, and of H-4, 3, 5, as

well as two methyl groups correlated with H-3 and 4 in the <sup>1</sup>H–<sup>1</sup>H COSY spectrum. Other evidence for structural elucidation comes from HMBC studies, the spectrum showing the couplings between H-2' and C-1, between H-2 and C-1, and between H-4' and C-5 suggested that the dihydrobenzo[*b*]furan was connected to a pentano moiety at 3'–4' positions. This evidence together with the signals of an oxoacetate and a 2-methoxy-2-oxoethylidene groups occurring in the NMR spectra suggested that 1 was the analogue of taiwanschirin C (2)<sup>9)</sup> which possesses a substituted dihydrobenzo[*b*]furan, a substituted cyclooctene, a substituted oxoacetate, and a substituted methoxy oxoethylidene moiety.<sup>2)</sup> However, proton signals ( $\delta_H$  2.22, 2.36, 1.52, 1.30 $\times$ 2, 0.87) and carbon signals [ $\delta_C$  33.7 (t), 24.5 (t), 22.4 (t), 31.3 (t), 13.9 (q), 173.9 (s)] for a caproxy group (*n*-hexanoic acid, C<sub>6</sub>H<sub>12</sub>O<sub>2</sub>) instead of a benzoyl ester group were observed in the NMR spectra.

Moreover, the mass spectrum of 1 exhibited a molecular ion at *m/z* 530 and an intense peak at *m/z* 327 [*M*<sup>+</sup> – CH<sub>3</sub>OCOCO – C<sub>6</sub>H<sub>12</sub>O<sub>2</sub>] corresponding to the fragmentation of an oxoacetate, and reflected the 1,2-elimination of an *n*-hexanoic acid *via* McLafferty ester rearrangement. In detailed inspection of the HMBC spectrum, cross signals between methylene-2''', H-5 and ester carbonyl carbon at  $\delta_C$  173.9 (C-1''') consequently suggested that the *n*-hexanoic acid ester is located at C-5. On the basis of the above corroboration, the entire structure of taiwanschirin D (1) was deduced to have a 3,4-{1-[(Z)-2-methoxy-2-oxoethylidene]}pentano (2,3-dihydrobenzo[*b*]furan)-3(2-oxoacetate) skeleton with a caproxy group at C-5.

These lignans and homolignans 1–4 were assayed for cytotoxicity against human epidermoid carcinoma of nasophar-



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Table 1.  $^1\text{H}$ - (300 MHz) and  $^{13}\text{C}$ - (75 MHz) NMR Data ( $\text{CDCl}_3$ ) for Compound **1**<sup>a)</sup>

|                        | Carbon  | Proton                 | C-H connectivities <sup>b)</sup> |
|------------------------|---------|------------------------|----------------------------------|
| 1                      | 154.9 s |                        | H-2, 2'                          |
| 2                      | 41.6 t  | 2.43 (m)               | H-1''', H-2                      |
| 3                      | 35.9 d  | 2.03 (m)               | H-5, 3-CH <sub>3</sub>           |
| 4                      | 43.6 d  | 1.92 (m)               | H-2, 3, 5                        |
| 5                      | 80.7 d  | 5.82 (s)               | H-5', 4-CH <sub>3</sub>          |
| CH <sub>3</sub> -3     | 19.9 q  | 1.03 (d, 8.0)          | H-5, 3                           |
| CH <sub>3</sub> -4     | 13.8 q  | 1.01 (d, 8.0)          | H-3, 5                           |
| 2'                     | 85.4 t  | 4.68, 5.92 (ABq, 10.2) |                                  |
| 3'                     | 64.9 s  |                        | H-1'''                           |
| 4'                     | 132.9 s |                        | H-5                              |
| 5'                     | 102.3 d | 6.32 (s)               | H-5                              |
| 6'                     | 130.4 s |                        | H-5'                             |
| 7'                     | 150.8 s |                        | H-10'                            |
| 8'                     | 145.5 s |                        | H-2'                             |
| 9'                     | 118.1 s |                        | H-5', 5                          |
| 10'                    | 102.3 t | 5.98 (s)               | —                                |
| 1''                    | 166.0 s |                        | 1''-OCH <sub>3</sub>             |
| 2''                    | 184.5 s |                        | H-2'a, b                         |
| OCH <sub>3</sub> -1''  | 51.4 q  | 3.57 (s)               | —                                |
| 1'''                   | 124.2 d | 6.09 (s)               | H-2                              |
| 2'''                   | 161.6 s |                        | 2'''-OCH <sub>3</sub>            |
| OCH <sub>3</sub> -2''' | 52.5 q  | 3.68 (s)               | —                                |
| 1'''                   | 173.9 s |                        | c)                               |
| 2'''                   | 33.7 t  | 2.22, 2.36 (m)         | c)                               |
| 3'''                   | 24.5 t  | 1.56 (m)               | c)                               |
| 4'''                   | 22.4 t  | 1.30 (m)               | c)                               |
| 5'''                   | 31.3 t  | 1.30 (m)               | c)                               |
| 6'''                   | 13.9 q  | 0.87 (t, 5.5)          | c)                               |

a) Multiplicity was determined from DEPT spectra. b) HMBC corresponded to two or three bonds. c) The assignments were explained in the text.

ynx (KB), colon carcinoma (COLO-205), hepatoma (Hepa-3B), and cervix (Hela) tumor cells. Compound **2** exhibited the cytotoxic effect against hepatoma ( $\text{ED}_{50}=2.3 \text{ g/ml}$ ) as previously reported,<sup>7)</sup> while  $\text{ED}_{50}$  values for compounds **1**, **3**, and **4** exceeded  $10 \mu\text{g/ml}$ . Moreover, biological evaluation of compounds **1**–**4** in anti-HBsAg and anti-HBeAg for the human type B hepatitis was also established. Only compound **1** showed the marginal inhibition in anti-HBeAg at a concentration of  $94.3 \mu\text{M}$  ( $50 \mu\text{g/ml}$ ). To our knowledge, this is the first report that the C<sub>19</sub> homolignan with 3,4-{1-[(Z)-2-methoxy-2-oxoethylidene]}pentano (2,3-dihydrobenzo[b]-furan)-3(2-oxoacetate) skeleton as compound **1** has anti-HBeAg activity.

## Experimental

**General Experimental Procedures** NMR spectra were measured at 300 MHz for  $^1\text{H}$  and 75 MHz for  $^{13}\text{C}$ . Heteronuclear long range correlation (HMBC) spectra were performed using coupling constants of 8 Hz. Samples for IR spectral measurements were prepared as KBr discs. Electron impact

(EI)-MS were performed in the electron impact mode (20 eV). HPLC was employed using the semipreparative  $5\text{C}_{18}$  column.

**Plant Material** The stems of *Kadsura matsudai* HAYATA were collected in July 1997 in Taichung County, Taiwan. A voucher specimen was deposited at the National Research Institute of Chinese Medicine, Taipei, Taiwan, R.O.C.

**Extraction and Isolation** The dried stems of *K. matsudai* HAYATA (6.2 kg) were extracted exhaustively with ethanol. The crude ethanol syrup was extracted five times with hexane. The ethanol layer was partitioned with EtOAc–H<sub>2</sub>O (1:1) three times to give EtOAc and H<sub>2</sub>O layers. After the EtOAc layer was evaporated *in vacuo*, its extract (82 g) was chromatographed on a silica gel column with *n*-hexane–EtOAc (8:1, 6:1, 4:1, 2:1, 1:1, EtOAc) to give 12 fractions, fr. 1–12. Bioactive fraction 5 was further separated by column chromatography on Silica gel eluting with  $\text{CHCl}_3$ :MeOH (15:1, 12:1, 10:1, 8:1, 6:1, 4:1) to yield 12 fractions, fr. 5-1–5-12. Compounds **1** (3.3 mg) and **4** (4.2 mg) were furnished from fr. 5-4 by HPLC ( $5\text{C}_{18}$ ,  $250 \times 10 \text{ mm}$ , MeOH–H<sub>2</sub>O=70:30). Compound **3** (186 mg) was obtained from fraction 5-5 after recrystallization.

Taiwanschirin D (**1**): Light yellow powder; mp 47–50 °C, IR (KBr), 1740, 1735, 1715, 1655, 1615  $\text{cm}^{-1}$ ;  $[\alpha]_D^{25} +315^\circ$  ( $\text{CH}_2\text{Cl}_2$ ,  $c=0.3$ ); EI-MS  $m/z$  (rel. intensity) 530 ( $\text{M}^+$ , 8), 443 (15), 327 (100), 295 (70), 267 (68), 253 (25); HR-EI-MS  $m/z$  530.2153 [ $\text{M}]^+$  (Calcd for  $\text{C}_{28}\text{H}_{34}\text{O}_{10}$ , 530.2152);  $^1\text{H}$ - and  $^{13}\text{C}$ - NMR, see Table 1.

**Cytotoxicity Assay** The *in vitro* cytotoxicity assay against KB (nasal pharyngeal carcinoma), Hepa-3B (hepatoma), Hela (cervix carcinoma), and COLO-205 (colon carcinoma) tumor cells by the methylene blue dying method was conducted as described in a previous paper.<sup>10)</sup>

**Anti-HBsAg and Anti-HBeAg Test** The assay for *in vitro* antiviral activity against hepatitis B virus (HBV) followed to our previously described procedure.<sup>11)</sup>

**Acknowledgments** We would like to thank Mr. Ting-Chuan Chung and Dr. Ge-Hsiung Tsai for collecting the plants, Mr. Shih-Jen Wang, NSC Regional Instrument Center of HSIN-CHU, for measuring the HR-MS spectrum and the National Science Council, Taiwan, Republic of China, for financial support (NSC 89-2113-M-077-003) to Y. H. Kuo.

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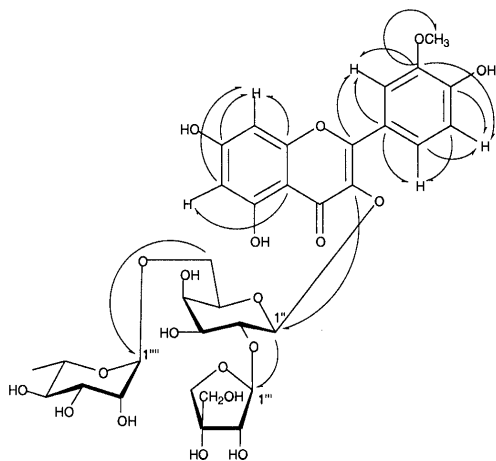


Chart 2. Key Heteronuclear Multiple-Bond Correlations (HMBC) of **1**

grouping to that found in kaempferol 3-*O*- $\beta$ -D-apiofuranosyl-(1 $\rightarrow$ 2)-[ $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 6)]- $\beta$ -D-galactopyranoside,<sup>21)</sup> confirming the proposed sugar sequence.

On the basis of these data, the structure of compound **1** was established as isorhamnetin 3-*O*- $\beta$ -D-apiofuranosyl-(1 $\rightarrow$ 2)-[ $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 6)]- $\beta$ -D-galactopyranoside.

From the aerial parts of *A. vulneraria* isorhamnetin 3-*O*- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-galactopyranoside (**2**) was also isolated and identified by comparison of its <sup>1</sup>H- and <sup>13</sup>C-NMR spectral data with literature values.<sup>22)</sup>

## Experimental

**General Experimental** A Bruker DRX-600 spectrometer operating at 599.19 MHz for <sup>1</sup>H and 150.86 for <sup>13</sup>C using the UXNMR software package was used for NMR measurements in CD<sub>3</sub>OD solutions. 2D experiments: <sup>1</sup>H-<sup>1</sup>H DQF-COSY<sup>16)</sup> inverse detected <sup>1</sup>H-<sup>13</sup>C HSQC<sup>17)</sup> and HMBC<sup>19)</sup> were obtained by employing the conventional pulse sequences as described previously. The selective excitation spectra, 1D TOCSY<sup>15)</sup> were acquired using waveform generator-based GAUSS shaped pulses, mixing time ranging from 100 to 120 ms and a MLEV-17 spin-lock field of 10 kHz preceded by a 2.5 ms trim pulse. Optical rotations were measured on a Perkin-Elmer 141 polarimeter using a sodium lamp operating at 589 nm in 0.1% w/v solutions in MeOH. FAB-MS spectra were recorded in a glycerol matrix in the negative ion mode on a VG ZAB instrument (XE atoms of energy of 2–6 kV).

**Plant Material** *Astragalus vulneraria* DC. was collected from Polatli, Ankara, Central Anatolia, Turkey in June 1998. Voucher specimens have been deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, Ankara.

**Extraction and Isolation** Air-dried aerial parts of *A. vulneraria* (380 g) were powdered and extracted with 80% EtOH (3.5 l  $\times$  2) under reflux, and then filtered. The filtrate was concentrated to dryness *in vacuo* (27.7 g). The extract was subjected to VLC using normal-phase silica gel, employing pentane (200 ml, fr. 1), petroleum ether (400 ml, fr. 2), diethylether (500 ml, fr. 3), CHCl<sub>3</sub> (400 ml, fr. 4), CHCl<sub>3</sub>-MeOH (90:10, 400 ml, fr. 5), and then CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O mixtures (80:20:2, fr. 6; 70:30:3, fr. 7; 61:32:7, fr. 8, each 500 ml). fr. 7 (2.47 g) was subjected to column chromatography (silica gel, 60 g) eluted with CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O mixtures (80:20:2, 100 ml; 70:30:3, 200 ml, 61:32:7, 400 ml) to give nine main fractions (Frs. A–I). Fraction B (55 mg) was chromatographed on a silica gel column (20 g), eluted with CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O (70:30:3, 200 ml) to give compound **2** (26 mg). Fractionation of fr. 6 (300 mg) by open column chromatography (silica gel, 30 g) using EtOAc-MeOH-H<sub>2</sub>O (90:17:13, 400 ml) gave compound **1** (16.5 mg).

Isorhamnetin-3-*O*- $\beta$ -D-apiofuranosyl-(1 $\rightarrow$ 2)-[ $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 6)]- $\beta$ -D-galactopyranoside (**1**): [ $\alpha$ ]<sub>D</sub><sup>25</sup> 38.4° (*c*=0.1, MeOH); <sup>1</sup>H-NMR

(600 MHz, CD<sub>3</sub>OD) aglycon moiety:  $\delta$ : 8.00 (1H, d, *J*=1.5 Hz, H-2'), 7.61 (1H, dd, *J*=1.5, 7.5 Hz, H-6'), 6.43 (1H, d, *J*=1.5 Hz, H-8), 6.22 (1H, d, *J*=1.5 Hz, H-6), 4.00 (3H, s, OCH<sub>3</sub>);  $\beta$ -D-galactopyranose moiety:  $\delta$ : 5.60 (1H, d, *J*=7.5 Hz, H-1''), 4.00 (1H, dd, *J*=7.5, 9.0 Hz, H-2''), 3.80 (1H, dd, *J*=4.0, 2.5 Hz, H-4''), 3.73 (1H, m, H-5''), 3.73 (1H, dd, *J*=12.0, 2.5 Hz, H-6a''), 3.67 (1H, dd, *J*=9.0, 4.0 Hz, H-3''), 3.47 (1H, dd, *J*=12.0, 4.5 Hz, H-6b'');  $\beta$ -D-apiofuranose moiety:  $\delta$ : 5.44 (1H, d, *J*=2.0 Hz, H-1'''), 4.04 (1H, d, *J*=2.0 Hz, H-2'''), 4.04 (1H, d, *J*=10.0 Hz, H-4a'''), 3.73 (1H, brs, H-5a'''), 3.67 (1H, d, *J*=10.0 Hz, H-4b'''), 3.65 (1H, brs, H-5b''');  $\alpha$ -L-rhamnopyranose moiety:  $\delta$ : 4.55 (1H, d, *J*=1.5 Hz, H-1'''), 3.59 (1H, dd, *J*=1.5, 2.5 Hz, H-2'''), 3.53 (1H, m, H-5'''), 3.51 (1H, dd, *J*=8.4, 2.5 Hz, H-3'''), 3.28 (t, *J*=8.4 Hz, H-4'''), 1.19 (d, *J*=6.5 Hz, H-6'''); <sup>13</sup>C-NMR (150 MHz, CD<sub>3</sub>OD) aglycon moiety:  $\delta$ : 179.5 (s, C-4), 166.1 (s, C-7), 163.0 (s, C-5), 158.9 (s, C-2), 158.5 (s, C-9), 150.9 (s, C-4'), 148.4 (s, C-3'), 135.5 (s, C-3), 123.8 (d, C-6'), 123.0 (s, C-1'), 116.0 (d, C-5'), 114.6 (d, C-2'), 105.5 (s, C-10), 99.8 (d, C-6), 94.9 (d, C-8), 57.0 (OCH<sub>3</sub>);  $\beta$ -D-galactopyranose moiety:  $\delta$ : 101.3 (d, C-1''), 76.4 (d, C-2''), 75.7 (d, C-5''), 70.4 (d, C-4''), 67.3 (t, C-6'');  $\beta$ -D-apiofuranose moiety:  $\delta$ : 110.7 (d, C-1'''), 79.5 (s, C-3'''), 78.0 (d, C-2'''), 75.7 (t, C-4'''), 66.5 (t, C-5''');  $\alpha$ -L-rhamnopyranose moiety:  $\delta$ : 101.9 (d, C-1'''), 73.5 (d, C-4'''), 72.8 (d, C-3'''), 72.1 (d, C-2'''), 69.8 (d, C-5'''), 18.1 (q, C-6'''); FAB-MS *m/z* 755 [M-H]<sup>+</sup>, 623 [(M-H)-(132)]<sup>+</sup>, 477 [(M-H)-(132+146)]<sup>+</sup>, 315 [(M-H)-(132+146+162)]<sup>+</sup>.

Isorhamnetin-3-*O*- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-galactopyranoside (**2**): <sup>1</sup>H- and <sup>13</sup>C-NMR (600 MHz, CD<sub>3</sub>OD) data were identical to those reported in the literature.<sup>22)</sup>

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## Microbial Transformation of Kawain and Methysticin

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Received June 5, 2000; accepted August 7, 2000

The styryl  $\alpha$ -pyrones, *d*-kawain (**1**) and *d*-methysticin (**2**) are two of the major kavalactone constituents of the anxiolytic herb *Piper methysticum*, commonly known as kava. The use of fungal models to mimic the mammalian metabolism of **1** resulted in the production of 4'-hydroxykawain (**1a**) from the culture broth of *Cunninghamella elegans* (ATCC 9245), the same metabolite identified in rat urine. The fungus *Torulopsis petrophilum* (ATCC 20225) biotransformed **2** to 3'-hydroxy-4'-methoxykawain (**2c**) which is analogous, but not identical, to a known rat metabolite of methysticin.

**Key words** biotransformation; microbial transformation; *Piper methysticum*; kawain; methysticin; metabolism

Kava-kava, or kava, (*Piper methysticum* Forst., Piperaceae) is a South Pacific plant that has been used by indigenous peoples as an intoxicating beverage since ancient times.<sup>1)</sup> At present, various preparations of kava are marketed in Europe and North America to manage mild anxiety.<sup>2)</sup> Additional reports indicate that kava preparations may have analgesic,<sup>3)</sup> spasmolytic,<sup>4)</sup> neuroprotective<sup>5)</sup> and antimitotic activities.<sup>6)</sup> The main active constituents believed to be responsible for the pharmacological effects of *P. methysticum* are the styryl  $\alpha$ -pyrones (kavalactones).<sup>7)</sup> The synthetic *dl*-form of one of the natural kavalactones, *d*-kawain (**1**), exhibits a synergistic sedative effect with kava extract and is marketed in Germany as a single entity for managing anxiety.<sup>2)</sup> *d*-Methysticin (**2**) is another major kavalactone that was reported to protect brain tissue against ischemic brain damage in a rat model.<sup>5)</sup> Various analytical techniques have been developed for the determination of **1**, **2**, and other kavalactones in kava products.<sup>8)</sup>

In addition to numerous pharmacological and mechanistic studies that aimed at corroborating the biological effects of *P. methysticum* extracts and isolates, two previous investigations have reported metabolites of **1** and **2** in rat and human urine.<sup>9,10)</sup> In both cases, however, unmetabolized kavalactones were detected in greater concentrations than any of their metabolites. The metabolites identified for **1** and for **2** were different for rats and humans. In rats, the metabolism of **1** and **2** occurred at the aromatic ring to produce the phenolic metabolites **1a** and **2a**, respectively. In humans, the major sites of metabolism were the styryl double bond ( $\Delta^7$ ) and the pyrone ring, resulting in the 3,4,7,8-tetrahydro-4-*O*-demethyl metabolites **1b** and **2b**, respectively (Chart 1).

In the present report, microbial models were utilized retrospectively in an attempt to generate the mammalian metabolites of **1** and **2**. Such metabolites can be used for further pharmacological evaluation alongside the already available kavalactones and also as analytical standards for detection and characterization of kava metabolites in biological fluids. Metabolite **1a** was identical to that detected in rat urine while metabolite **2c** was identical to a synthetic analog of **1**<sup>11)</sup> and analogous to the catechol rat metabolite **2b**.<sup>9)</sup> The analogy between **2b** and **2c** arises from the fact that both resulted from the hydrolysis of the methylenedioxy group of **2**, a well-documented metabolic reaction.<sup>12)</sup> It was not clear, however, whether **2c** resulted from a single step metabolic transformation, or *via* a multiple step pathway involving initial

production of **2b** followed by *O*-methylation to **2c**. Although both metabolites **1a** and **2c** have been previously reported, no spectroscopic data were published for either compound. In this publication, we report the production of **1a** and **2c** *via* an alternative route and provide their full structure elucidation and spectroscopic data.

Twenty-one cultures were screened for their ability to biotransform **1** and **2** according to a standard two-stage procedure.<sup>13)</sup> TLC results showed that three fungi, *Cunninghamella blakesleana* (ATCC 8688a), *C. echinulata* (NRRL 3655) and *C. elegans* (ATCC 9245), produced the same metabolite **1a** from **1**. Of these, *C. elegans* was chosen for the preparative stage based on its relatively higher biotransformational efficiency as detected by TLC. For **2**, only one organism, *Torulopsis petrophilum* (ATCC 20225) produced a metabolite (**2c**).

Preparative scale fermentation of **1** by *C. elegans* provided metabolite **1a** in *ca.* 22% yield (based on 145 mg of biotransformed substrate). High resolution electrospray ionization mass spectrometric (HR-ESI-MS) analysis of **1a** provided a molecular formula of C<sub>14</sub>H<sub>14</sub>O<sub>4</sub> corresponding to a monooxygenated product of **1**. The IR spectrum showed a hydroxyl band at 3277 cm<sup>-1</sup>. The <sup>1</sup>H-NMR spectrum of **1a** differed from that of **1** in the reduction of the total aromatic protons from 5 to 4 with the appearance of two doublets (AA'BB' system,  $\delta$  6.82, 7.33) characteristic of a 1,4-disubstituted benzene and confirming the *p*-hydroxylation of the aromatic ring. Moreover, the <sup>13</sup>C-NMR spectrum of **1a** showed a singlet carbon signal at  $\delta$  158.0 corresponding to C-4'. Metabolite **1a** was thus characterized as 4'-hydroxykawain.<sup>9)</sup>

Preparative scale fermentation of **2** by *T. petrophilum*

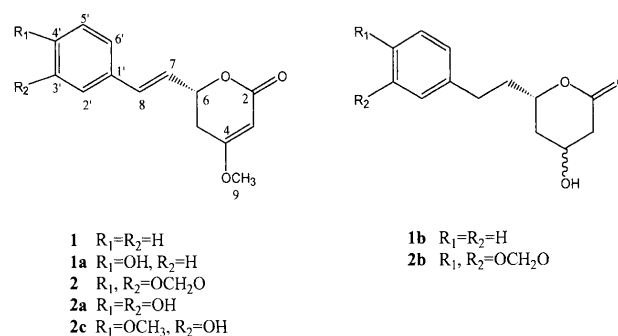


Chart 1. Structures and Major Metabolites of **1** and **2**

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Table 1.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR Data<sup>a)</sup> of Compounds **1a**, **2** and **2c**

| Position | <b>1a<sup>b)</sup></b> |                            | <b>2<sup>c)</sup></b> |                            | <b>2c<sup>b)</sup></b> |                               |
|----------|------------------------|----------------------------|-----------------------|----------------------------|------------------------|-------------------------------|
|          | $\delta_{\text{C}}$    | $\delta_{\text{H}}$ (J Hz) | $\delta_{\text{C}}$   | $\delta_{\text{H}}$ (J Hz) | $\delta_{\text{C}}$    | $\delta_{\text{H}}$ (m, J Hz) |
| 2        | 166.7 s                | —                          | 167.2 s               | —                          | 167.4 s                | —                             |
| 3        | 91.1 d                 | 5.15 s                     | 90.9 d                | 5.18 s                     | 90.9 d                 | 5.19 s                        |
| 4        | 173.5 s                | —                          | 172.8 s               | —                          | 172.9 s                | —                             |
| 5        | 34.0 t                 | 2.56 dd (17.1, 4.3)        | 33.8 t                | 2.51 dd (17.1, 4.4)        | 33.8 t                 | 2.52 dd (17.1, 4.2)           |
|          |                        | 2.68 dd (18.0, 10.7)       |                       | 2.64 ddd (17.1, 10.9, 0.8) |                        | 2.65 dd (17.1, 11.0)          |
| 6        | 77.0 d                 | 5.03 m                     | 76.4 d                | 5.01 m                     | 76.6 d                 | 5.02 m                        |
| 7        | 124.3 d                | 6.20 dd (16.0, 6.7)        | 124.0 d               | 6.07 dd (15.8, 6.4)        | 124.1 d                | 6.10 dd (15.8, 6.4)           |
| 8        | 133.5 d                | 6.68 d (16.0)              | 133.4 d               | 6.62 d (15.8)              | 133.4 d                | 6.61 d (15.9)                 |
| 9        | 56.7 q                 | 3.80 s                     | 56.5 q                | 3.75 s                     | 56.5 q                 | 3.75 s                        |
| 10       | —                      | —                          | 101.6 t               | 5.95 s                     | 56.4 q                 | 3.88 s                        |
| 1'       | 128.7 s                | —                          | 130.5 s               | —                          | 129.8 s                | —                             |
| 2'       | 129.0 d                | 7.33 d (8.5)               | 108.8 d               | 6.91 d (1.3)               | 112.5 d                | 7.00 d (1.7)                  |
| 3'       | 116.4 d                | 6.82 d (8.5)               | 148.3 s               | —                          | 146.2 s                | —                             |
| 4'       | 158.0 s                | —                          | 148.5 s               | —                          | 147.4 s                | —                             |
| 5'       | 116.4 d                | 6.82 d (8.5)               | 106.2 d               | 6.75 d (8.0)               | 111.0 d                | 6.79 d (8.3)                  |
| 6'       | 129.0 d                | 7.33 d (8.5)               | 122.2 d               | 6.81 dd (8.0, 1.3)         | 119.9 d                | 6.86 dd (8.3, 1.7)            |
| OH       | —                      | 8.64 s                     | —                     | —                          | —                      | 5.85 s                        |

Measured at 400 MHz ( $^1\text{H}$ ) and 100 MHz ( $^{13}\text{C}$ ).  $^{13}\text{C}$  Multiplicities were determined by DEPT 135 experiments. a) Full NMR data of **1** and NMR data of **2** is included for comparison **2** have been previously reported.<sup>15,16,17</sup> b) In acetone- $d_6$ . c) In  $\text{CDCl}_3$ .

yielded metabolite **2c** in 4% yield (based on 125 mg of bio-transformed substrate). HR-ESI-MS analysis of **2c** provided a molecular formula of  $\text{C}_{15}\text{H}_{16}\text{O}_5$  corresponding to a product of **2** with two more hydrogen atoms. The IR spectrum showed a strong hydroxyl band at  $3364\text{ cm}^{-1}$ . The  $^1\text{H}$ -NMR spectrum of **2c** resembled that of **2** in all aspects including the 3',4'-disubstitution of the aromatic ring. However, the methylenedioxy singlet at  $\delta$  5.95 in **2** was replaced by another methoxyl singlet at  $\delta$  3.88 indicating the reductive cleavage of the methylenedioxy group. Long-range heteronuclear multiple bond correlation (HMBC) showed that the hydroxy and the methoxy groups were attached to the singlet carbons C-3 and C-4' at  $\delta$  146.2 and 147.4, respectively. The exact position of the two substituents on the aromatic ring was determined by nuclear overhauser enhancement spectroscopy (NOESY) and HMBC experiments. The NOESY spectrum showed a correlation between the 4'-OMe protons (H-10) and H-5', indicating that the methoxyl group was attached to C-4'. Also, the presence of an HMBC correlation between H-6' and the singlet carbon at  $\delta$  147.4 (C-4') and the absence of any correlation with the other singlet carbon at  $\delta$  146.2 (C-3') further supported the NOESY data. Metabolite **2c** was thus determined as 3'-hydroxy-4'-methoxykawain.<sup>11)</sup>

## Experimental

**General Experimental Procedure** Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Optical rotations were measured using a Jasco DIP-370 digital polarimeter. UV spectra were obtained with a Hewlett Packard 8452A diode array spectrophotometer. The IR spectra were recorded with an ATI Mattson Genesis Series FTIR spectrophotometer. The  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra were obtained on a Bruker Avance DRX-400 FT spectrometer operating at 400 and 100 MHz, respectively. HR-ESI-MS analysis was conducted on a Bruker BioApex 3.0 mass spectrometer.

**Chromatographic Conditions** TLC: precoated Si 250F plates (Baker); developing system: hexane-EtOAc (3:7, v/v); visualization: UV light (254 nm), and *p*-anisaldehyde spray reagent. Column chromatography: Si gel 230—400 mesh (Merck).

**Organisms and Metabolism** Fungi were accessed from the culture collection housed in the National Center for Natural Products Research, Uni-

versity of Mississippi, and were originally obtained from the American Type Culture Collection (ATCC), Rockville, Maryland, or from the National Center for Agricultural Utilization Research (NCAUR) (formerly Northern Regional Research Laboratories (NRRL)), Peoria, Illinois. Stock cultures were maintained on agar slants of media recommended by ATCC and were stored at 4 °C. All screening and scale-up fermentations were run in a complex culture medium (medium  $\alpha$ ) of the following composition: 5 g yeast extract (Difco Labs, Detroit, MI), 5 g bacto-peptone (Difco Labs), 5 g NaCl, 5 g  $\text{K}_2\text{HPO}_4$ , 20 g dextrose, and distilled  $\text{H}_2\text{O}$  to 1 l. For screening, cultures were grown in 25 ml of medium- $\alpha$  held in 125-ml Erlenmeyer flasks and equipped with stainless steel caps. Compounds **1** and **2** were separately added to 1-day-old stage II culture media as a 10% acetone solution (0.2 mg of substrate/ml of culture medium). Cultures were incubated at room temperature on a rotary shaker (New Brunswick Model G10-21) at 150 rpm for a maximum period of 14 d with sampling and TLC monitoring at three-day intervals. The production of **1a** and **2c** started at day 6 and 12, respectively. Preparative scale fermentations followed the same general procedure with the difference that, for each organism, 2 ml of 10% solutions of **1** and **2** in acetone were equally divided on 4 1-l Erlenmeyer flasks, each containing 250 ml of stage II culture of the respective organism (50 mg/flask). Incubation periods for the preparative scale fermentations were 9 and 14 d for **1** and **2**, respectively. Work-up followed a routine procedure<sup>14)</sup> that comprised exhaustive EtOAc extraction of both culture filtrates and residues as determined by TLC. The combined biomass and filtrate extracts were concentrated *in vacuo* at 40 °C to yield residues that were subsequently subjected to column chromatography for metabolite isolation and purification. Appropriate substrate and culture controls were simultaneously run alongside each preparative scale fermentation.

**Substrates** Pure **1** and **2** were isolated from crude *P. methysticum* extract (Botanicals International, Long Beach, CA) and their identities were confirmed by comparing their chromatographic and spectroscopic characteristics with literature values.

**Biotransformation of 1 with C. elegans** At the end of the fermentation period, the media were filtered. Both the filtrate and biomass were exhaustively extracted with EtOAc to yield an orange residue (ca. 350 mg). The residue was chromatographed on a Si gel column using gradient elution (EtOAc in hexane, 10→100%). Unchanged **1** was recovered in earlier fractions (55 mg), then a white residue was obtained from later fractions. The residue was rechromatographed with the same solvent system and crystallized from hexane/EtOAc to yield metabolite **1a** (0.033 g, 22 %).

**Biotransformation of 2 with T. petrophilum** Fermentation media were filtered at the end of the 14-d fermentation period. Both the filtrate and biomass were exhaustively extracted with EtOAc to yield a brownish residue (ca. 300 mg), which was chromatographed on a silica gel column using the same gradient as before. Unchanged **2** was recovered in earlier fractions (75 mg). Late eluting semi-pure fractions were concentrated and purified by

preparative TLC to yield metabolite **2c** (0.005 g, 4%).

Metabolite **1a**, 4'-Hydroxykawain [6-(4'-Hydroxystyryl)-4-methoxy-5,6-dihydropyran-2-one]: Colorless needles; mp 165–166 °C; *R*<sub>f</sub> 0.70; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +35.1° (*c*=0.02, MeOH); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 264 (3.85) nm; IR (CHCl<sub>3</sub>)  $\nu_{\text{max}}$  3277, 3028, 2924, 2858, 1688, 1618, 1516 cm<sup>-1</sup>; <sup>1</sup>H- and <sup>13</sup>C-NMR: see Table 1; HR-ESI-MS *m/z* [M+Na]<sup>+</sup>: 269.0788 (Calcd for C<sub>14</sub>H<sub>14</sub>O<sub>4</sub>Na: 269.0789).

Metabolite **2c**, 3'-Hydroxy-4'-methoxykawain [6-(3'-Hydroxy-4'-methoxystyryl)-4-methoxy-5,6-dihydropyran-2-one]: White residue; mp 139–140 °C; *R*<sub>f</sub> 0.60; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +73.5° (*c*=0.02, MeOH); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 236 (4.05), 264 (4.01) nm; IR (CHCl<sub>3</sub>)  $\nu_{\text{max}}$  3364, 3011, 2934, 2851, 1692, 1621, 1511 cm<sup>-1</sup>; <sup>1</sup>H- and <sup>13</sup>C-NMR: see Table 1; HR-ESI-MS *m/z* [M+H]<sup>+</sup>: 277.1092 (Calcd for C<sub>15</sub>H<sub>17</sub>O<sub>5</sub>: 277.1076).

**Acknowledgments** We are grateful to Ms. Rangavalli B. Manyam for assistance with the fermentation and isolation procedures. Ms. Julie R. Mikell is acknowledged for providing the kavalactone substrates. This work was supported, in part, by the United States Department of Agriculture, Agricultural Research Service Specific Cooperative Agreement No. 58-6408-7-012.

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## Trifluoromethanesulfonic Anhydride-Promoted $\alpha$ -Bromination of Ketones with Grignard Reagent or Magnesium Bromide

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**The direct  $\alpha$ -bromination of various ketones using trifluoromethanesulfonic anhydride and Grignard reagent or magnesium bromide in ether gave the corresponding  $\alpha$ -bromo ketones in moderate to good yields under mild reaction conditions.**

**Key words** trifluoromethanesulfonic anhydride;  $\alpha$ -bromo ketones;  $\alpha$ -bromo- $\alpha,\beta$ -enones

Halogenation of ketones has been widely reported in organic syntheses. Therefore, a number of methods have been developed for this purpose. Classical methods for the bromination of ketones include the use of molecular bromine<sup>1)</sup> and copper(II) bromide.<sup>2)</sup> Halogenation is characterized by selectivity at the  $\alpha$ -carbon of the ketones in the side favorable to enolization. Therefore, the reaction is carried out in the presence of acid to increase the rate of halogenation. Under these acidic conditions,  $\alpha$ -chlorination of an unsymmetrical ketone takes place at the more highly substituted  $\alpha$ -carbon atom.<sup>3)</sup> To effect the  $\alpha$ -chlorination of a ketone at the less substituted carbon, several methods have been studied, including the initial formation of the kinetic enolate of a ketone<sup>4)</sup> and the bromination of enol trimethylsilyl ethers.<sup>5)</sup>

Selective bromination of ketones at the  $\alpha$ -position in the presence of a double bond has been achieved through the action of phenyltrimethylammonium tribromide,<sup>6)</sup> cupric bromide,<sup>7)</sup> and pyrrolidine hydrotribromide.<sup>8)</sup> Other methods reported for the preparations of  $\alpha$ -halo ketones include sulfonyl chloride,<sup>4)</sup> 2-bromo-2-cyano-*N,N*-dimethylacetamide,<sup>9)</sup> and sulfonyl chloride.<sup>10)</sup> Recently, Righi and co-workers have reported the selective transformation of cyclic enones into  $\alpha$ -halo- $\alpha,\beta$ -enones using dimethyldioxirane and metal halides/Amberlyst.<sup>11)</sup>

We have recently reported a new simple procedure for the homo-coupling reaction of Grignard reagents using trifluoromethanesulfonic anhydride ( $\text{ Tf}_2\text{O}$ ).<sup>12)</sup> On the other hand, Kotsuki has reported a strange reaction in which a triflate reacts with methylmagnesium iodide in the presence of copper(I) bromide to give the corresponding iodinated product in 54% yield.<sup>13)</sup> From these results, we envisaged that new brominating reagents could be developed by using  $\text{ Tf}_2\text{O}$  and Grignard reagents.

In this paper, we describe a convenient synthesis of  $\alpha$ -bromo ketones from the corresponding ketones using  $\text{ Tf}_2\text{O}$  and Grignard reagent or  $\text{ MgBr}_2$ . The scope and limitations of this procedure are also discussed.

### Results and Discussion

In an effort to arrive at the optimum reaction conditions for the bromination, different reaction conditions (addition order of ketone, solvent and brominating reagent) were varied for the bromination of cyclohexanone. Method A:  $\text{ Tf}_2\text{O}$  was added over a few minutes to a stirred  $\text{ CH}_2\text{Cl}_2$  or ether solution of cyclohexanone kept at 0 °C under a balloon of nitrogen. After 1 h, methylmagnesium bromide ( $\text{ MeMgBr}$ ) was

added, and the mixture was stirred for 6 h at room temperature. Method B: A mixture of  $\text{ Tf}_2\text{O}$  and brominating reagent in  $\text{ CH}_2\text{Cl}_2$  or ether was stirred under a balloon of nitrogen. After 10 min, cyclohexanone was added to the mixture.

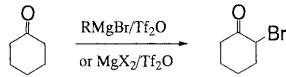
Table 1 summarizes the results of the  $\alpha$ -bromination of cyclohexanone under various reaction conditions. When cyclohexanone was treated with  $\text{ MeMgBr}$  in the presence of  $\text{ Tf}_2\text{O}$  in  $\text{ CH}_2\text{Cl}_2$  in method A, a low yield of 2-bromocyclohexanone was obtained (10%); the major product of the reaction was 1-cyclohexenyl triflate (41%, entry 1).<sup>14)</sup> The solvent was critical to the success of this reaction. Thus, the reaction in ether in place of  $\text{ CH}_2\text{Cl}_2$  proceeded much more smoothly, giving the 2-bromocyclohexanone in a yield of 53% (entry 2). However, the reaction of cyclohexanone with  $\text{ MeMgBr}/\text{ Tf}_2\text{O}$  in ether (method B) gave 2-bromocyclohexanone in 92% yield within 10 min at 0 °C (entry 3). A stoichiometric amount of  $\text{ Tf}_2\text{O}$  was necessary for the reaction. After treatment of cyclohexanone with 0.5 and 0.2 eq of  $\text{ Tf}_2\text{O}$ , the yields of 2-bromocyclohexanone decreased to 44 and 6%, respectively (entries 4, 5). In place of ether the use of  $\text{ CH}_2\text{Cl}_2$  gave only 2-bromocyclohexanone in 3% yield and unreacted cyclohexanone in 97% yield, even when the reaction was carried out at room temperature for 1 h (entry 6). On the other hand, treatment of cyclohexanone with phenylmagnesium bromide ( $\text{ PhMgBr}$ ) gave 2-bromocyclohexanone in 33% yield along with biphenyl (entry 7).<sup>12)</sup>

Many authors have reported that the Grignard reagent in solution either consists in part of a dimeric species or contains a mixture of dialkylmagnesium and  $\text{ MgX}_2$ .<sup>15,16)</sup> Therefore, we attempted to brominate cyclohexanone using  $\text{ MgBr}_2$  in the presence of  $\text{ Tf}_2\text{O}$ . In the first example, the treatment of cyclohexanone in ether with 1.1 eq of  $\text{ MgBr}_2$  and 0.5 eq of  $\text{ Tf}_2\text{O}$  at 0 °C for 10 min gave 2-bromocyclohexanone in 92% yield (entry 8). The use of 0.5 eq of  $\text{ MgBr}_2$  and 0.5 eq of  $\text{ Tf}_2\text{O}$  resulted in a lower yield of 2-bromocyclohexanone. Attempts to effect the bromination of cyclohexanone using  $\text{ CH}_2\text{Cl}_2$  resulted in a low yield (entry 10). Unfortunately, in the case of a chlorination of cyclohexanone with  $\text{ Tf}_2\text{O}/\text{ MgCl}_2$  in ether, no chlorination product is obtained, and only the starting cyclohexanone was recovered (entry 11).

In order to show the efficiency of method B in ether, we extended the reaction to other substrates and explored the generality of the reaction. As shown in Table 2, the reaction of acetophenone with 1.1 eq of  $\text{ MgBr}_2$  and 0.5 eq of  $\text{ Tf}_2\text{O}$  at 0 °C gave  $\alpha$ -bromoacetophenone in 79% yield (entry 2). The reaction was completed in 10 min. On the other hand, treat-

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Table 1.  $\alpha$ -Bromination of Cyclohexanone with  $\text{TiF}_2\text{O}$  and Grignard Reagents or  $\text{MgX}_2$ 

|  |                          |        |                           |                           |                                |               |                         |
|-----------------------------------------------------------------------------------|--------------------------|--------|---------------------------|---------------------------|--------------------------------|---------------|-------------------------|
| Entry                                                                             | Solvent                  | Method | Halogenating reagent (eq) | $\text{TiF}_2\text{O}$ eq | Reaction temp $^\circ\text{C}$ | Reaction time | Yield <sup>a)</sup> (%) |
| 1                                                                                 | $\text{CH}_2\text{Cl}_2$ | A      | $\text{MeMgBr}$ (1.5)     | 1.5                       | r.t.                           | 6 h           | 10 <sup>b)</sup>        |
| 2                                                                                 | $\text{Et}_2\text{O}$    | A      | $\text{MeMgBr}$ (1.5)     | 1.5                       | r.t.                           | 6 h           | 53                      |
| 3                                                                                 | $\text{Et}_2\text{O}$    | B      | $\text{MeMgBr}$ (1.0)     | 1.2                       | 0                              | 10 min        | 92                      |
| 4                                                                                 | $\text{Et}_2\text{O}$    | B      | $\text{MeMgBr}$ (1.0)     | 0.5                       | 0                              | 10 min        | 44                      |
| 5                                                                                 | $\text{Et}_2\text{O}$    | B      | $\text{MeMgBr}$ (1.0)     | 0.2                       | 0                              | 10 min        | 6                       |
| 6                                                                                 | $\text{CH}_2\text{Cl}_2$ | B      | $\text{MeMgBr}$ (1.0)     | 1.2                       | r.t.                           | 1 h           | 3                       |
| 7                                                                                 | $\text{Et}_2\text{O}$    | B      | $\text{PhMgBr}$ (1.0)     | 1.2                       | 0                              | 10 min        | 33 <sup>c)</sup>        |
| 8                                                                                 | $\text{Et}_2\text{O}$    | B      | $\text{MgBr}_2$ (1.1)     | 0.5                       | 0                              | 10 min        | 92 (89)                 |
| 9                                                                                 | $\text{Et}_2\text{O}$    | B      | $\text{MgBr}_2$ (0.5)     | 0.5                       | 0                              | 10 min        | 50                      |
| 10                                                                                | $\text{CH}_2\text{Cl}_2$ | B      | $\text{MgBr}_2$ (1.1)     | 0.5                       | 0                              | 10 min        | 11                      |
| 11                                                                                | $\text{Et}_2\text{O}$    | B      | $\text{MgCl}_2$ (1.1)     | 0.5                       | 0                              | 10 min        | 0 <sup>d)</sup>         |
| 12                                                                                | $\text{Et}_2\text{O}$    | B      | $\text{MgBr}_2$ (1.1)     | —                         | 0                              | 20 min        | 0 <sup>d)</sup>         |

a) Determined by GLC analysis. Value in parenthesis indicates yield after purification. b) The major product is 1-cyclohexenyl triflate (41%). c) A small amount of biphenyl (6%) was produced. d) No reaction occurred; only starting materials remained according to GLC.

Table 2. Bromination of Ketones with  $\text{TiF}_2\text{O}$  and  $\text{MeMgBr}$  or  $\text{MgBr}_2$ 

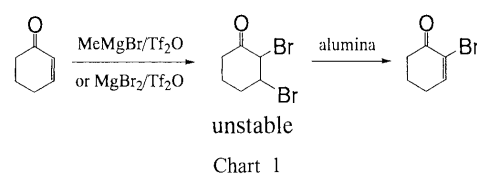
| Entry | Substrate                                                                           | Brominating reagent (eq) | $\text{TiF}_2\text{O}$ eq | Reaction temp $^\circ\text{C}$ | Reaction time | Yield <sup>a)</sup> (%)             |
|-------|-------------------------------------------------------------------------------------|--------------------------|---------------------------|--------------------------------|---------------|-------------------------------------|
| 1     |   | $\text{MeMgBr}$ (1.2)    | 1.2                       | r. t.                          | 3 h           | 74                                  |
| 2     |  | $\text{MgBr}_2$ (1.1)    | 0.5                       | 0                              | 10 min        | 79                                  |
| 3     |  | $\text{MeMgBr}$ (2.0)    | 2.4                       | r. t.                          | 10 min        | 85                                  |
| 4     |  | $\text{MgBr}_2$ (1.1)    | 0.5                       | 0                              | 10 min        | 79                                  |
| 5     |  | $\text{MeMgBr}$ (1.0)    | 1.2                       | r. t.                          | 4 h           | 88                                  |
| 6     |  | $\text{MgBr}_2$ (1.1)    | 0.5                       | 0                              | 10 min        | 91                                  |
| 7     |  | $\text{MeMgBr}$ (2.0)    | 2.4                       | 30                             | 2 h           | 84                                  |
| 8     |  | $\text{MgBr}_2$ (1.1)    | 0.5                       | 0                              | 10 min        | 77                                  |
| 9     |  | $\text{MeMgBr}$ (1.2)    | 2.4                       | r. t.                          | 40 h          | 20 <sup>b)</sup> (25) <sup>c)</sup> |
| 10    |  | $\text{MgBr}_2$ (1.1)    | 0.5                       | 0                              | 10 min        | 92 <sup>b)</sup> (98) <sup>c)</sup> |
| 11    |  | $\text{MgBr}_2$ (1.1)    | 0.5                       | r. t.                          | 10 min        | 80 <sup>b)</sup> (86) <sup>c)</sup> |
| 12    |  | $\text{MgBr}_2$ (1.1)    | 0.5                       | r. t.                          | 10 min        | 80 <sup>b)</sup> (82) <sup>c)</sup> |
| 13    |  | $\text{MgBr}_2$ (1.1)    | —                         | r. t.                          | 20 min        | 0 <sup>d)</sup>                     |
| 14    |  | $\text{MgBr}_2$ (1.1)    | 0.5                       | r. t.                          | 10 min        | 82 <sup>e)</sup>                    |

a) Isolated yields. b) Isolated yields of  $\alpha$ -bromo- $\alpha,\beta$ -enones. c) Values in parentheses indicate GLC yield of crude  $\alpha,\beta$ -dibromo ketones. These compounds are unstable. d) No reaction occurred; only starting materials remained according to GLC. e) Yield of 1,2-dibromocyclohexane.

ment with  $\text{MeMgBr}$  as a brominating reagent was less effective, and the reaction required longer reaction times and a higher temperature to attain a comparable yield (entry 1). The treatment of cycloheptanone or 4-heptanone with  $\text{TiF}_2\text{O}/\text{MeMgBr}$  or  $\text{TiF}_2\text{O}/\text{MgBr}_2$  afforded the corresponding  $\alpha$ -bromo ketones in a good yield (entries 3—6).

The substituent effects in the  $\alpha$ -position of ketone on the  $\alpha$ -bromination were also examined. The bromination of 2-methylcyclohexanone with  $\text{MeMgBr}$  or  $\text{MgBr}_2$  in the presence of  $\text{TiF}_2\text{O}$  provided 2-bromo-2-methylcyclohexanone in 84 and 77% yields, respectively. In each case, 2-bromo-6-methylcyclohexanone was formed in 6 and 9% yields.

Entries 9—12 show the results for the bromination of  $\alpha,\beta$ -enones. The treatment of 2-cyclohexen-1-one with  $\text{MgBr}_2/\text{TiF}_2\text{O}$  afforded 2,3-dibromocyclohexanone (entry 10). Separation



tion by alumina chromatography resulted in 2-bromo-2-cyclohexen-1-one obtained by dehydrobromination in 92% yield.  $\alpha,\beta$ -Dibromo ketones showed a strong tendency to decompose and were not isolated in analytically pure forms. In most cases, the  $\alpha,\beta$ -dibromo ketones were fairly pure; their structures were determined unambiguously by spectral methods. A similar dehydrobromination has been observed for the bromination of  $\alpha,\beta$ -enones with bromine<sup>17)</sup> and oxone (2

$\text{KHSO}_5 \cdot \text{KHSO}_4 \cdot \text{K}_2\text{SO}_4/\text{NaBr}$ ).<sup>18)</sup> On the other hand, bromination with  $\text{MeMgBr}/\text{Tf}_2\text{O}$  was less effective than with  $\text{MgBr}_2/\text{Tf}_2\text{O}$  for the formation of  $\alpha$ -bromo- $\alpha,\beta$ -enones (entry 9).

In order to determine the active species of the present method, we examined the bromination of simple alkene with the two methods (entries 13, 14). Cyclohexene afforded excellent yields of the 1,2-dibromocyclohexane upon treatment with  $\text{MgBr}_2/\text{Tf}_2\text{O}$ . Direct treatment of cyclohexene with  $\text{MgBr}_2$  without  $\text{Tf}_2\text{O}$  did not afford any 1,2-dibromocyclohexane. The addition of  $\text{Br}_2$  to cyclohexene suggests that this combination of  $\text{MgBr}_2/\text{Tf}_2\text{O}$  provides an *in situ* generation of  $\text{Br}_2$ .

Finally, we examined the addition of  $\text{Br}_2$  alone to ketone. The reaction of cyclohexanone in ether with 1.1 eq of  $\text{Br}_2$  in  $\text{CHCl}_3$  at  $0^\circ\text{C}$  for 10 min gave 2-bromocyclohexanone and dibrominated cyclohexanone as a 1 : 2 mixture.

## Conclusion

We have described a new procedure for the bromination of ketones in a regioselective fashion by reaction of  $\text{Tf}_2\text{O}$  with  $\text{MeMgBr}$  or  $\text{MgBr}_2$  followed by addition of the ketone. The bromination occurs at the more substituted carbon atom of an unsymmetrical ketone and yields are good. The procedure can also be used to convert  $\alpha,\beta$ -enones into  $\alpha$ -bromo- $\alpha,\beta$ -enones by addition of  $\text{Br}_2$  followed by elimination of  $\text{HBr}$ .

The mechanism of this reaction is not yet understood. The addition of  $\text{Br}_2$  to cyclohexene suggests that the combination of  $\text{MeMgBr}/\text{Tf}_2\text{O}$  or  $\text{MgBr}_2/\text{Tf}_2\text{O}$  reagents provides an *in situ* generation of  $\text{Br}_2$ . Further studies towards identifying the actual reactive species of this bromination reaction of ketones are under current investigation.

## Experimental

**General Methods** NMR spectra were recorded in  $\text{CDCl}_3$  on a JEOL GSX-400 spectrometer operating at 400 and 100 MHz for  $^1\text{H}$  and  $^{13}\text{C}$ , respectively. Chemical shifts for NMR spectra were referenced to internal  $(\text{CH}_3)_4\text{Si}$ . Mass spectra were measured with a Perkin-Elmer model 910 spectrometer operating in the electron impact mode (70 eV). IR spectra were recorded on a Perkin-Elmer model 1600 spectrophotometer. Gas chromatography was carried out on a 20 m  $\times$  0.22 mm capillary column packed with methyl silicone.

**General Procedure** To a solution of  $\text{MgBr}_2 \cdot \text{Et}_2\text{O}$  (1.1 mmol) in 5 ml of dry ether was added  $\text{Tf}_2\text{O}$  (0.5 mmol) under stirring at  $0^\circ\text{C}$ . The clear solution immediately turned yellow-orange. After stirring at  $0^\circ\text{C}$  for 10 min, cycloheptanone (1 mmol) was slowly added in 5 ml of dry ether and further stirred for 10 min at  $0^\circ\text{C}$ . The reaction was quenched with water and the organic portion was extracted with ether, washed with  $\text{NaHCO}_3$  solution, and dried over  $\text{MgSO}_4$ . The solvent was evaporated under reduced pressure to give a light yellow oil, and the crude oil was purified by preparative TLC (alumina, ether:hexane = 1 : 1) to yield 2-bromocycloheptanone (0.15 g, 79%).<sup>19)</sup>  $^1\text{H}$ -NMR  $\delta$ : 1.33—1.43 (m, 1H), 1.51—1.63 (m, 2H), 1.72—1.81 (m, 1H), 1.91—2.06 (m, 3H), 2.33—2.41 (m, 1H), 2.47—2.53 (m, 1H), 4.38 (dd,  $J=9.5, 5.1$  Hz, 1H);  $^{13}\text{C}$ -NMR  $\delta$ : 25.0, 26.8, 29.6, 34.3, 39.4, 53.7, 206.2;  $m/z$  (rel. intensity): 192 ( $\text{M}^+$ , 7), 190 ( $\text{M}^+$ , 7), 112 (12), 111 (74), 93 (22), 84 (44), 83 (66), 69 (35), 67 (35), 56 (33), 55 (100).

**2-Bromocyclohexanone**<sup>9)</sup> Pale yellow oil.  $^1\text{H}$ -NMR  $\delta$ : 1.70—1.78 (m, 1H), 1.80—1.88 (m, 1H), 1.91—2.08 (m, 2H), 2.18—2.27 (m, 1H), 2.30—2.38 (m, 2H), 2.93—3.01 (m, 1H), 4.44—4.48 (m, 1H);  $^{13}\text{C}$ -NMR  $\delta$ : 22.2, 26.8, 36.8, 38.0, 53.5, 203.4; MS  $m/z$  (rel. intensity): 178 ( $\text{M}^+$ , 11), 176 ( $\text{M}^+$ , 11), 134 (9), 132 (9), 97 (62), 69 (46), 55 (100).

**2-Bromoacetophenone**<sup>9)</sup> mp  $49.2$ — $50.6^\circ\text{C}$ .  $^1\text{H}$ -NMR  $\delta$ : 4.48 (s, 2H), 7.46—7.50 (m, 2H), 7.58—7.61 (m, 1H), 7.96—7.98 (m, 2H);  $^{13}\text{C}$ -NMR  $\delta$ : 31.1, 128.8, 128.9, 133.9, 191.2; MS  $m/z$  (rel. intensity): 200 ( $\text{M}^+$ , 3), 198 ( $\text{M}^+$ , 3), 106 (8), 105 (100), 91 (10), 77 (48).

**3-Bromo-4-heptanone**<sup>20)</sup> Pale yellow oil.  $^1\text{H}$ -NMR  $\delta$ : 0.94 (t,  $J=7.3$  Hz, 3H), 1.02 (t,  $J=7.3$  Hz, 3H), 1.61—1.70 (m, 2H), 1.91—2.00 (m, 1H),

2.00—2.08 (m, 1H), 2.57—2.74 (m, 2H), 4.19 (dd,  $J=8.1, 6.6$  Hz, 1H);  $^{13}\text{C}$ -NMR  $\delta$ : 12.0, 13.6, 17.4, 26.9, 40.9, 55.5, 204.2; MS  $m/z$  (rel. intensity): 71 (89), 55 (13), 43 (100).

**2-Bromo-2-methylcyclohexanone**<sup>5)</sup> Pale yellow oil.  $^1\text{H}$ -NMR  $\delta$ : 1.57—1.65 (m, 1H), 1.75—1.81 (m, 2H), 1.82 (s, 3H), 2.03—2.10 (m, 2H), 2.33—2.41 (m, 2H), 3.17—3.26 (m, 1H);  $^{13}\text{C}$ -NMR  $\delta$ : 22.3, 26.8, 28.1, 36.7, 43.6, 65.8, 204.6; MS  $m/z$  (rel. intensity): 192 ( $\text{M}^+$ , 3), 190 ( $\text{M}^+$ , 3), 148 (13), 146 (13), 111 (20), 83 (17), 69 (10), 67 (22), 55 (100).

**2,3-Dibromocyclohexanone**<sup>21)</sup> Pale yellow oil.  $^1\text{H}$ -NMR  $\delta$ : 1.65—2.07 (m, 1H), 2.08—2.11 (m, 1H), 2.23—2.41 (m, 2H), 2.62—2.72 (m, 1H), 3.04—3.13 (m, 1H), 4.50—4.51 (m, 1H), 4.72—4.75 (m, 1H);  $^{13}\text{C}$ -NMR  $\delta$ : 21.5, 27.2, 35.1, 50.6, 53.1, 200.6; MS  $m/z$  (rel. intensity): 177 (53), 175 (55), 121 (16), 119 (16), 95 (19), 66 (63), 67 (100).

**2-Bromo-2-cyclohexen-1-one**<sup>22)</sup> mp  $75.2$ — $76.1^\circ\text{C}$ .  $^1\text{H}$ -NMR  $\delta$ : 2.06—2.12 (m, 2H), 2.46—2.50 (m, 2H), 2.61—2.65 (m, 2H), 7.45 (t,  $J=4.8$  Hz, 1H);  $^{13}\text{C}$ -NMR  $\delta$ : 22.7, 28.4, 38.4, 123.8, 151.3, 191.2; MS  $m/z$  (rel. intensity): 176 ( $\text{M}^+$ , 31), 174 ( $\text{M}^+$ , 33), 148 (33), 146 (33), 135 (9), 133 (10), 68 (9), 67 (67), 55 (44), 41 (27), 39 (100).

**3,4-Dibromo-2-heptanone** Pale yellow oil.  $^1\text{H}$ -NMR  $\delta$ : 0.98 (t,  $J=7.3$  Hz, 3H), 1.48—1.56 (m, 1H), 1.60—1.72 (m, 1H), 1.77—1.87 (m, 1H), 2.17—2.25 (m, 1H), 2.37 (s, 3H), 4.34—4.39 (m, 1H), 4.50 (d,  $J=11.0$  Hz, 1H);  $^{13}\text{C}$ -NMR  $\delta$ : 13.3, 19.6, 26.5, 37.4, 51.5, 54.2, 198.5; MS  $m/z$  (rel. intensity): 150 (25), 148 (25), 121 (6), 119 (6), 97 (11), 93 (5), 70 (6), 69 (100).

**3-Bromo-3-hepten-2-one** Pale yellow oil.  $^1\text{H}$ -NMR  $\delta$ : 0.93 (t,  $J=7.3$  Hz, 3H), 1.53—1.62 (m, 2H), 2.37—2.45 (m, 2H), 2.46 (s, 3H), 7.16 (t,  $J=7.0$  Hz, 1H);  $^{13}\text{C}$ -NMR  $\delta$ : 13.9, 21.1, 26.5, 34.6, 127.7, 145.9, 191.8; MS  $m/z$  (rel. intensity): 192 ( $\text{M}^+$ , 60), 190 ( $\text{M}^+$ , 61), 177 (52), 175 (53), 151 (61), 149 (62), 135 (28), 133 (29), 111 (36), 96 (41), 95 (44), 82 (23), 69 (20), 67 (50), 55 (19), 53 (41), 43 (100); IR (neat,  $\text{cm}^{-1}$ ): 2960, 2928, 2896, 1686, 1617, 1609, 1458, 1357, 1242, 1214; Anal. Calcd. for  $\text{C}_7\text{H}_{11}\text{OBr}$ : C 44.00, H 5.80; found: C 44.28, H 5.74.

**2,3-Dibromo-1-phenyl-1-butanone**<sup>23)</sup> Pale yellow oil.  $^1\text{H}$ -NMR  $\delta$ : 2.01 (d,  $J=7.0$  Hz, 3H), 4.73—4.77 (m, 1H), 5.40 (d,  $J=10.6$  Hz, 1H), 7.47—7.51 (m, 2H), 7.59—7.63 (m, 1H), 8.01—8.02 (m, 2H);  $^{13}\text{C}$ -NMR  $\delta$ : 24.3, 44.9, 128.8, 128.9, 133.9, 191.3; MS  $m/z$  (rel. intensity): 106 (8), 105 (100), 77 (31).

**2-Bromo-1-phenyl-2-buten-1-one**<sup>24)</sup> Pale yellow oil.  $^1\text{H}$ -NMR  $\delta$ : 2.05 (d,  $J=7.0$  Hz, 3H), 6.91 (q,  $J=7.0$  Hz, 1H), 7.41—7.68 (m, 5H);  $^{13}\text{C}$ -NMR  $\delta$ : 18.4, 128.4, 129.5, 132.4, 136.7, 143.6, 190.5; MS  $m/z$  (rel. intensity): 226 ( $\text{M}^+$ , 7), 224 ( $\text{M}^+$ , 7), 145 (29), 104 (8), 105 (100), 77 (51).

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## Synthesis and Evaluation of Novel 2-Oxo-1,2-dihydro-3-quinolinecarboxamide Derivatives as Serotonin 5-HT<sub>4</sub> Receptor Agonists

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Received June 22, 2000; accepted September 8, 2000

A series of *N*-azabicycloalkyl-1-alkyl-2-oxo-1,2-dihydro-3-quinolinecarboxamides were synthesized and tested for serotonin 5-HT<sub>4</sub> receptor-stimulating effects in the regulation of electrically-evoked contraction in guinea pig muscle. Among them, *N*-azabicycloalkyl-1-isopropyl-2-oxo-1,2-dihydro-3-quinolinecarboxamide (8c, 9c, 10c, 11c, 12c) exhibited potent serotonin 5-HT<sub>4</sub> receptor-stimulating activity. The most potent compound, *N*-(endo-8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-1-isopropyl-2-oxo-1,2-dihydro-3-quinolinecarboxamide (8c, ED<sub>50</sub> = 36.3 nM), was seven times as active as cisapride, while 8c had no affinity for 5-HT<sub>1A</sub>, 5-HT<sub>1D</sub>, D<sub>2</sub>, muscarinic M<sub>2</sub> or muscarinic M<sub>3</sub> receptors even at 10 μM. Compound 8c stimulated digestive tract motility in conscious fed dogs (1.0 mg/kg *p.o.*).

**Key words** Quinolinecarboxamide; serotonin 5-HT<sub>4</sub> receptor-stimulating activity; structure-activity relationship

Serotonin (5-HT) is a neurotransmitter which is widely distributed in humans and has a remarkable variety of physiological effects. Seven subtypes of 5-HT receptors have been reported.<sup>1,2)</sup> 5-HT<sub>4</sub> receptors<sup>3)</sup> modulate cholinergic nerve-mediated contraction in the guinea pig ileum<sup>4,5)</sup> and in the proximal colon, potentiate electrical field stimulation in the guinea pig ileum,<sup>6)</sup> and induce of chloride secretion in rat distal colon.<sup>7)</sup> These findings suggest that the 5-HT<sub>4</sub> receptors in the gut play roles in the induction and maintenance of gastrointestinal motility, and that 5-HT<sub>4</sub> receptor agonists activate gastrointestinal motor function and improve gastrointestinal dysfunction or conditions accompanied by motility failure. In fact, cisapride<sup>8)</sup> and renzapride,<sup>9)</sup> which stimulate 5-HT<sub>4</sub> receptors, are reported to increase gastrointestinal motor function and to improve gastrointestinal conditions such as heartburn, anorexia, bowel pain, and abdominal distension accompanied by chronic gastritis, diabetes mellitus or postoperative gastroparesis.

Three classes of compounds stimulating 5-HT<sub>4</sub> receptors are known,<sup>10)</sup> 1) the indolealkylamines (5-HT, 5-methoxytryptamine), 2) the benzamides (metoclopramide,<sup>11)</sup> cisapride, renzapride, zacopride<sup>12,13)</sup>, and 3) the benzimidazolones (BIMU-8<sup>14)</sup>) (Fig. 1). Generally, these agents act on other monoamine receptors as well. For example, cisapride and metoclopramide are used clinically as 5-HT<sub>4</sub> agonists, but both are apt to induce extrapyramidal syndrome as a result of D<sub>2</sub> receptor antagonism.<sup>15,16)</sup> Mosapride is modified at a tertiary amine in order to reduce extrapyramidal syndrome.<sup>17)</sup> There are many reports on the modification of tertiary amine moieties such as alkylamine, cyclic amine and azabicyclo structures. Among these amino moieties, azabicyclo structures exhibit potent and selective serotonin 5-HT<sub>4</sub> receptor-stimulating activity.<sup>18)</sup> There are few reports, however, concerning modifications in the aromatic ring moiety of compounds stimulating 5-HT<sub>4</sub> receptors. In the present study, we report the synthesis and 5-HT<sub>4</sub> receptor-stimulating activity of 2-oxo-1,2-dihydro-3-quinolinecarboxamide derivatives having azabicycloamine structures with the aim of finding a new class of heterocyclic compounds that stimulate 5-HT<sub>4</sub> receptors.

**Chemistry** Compounds **5a–e** were prepared by the following synthetic pathway depicted in Chart 1. 2-Nitrobenzaldehyde **1** was condensed with diethyl malonate followed by a reduction of the nitro group to give 2-oxo-1,2-dihydro-3-quinolinecarboxylate **3**. Alkylation of **3** with appropriate

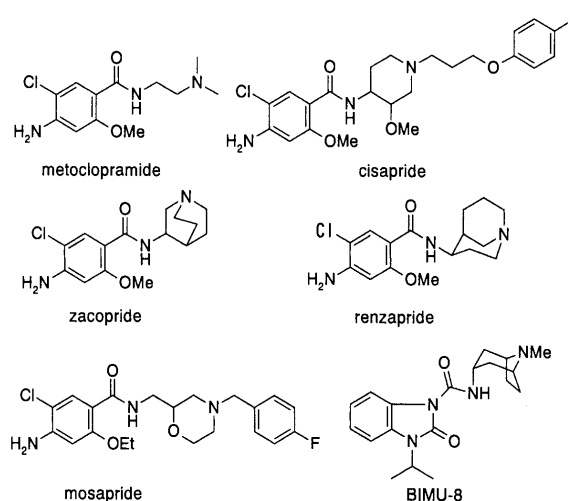
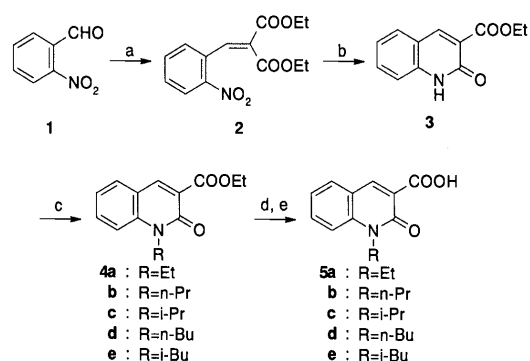


Fig. 1



Reagents: (a) diethyl malonate, NaHCO<sub>3</sub>, Ac<sub>2</sub>O; (b) Fe, AcOH;  
(c) R-I, NaH, DMF; (d) NaOH, aq. MeOH

Chart 1

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alkylhalides and sodium hydride in DMF led to a mixture of the *N*-alkyl isomers **4a—e** and *O*-alkyl isomers, which were separated by column chromatography. The *N*-alkyl isomers **4a**, **4b** and **4d** were obtained as major products. When isopropyl and isobutyl iodides were used as alkylating agents, *O*-alkyl isomers were obtained as the major products; the reaction of **3** and isopropyl iodide yielded the *N*-isopropyl isomer **4c** in 6.5% yield, along with the *O*-isopropyl isomer **6c**

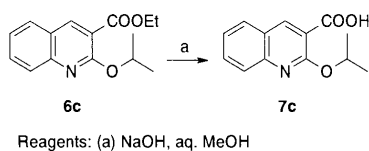
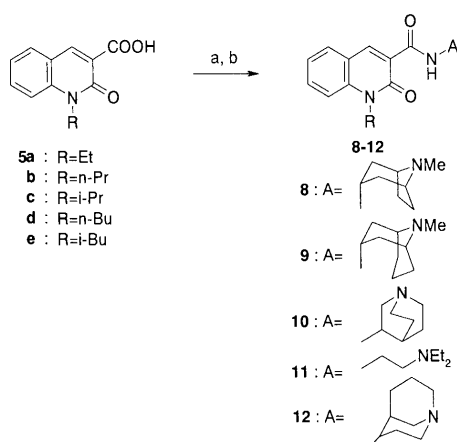
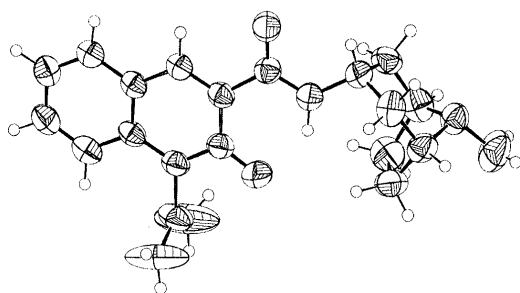


Chart 2



Reagents: (a) SOCl<sub>2</sub>, toluene; (b) H<sub>2</sub>N-A

Chart 3

Fig. 2. X-Ray Structure of Compound **8c**

in 53.0% yield. Both isomers were hydrolyzed to the corresponding carboxylic acids (Chart 1 and Chart 2). Carboxylic acids **5c** and **7c** could be distinguished by IR spectra. Carboxylic acid **5c** exhibited absorption around 1734 cm<sup>-1</sup>, whereas **7c** exhibited absorption around 1709 cm<sup>-1</sup>. Treatment of carboxylic acids (**5a—e**) with thionyl chloride in toluene, followed by coupling with appropriate amines yielded the carboxamide derivatives (**8a—e**, **9c**, **10c**, **11c**, **12c**), as depicted in Chart 3.

As mentioned above, alkylation yielded two isomers, *O*-alkyl and *N*-alkyl compounds. In order to clarify the structures of the alkylated compounds, compound **8c** was examined by X-ray crystallographic analysis. As shown in Fig. 2, **8c** was determined to be a *N*-isopropyl compound. Taking into account the IR spectra, **5a**, **5b**, **5d**, **5e** were shown to be *N*-alkyl isomers.

## Results and Discussion

The 5-HT<sub>4</sub> receptor-stimulating activities of the carboxamide derivatives were evaluated in the regulation of electrically-evoked contractions in guinea pig muscle. The 50% effective doses (ED<sub>50</sub>) of these compounds are shown in Table 1. Four azabicyclo structures, 8-azabicyclo[3.2.1]oct-3-yl (a), 9-azabicyclo[3.3.1]non-3-yl (b), 3-quinuclidinyl (c) and 1-azabicyclo[3.3.1]non-4-yl (e), and one straight chain, *N,N*-diethylaminoethyl (d), were selected for examination. Azabicyclo[3.3.1]non-3-yl (b) is an amine moiety of granisetron,<sup>19)</sup> a 5-HT<sub>3</sub> receptor antagonist. We selected its 9-azabicyclo[3.3.1]non-3-yl moiety as an azabicyclo structure because of its structural similarity to 8-azabicyclo[3.2.1]octane. We selected the 8-azabicyclo[3.2.1]oct-3-yl (a) moiety as an amine moiety to examine the effect of alkyl substituents on the 1-position of the quinoline ring. 1-Alkyl groups strongly affect ED<sub>50</sub> values. When operated with straight-chain alkyl groups, **8a** having an ethyl group showed potent activity (ED<sub>50</sub>=138 nm). Branched-chain alkyl groups yielded better ED<sub>50</sub> values than straight-chain alkyl groups. Iso-butyl (**8e**, ED<sub>50</sub>=974 nm) and iso-propyl (**8c**, ED<sub>50</sub>=36.3 nm) groups yielded better ED<sub>50</sub> values than the corresponding *n*-butyl (**8d**, ED<sub>50</sub>>3000 nm) and *n*-propyl (**8b**, ED<sub>50</sub>=413 nm). The iso-propyl group appeared to be the best substituent for the 1-position. We next investigated the effect of the tertiary amine moiety. With iso-propyl as the alkyl group on the quinoline 1-position, all of five derivatives (**8c**, **9c**, **10c**, **11c**, **12c**) exhibited good 5-HT<sub>4</sub> receptor-stimulating activity. The

Table 1. Physicochemical Data and 5-HT<sub>4</sub> Receptor-Stimulating Activities of the Synthesized Compounds (**8a—e**, **9c**, **10c**, **11c** and **12c**)

| Compound   | mp<br>(°C) | Recrystallization<br>solvent | Formula                                                                                                                                          | Calcd |      |       | Found |      |       | ED <sub>50</sub><br>(nm) |
|------------|------------|------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|-------|------|-------|-------|------|-------|--------------------------|
|            |            |                              |                                                                                                                                                  | C     | H    | N     | C     | H    | N     |                          |
| <b>8a</b>  | 142—144    | AcOEt                        | C <sub>20</sub> H <sub>25</sub> N <sub>3</sub> O <sub>2</sub> ·0.4 H <sub>2</sub> O                                                              | 69.29 | 7.50 | 12.12 | 69.23 | 7.43 | 12.17 | 138                      |
| <b>8b</b>  | 151—153    | AcOEt                        | C <sub>21</sub> H <sub>27</sub> N <sub>3</sub> O <sub>2</sub>                                                                                    | 71.36 | 7.70 | 11.88 | 71.20 | 7.61 | 11.80 | 413                      |
| <b>8c</b>  | 173—177    | AcOEt                        | C <sub>21</sub> H <sub>27</sub> N <sub>3</sub> O <sub>2</sub>                                                                                    | 71.36 | 7.70 | 11.88 | 71.21 | 7.67 | 11.84 | 36.3                     |
| <b>8d</b>  | 150—152    | AcOEt                        | C <sub>22</sub> H <sub>29</sub> N <sub>3</sub> O <sub>2</sub>                                                                                    | 71.90 | 7.95 | 11.43 | 71.63 | 7.80 | 11.16 | >3000                    |
| <b>8e</b>  | 234—237    | IPA-hexane                   | C <sub>22</sub> H <sub>29</sub> N <sub>3</sub> O <sub>2</sub> ·C <sub>4</sub> H <sub>4</sub> O <sub>4</sub> ·0.25 H <sub>2</sub> O <sup>a)</sup> | 63.98 | 6.92 | 8.61  | 64.07 | 6.92 | 8.38  | 974                      |
| <b>9c</b>  | 184—187    | AcOEt                        | C <sub>22</sub> H <sub>29</sub> N <sub>3</sub> O <sub>2</sub> ·0.2 H <sub>2</sub> O                                                              | 71.21 | 7.99 | 11.32 | 70.97 | 7.93 | 11.20 | 122                      |
| <b>10c</b> | 127—129    | AcOEt                        | C <sub>20</sub> H <sub>25</sub> N <sub>3</sub> O <sub>2</sub> ·0.3 H <sub>2</sub> O                                                              | 69.66 | 7.48 | 12.19 | 69.89 | 7.22 | 11.79 | 330                      |
| <b>11c</b> | 209—213    | MeOH-AcOEt                   | C <sub>19</sub> H <sub>27</sub> N <sub>3</sub> O <sub>2</sub> ·HCl·0.3 H <sub>2</sub> O                                                          | 61.46 | 7.76 | 11.31 | 61.70 | 7.46 | 11.23 | 433                      |
| <b>12c</b> | 229—231    | AcOEt                        | C <sub>21</sub> H <sub>27</sub> N <sub>3</sub> O <sub>2</sub>                                                                                    | 71.36 | 7.69 | 11.88 | 71.07 | 7.60 | 11.69 | 390                      |
| Cisapride  |            |                              |                                                                                                                                                  |       |      |       |       |      |       | 271                      |

a) Maleic acid salt.

Table 2. Physicochemical Data of the Synthesized Compounds (**8a–e**, **9c**, **10c**, **11c** and **12c**)

| Compound   | IR (KBr)<br>(cm <sup>-1</sup> )                      | MS <sup>a)</sup><br>(m/z)   | <sup>1</sup> H-NMR $\delta$<br>(ppm)                                                                                                                                                                                                                                                                                                                                                                                  |
|------------|------------------------------------------------------|-----------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>8a</b>  | 3245, 2935, 1675,<br>1619, 1541                      | 339 (M <sup>+</sup> )       | (CDCl <sub>3</sub> ): 1.41 (3H, t, <i>J</i> =7.2 Hz), 1.75–1.88 (2H, m), 2.01–2.27 (4H, m), 2.27–2.45 (2H, m), 2.36 (3H, s), 3.25–3.35 (2H, m), 4.30 (1H, q, <i>J</i> =7.0 Hz), 4.44 (2H, q, <i>J</i> =7.2 Hz), 7.31 (1H, dt, <i>J</i> =1.6, 7.2 Hz), 7.43 (1H, d, <i>J</i> =8.6 Hz), 7.68 (1H, ddd, <i>J</i> =1.6, 7.2, 8.6 Hz), 7.77 (1H, dd, <i>J</i> =1.6, 7.2 Hz), 8.88 (1H, s), 10.62 (1H, d, <i>J</i> =7.4 Hz) |
| <b>8b</b>  | 3240, 2958, 2927,<br>1666, 1611, 1583,<br>1566, 1529 | 353 (M <sup>+</sup> )       | (CDCl <sub>3</sub> ): 1.08 (3H, t, <i>J</i> =7.2 Hz), 1.70–1.95 (4H, m), 2.00–2.27 (4H, m), 2.27–2.48 (2H, m), 2.37 (3H, s), 3.15–3.30 (2H, m), 4.20–4.40 (3H, m), 7.30 (1H, dt, <i>J</i> =1.6, 7.2 Hz), 7.39 (1H, d, <i>J</i> =8.6 Hz), 7.67 (1H, ddd, <i>J</i> =1.6, 7.2, 8.6 Hz), 7.77 (1H, dd, <i>J</i> =1.6, 7.2 Hz), 8.88 (1H, s), 10.48 (1H, d, <i>J</i> =6.8 Hz)                                              |
| <b>8c</b>  | 3263, 2961, 1673,<br>1616, 1585, 1568,<br>1528       | 353 (M <sup>+</sup> )       | (CDCl <sub>3</sub> ): 1.68 (6H, d, <i>J</i> =7.2 Hz), 1.76–1.83 (2H, m), 2.00–2.40 (6H, m), 2.34 (3H, s), 3.10–3.28 (2H, m), 4.30 (1H, q, <i>J</i> =7.0 Hz), 5.40–5.90 (1H, br), 7.22–7.33 (1H, m), 7.55–7.70 (2H, m), 7.75 (1H, d, <i>J</i> =7.2 Hz), 8.83 (1H, s), 10.48 (1H, d, <i>J</i> =7.2 Hz)                                                                                                                  |
| <b>8d</b>  | 3240, 2955, 2928,<br>1668, 1612, 1585,<br>1567, 1527 | 367 (M <sup>+</sup> )       | (CDCl <sub>3</sub> ): 1.02 (3H, t, <i>J</i> =7.2 Hz), 1.40–1.65 (2H, m), 1.65–1.95 (4H, m), 2.00–2.25 (4H, m), 2.25–2.45 (2H, m), 2.36 (3H, s), 3.15–3.30 (2H, m), 4.22–4.45 (3H, m), 7.30 (1H, dt, <i>J</i> =1.6, 7.2 Hz), 7.40 (1H, d, <i>J</i> =8.6 Hz), 7.67 (1H, ddd, <i>J</i> =1.6, 7.2, 8.6 Hz), 7.77 (1H, dd, <i>J</i> =1.6, 7.2 Hz), 8.88 (1H, s), 10.48 (1H, d, <i>J</i> =7.2 Hz)                           |
| <b>8e</b>  | 3436, 1713, 1673,<br>1618                            | 367 (M <sup>+</sup> ) (Fab) | (DMSO- <i>d</i> <sub>6</sub> ): 0.92 (6H, d, <i>J</i> =7.5 Hz), 1.76–2.64 (12H, m), 3.48–3.65 (2H, m), 4.06–4.21 (1H, m), 4.29 (2H, d, <i>J</i> =7.5 Hz), 6.51 (2H, s), 7.32–7.43 (1H, m), 7.67–7.84 (2H, m), 7.99–8.09 (1H, m), 8.87 (1H, s), 10.46 (1H, d, <i>J</i> =8.8 Hz)                                                                                                                                        |
| <b>9c</b>  | 3248, 2924, 1661,<br>1581, 1566, 1531                | 367 (M <sup>+</sup> )       | (CDCl <sub>3</sub> ): 1.05–1.30 (2H, m), 1.68 (6H, d, <i>J</i> =7.2 Hz), 1.40–1.75 (3H, m), 1.90–2.20 (3H, m), 2.48–2.70 (2H, m), 2.57 (3H, s), 3.05–3.25 (2H, m), 4.40–4.65 (1H, m), 5.35–5.80 (1H, br), 7.23–7.35 (1H, m), 7.55–7.69 (2H, m), 7.74 (1H, d, <i>J</i> =7.8 Hz), 8.85 (1H, s), 9.83 (1H, d, <i>J</i> =7.4 Hz)                                                                                          |
| <b>10c</b> | 3436, 2938, 1671,<br>1613, 1585, 1567,<br>1525       | 339 (M <sup>+</sup> )       | (CDCl <sub>3</sub> ): 1.45–1.80 (3H, m), 1.68 (6H, d, <i>J</i> =7.0 Hz), 1.80–2.10 (2H, m), 2.65–3.12 (5H, m), 3.45 (1H, ddd, <i>J</i> =2.0, 9.4, 14.2 Hz), 4.10–4.28 (1H, m), 5.30–5.90 (1H, br), 7.24–7.35 (1H, m), 7.58–7.70 (2H, m), 7.77 (1H, d, <i>J</i> =7.8 Hz), 8.84 (1H, s), 10.31 (1H, d, <i>J</i> =7.2 Hz)                                                                                                |
| <b>11c</b> | 3249, 2976, 2433,<br>1676, 1619, 1567,<br>1540       | 329 (M <sup>+</sup> ) (Fab) | (DMSO- <i>d</i> <sub>6</sub> ): 1.47 (6H, t, <i>J</i> =7.0 Hz), 1.67 (6H, d, <i>J</i> =7.0 Hz), 3.11–3.39 (6H, m), 3.92–4.08 (2H, m), 5.30–5.70 (1H, br), 7.26–7.38 (1H, m), 7.60–7.70 (2H, m), 7.75 (1H, d, <i>J</i> =7.8 Hz), 8.78 (1H, s), 10.28 (1H, t, <i>J</i> =5.6 Hz), 12.30–12.60 (1H, br)                                                                                                                   |
| <b>12c</b> | 3258, 2933, 1672,<br>1615, 1584, 1567<br>1525        | 353 (M <sup>+</sup> )       | (CDCl <sub>3</sub> ): 1.40–1.55 (1H, m), 1.68 (6H, d, <i>J</i> =7.0 Hz), 1.77–2.20 (6H, m), 2.90–3.30 (6H, m), 4.40–4.60 (1H, m), 5.40–5.90 (1H, br), 7.23–7.35 (1H, m), 7.56–7.70 (2H, m), 7.75 (1H, dd, <i>J</i> =1.0, 7.8 Hz), 8.85 (1H, s), 10.08 (1H, d, <i>J</i> =8.0 Hz)                                                                                                                                       |

a) EI mass spectra was measured, unless otherwise noted.

8-azabicyclo[3.2.1]oct-3-yl (a) group yielded the highest activity within synthesized compounds. Compound **8c** had 7 times the 5-HT<sub>4</sub> receptor-stimulating activity of cisapride (ED<sub>50</sub>=271 nM).

Using **8c**, which had the highest 5-HT<sub>4</sub> receptor-stimulating activity, effects on digestive tract motility in conscious fed dogs were tested. Fig. 3 shows typical effects of **8c** (1.0 mg/kg *p.o.*). In the radioligand binding assay, **8c** had high affinity for the 5-HT<sub>4</sub> receptor which inhibited binding of [<sup>3</sup>H] GR113808 on guinea pig striatum (IC<sub>50</sub>=67.7 nM).<sup>20)</sup> **8c** exhibited no affinity for dopamine D<sub>2</sub> receptors ([<sup>3</sup>H] raclopride, rat striatum),<sup>21)</sup> 5-HT<sub>1A</sub> receptors ([<sup>3</sup>H] 8OH-DPAT, guinea pig cortex),<sup>22)</sup> 5-HT<sub>1D</sub> receptors ([<sup>3</sup>H] L-694247, guinea pig striatum),<sup>23)</sup> muscarinic M<sub>2</sub> receptors ([<sup>3</sup>H] NMS, rat heart),<sup>24)</sup> or muscarinic M<sub>3</sub> receptors ([<sup>3</sup>H] 4-DAMP, guinea pig bladder),<sup>25)</sup> even at a concentration of 10  $\mu$ M. Thus, Compound **8c** appears to be free of binding to these receptors.<sup>26)</sup>

In conclusion, we investigated 2-oxo-1,2-dihydro-3-quinolinecarboxamide derivatives as possible 5-HT<sub>4</sub> receptor agonists. Of the compounds synthesized, *N*-endo-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-1-isopropyl-2-oxo-1,2-dihydro-3-quinolinecarboxamide (**8c**) (Fig. 4) was optimal, and had 7 times the 5-HT<sub>4</sub> receptor-stimulating activity of cisapride, as measured by its effect on the regulation of electrically-evoked contractions in guinea pig muscle, and exhibited no 5-HT<sub>1A</sub>, 5-HT<sub>1D</sub>, D<sub>2</sub>, muscarinic M<sub>2</sub> or muscarinic M<sub>3</sub> receptor binding. Compound **8c** thus exhibited high and specific 5-

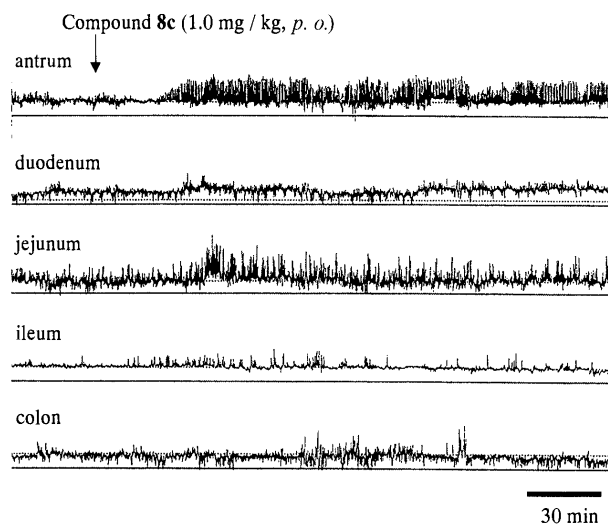
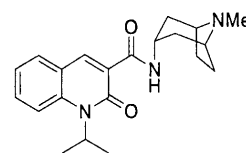


Fig. 3. Effect of **8c** on Digestive Tract Motility in Conscious Fed Dog



**8c**

Fig. 4

HT<sub>4</sub> receptor-stimulating activity, and is a promising compound for the improvement of gastrointestinal dysfunction.

### Experimental

Melting points were determined by a Buchi 535 melting point apparatus and are uncorrected. IR spectra were obtained on a Perkin-Elmer 1760 spectrometer. <sup>1</sup>H-NMR spectra were recorded on a Varian VXL-200 spectrometer. Chemical shifts are reported in ppm ( $\delta$ ) values, based on tetramethylsilane as an internal standard. MS were obtained on a JEOL JMS-SX102 spectrometer. Elemental analyses were performed on a Perkin-Elmer 2400. TLC was performed on silica gel pre-coated plates (Merck, Kieselgel 60F-254). Column chromatography was performed over silica gel (Asahi glass, M. S. GEL. SIL.). 3-Amino-8-methyl-8-azabicyclo [3.2.1] octane (a),<sup>27</sup> 3-amino-9-azabicyclo [3.3.1] nonane (b),<sup>28</sup> and 4-amino-1-azabicyclo[3.3.1]-nonane (e)<sup>29</sup> were synthesized according to references. 3-Aminoquinuclidine (c) and 2-diethylaminoethylamine (d) were purchased from Tokyo Kasei Organic Chemicals.

**Ethyl 3-(2-Nitrophenyl)-2-ethoxycarbonylpropionate (2)** Diethyl malonate (70.3 ml, 0.46 mol) and NaHCO<sub>3</sub> (58.4 g, 0.70 mol) were added to a solution of *o*-nitrobenzaldehyde **1** (70.0 g, 0.46 mol) in acetic anhydride (175 ml). The reaction mixture was stirred at 100 °C for 6 h. After being cooled, the reaction mixture was partitioned between AcOEt and water. The organic layer was washed successively with water, 5% Na<sub>2</sub>CO<sub>3</sub> and brine, then dried over Na<sub>2</sub>SO<sub>4</sub>. The solvent was removed *in vacuo* to give a residue, which was triturated in EtOH to give 77.1 g (56.7%) of **2** as a pale yellow solid. <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 1.02 (3H, t, *J*=7.2 Hz), 1.36 (3H, t, *J*=7.2 Hz), 4.08 (2H, q, *J*=7.2 Hz), 4.35 (2H, q, *J*=7.2 Hz), 7.39–7.48 (1H, m), 7.50–7.70 (2H, m), 8.20 (1H, s), 8.16–8.26 (1H, m). MS *m/z* (EI): 293 (M<sup>+</sup>).

**Ethyl 2-Oxo-1,2-dihydro-3-quinolinecarboxylate (3)** Iron powder (53 g, 1.0 mol) was added to a pre-heated (80 °C) solution of **2** (46 g, 0.15 mol) in acetic acid. The reaction mixture was heated at 80 °C for 6.5 h, cooled and filtered through a Celite pad. The filtrate was evaporated *in vacuo* to leave a residue, which was purified by column chromatography using CHCl<sub>3</sub>/MeOH=10:1 to give 21.3 g (62.5%) of **3** as a yellow solid. mp 175–179 °C (recrystallized from AcOEt–hexane). <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 1.45 (3H, t, *J*=7.2 Hz), 4.46 (2H, q, *J*=7.2 Hz), 7.20–7.30 (1H, m), 7.50 (1H, d, *J*=7.8 Hz), 7.61 (1H, ddd, *J*=1.4, 7.0, 8.4 Hz), 7.65 (1H, d, *J*=8.0 Hz), 8.56 (1H, s), 12.51 (1H, s). IR (KBr) cm<sup>-1</sup>: 3009, 2901, 1732, 1641, 1211. MS *m/z* (EI): 217 (M<sup>+</sup>). *Anal.* Calcd for C<sub>12</sub>H<sub>11</sub>NO<sub>3</sub>: C, 66.35; H, 5.10; N, 6.45. Found: C, 66.11; H, 5.24; N, 6.20.

**Ethyl 1-Isopropyl-2-oxo-1,2-dihydro-3-quinolinecarboxylate (4c) and Ethyl 2-Isopropoxy-3-quinolinecarboxylate (6c)** Compound **3** (40 g, 0.18 mol) and isopropyl iodide (62.6 g, 0.36 mol) were added to a suspension of sodium hydride (60% mineral oil suspension, 8.9 g, 0.38 mol) in DMF (200 ml). The reaction mixture was heated at 70 °C for 8 h. It was then diluted with water and extracted with AcOEt. The extract was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated *in vacuo* to afford a residue, which was purified by column chromatography using AcOEt/hexane=1:4 to give 3.1 g (6.5%) of **4c** as a yellow solid and 25.3 g (53.0%) of **6c** as a colorless oil. Physicochemical data of **4c** and **6c** are summarized in Tables 3 and 4.

**Ethyl 1-alkyl-2-oxo-1,2-dihydro-3-quinolinecarboxylate (4a, 4b, 4d, 4e)** The syntheses of compounds **4a**, **4b**, **4d** and **4e** were conducted using

procedures similar to that employed in the synthesis of **4c**. Chemical yield and physicochemical data of **4a**, **4b**, **4d** and **4e** are summarized in Tables 3 and 4.

**1-Isopropyl-2-oxo-1,2-dihydro-3-quinolinecarboxylic Acid (5c)** 5% NaOH solution (20 ml) was added to a solution of **4c** (1.55 g, 6.0 mmol) in MeOH (20 ml) at room temperature. The reaction mixture was stirred for 18 h and then acidified with concentrated HCl. The resulting precipitates were collected by filtration and dried under a vacuum to give 1.20 g (87.0%) of **5c** as a colorless powder. Physicochemical data of **5c** are summarized in Tables 3 and 4.

**1-Alkyl-2-oxo-1,2-dihydro-3-quinolinecarboxylic Acid (5a, 5b, 5d, 5e) and 2-Isopropoxy-3-quinolinecarboxylic Acid (7c)** The synthesis of compounds **5a**, **5b**, **5d**, **5e** and **7c** was conducted using procedures similar to that employed in the synthesis of **5c**. Chemical yield and physicochemical data of **5a**, **5b**, **5d**, **5e** and **7c** are summarized in Tables 3 and 4.

**N-(endo-8-Methyl-8-azabicyclo[3.2.1]oct-3-yl)-1-isopropyl-2-oxo-1,2-dihydro-3-quinolinecarboxamide (8c)** A solution of **5c** (500 mg, 2.7 mmol) and thionyl chloride (5 ml) was heated at 100 °C for 1.5 h. The reaction mixture was concentrated to dryness *in vacuo*, and the residue was dissolved in toluene. The solvent was evaporated *in vacuo* again and the residue was dissolved in toluene. Then, 3-amino-8-methyl-8-azabicyclo[3.2.1]octane (404 mg, 2.8 mmol) in toluene (3 ml) was added to the above toluene solution, and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was then poured into 5% NaHCO<sub>3</sub> and extracted with CHCl<sub>3</sub>. The extract was washed with brine, dried and evaporated *in vacuo*. The residue was purified by column chromatography using CHCl<sub>3</sub>, then recrystallized from AcOEt to give 391 mg (51%) of **8c** as a colorless solid. Physicochemical data of **8c** are summarized in Tables 1 and 2.

**1-Alkyl-2-oxo-1,2-dihydro-3-quinolinecarboxamide (8a, 8b, 8d, 9c, 10c, 12c)** The synthesis of compounds **8a**, **8b**, **8d**, **9c**, **10c** and **12c** was conducted using procedures similar to that employed in the synthesis of **8c** to give **8a** (45.0%, colorless solid), **8b** (47.1%, colorless solid), **8d** (41.9%, colorless solid), **9c** (69.7%, colorless solid), **10c** (57.6%, colorless solid) and **12c** (20%, colorless solid). Physicochemical data of **8a**, **8b**, **8d**, **9c**, **10c** and **12c** are summarized in Tables 1 and 2.

**N-(endo-8-Methyl-8-azabicyclo[3.2.1]oct-3-yl)-1-isobutyl-2-oxo-1,2-dihydro-3-quinolinecarboxamide Maleate (8e)** A solution of **5e** (720 mg, 2.9 mmol) and thionyl chloride (5 ml) was heated at 100 °C for 1.5 h. The reaction mixture was concentrated to dryness *in vacuo* and the residue was dissolved in toluene. The solvent was evaporated *in vacuo* again and the residue again dissolved in toluene. Then, 3-amino-8-methyl-8-azabicyclo[3.2.1]octane (535 mg, 3.8 mmol) in toluene (3 ml) was added to the toluene solution and the reaction mixture was stirred at room temperature overnight. The reaction mixture was poured into 5% NaHCO<sub>3</sub> and extracted with CHCl<sub>3</sub>. The extract was washed with brine, dried and evaporated *in vacuo*. The residue was purified by column chromatography using CHCl<sub>3</sub>/MeOH saturated with NH<sub>3</sub>=20:1 to leave a solid (580 mg), which was dissolved in IPA (30 ml). A solution of maleic acid (158 mg, 1.4 mmol) was added to the solution and stirred at room temperature for 30 min. To the mixture was added hexane (100 ml), and the resulting precipitates were collected by filtration and recrystallized from IPA–hexane to give 405 mg (61.5%) of **8e** as a colorless solid. Physicochemical data of **8e** are summarized in Tables 1 and 2.

Table 3. Physicochemical Data of the Synthesized Compounds (**4a–e**, **5a–e**, **6c** and **7c**)

| Compound  | Yield (%) | Appearance        | mp (°C) | Recrystallization solvent | Formula                                         | Calcd |      |      | Found |      |      |
|-----------|-----------|-------------------|---------|---------------------------|-------------------------------------------------|-------|------|------|-------|------|------|
|           |           |                   |         |                           |                                                 | C     | H    | N    | C     | H    | N    |
| <b>4a</b> | 63.2      | Pale yellow prism | 73–74   | AcOEt–hexane              | C <sub>14</sub> H <sub>15</sub> NO <sub>3</sub> | 68.56 | 6.16 | 5.71 | 68.33 | 6.21 | 5.58 |
| <b>4b</b> | 75.4      | Colorless gum     | —       | —                         | C <sub>15</sub> H <sub>17</sub> NO <sub>3</sub> | —     | —    | —    | —     | —    | —    |
| <b>4c</b> | 6.5       | Pale yellow prism | 54–57   | AcOEt–hexane              | C <sub>15</sub> H <sub>17</sub> NO <sub>3</sub> | 69.48 | 6.61 | 5.40 | 69.28 | 6.72 | 5.18 |
| <b>4d</b> | 50.6      | Pale yellow prism | 50–52   | AcOEt–hexane              | C <sub>16</sub> H <sub>19</sub> NO <sub>3</sub> | 70.31 | 7.01 | 5.12 | 70.11 | 7.03 | 4.97 |
| <b>4e</b> | 10.5      | Pale yellow gum   | —       | —                         | C <sub>16</sub> H <sub>19</sub> NO <sub>3</sub> | —     | —    | —    | —     | —    | —    |
| <b>5a</b> | 87.6      | Colorless solid   | 186–188 | Acetone                   | C <sub>12</sub> H <sub>11</sub> NO <sub>3</sub> | 66.35 | 5.10 | 6.45 | 66.20 | 5.08 | 6.31 |
| <b>5b</b> | 87.9      | Colorless solid   | 165–167 | Acetone–H <sub>2</sub> O  | C <sub>13</sub> H <sub>13</sub> NO <sub>3</sub> | 67.52 | 5.67 | 6.06 | 67.47 | 5.60 | 6.01 |
| <b>5c</b> | 87.0      | Colorless solid   | 167–169 | Acetone–H <sub>2</sub> O  | C <sub>13</sub> H <sub>13</sub> NO <sub>3</sub> | 67.52 | 5.67 | 6.06 | 67.42 | 5.54 | 6.07 |
| <b>5d</b> | 94.3      | Colorless needle  | 160–162 | Acetone–H <sub>2</sub> O  | C <sub>14</sub> H <sub>15</sub> NO <sub>3</sub> | 68.56 | 6.16 | 5.71 | 68.35 | 6.10 | 5.51 |
| <b>5e</b> | 86.2      | Colorless needle  | 167–169 | Acetone–H <sub>2</sub> O  | C <sub>14</sub> H <sub>15</sub> NO <sub>3</sub> | 68.56 | 6.16 | 5.71 | 68.40 | 6.11 | 5.59 |
| <b>6c</b> | 53.0      | Colorless oil     | —       | —                         | C <sub>15</sub> H <sub>17</sub> NO <sub>3</sub> | —     | —    | —    | —     | —    | —    |
| <b>7c</b> | 86.8      | Colorless solid   | 103–104 | AcOEt–hexane              | C <sub>13</sub> H <sub>13</sub> NO <sub>3</sub> | 67.52 | 5.67 | 6.06 | 67.27 | 5.68 | 5.84 |

Table 4. Physicochemical Data of the Synthesized Compounds (**4a**—**e**, **5a**—**e**, **6c** and **7c**)

| Compound  | IR (KBr)<br>(cm <sup>-1</sup> ) | MS (EI)<br>(m/z)      | <sup>1</sup> H-NMR ( <b>4a</b> — <b>e</b> , <b>6c</b> : CDCl <sub>3</sub> , <b>5a</b> — <b>e</b> , <b>7c</b> : DMSO- <i>d</i> <sub>6</sub> ) $\delta$<br>(ppm)                                                          |
|-----------|---------------------------------|-----------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>4a</b> | 1733, 1641                      | 245 (M <sup>+</sup> ) | 1.37 (3H, t, <i>J</i> =7.4 Hz), 1.43 (3H, t, <i>J</i> =7.4 Hz), 4.40 (2H, q, <i>J</i> =7.4 Hz), 4.44 (2H, q, <i>J</i> =7.4 Hz), 7.21—7.43 (2H, m), 7.60—7.72 (2H, m), 8.40 (1H, s)                                      |
| <b>4b</b> | 1738, 1651                      | 259 (M <sup>+</sup> ) | 1.05 (3H, t, <i>J</i> =7.4 Hz), 1.41 (3H, t, <i>J</i> =7.4 Hz), 1.68—1.90 (2H, m), 4.20—4.34 (2H, m), 4.92 (2H, t, <i>J</i> =7.4 Hz), 7.20—7.40 (2H, m), 7.59—7.72 (2H, m), 8.39 (1H, s)                                |
| <b>4c</b> | 1732, 1646                      | 259 (M <sup>+</sup> ) | 1.42 (3H, t, <i>J</i> =7.4 Hz), 1.66 (6H, d, <i>J</i> =7.2 Hz), 4.41 (2H, q, <i>J</i> =7.4 Hz), 5.15—5.80 (1H, br), 7.17—7.27 (1H, m), 7.54—7.69 (3H, m), 8.30 (1H, s)                                                  |
| <b>4d</b> | 1738, 1651                      | 273 (M <sup>+</sup> ) | 1.00 (3H, t, <i>J</i> =7.2 Hz), 1.42 (3H, t, <i>J</i> =7.2 Hz), 1.40—1.60 (2H, m), 1.65—1.84 (2H, m), 4.23—4.35 (2H, m), 4.43 (2H, q, <i>J</i> =7.2 Hz), 7.21—7.39 (2H, m), 7.58—7.70 (2H, m), 8.39 (1H, s)             |
| <b>4e</b> | 1739, 1654                      | 273 (M <sup>+</sup> ) | 1.00 (6H, d, <i>J</i> =7.5 Hz), 1.41 (3H, t, <i>J</i> =7.5 Hz), 2.27 (1H, septet, <i>J</i> =7.5 Hz), 4.21 (2H, d, <i>J</i> =7.5 Hz), 4.42 (2H, q, <i>J</i> =7.5 Hz), 7.20—7.40 (2H, m), 7.56—7.70 (2H, m), 8.38 (1H, s) |
| <b>5a</b> | 1730, 1624                      | 217 (M <sup>+</sup> ) | 1.44 (3H, t, <i>J</i> =7.4 Hz), 4.49 (2H, q, <i>J</i> =7.4 Hz), 7.38—7.59 (2H, m), 7.76—7.89 (2H, m), 8.91 (1H, s), 14.62 (1H, s)                                                                                       |
| <b>5b</b> | 1720, 1625                      | 231 (M <sup>+</sup> ) | 1.09 (3H, t, <i>J</i> =7.4 Hz), 1.75—1.96 (2H, m), 4.31—4.45 (2H, m), 7.38—7.55 (2H, m), 7.74—7.87 (2H, m), 8.92 (1H, s), 14.62 (1H, s)                                                                                 |
| <b>5c</b> | 1734, 1631                      | 231 (M <sup>+</sup> ) | 1.71 (6H, d, <i>J</i> =7.2 Hz), 5.25—5.75 (1H, br), 7.36—7.45 (1H, m), 7.69—7.85 (3H, m), 8.88 (1H, s), 14.76 (1H, s)                                                                                                   |
| <b>5d</b> | 1737, 1622                      | 245 (M <sup>+</sup> ) | 1.02 (3H, t, <i>J</i> =7.4 Hz), 1.43—1.68 (2H, m), 1.70—1.89 (2H, m), 4.34—4.47 (2H, m), 7.39—7.56 (2H, m), 7.75—7.88 (2H, m), 8.92 (1H, s), 14.63 (1H, s)                                                              |
| <b>5e</b> | 1737, 1627                      | 245 (M <sup>+</sup> ) | 1.02 (6H, d, <i>J</i> =7.5 Hz), 2.30 (1H, septet, <i>J</i> =7.5 Hz), 4.31 (2H, d, <i>J</i> =7.5 Hz), 7.37—7.55 (2H, m), 7.73—7.86 (2H, m), 8.92 (1H, s)                                                                 |
| <b>6c</b> | 1738, 1710, 1623                | 259 (M <sup>+</sup> ) | 1.42 (3H, t, <i>J</i> =7.4 Hz), 1.46 (6H, d, <i>J</i> =7.2 Hz), 4.43 (2H, q, <i>J</i> =7.4 Hz), 5.64 (1H, septet, <i>J</i> =7.2 Hz), 7.39 (1H, ddd, <i>J</i> =1.2, 1.6, 7.8 Hz), 7.62—7.86 (3H, m), 8.54 (1H, s)        |
| <b>7c</b> | 1709, 1622                      | 231 (M <sup>+</sup> ) | 1.55 (6H, d, <i>J</i> =7.2 Hz), 5.88 (1H, septet, <i>J</i> =7.2 Hz), 7.49 (1H, ddd, <i>J</i> =1.2, 1.6, 7.8 Hz), 7.73—7.92 (3H, m), 9.01 (1H, s), 11.05—11.27 (1H, br)                                                  |

**N-(2-Diethylaminoethyl)-1-isopropyl-2-oxo-1,2-dihydro-3-quinolinecarboxamide Hydrochloride (**11c**)** A solution of **5c** (500 mg, 2.7 mmol) and thionyl chloride (5 ml) was heated at 100 °C for 1.5 h. The reaction mixture was concentrated to dryness *in vacuo* and the residue was dissolved in toluene. The solvent was evaporated *in vacuo* again and the residue again dissolved in toluene. Then, 2-diethylaminoethylamine (330 mg) was added to the above toluene solution, and the reaction mixture stirred at room temperature for 1 h. The reaction mixture was then poured into 5% NaHCO<sub>3</sub> and extracted with CHCl<sub>3</sub>. The extract was dried and evaporated *in vacuo*. The residue was dissolved in EtOH (10 ml), then concentrated HCl (230 mg) was added. The mixture was evaporated *in vacuo* to afford a residue, which was recrystallized from MeOH–AcOEt to give 549 mg (69.3%) of **11c** as a colorless solid. Physicochemical data of **11c** are summarized in Tables 1 and 2.

**Single-Crystal X-Ray Analysis of **8c**** Crystals of compound **8c** were grown from AcOEt as yellow prisms. Data was collected from a crystal with the dimensions 0.50×0.30×0.10 mm<sup>3</sup> on a Mac Science MXC18 diffractometer and corrected for Lorentz and polarization factors. The structure was determined by a direct method using the SHELXS86 program and refined using the crystal-GM program.<sup>30</sup> The final refinement was achieved by the full-matrix least-squares method, with anisotropic thermal parameters for all non-hydrogen atoms and fixed isotropic thermal parameters for hydrogen atoms. Crystal data: C<sub>21</sub>H<sub>27</sub>N<sub>3</sub>O<sub>2</sub>, M.W.=353.46, triclinic, space group P1, *a*=9.828 (2) Å, *b*=12.160 (3) Å, *c*=7.998 (4) Å,  $\alpha$ =98.33 (3) Å,  $\beta$ =93.32 (3) Å,  $\gamma$ =90.26 (2) Å, *V*=944.0 (6) Å<sup>3</sup>, *Z*=2, *D*<sub>c</sub>=1.24 g/cm<sup>3</sup>, *F*<sub>000</sub>=380, temperature 15 °C, CuK $\alpha$  ( $\lambda$ =1.54178 Å), 3157 observed reflections (*I*>3.00  $\sigma$  (*I*)), 313 variable parameters, *R*=0.074, *R*<sub>w</sub>=0.091.

**Serotonin 5-HT<sub>4</sub> Receptor Agonistic Activity<sup>31</sup>** Male guinea pigs of the Hartley strain (Nihon SLC Inc., Shizuoka) weighing 200–400 g were housed at 20–26 °C on a 12 h light/dark cycle with free access to food and water. Guinea pigs were killed by a blow to the head and by cutting of the carotid arteries. The ileum was excised 10–20 cm from the ileo-caecal junction and divided longitudinally into segments approximately 3 cm in length. The longitudinal muscle strips with the myenteric plexus attached were removed by gentle stroking with a cotton swab. The tissue was vertically suspended in an organ bath containing Krebs' solution (composition in mmol/l: NaCl, 120; KCl, 5.9; NaHCO<sub>3</sub>, 15.5; NaH<sub>2</sub>PO<sub>4</sub>, 1.2; CaCl<sub>2</sub>, 2.5; MgCl<sub>2</sub>, 1.2; glucose, 11.5) aerated with a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub> and maintained at 32 °C. Twitch responses were evoked by transmural electrical stimulation of the enteric cholinergic nerves using square-wave pulses (0.2 Hz, 1 ms pulse duration). Twitch responses were recorded isometrically with a resting tension of 0.8 g. The tissue was stimulated at supramaximal

voltage to equilibration for 2–3 h. The twitch response was then decreased by voltage reduction. After obtaining a stable submaximal response, the tissue was exposed to 10 nmol/l of 5-HT. This concentration of 5-HT produced a sustained increase in the twitch response. Concentration–effect curves to agonists were constructed by exposing the strips to a cumulative addition of agonists. The responses to agonists were measured in terms of their ability to restore the twitch response to that obtained at supramaximal voltage.

**Gastrointestinal Motility** Dogs with strain gauge force transducers sutured to the serosa of the gastric antrum, small intestine or colon were prepared to measure circular muscle contractions. The experiments were started at least 14 d after surgery. The animals were deprived of food, but not water, for 18 h before each experiment. Before the application of test drugs, control recordings were performed in each dog. During this recording, a previously reported motility pattern, *i.e.*, alternating contractile and quiescent states, was confirmed at the antral site. Compound **8c** and vehicle were given 5–10 min after termination of the contractile state in the antrum, and a meal (100 kcal) was then given immediately.

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## Two New 3-Methoxy-4-quinolone Alkaloids from the Bark of *Sarcomelicope megistophylla*

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**Two new alkaloids, megistonine I (1) and megistonine II (2), were isolated from the bark of *Sarcomelicope megistophylla*. Their structures, which are derived from the 3-methoxy-4-quinolone basic skeleton, were elucidated on the basis of MS and extensive NMR studies.**

**Key words** quinolone; alkaloid; *Sarcomelicope megistophylla*; Rutaceae

*Sarcomelicope megistophylla* HARTLEY (Rutaceae) is a small to medium sized tree, 8–12 m high, characterized by its large (up to 34 cm long) pubescent leaves. Hartley<sup>1)</sup> described it as endemic to the region of Nouméa, New Caledonia. Recently, we described the chemical constituents of its leaves<sup>2,3)</sup> and the major alkaloids of the bark.<sup>4)</sup> In a continuation of our studies of the genus *Sarcomelicope*<sup>5–12)</sup> we report here the isolation and structural elucidation of two new quinolone alkaloids, megistonine I (1) and megistonine II (2), isolated from the ether extract of the bark of *Sarcomelicope megistophylla*.

Megistonine I (1) was obtained as a yellow amorphous compound. The empirical formula was determined by HR-MS to be C<sub>18</sub>H<sub>21</sub>NO<sub>5</sub>. The UV spectrum was suggestive of a quinolone derivative. A typical hypsochromic shift observed upon the addition of acid gave evidence of a 4-quinolone basic skeleton.<sup>13,14)</sup> In the aromatic region of the <sup>1</sup>H-NMR spectrum, two *ortho*-coupled signals, at 8.17 and 6.98 (each 1H, d, *J*=8.8 Hz), were associated with the A ring of a disubstituted-4-quinolone. A broad singlet at 10.5 was indicative of one phenolic OH group. At a higher field, typical signals accounted for one 1,1-dimethylallyl side chain, two OMe groups and one NMe group. The <sup>13</sup>C-NMR spectrum showed two carbonyl resonances, at δ 173.7 and 163.5 ppm, the former confirming the presence of the 4-quinolone system and the latter characterizing a methyl ester group. Further information on the structure of 1 was obtained from the long range C–H correlations in the <sup>1</sup>H-detected heteronuclear multibond connectivity (HMBC) spectrum (Fig. 2). Three bond correlations observed between the 3H-singlet of the NMe group and two quaternary aromatic carbons (δ 144.7, 140.5) permitted the assignment of C-8a and C-2, respectively. The methylene protons of the side chain, at δ 3.53, showed a correlation with the 8a-quaternary carbon on one hand, and the oxygenated aromatic carbon C-7 at δ 160.3, on the other hand. These observations permitted location of the 1,1-dimethylallyl side chain at C-8 of the quinolone A ring. Additionally, a three bond correlation between the signal of the tertiary C-3 at δ 140.2 and the 3H-singlet of the OMe ether group placed this latter at position 3 and, consequently, the COOCH<sub>3</sub> group at position 2 and the phenolic hydroxyl group at position 7. Therefore, the structure of this new alkaloid was depicted as 1. This structure was in full agreement

with the chemical shift effects observed for C-2 and C-3, when compared with the homologous signals of 1-methyl-4-quinolone-2,3-dicarboxylic acid esters.<sup>5,15)</sup>

Megistonine II (2) was obtained as a yellow amorphous compound with the molecular formula C<sub>14</sub>H<sub>15</sub>NO<sub>6</sub>. The UV spectrum was similar to that of 1. Comparison of the <sup>1</sup>H- and <sup>13</sup>C-NMR data of 2 with those of 1 indicated that the 1,1-dimethylallyl side chain in 1 was replaced by a methoxy group in 2. The position of the methoxy group was further confirmed by the HMBC spectrum in which the 3H-singlet of the OMe at δ 3.77 had a three bond correlation with C-8, at δ 134.9. The other cross peaks were essentially similar to those observed in the HMBC spectrum of 1, except those referring to the 1,1-dimethylallyl side chain. Consequently, structure 2 was attributed to this new natural product for which we propose the trivial name megistonine II.

It is interesting to point out that megistonine I (1) and megistonine II (2) are the second examples of natural 4-quinolones oxygenated in position 3. To the best of our knowledge there is only one previous account describing a natural compound of this type, *i.e.*, japonine (1-methyl-2-phenyl-3,6-dimethoxy-4-quinolone), isolated from *Orixa*

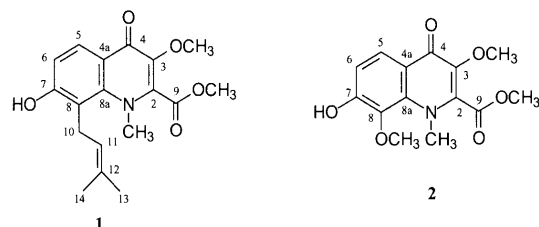


Fig. 1. Structures of Megistonine I (1), Megistonine II (2)

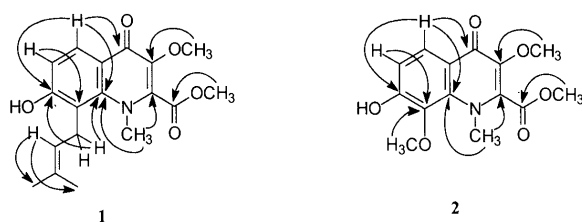


Fig. 2. Selected HMBC Correlations for Megistonine I (1) and Megistonine II (2)

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*japonica*, which also belongs to the Rutaceae family.<sup>14)</sup>

## Experimental

**General Experimental Procedures** UV spectra were recorded in spectroscopic grade MeOH on a Shimadzu-160A spectrophotometer. NMR spectra were recorded on Bruker DRX 400 and Bruker AC 200 spectrometers [<sup>1</sup>H (400 and 200 MHz) and <sup>13</sup>C (50 MHz)], and chemical shifts are expressed in ppm downfield to TMS. The 2D NMR experiments were performed using standard Bruker microprograms. EI-MS spectra were determined on a HP-6890 and HR-MS on an AEI MS-902 spectrometer.

**Plant Material** The plant material was collected at Nouméa (New Caledonia) in May 1984. A voucher sample (Pusset-Chauvière 261) is deposited in the herbarium of the Centre ORSTOM at Nouméa, New Caledonia.

**Extraction and Isolation** Extraction of the alkaloids was as described.<sup>4)</sup> The ether bark extract was chromatographed over a column containing Si gel (Merck 0.04–0.06 mm; flash) using a cyclohexane/EtOAc gradient to give 7 fractions. Fraction 6 was submitted to flash chromatography on silica gel with CH<sub>2</sub>Cl<sub>2</sub>/MeOH (99 : 1) to afford megistonine I (7 mg) and megistonine II (5 mg).

Megistonine I (**1**): <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz) δ: 10.5 (1H, br s, HO-C7), 8.17 (1H, d, *J*=8.8 Hz, H-5), 6.98 (1H, d, *J*=8.8 Hz, H-6), 5.27 (1H, t, *J*=8.3 Hz, H-11), 3.99 (3H, s, OMe-C9), 3.92 (3H, s, OMe-C3), 3.65 (3H, s, NMe), 3.53 (2H, d, *J*=8.3 Hz, H-10), 1.76 (6H, s, H-13, 14). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 50 MHz) δ: 173.7 (C-4), 163.5 (C-9), 160.3 (C-7), 144.7 (C-8a), 140.5 (C-2), 140.2 (C-3), 135.1 (C-12), 126.2 (C-5), 122.8 (C-4a), 122.0 (C-11), 114.5 (C-6), 114.2 (C-8), 61.1 (OMe-C3), 53.2 (OMe-C9), 42.5 (NMe), 27.4 (C-10), 25.7 (C-13), 18.2 (C-14). UV λ<sub>max</sub> (MeOH) nm (log ε): 243 (3.91), 267 (sh.), 335 (3.48). UV λ<sub>max</sub> (MeOH+HCl) nm (log ε): 258 (4.07), 315 (3.48). HRMS *m/z*: 331.1415 (Calcd for C<sub>18</sub>H<sub>21</sub>NO<sub>5</sub>: 331.1419); MS-DCI *m/z*: 332 [M+H]<sup>+</sup>; EI *m/z* (rel.int.): 331 (90), 316 (100).

Megistonine II (**2**): <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz) δ: 10.6 (1H, br s, HO-C7), 8.16 (1H, d, *J*=8.8 Hz, H-5), 7.92 (1H, d, *J*=8.8 Hz, H-6), 4.02 (3H, s, OMe-C9), 3.93 (3H, s, OMe-C3), 3.84 (3H, s, NMe), 3.77 (3H, s, OMe-C8). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 50 MHz) δ: 172.6 (C-4), 163.3 (C-9), 153.3 (C-7), 140.2 (C-2), 140.1 (C-3), 135.2 (C-8a), 134.9 (C-8), 123.9 (C-5), 123.8 (C-4a), 113.6 (C-6), 62.0 (OMe-C8), 60.9 (OMe-C3), 53.3 (OMe-C9), 39.4 (NMe).

UV λ<sub>max</sub> (MeOH) nm (log ε): 241 (3.99), 268.5 (3.85), 337.5 (3.53). UV λ<sub>max</sub> (MeOH+HCl) nm (log ε): 257 (4.05), 317 (3.53). HR-MS *m/z*: 293.0897 (Calcd for C<sub>14</sub>H<sub>15</sub>NO<sub>6</sub>: 293.0899); MS-DCI *m/z*: 294 [M+H]<sup>+</sup>; EI *m/z* (rel.int.): 293 (60), 278 (100), 260 (20), 248 (30), 234 (18), 218 (50).

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## Chemiluminescence Characteristics of *N*-(4-Substituted Benzyl)isoluminol

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Received July 26, 2000; accepted September 7, 2000

We synthesized *N*-(4-substituted benzyl)isoluminol which has 4-bromo-, 4-methyl-, 4-methoxy-, 4-nitro-groups. These compounds produced chemiluminescence by the reaction with the oxidizing agent, potassium hexacyanoferrate and hydrogen peroxide, in an alkaline medium. The chemiluminescence intensities of these compounds were 0.03–4.7 times that of isoluminol. We used Hamett substituent constants as a parameter for the electronic substituent effects. The relationship between the amino-H chemical shift value and the Hamett substituent constants showed a good linear correlation. The relationship between the chemiluminescence intensities and the Hamett substituent constants showed a good linear correlation. The relationship between the fluorescence intensities and the Hamett substituent constants also showed a good linear correlation. These results suggest that the change in the electron density around the amino group strongly influences the fluorescence intensities and corresponding chemiluminescence intensities of these derivatives.

**Key words** chemiluminescence; *N*-(4-substituted benzyl)isoluminol; luminol; isoluminol derivative

5-Amino-2,3-dihydro-1,4-phthalazine-1,4-dione (luminol) and 6-amino-2,3-dihydro-1,4-phthalazine-1,4-dione (isoluminol) are well-known chemiluminescence (CL) compounds, and many synthesized luminol and isoluminol derivatives have been widely used for various biomicroanalyses due to their high sensitivity. A practical labelling reagent having a luminol moiety has not been reported due to the difficulty of its synthesis and low CL intensity. On the other hand, various reagents having an isoluminol moiety, such as isoluminol-isothiocyanate (ILITC),<sup>1</sup> *N*-aminobutyl-*N*-ethylisoluminol (ABEI),<sup>2,3</sup> 4,5-diaminophthalhydrazide (DPH)<sup>4</sup> and 6-aminomethylphthalhydrazide (6-AMP)<sup>5</sup> have been used for the microanalyses of biological compounds. Most of these reagents react with the amino group or carboxylic acid of the analytes and a new CL labelling reagent which reacts with other functional groups has been required. The modification of the amino group of luminol with the electron donating group such as an alkyl group causes a decrease in the CL intensity. On the other hand, that of isoluminol with an electron donating group causes an increase in the intensity.<sup>6,7</sup> Thus we synthesized *N*-(4-substituted benzyl)isoluminols (**3a–e**) and the CL characteristic (relative CL intensity) of these isoluminol derivatives were studied. To clarify the relationship between the substituent groups and CL intensities, we used the Hamett substituent constants ( $\sigma$ ) as a parameter for the electronic effects of the substituent groups.

### Experimental

**Reagents and Solutions** Deionized H<sub>2</sub>O purified with a Milli-Q II system (Japan Millipore, Tokyo, Japan) was used for the preparation of the aqueous solutions. Hydrogen peroxide (31%, v/v) was purchased from Mitsubishi Gas Kagaku (Tokyo, Japan). Luminol and isoluminol were purchased from Tokyo Kasei Industry (Tokyo, Japan). Luminol was purified as sodium luminol by 3 successive salting outs with 5% NaOH.<sup>8</sup> All other chemicals were of reagent grade and used without further purification. 4-Amino-*N*-methylphthalimide (**1**) was synthesized from 4-nitrophthalimide. The *N*-methylation of 4-nitrophthalimide was done using a method similar to that of Roswell *et al.*<sup>9</sup> *N*-methyl-4-nitrophthalimide was reduced to **1** by the method of Drew and Pearmann.<sup>10</sup> Compound **1** was recrystallized from MeOH. Stock solutions of the CL compounds were prepared as follows: sodium luminol in 0.1 M sodium carbonate, and isoluminol and compounds **3a–e** in dimethyl sulfoxide (DMSO). These stock solutions were diluted to 1 mM or 10 nM solution with 0.1 M sodium carbonate.

**Apparatus** The CL reactions were carried out in a 75×12 mm round-

bottom glass tube. The CL measurements were performed using a Lumat LB9507 luminometer (Berthold, Wildbad, Germany). Uncorrected fluorescence excitation and emission spectra were obtained using a Hitachi F2000 fluorescence spectrometer (Tokyo, Japan). FAB-MS were taken with a JEOL (Tokyo, Japan) JMS600 spectrometer. <sup>1</sup>H-NMR spectra were taken in chloroform-*d*<sub>1</sub> (CDCl<sub>3</sub>) and DMSO-*d*<sub>6</sub> ((CD<sub>3</sub>)<sub>2</sub>SO) with a Varian UNITY plus spectrometer (U.S.A.) at 500 MHz.

**Synthesis of 4-(*p*-Substituted Benzyl)amino-*N*-methylphthalimide (**2a–e**)** Compound **1** (0.352 g, 2 mmol) and *p*-substituted benzyl bromide or chloride (2 mmol) were dissolved in *N,N*-dimethyl formamide (DMF) (5 ml). The mixture was refluxed with stirring for 8 h. The reaction mixture was purified by column chromatography on silica gel with CHCl<sub>3</sub>–Me<sub>2</sub>CO (15:1, v/v) as the eluent. Each fraction corresponding to **2a–e** was collected and evaporated to dryness under reduced pressure. The residue was recrystallized from MeOH–H<sub>2</sub>O to give **2a–c**, **e** (yellow crystal), and **2d** (orange crystal).

**2a:** Yield 61%. mp=151–152 °C. <sup>1</sup>H-NMR 500 MHz (CDCl<sub>3</sub>)  $\delta$ : 3.09 (s, 3H, CH<sub>3</sub>), 4.41 (d, 2H, CH<sub>2</sub>, *J*=5.5 Hz), 4.76 (s, 1H, NH), 6.72 (q, 1H, ArH), 6.99 (d, 1H, ArH), 7.28–7.37 (m, 5H, ArH), 7.57 (d, 1H, ArH). FAB-MS (positive ion mode) *m/z*: 267.2 [M+1]<sup>+</sup>.

**2b:** Yield 51%. mp=163–164 °C. <sup>1</sup>H-NMR 500 MHz (CDCl<sub>3</sub>)  $\delta$ : 2.33 (s, 3H, Ar-CH<sub>3</sub>), 3.10 (s, 3H, CH<sub>3</sub>), 4.36 (d, 2H, CH<sub>2</sub>, *J*=5.5 Hz), 4.70 (s, 1H, NH), 6.71 (q, 1H, ArH), 6.98 (d, 1H, ArH), 7.15 (d, 2H, ArH, *J*=7.5 Hz), 7.20 (d, 2H, ArH, *J*=8 Hz), 7.56 (d, 1H, ArH). FAB-MS *m/z*: 281.2 [M+1]<sup>+</sup>.

**2c:** Yield 41%. mp=177–178 °C. <sup>1</sup>H-NMR 500 MHz (CDCl<sub>3</sub>)  $\delta$ : 3.09 (s, 3H, CH<sub>3</sub>), 3.79 (s, 3H, OCH<sub>3</sub>), 4.33 (d, 2H, CH<sub>2</sub>, *J*=5.5 Hz), 4.66 (s, 1H, NH), 6.71 (q, 1H, ArH), 6.87 (q, 2H, ArH), 6.98 (d, 1H, ArH, *J*=4 Hz), 7.26 (2H, ArH), 7.56 (d, 1H, ArH). FAB-MS *m/z*: 297.2 [M+1]<sup>+</sup>.

**2d:** Yield 33%. mp=236–238 °C. <sup>1</sup>H-NMR 500 MHz (CDCl<sub>3</sub>)  $\delta$ : 3.09 (s, 3H, CH<sub>3</sub>), 4.57 (d, 2H, CH<sub>2</sub>, *J*=6 Hz), 4.91 (s, 1H, NH), 6.71 (q, 1H, ArH), 6.94 (d, 1H, ArH, *J*=2 Hz), 7.48 (d, 2H, ArH, *J*=8.5 Hz), 7.58 (d, 1H, ArH,

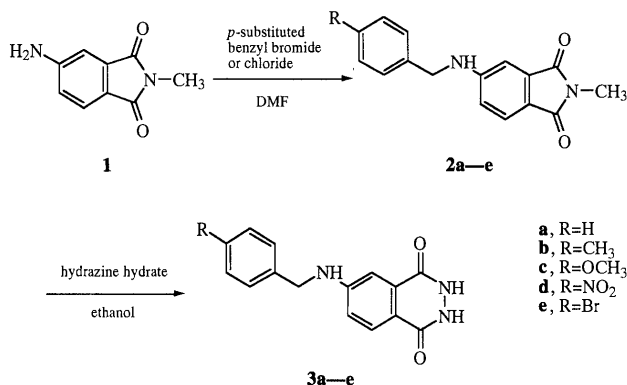


Fig. 1. Synthetic Route of **3a–e**

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$J=8.5$  Hz), 8.20 (d, 2H, ArH,  $J=9$  Hz). FAB-MS  $m/z$ : 312.2  $[M+1]^+$ .

**2e**: Yield 52%. mp=207–208 °C.  $^1\text{H-NMR}$  500 MHz ( $\text{CDCl}_3$ )  $\delta$ : 3.09 (s, 3H,  $\text{CH}_3$ ), 4.38 (d, 2H,  $\text{CH}_2$ ,  $J=5.5$  Hz), 4.76 (s, H, NH), 6.70 (q, H, ArH), 6.96 (d, H, ArH), 7.19 (d, 2H, ArH,  $J=8.5$  Hz), 7.46 (d, 2H, ArH,  $J=8.5$  Hz), 7.57 (d, H, ArH). FAB-MS  $m/z$ : 345.1  $[M+1]^+$ .

**Synthesis of *N*-(4-Substituted Benzyl)isoluminol (3a–e)** Hydrazine hydrate (500  $\mu\text{l}$ ) was added to a suspension of **2a–e** (1 mmol) in EtOH (5 ml). The mixture was refluxed for 3 h. The mixture was then added to 1 M HCl (100 ml) and stirred at room temperature for 1 h. The resulting precipitate was collected and recrystallized from aqueous acetic acid to give compounds **3a–e** as white crystals **3a, b** and yellow crystals **3c–e**, respectively.

**6-Benzylamino-2,3-dihydrophthalazine-1,4-dione (3a)**: Yield 23% from **2a**. mp=223–225 °C.  $^1\text{H-NMR}$  500 MHz ( $\text{DMSO}-d_6$ )  $\delta$ : 4.39 (d, 2H,  $\text{CH}_2$ ,  $J=5.5$  Hz), 6.95 (s, H, ArH), 7.09 (dd, H, ArH,  $J=8.5$ , 2 Hz), 7.22 (t, H, ArNH,  $J=7$  Hz), 7.28–7.37 (m, 5H, ArH), 7.75 (s, H, ArH). FAB-MS  $m/z$ : 268.2  $[M+1]^+$ . *Anal.* Calcd for  $\text{C}_{15}\text{H}_{13}\text{N}_3\text{O}_2 \cdot 1/2 \text{H}_2\text{O}$ : C, 65.20; H, 5.11; N, 15.21. Found: C, 65.35; H, 5.13; N, 15.04.

**6-[(4-Methylphenyl)methyl]amino-2,3-dihydrophthalazine-1,4-dione (3b)**: Yield 86% from **2b**. mp=205 °C.  $^1\text{H-NMR}$  500 MHz ( $\text{DMSO}-d_6$ )  $\delta$ : 2.26 (s, 3H,  $\text{CH}_3$ ), 4.33 (d, 2H,  $\text{CH}_2$ ,  $J=6$  Hz), 6.93 (s, H, ArH), 7.07 (dd, H, ArH,  $J=8.5$ , 2 Hz), 7.12 (d, 2H, ArH,  $J=8$  Hz), 7.23 (d, 2H, ArH,  $J=8$  Hz), 7.24 (t, H, ArNH,  $J=5.5$  Hz), 7.74 (s, H, ArH). FAB-MS  $m/z$ : 282.2  $[M+1]^+$ . *Anal.* Calcd for  $\text{C}_{16}\text{H}_{15}\text{N}_3\text{O}_2 \cdot \text{H}_2\text{O}$ : C, 64.20; H, 5.72; N, 14.04. Found: C, 64.24; H, 5.59; N, 13.96.

**6-[(4-Methoxyphenyl)methyl]amino-2,3-dihydrophthalazine-1,4-dione (3c)**: Yield 69% from **2c**. mp=224–225 °C.  $^1\text{H-NMR}$  500 MHz ( $\text{DMSO}-d_6$ )  $\delta$ : 3.71 (s, 3H,  $\text{OCH}_3$ ), 4.30 (d, 2H,  $\text{CH}_2$ ,  $J=5.5$  Hz), 6.88 (d, 2H, ArH,  $J=8.5$  Hz), 6.94 (s, H, ArH), 7.07 (dd, H, ArH,  $J=8.5$ , 2.5 Hz), 7.21 (t, H, ArNH,  $J=6$  Hz), 7.27 (d, 2H, ArH,  $J=8.5$  Hz), 7.73 (s, H, ArH). FAB-MS  $m/z$ : 298.2  $[M+1]^+$ . *Anal.* Calcd for  $\text{C}_{16}\text{H}_{15}\text{N}_3\text{O}_3 \cdot 1/2 \text{H}_2\text{O}$ : C, 62.73; H, 5.26; N, 13.71. Found: C, 62.62; H, 5.35; N, 13.63.

**6-[(4-Nitrophenyl)methyl]amino-2,3-dihydrophthalazine-1,4-dione (3d)**: Yield 76% from **2d**. mp>300 °C.  $^1\text{H-NMR}$  500 MHz ( $\text{DMSO}-d_6$ )  $\delta$ : 4.57 (d, 2H,  $\text{CH}_2$ ,  $J=6$  Hz), 6.92 (s, H, ArH), 7.09 (dd, H, ArH,  $J=9$ , 2.5 Hz), 7.44 (t, H, ArNH,  $J=6$  Hz), 7.61 (d, 2H, ArH,  $J=8.5$  Hz), 7.76 (d, H, ArH,  $J=9$  Hz), 8.19 (d, 2H, ArH,  $J=9$  Hz). FAB-MS  $m/z$ : 313.2  $[M+1]^+$ . *Anal.* Calcd for  $\text{C}_{15}\text{H}_{12}\text{N}_4\text{O}_4 \cdot \text{CH}_3\text{COOH}$ : C, 54.84; H, 4.33; N, 15.05. Found: C, 54.88; H, 4.42; N, 14.94.

**6-[(4-Bromophenyl)methyl]amino-2,3-dihydrophthalazine-1,4-dione (3e)**: Yield 58% from **2e**. mp>300 °C.  $^1\text{H-NMR}$  500 MHz ( $\text{DMSO}-d_6$ )  $\delta$ : 4.37 (d, 2H,  $\text{CH}_2$ ,  $J=5.5$  Hz), 6.91 (s, H, ArH), 7.07 (dd, H, ArH,  $J=9$ , 2.5 Hz), 7.30 (d, 2H, ArH,  $J=8.5$  Hz), 7.33 (t, H, ArNH), 7.51 (d, 2H, ArH,  $J=8$  Hz), 7.74 (d, H, ArH,  $J=9$  Hz). FAB-MS  $m/z$ : 346.0, 348.0  $[M+1]^+$ . *Anal.* Calcd for  $\text{C}_{15}\text{H}_{12}\text{BrN}_3\text{O}_2 \cdot 1/2 \text{H}_2\text{O}$ : C, 50.72; H, 3.69; N, 11.83. Found: C, 50.67; H, 5.74; N, 11.77.

**CL Property** To 200  $\mu\text{l}$  of a 10 mM stock solution, 50  $\mu\text{l}$  of 40 mM potassium hexacyanoferrate(III) in 0.1 M  $\text{Na}_2\text{CO}_3$  and 50  $\mu\text{l}$  of 20 mM hydrogen peroxide were added by automatic injection. The CL intensities were measured for 1 min and the integral photon counts were used.

**Fluorescence of the CL Reaction Product** To 400  $\mu\text{l}$  of the 1 mM stock solution, 100  $\mu\text{l}$  of 40 mM potassium hexacyanoferrate(III) in 0.1 M  $\text{Na}_2\text{CO}_3$  and 100  $\mu\text{l}$  of 20 mM hydrogen peroxide were added and allowed to stand at room temperature for 1 h. The fluorescence excitation, emission maxima and relative intensities of the resulting mixtures were measured by a fluorescence spectrometer.

**Hammett Substituent Constants** The Hammett substituent constants ( $\sigma_p$ ) used in this study were cited from the review written by Hansch *et al.*<sup>(11)</sup> as follows; **3a** (H, 0.00), **3b** (Me, −0.17), **3c** (OMe, −0.27), **3d** ( $\text{NO}_2$ , 0.78), **3e** (Br, 0.23).

## Results and Discussion

We synthesized isoluminol derivatives which were modified with a 4-substituted benzyl group through the amino group of isoluminol. By this modification, a change in the CL intensities was expected. Compound **3a–e** produce CL by the reaction with hydrogen peroxide and potassium hexacyanoferrate(III) in the presence of alkaline medium. The CL intensity reached a maximum in a few seconds after the addition of hydrogen peroxide and potassium hexacyanoferrate(III) and then almost disappeared within one minute.

The effects of the concentrations of hydrogen peroxide and

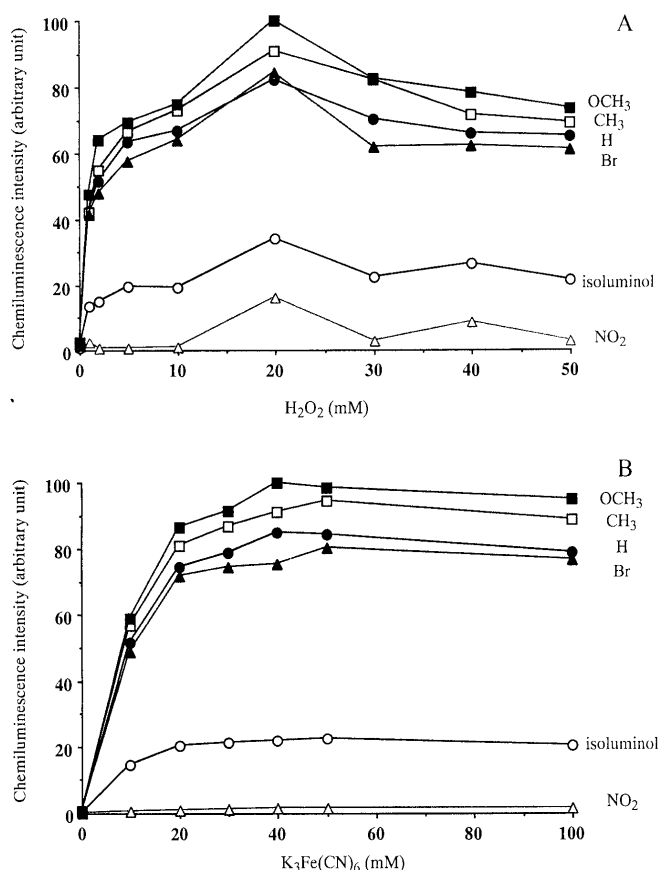


Fig. 2. Effects of Concentrations of (A) Hydrogen Peroxide and (B) Potassium Hexacyanoferrate(III) on the CL Intensities

Table 1. Relative CL Intensities (RCI) of *N*-(4-Substituted benzyl)isoluminols

| Compound                 |                     |
|--------------------------|---------------------|
| Sodium luminol           | 100.0 <sup>a)</sup> |
| Isoluminol               | 3.7                 |
| 3a (R=H)                 | 15.2                |
| 3b (R=CH <sub>3</sub> )  | 17.0                |
| 3c (R=OCH <sub>3</sub> ) | 17.6                |
| 3d (R=NO <sub>2</sub> )  | 0.1                 |
| 3e (R=Br)                | 14.6                |

a) Integrated CL intensity of luminol was taken as 100.

potassium hexacyanoferrate(III) on the CL intensity were examined (Fig. 2). A 20 mM hydrogen peroxide and 40 mM potassium hexacyanoferrate(III) produced almost the maximum intensity. Under these reaction conditions, the CL intensities obtained from the seven derivatives were determined (Table 1). The CL intensities of **3a–e** were 0.03–4.7 times of isoluminol, and the intensities corresponded to 1/1000–9/50 of that obtained with luminol. In the CL of luminol, the 3-aminophthalate ion produced during the oxidizing reaction, has been shown to be the light emitter. Therefore, in the case of **3a–e**, the corresponding dicarboxylate ions should be emitting species. The fluorescence properties (excitation and emission maximum wavelengths of the fluorescence and relative intensities) of the species were measured after the CL reactions were completed (Table 2). The fluorescence intensities of **3a–e** were 0.02–1.9 times larger than that of luminol. The fluorescence excitation and emission maximum

Table 2. Fluorescence Excitation (Ex), Emission (Em) Maxima and Relative Intensities (RFI) of *N*-(4-Substituted benzyl)isoluminol after the CL Reaction

| Compound<br>(CL reactant) | Ex<br>(nm) | Em<br>(nm) | RFI <sup>a)</sup> |
|---------------------------|------------|------------|-------------------|
| Sodium luminol            | 307        | 465        | 100.0             |
| Isoluminol                | 274        | 467        | 106.3             |
| 3a (R=H)                  | 278        | 467        | 146.2             |
| 3b (R=CH <sub>3</sub> )   | 279        | 466        | 168.8             |
| 3c (R=OCH <sub>3</sub> )  | 278        | 465        | 190.7             |
| 3d (R=NO <sub>2</sub> )   | 270        | 466        | 2.1               |
| 3e (R=Br)                 | 285        | 466        | 78.6              |

a) Fluorescence intensity of luminol was taken as 100.

wavelengths of **3a–e** were almost the same as that of isoluminol.

The relationship between the amino-H chemical shift value and  $\sigma_p$  is shown in Fig. 3A. The electron donating substituent groups such as the methyl and methoxy groups cause upfield shifts of  $\delta$  for the amine proton. On the other hand, the electron accepting groups such as the bromo and nitro groups cause downfield shifts of  $\delta$  for the amine proton. Figure 3A showed a good linear correlation between the Hamett substituent constants and the amino-H chemical shift values. This result suggests that the change in the substituent group influences the electron density around the amino group. It is known that *N*-alkylated isoluminol have higher CL intensities than that of isoluminol. To explain this result, we examined the relationship between the CL intensities and the values of  $\sigma_p$  (Fig. 3B). Introduction of the electron donating groups onto the amino nitrogen caused an increase in the CL intensity. On the other hand, the electron accepting group caused a decrease in the intensity. The intensities are proportional to the fluorescence intensities of the final products of the CL reaction. Thus, we examined the relationship between the fluorescence intensities and the values of  $\sigma_p$  (Fig. 3C). Introduction of electron donating groups onto the amino nitrogen caused an increase in the fluorescence intensity, while the substitution of an electron accepting group onto the nitrogen caused a decrease in the intensity. These results suggest that the change in the electron density around the amino group strongly influences the fluorescence intensities and corresponding CL intensities of these derivatives. Thus, introduction of the electron donating groups onto the *p*-position of the benzyl group of the *N*-benzyl isoluminol enables us to develop luminol-type labelling reagents without any decrease in the CL intensity of the isoluminol. *N*-benzyl isoluminol can be easily synthesized and it can have various reactive substituent groups on the *p*-position of the benzyl group. The labelling reagent which has an electron donating group on the *p*-position of the benzyl group after the labelling reaction should have sufficient CL intensities and be useful for CL detection of biomicroanalyses. The synthesis of such labelling reagents for HPLC–CL analyses and their application are now in progress.

**Acknowledgments** The authors would like to thank Dr. Shigeki Sasaki and Dr. Masakazu Tanaka, Graduate School of Pharmaceutical Sciences, Kyushu University, for their helpful suggestions.

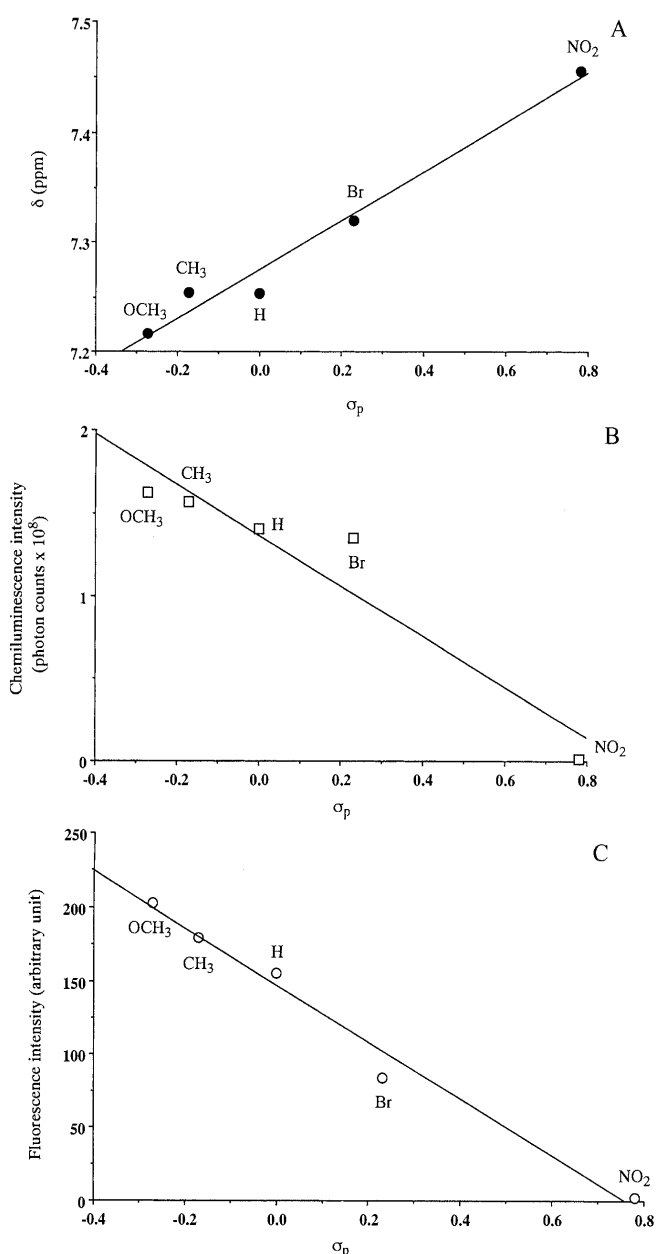


Fig. 3. The Relationship between the Hamett Substituent Constant and the Amine-H Chemical Shift Values (A), the Relative Chemiluminescence Intensities (B), and the Relative Fluorescence Intensities (C)

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# Conformational Difference between Mono- and Diprotonated *cis*-2,5-Diphenylpiperazinium Salts in the Solid State

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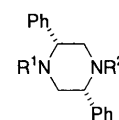
**Monohydrochlorides of *cis*-2,5-diphenylpiperazine assume a chair conformation, while the corresponding dihydrochlorides assume a boat form regardless of the substituent(s) at the nitrogen atom.**

**Key words** piperazine; conformation; X-ray analysis; <sup>15</sup>N-NMR

The conformation of saturated six-membered azacycles has been the subject of considerable interest,<sup>3)</sup> since they exhibit different types of characteristic motion, such as pyramidal nitrogen inversion, ring inversion, and rotation around single bonds of the ring substituents. Several molecules containing a piperazine moiety have recently attracted attention from the viewpoint of organic reactions,<sup>4)</sup> coordination chemistry<sup>5)</sup> and medicinal chemistry.<sup>6)</sup> Previously, we reported that protonation of the lithium enolate of 2-benzylcyclohexanone with (2*R*,5*R*)-diphenylpiperazine monohydrochloride (**1**·HCl) resulted in the formation of the corresponding (*S*)-ketone with up to 70% ee.<sup>4b)</sup> Most of the reactions were carried out under heterogeneous conditions. An important conclusion drawn from this study was that variation of a substituent on the nitrogen atom did not play a significant role in asymmetric protonation, while the piperazine

ring itself did. Thus, elucidation of the crystal structure of the piperazinium salts is needed to understand the reaction mechanism. This paper describes the solid-state structure of (2*R*,5*R*)-diphenylpiperazinium salts.

Single crystals of monohydrochlorides and dihydrochlorides of **1** and **2** were obtained from ethanol–water solution by slow evaporation of the solvent. Their crystal structures are given in Fig. 1.<sup>7)</sup> Interestingly, monohydrochlorides of **1**



**1**; R<sup>1</sup> = R<sup>2</sup> = H

**2**; R<sup>1</sup> = Me, R<sup>2</sup> = H

**3**; R<sup>1</sup> = R<sup>2</sup> = CH<sub>2</sub>CH<sub>2</sub>C(CH<sub>3</sub>)<sub>3</sub>

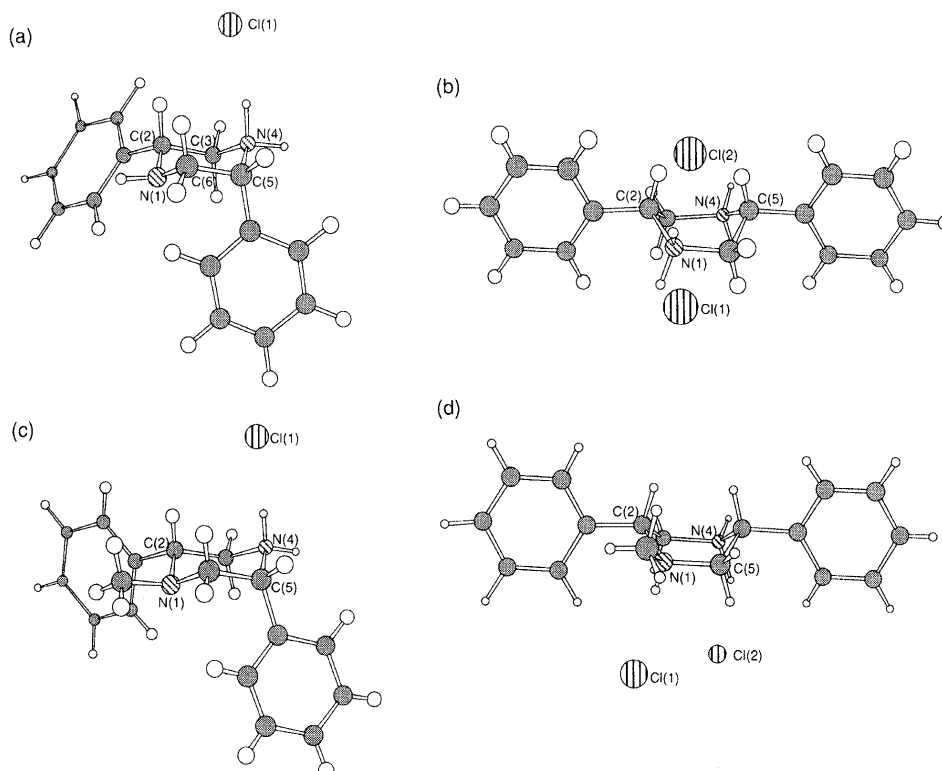


Fig. 1. Crystal Structures of **1**·HCl (a), **1**·2HCl (b), **2**·HCl (c), and **2**·2HCl (d)  
Chlorine atoms are omitted for clarity.

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Table 1. Ring Torsion Angles ( $^{\circ}$ ) of  $\mathbf{1} \cdot \text{HCl}$ ,  $\mathbf{1} \cdot 2\text{HCl}$ ,  $\mathbf{2} \cdot \text{HCl}$ , and  $\mathbf{2} \cdot 2\text{HCl}$ 

| Torsion angle       | $\mathbf{1} \cdot \text{HCl}$ | $\mathbf{1} \cdot 2\text{HCl}$ | $\mathbf{2} \cdot \text{HCl}$ | $\mathbf{2} \cdot 2\text{HCl}$ |
|---------------------|-------------------------------|--------------------------------|-------------------------------|--------------------------------|
| C(6)–N(1)–C(2)–C(3) | 70.2                          | 49.6                           | 61.2                          | 51.9                           |
| N(1)–C(2)–C(3)–N(4) | –60.2                         | –58.7                          | –57.5                         | –52.2                          |
| C(2)–C(3)–N(4)–C(5) | 58.6                          | 9.0                            | 55.6                          | –3.0                           |
| C(3)–N(4)–C(5)–C(6) | –54.7                         | 48.3                           | –53.7                         | 56.9                           |
| N(4)–C(5)–C(6)–N(1) | 51.4                          | –57.3                          | 57.7                          | –56.6                          |
| C(5)–C(6)–N(1)–C(2) | –67.7                         | 8.0                            | –63.7                         | 1.8                            |

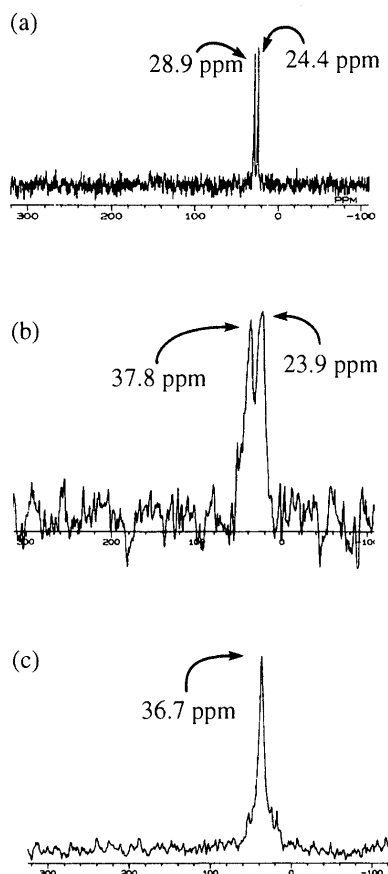


Fig. 2. Solid-State  $^{15}\text{N}$ -NMR Spectra of  $\mathbf{3}$  (a),  $\mathbf{3} \cdot \text{HCl}$  (b) and  $\mathbf{3} \cdot 2\text{HCl}$  (c). Chemical shifts are expressed in ppm from  $\text{NH}_4\text{Cl}$  as an external standard.

and  $\mathbf{2}$  exist in the chair conformation, while the corresponding dichlorides adopt a boat conformation, although each conformation is slightly distorted (Table 1). The position of the ammonium proton in monohydrochlorides of  $\mathbf{1}$  and  $\mathbf{2}$  was determined by assuming that the chloride anion is located in the neighborhood of the positively charged nitrogen atom due to electrostatic interaction. The distances from Cl(1) to N(4) and N(1) are 3.154 and 4.407 Å for  $\mathbf{1} \cdot \text{HCl}$  and 3.082 and 4.482 Å for  $\mathbf{2} \cdot \text{HCl}$ , respectively. Another interesting feature of monohydrochlorides of  $\mathbf{1}$  and  $\mathbf{2}$  is that protonation occurs at the nitrogen atom next to the carbon bearing the axial phenyl substituent.

(2*R*,5*R*)-1,4-Di-*neo*-hexyl-2,5-diphenylpiperazine  $\mathbf{3}$  and its hydrochloride salts,  $\mathbf{3} \cdot \text{HCl}$  and  $\mathbf{3} \cdot 2\text{HCl}$ , were prepared from  $\mathbf{1}$ . Solid-state  $^{15}\text{N}$ -NMR spectra of  $\mathbf{3}$ ,  $\mathbf{3} \cdot \text{HCl}$ , and  $\mathbf{3} \cdot 2\text{HCl}$  were measured by using the cross polarization magic angle spinning (CPMAS) method.<sup>8)</sup> Two nitrogens of  $\mathbf{3}$  were observed at  $\delta$  28.9 and 24.4 ppm (Fig. 2a). On the other hand, only one signal was detected for  $\mathbf{3} \cdot 2\text{HCl}$  at  $\delta$  36.7 ppm (Fig.

2c). These results indicate that the two nitrogen atoms of  $\mathbf{3}$  were exposed to different environments, while the nitrogens of  $\mathbf{3} \cdot 2\text{HCl}$  were equivalent; *i.e.*,  $\mathbf{3}$  assumes a chair conformation while  $\mathbf{3} \cdot 2\text{HCl}$  exists as a boat conformer in the solid state, which is consistent with the crystal structures of  $\mathbf{1} \cdot 2\text{HCl}$  and  $\mathbf{2} \cdot 2\text{HCl}$ .  $^{15}\text{N}$  signals of  $\mathbf{3} \cdot \text{HCl}$  appeared at  $\delta$  37.8 for the protonated nitrogen atom and at 23.9 ppm for the free nitrogen atom (Fig. 2b). Although it is difficult to determine the ring structure from the chemical shifts, we conjecture a chair conformation for  $\mathbf{3} \cdot \text{HCl}$  by analogy with  $\mathbf{1} \cdot \text{HCl}$  and  $\mathbf{2} \cdot \text{HCl}$ .

In conclusion, conformational studies of *cis*-2,5-diphenylpiperazinium salts revealed that the piperazine rings of mono- and dihydrochlorides adopt different conformations in the solid state; the former has a chair conformation, while the latter has a boat form. It is possible that monoprotonated *cis*-2,5-disubstituted piperazines generally exist in a chair conformation while diprotonated *cis*-2,5-disubstituted piperazines have a boat conformation, although more examples should be collected before we generalize the present conclusion.

## Experimental

Melting points were measured on a Yanagimoto micro melting point apparatus and are uncorrected. Specific rotations were obtained at Horiba SEPA-200 polarimeter.  $^1\text{H}$ -NMR spectra were recorded on a Varian Gemini 200 (200 MHz) spectrometer and chemical shifts are given in  $\delta$  (ppm) values with trimethylsilane as an internal standard. Solid state  $^{15}\text{N}$ -NMR spectra were recorded on a JEOL JNM-CMX 400 (400 MHz) spectrometer and chemical shifts are given in  $\delta$  (ppm) values with ammonium chloride as an external standard. IR spectra were determined with a JASCO FT/IR-300.

**(2*R*,5*R*)-1,4-Di-*neo*-hexyl-2,5-diphenylpiperazine ( $\mathbf{3}$ )** To a solution of  $\mathbf{1}$  (0.96 g, 2.4 mmol) and  $\text{NEt}_3$  (2.3 ml, 16 mmol) in  $\text{CH}_2\text{Cl}_2$  (20 ml) was added *tert*-butylacetylchloride (2.0 g, 15 mmol) dropwise at room temperature, and the mixture was stirred for 4 h. After addition of 1 *N* HCl, the mixture was extracted with AcOEt. The organic layer was washed successively with saturated  $\text{K}_2\text{CO}_3$  and brine, dried over  $\text{MgSO}_4$ , and concentrated. Column chromatography of the residue on silica gel (hexane:AcOEt=1:2 w/w) gave the corresponding diamide (1.3 g, 75%) as white amorphous. To a suspension of the above amide (1.1 g, 2.5 mmol) and  $\text{NaBH}_4$  (0.58 g, 15 mmol) in (THF) (30 ml) was slowly added  $\text{BF}_3 \cdot \text{OEt}_2$  (2.3 ml, 20 mmol) at  $0^\circ\text{C}$ , and then refluxed for 2 h. After being cooled to room temperature, the mixture was carefully quenched by water (3 ml), and concentrated under reduced pressure. To the residue was added 15% HCl (12 ml), and the mixture was refluxed for 2 h. After the neutralization by 28%  $\text{NH}_4\text{OH}$ , the reaction mixture was extracted with AcOEt, which was washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , and concentrated. The resulting residue was purified by recrystallization from hexane to give  $\mathbf{3}$  (0.98 g, 96%) as colorless plates. mp  $64\text{--}66^\circ\text{C}$ ;  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ )  $\delta$ : 0.76 (18H, s), 1.29 (2H, dt,  $J=12.1$ , 4.9 Hz), 1.46 (2H, dt,  $J=12.1$ , 4.9 Hz), 2.13 (2H, dt,  $J=11.8$ , 4.9 Hz), 2.31 (2H, dt,  $J=11.8$ , 4.9 Hz), 2.65 (2H, dd,  $J=11.8$ , 3.5 Hz), 2.98 (2H, dd,  $J=11.8$ , 6.3 Hz), 3.71 (2H, dd,  $J=6.3$ , 3.5 Hz), 7.25–7.40 (6H), 7.69 (4H, d,  $J=7.4$  Hz).  $^{15}\text{N}$ -NMR (solid)  $\delta$ : 24.4, 28.9. IR (KBr)  $\text{cm}^{-1}$ : 2960–2800, 1490, 1475, 1360, 1120, 760, 705.  $[\alpha]_D^{18} -54.0^\circ$  ( $c=1.0$ ,  $\text{CHCl}_3$ ). Anal. Calcd for  $\text{C}_{28}\text{H}_{42}\text{N}_2$ : C, 82.70; H, 10.41; N, 6.89. Found: C, 82.42; H, 10.51; N, 6.94.

**(2*R*,5*R*)-1,4-Di-*neo*-hexyl-2,5-diphenylpiperazine Monohydrochloride ( $\mathbf{3} \cdot \text{HCl}$ )** Obtained by the same procedure as described for the preparation of  $\mathbf{2} \cdot \text{HCl}$ .<sup>4b)</sup> White solids; mp  $87\text{--}88^\circ\text{C}$  (dec.) (from EtOH– $\text{H}_2\text{O}$ );  $^{15}\text{N}$ -NMR (solid)  $\delta$ : 23.9, 37.8. IR (KBr)  $\text{cm}^{-1}$ : 3450, 2960, 2400, 1680, 1475, 1365, 1200, 1115, 760, 700.  $[\alpha]_D^{18} -75.4^\circ$  ( $c=1.1$ ,  $\text{CHCl}_3$ ). This compound was too hygroscopic to get the correct analytical data.

**(2*R*,5*R*)-1,4-Di-*neo*-hexyl-2,5-diphenylpiperazine Dihydrochloride ( $\mathbf{3} \cdot 2\text{HCl}$ )** Obtained by the same procedure as described for the preparation of  $\mathbf{2} \cdot 2\text{HCl}$ .<sup>4b)</sup> White solids; mp  $136\text{--}137^\circ\text{C}$  (dec.) (from EtOH– $\text{H}_2\text{O}$ );  $^{15}\text{N}$ -NMR (solid)  $\delta$ : 36.7. IR (KBr)  $\text{cm}^{-1}$ : 2960, 2240, 1675, 1465, 1360, 1200, 760, 700.  $[\alpha]_D^{18} -92.5^\circ$  ( $c=0.32$ ,  $\text{CHCl}_3$ ). Anal. Calcd for  $\text{C}_{28}\text{H}_{44}\text{N}_2\text{Cl}_2$ : C, 70.13; H, 9.25; N, 5.84. Found: C, 70.12; H, 9.34; N, 5.75.

**X-Ray Crystallographic Data.** (2*R*,5*R*)-Diphenylpiperazine Monohydrochloride<sup>4b)</sup> ( $\mathbf{1} \cdot \text{HCl}$ )  $\text{C}_{32}\text{H}_{44}\text{Cl}_2\text{N}_4\text{O}_2$  [as  $2(\text{C}_{16}\text{H}_{18}\text{N}_2) \cdot 2\text{HCl} \cdot 2\text{H}_2\text{O}$ ].<sup>7)</sup>

FW=585.62, colorless, prismatic, orthorhombic, space group C222<sub>1</sub>,  $a=10.07(1)\text{Å}$ ,  $b=23.02(3)\text{Å}$ ,  $c=28.73(1)\text{Å}$ ,  $V=6658\text{Å}^3$ ,  $Z=16$ ,  $D_{\text{calc}}=1.168\text{g/cm}^3$ . The structure was refined to  $R=0.116$ ,  $R_w=0.164$ .

**(2R,5R)-Diphenylpiperazine Dihydrochloride<sup>4b)</sup> (1·2HCl)** C<sub>16</sub>H<sub>20</sub>Cl<sub>2</sub>N<sub>2</sub>, FW=311.12, colorless, prismatic, orthorhombic, space group P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub>,  $a=19.681(6)\text{Å}$ ,  $b=11.201(2)\text{Å}$ ,  $c=7.299(2)\text{Å}$ ,  $V=1583.2\text{Å}^3$ ,  $Z=4$ ,  $D_{\text{calc}}=1.306\text{g/cm}^3$ . The structure was refined to  $R=0.038$ ,  $R_w=0.050$ .

**(2R,5R)-1-Methyl-2,5-diphenylpiperazine Monohydrochloride<sup>4b)</sup> (2·HCl)** C<sub>17</sub>H<sub>21</sub>ClN<sub>2</sub>, FW=288.80, colorless, prismatic, monoclinic, space group P2<sub>1</sub>,  $a=11.985(3)\text{Å}$ ,  $b=6.261(1)\text{Å}$ ,  $c=11.124(3)\text{Å}$ ,  $\beta=107.19^\circ$ ,  $V=797.5\text{Å}^3$ ,  $Z=2$ ,  $D_{\text{calc}}=1.203\text{g/cm}^3$ . The structure was refined to  $R=0.035$ ,  $R_w=0.047$ .

**(2R,5R)-1-Methyl-2,5-diphenylpiperazine Dihydrochloride<sup>4b)</sup> (2·2HCl)** C<sub>17</sub>H<sub>22</sub>Cl<sub>2</sub>N<sub>2</sub>, FW=325.25, colorless, prismatic, orthorhombic, space group P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub>,  $a=18.9720(10)\text{Å}$ ,  $b=11.747(6)\text{Å}$ ,  $c=7.560(4)\text{Å}$ ,  $V=1680.2\text{Å}^3$ ,  $Z=4$ ,  $D_{\text{calc}}=1.286\text{g/cm}^3$ . The structure was refined to  $R=0.081$ ,  $R_w=0.101$ .

## References and Notes

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## Stereocontrolled Synthesis of Optically Active $\beta$ -D-Glucopyranosides of 3-Hydroxy-7,8-didehydro- $\beta$ -ionol

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Received September 18, 2000; accepted October 23, 2000

**A stereocontrolled synthesis of optically active  $\beta$ -D-glucopyranosides 1–4 of 3-hydroxy-7,8-didehydro- $\beta$ -ionol utilizing an asymmetric transfer hydrogenation to  $\alpha,\beta$ -acetylenic ketones catalyzed by chiral ruthenium complexes as the key step is described.**

**Key words**  $\beta$ -glucosides; 3-hydroxy-7,8-didehydro- $\beta$ -ionol; asymmetric transfer hydrogenation; aroma precursor; chiral ruthenium complexes

Damascenone and the related C<sub>13</sub>-norisoprenoid aroma compounds have been suggested<sup>1)</sup> to be produced through biodegradation of carotenoids in plants; however, the direct progenitors were not completely clarified. The titled compounds have been isolated from rose petals<sup>2)</sup> and Riesling wine<sup>3)</sup> as glucosidic aroma precursors. Especially, the 9-*O*-glucoside was considered<sup>3)</sup> to be the more important damascenone progenitor, since its acid-treatment yielded a higher portion of damascenone compared to the free aglycon. The role of the glucosyl moiety in these glucosides is recognized<sup>2,3)</sup> as stabilization of the hydroxy group. In intact plants, not only the position of the glucosyl moiety but also the stereochemistries of the hydroxy groups in these glucosides are considered to have influence on the behavior against enzymatic hydrolysis. Their stereochemistries at C-3 are considered to be *R*, because most xanthophylls have *R* configuration for C-3.<sup>4)</sup> However, their stereochemistries at C-9 have not been confirmed yet. Although the 9-*O*- $\beta$ -glucoside of 3-hydroxy-7,8-didehydro- $\beta$ -ionol has been prepared<sup>5)</sup> as a diastereoisomeric mixture, there has been so far reported no stereocontrolled synthesis for the optically active glucoside. In order to clarify their roles as aroma precursors in plants, the confirmation of their stereochemistries including absolute configurations and the preparation of a diastereomeric pure sample is required. We have now accomplished the stereocontrolled synthesis of (3*R*,9*S*)- and (3*R*,9*R*)-9-*O*- $\beta$ -D-glucopyranosides **1** and **2** as well as the corresponding 3-*O*- $\beta$ -D-glucopyranosides **3** and **4** utilizing an asymmetric transfer hydrogenation<sup>6)</sup> to  $\alpha,\beta$ -acetylenic ketones catalyzed by chiral ruthenium complexes as the key step.

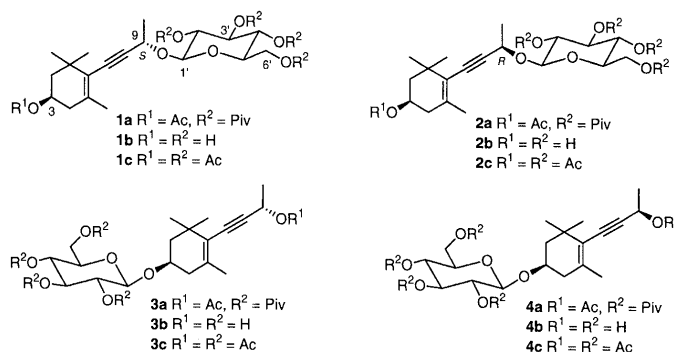
The known<sup>7)</sup> (3*R*)-3-acetoxy terminal alkyne **5**, prepared (67%) from (4*R*,6*R*)-4-hydroxy-2,2,6-trimethylcyclohexanone,<sup>8)</sup> was reduced with LiAlH<sub>4</sub> (93%) and then silylated (99%) to give the *tert*-butyldimethylsilyl (TBS) ether **6**. Reaction of the lithium derivative prepared from *n*-BuLi and **6** with acetaldehyde, followed by oxidation with MnO<sub>2</sub> provided  $\alpha,\beta$ -acetylenic ketone **7** (88%), which was deprotected (99%) and then acetylated (96%) to give the acetate **8**. Asymmetric transfer hydrogenation<sup>6)</sup> of  $\alpha,\beta$ -acetylenic ketones **7** and **8** using chiral Ru(II) catalysts **15** or **16**<sup>9)</sup> and 2-

propanol as the hydrogen donor afforded the diastereomeric pure (>98% ee)<sup>10)</sup> alcohol **9** or **10** as well as **11** or **12** in good isolated yields (>95%), respectively. The stereochemistries at C-9 of these alcohols were determined by the modified Mosher's method.<sup>11)</sup> The (*S*)- and (*R*)-MTPA [ $\alpha$ -methoxy- $\alpha$ -(trifluoromethyl)phenyl acetic acid] esters of **9** and **11** obtained by use of (*S,S*)-**15** were prepared. The positive  $\Delta\delta$  values of  $\delta_S - \delta_R$  were observed on all protons except for the 9-methyl protons, indicating that **9** and **11** had *S* configuration for C-9. Thus, it was found that **10** and **12** obtained by use of (*R,R*)-**16** had *R* configuration for C-9. In the case of the TBS ether **7**, the (*S*)- or (*R*)-alcohol **11** or **12** could be also prepared *via in situ* formation of **15** or **16** by mixing [RuCl<sub>2</sub>(*p*-cymene)]<sub>2</sub>, (1*S*,2*S*)- or (1*R*,2*R*)-*N*-(*p*-toluenesulfonyl)-1,2-diphenylethylenediamine (TsDPEN) and KOH in 2-propanol (Ru : diamine : KOH = 1 : 1 : 2.5).<sup>6)</sup>

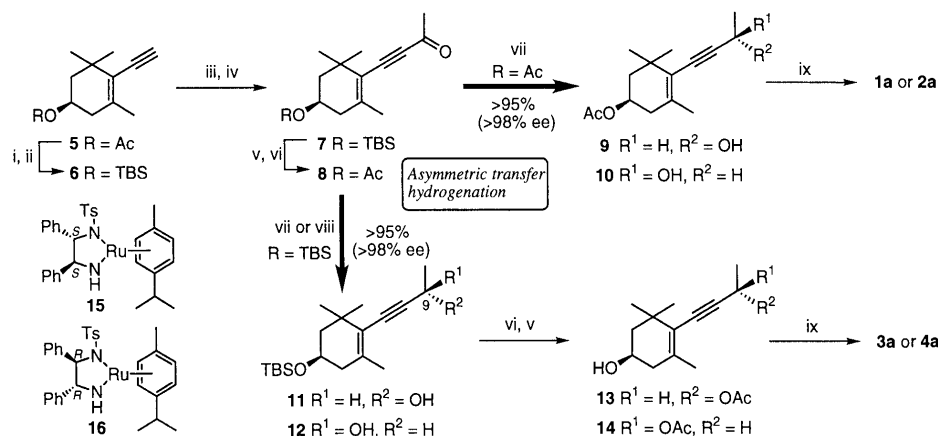
We next examined the glucosidation of alcohols **9** and **10** to prepare 9-*O*- $\beta$ -D-glucopyranosides **1** and **2**. We have previously reported<sup>12)</sup> that the reaction of 3-hydroxy- $\beta$ -ionone with tetra-*O*-benzoyl- $\alpha$ -D-glucopyranosyl bromide using silver triflate as an activator and *N,N*-tetramethylurea as a proton acceptor gave the ortho ester, whereas the desired  $\beta$ -glucoside was obtained in the absence of *N,N*-tetramethylurea. In the cases of alcohols **9** and **10**, reaction with tetra-*O*-benzoyl- $\alpha$ -D-glucopyranosyl bromide using silver triflate in the absence of *N,N*-tetramethylurea provided complex mixtures, probably due to their instability against acidic conditions.  $\beta$ -Glucosidation of **9** and **10** was achieved<sup>13)</sup> (**1a**: 72%; **2a**: 72%) by use of tetra-*O*-pivaloyl- $\alpha$ -D-glucopyranosyl bromide<sup>14)</sup> possessing a sterically bulky acyl group at C-2 position as a glucosyl donor and silver triflate as an activator in the presence of *N,N*-tetramethylurea. The acyl groups of **1a** and **2a** were removed under basic conditions to give the free alcohols (**1b**: 98%; **2b**: 99%), which were acetylated to provide pentaacetates **1c** (95%) and **2c** (87%).

3-*O*- $\beta$ -D-Glucopyranosides **3a** (64%) and **4a** (76%) were also prepared<sup>13)</sup> by a similar glucosidation of alcohols **13** and **14**, which were obtained (**13**: 97% from **11**; **14**: 91% from **12**) by acetylation of **11** and **12** and subsequent desilylation. Deacylation (**3b**: 93%; **4b**: 99%) of **3a** and **4a** followed by acetylation afforded pentaacetates **3c** (82%) and **4c** (91%).

<sup>1</sup>H- and <sup>13</sup>C-NMR spectra of 9-*O*-glucosides **1c** and **2c** were similar to each other but showed characteristic differences around C-9 as shown in the Table. Spectral data of the (9*S*)-glucoside **1c** were identical with those of the 9-*O*-glucoside isolated<sup>3)</sup> from Riesling wine, while those of the (9*R*)-glucoside **2c** were accordance with those of the 9-*O*-glucoside isolated<sup>2)</sup> from rose petals.



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Reagents: i,  $\text{LiAlH}_4$ ; ii,  $\text{TBSCl}$ ,  $\text{DMAP}$ ,  $\text{Et}_3\text{N}$ ; iii,  $n\text{-BuLi}$  then  $\text{CH}_3\text{CHO}$ ; iv,  $\text{MnO}_2$ ; v,  $\text{HF}$ ; vi,  $\text{Ac}_2\text{O}$ ,  $\text{Py}$ ; vii, cat. (*S,S*)-**15** or (*R,R*)-**16**, 2- $\text{PrOH}$ ; viii, cat.  $[\text{RuCl}_2(p\text{-cymene})]_2$ , cat. (*S,S*)- or (*R,R*)- $\text{TsDPEN}$ , cat.  $\text{KOH}$ , 2- $\text{PrOH}$ ; ix, tetra-*O*- $\text{pi}$ -valoyl- $\alpha$ - $\text{D}$ -glucopyranosyl bromide,  $\text{AgOTf}$ ,  $\text{Me}_2\text{NC(O)NMe}_2$ .

Chart 1

Table 1. Characteristic Spectral Data for Glucosides **1c**–**4c**

|                                                             |                | <b>1c</b> (9 <i>S</i> ) | <b>2c</b> (9 <i>R</i> ) | $\delta$ ( <b>1c</b> )— $\delta$ ( <b>2c</b> ) | <b>3c</b> (9 <i>S</i> ) | <b>4c</b> (9 <i>R</i> ) |
|-------------------------------------------------------------|----------------|-------------------------|-------------------------|------------------------------------------------|-------------------------|-------------------------|
| $^1\text{H-NMR}$<br>( $\text{CDCl}_3$ , 500 MHz)            | 3-H            | 5.03                    | 5.01                    | +0.02                                          | 3.92                    | 3.92                    |
|                                                             | 9-H            | 4.80                    | 4.74                    | +0.06                                          | 5.60                    | 5.59                    |
|                                                             | 9-Me           | 1.48                    | 1.51                    | −0.03                                          | 1.52                    | 1.52                    |
|                                                             | $\delta$ : ppm | 1'-H                    | 4.88                    | +0.02                                          | 4.62                    | 4.62                    |
| $^{13}\text{C-NMR}$<br>( $\text{CDCl}_3$ , 125 MHz)         | C3             | 67.74                   | 67.77                   | −0.03                                          | 73.07                   | 73.07                   |
|                                                             | C9             | 64.30                   | 67.52                   | −3.22                                          | 61.10                   | 61.10                   |
|                                                             | 9-Me           | 22.35                   | 23.12                   | +0.23                                          | 21.67                   | 21.68                   |
|                                                             | $\delta$ : ppm | C1'                     | 97.78                   | −1.00                                          | 99.61                   | 99.60                   |
| $[\alpha]_D^{26-27}$<br>( $c=0.8$ – $1.0$ , $\text{MeOH}$ ) |                | −62.1°                  | −6.8°                   | —                                              | −108.6°                 | +14.6°                  |

On the other hand, both  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra of 3-*O*-glucosides **3c** and **4c** did not show any differences, but their optical rotation data were quite different (Table). Although the  $^1\text{H}$ -NMR data for the 3-*O*-glucoside isolated<sup>3)</sup> from Riesling wine were in good agreement with those of **3c** and **4c**, the stereochemistry at C-9 is not confirmed yet as its optical rotation was not measured.

In conclusion, we have achieved a stereocontrolled synthesis of optically active  $\beta$ -*D*-glucopyranosides **1**–**4** of 3-hydroxy-7,8-didehydro- $\beta$ -ionol. Synthetic studies of related  $\text{C}_{13}$ -norisoprenoid-glucosides and investigation toward the clarification of their role as aroma precursors are now in progress.

**Acknowledgements** We are indebted to Drs. U. Hengartner and K. Bernhard, Hoffmann-La Roche Ltd., Basel, Switzerland for chemical support. We thank Dr. T. Yamano, Mr. M. Yamashita and Dr. M. Kawada, Takeda Chemical Industries, Ltd. for valuable discussions and Misses Y. Kaneko and T. Nokura for technical assistance.

#### References and Notes

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