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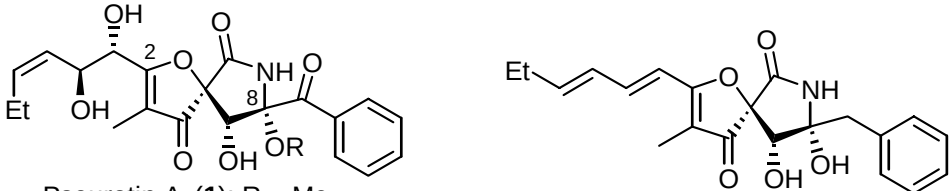
TOTAL SYNTHESES OF NATURAL PSEUROTINS A AND F_2 AND AZASPIRENE‡

Shin-ya Aoki, Takahiro Oi, Kazuya Shimizu, Ryota Shiraki, Ken-ichi Takao, and Kin-ichi Tadano*

Department of Applied Chemistry, Keio University, Hiyoshi, Kohoku-ku Yokohama 223-8522, Japan
tadano@applc.keio.ac.jp

Abstract — Total syntheses of natural pseurotins A (**1**) and F_2 (8-*O*-demethylpseurotin A) (**2**) and structurally related azaspirene (**3**), each possessing a novel heterospirocyclic system, i.e., 1-oxa-7-azaspiro[4.4]non-2-ene-4,6-dione skeleton, have been accomplished starting from D-glucose.

Pseurotin A (**1**) (Figure 1) was isolated from the cultures of *Pseudeurotium ovalis* (Ascomycetes) in 1976 by Tamm *et al.*[1] The structure of **1**, including its absolute stereochemistry, was determined by a combination of spectroscopic analysis, chemical modification, and finally by single-crystal X−Ray analysis of its dibromo derivative.[1b] Later, pseurotin F_2 (8-*O*-demethylpseurotin A) (**2**) was isolated from *Aspergillus fumigatus* DSM 6598.[2] Pseurotins are characterized by a highly functionalized spirocyclic core skeleton, i.e., 1-oxa-7-azaspiro[4.4]non-2-ene-4,6-dione structure, with three contiguous stereogenic centers, which attaches an oxygenated six-carbon olefinic side chain at C2 and a benzoyl group at C8. Pseurotins A (**1**) and F_2 (**2**) inhibit chitin synthase activity,[3] and compound (**1**) exhibits strong neurite formation activity to PC12 cells, a rat pheochromocytoma cell line.[4] In 2002, as a natural product structurally resembling pseurotins,



Pseurotin A (**1**): R = Me
Pseurotin F_2 (**2**): R = H

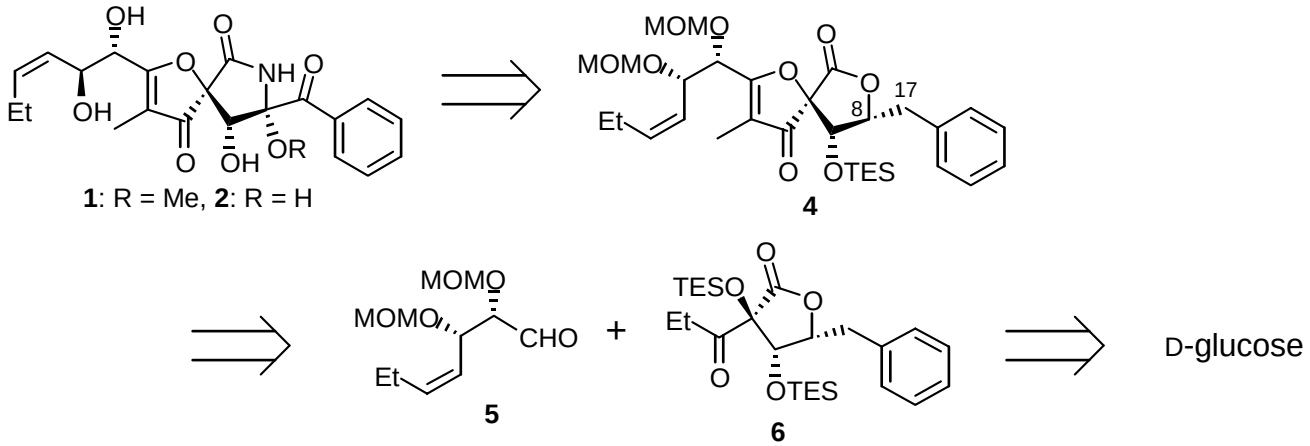
Azaspirene (**3**)

Figure 1. Structures of pseurotins A and F_2 and azaspirene

‡ This paper is dedicated to Professor Leo A. Paquette with respect and admiration on the occasion of his 70th birthday.

azaspirene (**3**) was isolated by Osada and Kakeya *et al.* from a fungus *Neosartorya* sp.,[5] which inhibits the endothelial migration induced by the vascular endothelial growth factor. Although the core spirocyclic framework of **3** is similar to that of pseurotins, the structure of **3** is characterized by an *E,E*-conjugate hexadiene unit at C2 and a benzyl group at C8 instead of the benzoyl group in **1** and **2**. Several synthetic approaches toward pseurotin A (**1**) have been reported so far by the Tamm's group.[6] Recently we reported the stereoselective synthesis of a highly functionalized spiro-furanone framework (**4**) (Scheme 1).[7] Herein, we report the aymmetric total syntheses of natural pseurotins A (**1**) and F_2 (**2**) and azaspirene (**3**).[8] Recently Hayashi and co-workers also reported the asymmetric total synthesis of **3** from methyl (*E*)-2-pentenoate.[9] Furthermore, quite recently, Hayashi's group also reported the asymmetric total syntheses of **1** and **2** from the same starting material.[10]

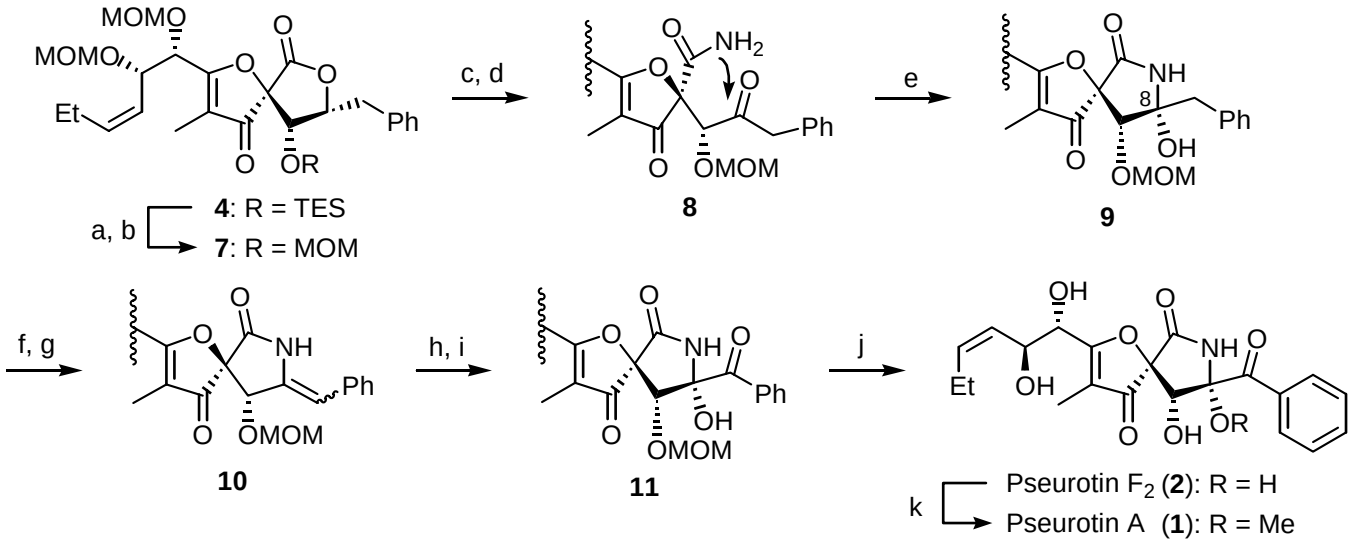
Our retrosynthesis to the pseurotins (**1**) and (**2**) is outlined in Scheme 1. We envisioned that the final transformation of the key intermediate (**4**) into the target natural products (**1**) and (**2**) would be achieved by 1) transformation of the γ-lactone to a ring-opened amide by ammonolysis, 2) oxidation of the C8 hydroxy group and simultaneous γ-lactam formation, and 3) oxidation at C17 to construct the benzoyl group in **1** and **2**. The stereoselective synthesis of the spirocyclic intermediate (**4**), featured by the connection of the ethyl ketone side chain of the highly functionalized γ-lactone (**6**) to the aldehyde (**5**), both prepared from D-glucose, has been previously reported.[7]



Scheme 1

The total syntheses of **1** and **2** are summarized in Scheme 2. The ammonolysis with liquid NH_3 or saturated NH_3 in *i*-PrOH resulted in the desired amide formation accompanied with the cleavage of the triethylsilyl (TES) group. Thus, we modified the synthetic route by the replacement of the TES group by a methoxymethyl (MOM) group. Treatment of **4** with HF·pyridine deprotected the TES group, and subsequent MOM ether formation with dimethoxymethane in the presence of phosphorus pentoxide[11] afforded the MOM ether (**7**).[12] The ammonolysis of **7** with saturated NH_3 in *i*-PrOH followed by Dess−Martin oxidation[13] provided the ring-opened amide-ketone (**8**). By exposure of **8** to saturated aqueous Na_2CO_3, intramolecular attack of the amide to the carbonyl occurred to form the aminal (**9**) (a

γ-hydroxy-γ-lactam) as the predominant α-anomer along with the β-anomer (the ratio was approximately 5:1), which was separable by column chromatography on silica gel. Heating **9** in MeOH at 60 °C followed by treatment with hot pyridine provided the enamide (**10**) as a geometrical mixture.[14,15] The enamide (**10**) was presumably obtained *via* the β-elimination of an intermediary methyl acetal (not shown). The formation of the γ-hydroxy-γ-lactam carrying a benzoyl group was successfully achieved by the regioselective epoxidation of the enamide double bond in **10** with *m*-chloroperoxybenzoic acid,[16] followed by Dess−Martin oxidation of the benzylic alcohols (not shown) formed by the ring-opening of the epoxide by attack of water. Acid hydrolysis of all the MOM groups in **11** completed the total synthesis of pseurotin F_2 (**2**). The spectroscopic data of synthetic **2**[17] matched well those reported for natural **2**. Finally, treatment of **2** with CSA in MeOH gave pseurotin A (**1**). Synthetic **1** was identical with an authentic sample of natural **1** in all respects (mp, IR, ^{1}H and ^{13}C NMR, HRMS spectrum, TLC).[18] The optical sign of the synthetic **1** established the absolute stereochemistry as shown {for synthetic **1**; $[\alpha]_D^{24}$ −8.1° (*c* 0.110, MeOH), for authentic natural **1**; $[\alpha]_D^{25}$ −9.0° (*c* 0.290, MeOH)}.

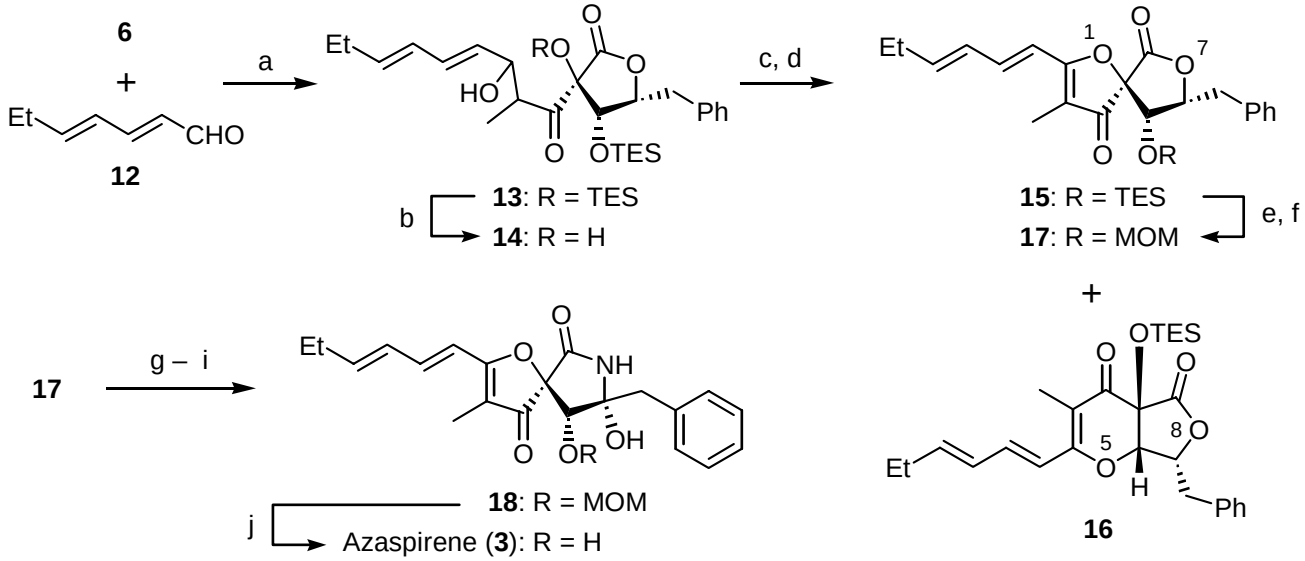


Reagents and conditions: a) HF·Py, Py, THF; b) $CH_2(OMe)_2$, P_2O_5, CH_2Cl_2, 0 °C (95% for 2 steps); c) saturated NH_3 in *i*-PrOH; d) Dess−Martin periodinane, CH_2Cl_2; e) saturated aqueous Na_2CO_3 (81% for **9** and 16% for C8-β-isomer from **7**); f) MeOH, 60 °C; g) Py, 80 °C; h) *m*-CPBA, $NaHCO_3$, CH_2Cl_2; i) Dess−Martin periodinane, CH_2Cl_2 (37% from **9**); j) 6 M HCl / MeOH (1:1, v/v) (87%); k) CSA, MeOH, 40 °C (41%).

Scheme 2

The total synthesis of natural azaspirene (**3**) was accomplished starting from the union of the intermediate (**6**) and commercially available (*E*,*E*)-2,4-heptadienal (**12**) (Scheme 3). The ethyl ketone (**6**) was deprotonated using 1.0 molar equiv. of potassium bis(trimethylsilyl)amide (KHMDS) in THF at −78 °C. The aldol reaction of the resulting enolate with **12** was best achieved in the presence of 5.0 molar equiv. of LiBr,[19] providing the aldol adduct (**13**) as a sole product. The stereochemistry of **13** was not determined.

When the reaction was conducted in the absence of LiBr, **13** was not obtained.[20] Exposure of **13** to HF·pyridine in pyridine selectively deprotected the TES ether attached to the less-hindered tertiary alcohol to provide **14**. Dess−Martin oxidation of **14** followed by dehydration of the resulting hemiketal with thionyl chloride provided the desired 1,7-dioxaspiro[4.4]non-2-ene-4,6-dione (**15**), along with a 5,8-dioxabicyclo[4.3.0]non-3-ene-2,9-dione derivative (**16**).[21] The γ-lactone (**15**) was converted into the aminal (**18**) (the γ-hydroxy-γ-lactam form) *via* the same reaction sequence used for the conversion of **4** into **9**. Hydrolysis of the MOM group completed the total synthesis of azaspirene (**3**). Synthetic **3** was identical with an authentic sample of natural **3** in all respects {(mp, IR, ^{1}H and ^{13}C NMR, HRMS spectrum, TLC),[22] for synthetic **3**; $[\alpha]_D^{23}$ −204° (*c* 0.100, MeOH), for natural **3**; $[\alpha]_D^{25}$ −204.4° (*c* 0.158, MeOH)[5]}. Thus, the absolute stereochemistry of **3** was established as shown.



Reagents and conditions: a) KHMDS, THF, −78 °C; **12**, LiBr; b) HF·Py, Py, THF, 0 °C (59% from **6**); c) Dess−Martin periodinane, CH_2Cl_2; d) $SOCl_2$, Py, 0 °C (42% for **15** and 24% for **16** from **14**); e) HF·py, py, THF; f) $CH_2(OMe)_2$, P_2O_5, CH_2Cl_2, 0 °C (72% for 2 steps); g) saturated NH_3 in *i*-PrOH; h) Dess−Martin periodinane, CH_2Cl_2; i) saturated aqueous Na_2CO_3 (85% from **17**); j) 6 M HCl / MeOH (1:1, v/v) (52%).

Scheme 3

In conclusion, we completed the asymmetric total syntheses of natural pseurotins A (**1**) and F_2 (**2**) using D-glucose as an enantiopure starting material. The formation of 1-oxa-7-azaspiro[4.4]non-2-ene-4,6-dione skeleton was achieved in a stereoselective manner. By a similar synthetic approach, the asymmetric total synthesis of natural azaspirene (**3**) was also completed.

ACKNOWLEDGMENTS

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and Taisho Pharmaceutical Co., Ltd. for participating in useful discussions. We also thank Drs. H. Osada and H. Kakeya (RIKEN) for providing the spectral data and a sample of natural azaspirene (**3**).

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12. All new compounds were fully characterized by spectroscopic means [^{1}H (300 MHz in $CDCl_3$) and ^{13}C (75 MHz in $CDCl_3$) NMR, IR] and gave satisfactory HRMS. Yields refer to homogeneous samples purified by chromatography on silica gel.
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14. The geometrical ratio (*Z/E* or *E/Z*) of enamide (**10**) was *ca.* 5:4 (determined by ^{1}H NMR at 300 MHz).
15. The C8-β-isomer of **9** also provided enamide (**10**) under similar conditions.
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17. Pseurotin F_2 (**2**) was obtained as white crystals: mp 94.4−95.0 °C [CH_2Cl_2/hexane (1:3)]; TLC R_f 0.29 (acetone/PhMe, 1:2); $[\alpha]_D^{25}$ +78.0° (*c* 0.165, $CHCl_3$), $[\alpha]_D^{20}$ −31.4° (*c* 0.100, MeOH); IR (neat) 3380,

3300, 1730, 1695, 1630 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.01 (t, 3 H, *J* = 7.6 Hz), 1.69 (s, 3 H), 2.03−2.24 (m, 2 H), 4.64 (d, 1 H, *J* = 4.2 Hz), 4.78 (dd, 1 H, *J* = 4.2, 8.9 Hz), 4.87 (s, 1 H), 5.16 (dd, 1 H, *J* = 8.9, 11.0 Hz), 5.57 (dt, 1 H, *J* = 11.0, 7.3 Hz), 6.83 (s, 1 H, O*H*), 7.49 (t, 2 H, *J* = 7.3 Hz), 7.64 (t, 1 H, *J* = 7.3 Hz), 8.40 (d, 2 H, *J* = 7.3 Hz), 8.55 (br s, 1 H, N*H*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 6.3, 14.1, 21.4, 70.8, 71.6, 71.7, 89.1, 94.8, 113.0, 126.2, 128.6 × 2, 131.4 × 2, 133.0, 134.6, 136.5, 164.8, 188.9, 193.8, 198.8; HRMS calcd for $C_{21}H_{21}NO_7$ ($M^+ - H_2O$) *m/z* 399.1318, found 399.1318.

18. Pseurotin A (**1**) was obtained as white crystals: mp 126.0−126.9 °C [CH_2Cl_2/hexane (1:3)]; TLC R_f 0.50 (acetone/PhMe, 1:1); $[\alpha]_D^{25}$ +70.8° (*c* 0.110, $CHCl_3$), for authentic natural **1**; $[\alpha]_D^{25}$ +70.8° (*c* 0.290, $CHCl_3$); IR (neat) 3400, 3280, 1730, 1680, 1635 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.99 (t, 3 H, *J* = 7.6 Hz), 1.68 (s, 3 H), 2.05−2.24 (m, 2 H), 3.44 (s, 3 H), 4.59 (d, 1 H, *J* = 4.4 Hz), 4.70 (s, 1 H), 4.75 (dd, 1 H, *J* = 4.4, 9.0 Hz), 5.28 (dd, 1 H, *J* = 9.0, 11.0 Hz), 5.60 (dt, 1 H, *J* = 11.0, 7.6 Hz), 7.49 (t, 2 H, *J* = 7.3 Hz), 7.65 (t, 1 H, *J* = 7.3 Hz), 8.27 (br s, 1 H, N*H*), 8.32 (d, 2 H, *J* = 7.3 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 6.1, 14.1, 21.4, 51.7, 70.6, 70.9, 73.0, 90.3, 92.8, 113.4, 126.4, 128.7 × 2, 130.7 × 2, 132.3, 134.8, 136.8, 166.6, 185.8, 195.1, 196.3; HRMS calcd for $C_{21}H_{21}NO_7$ ($M^+ - CH_3OH$) *m/z* 399.1318, found 399.1318.

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20. We also explored the following conditions. After treating **6** with 1.0 molar equiv. of KHMDS, 5.0 molar equiv. of the dienal (**12**) was added with 5.0 molar of chlorotriethylsilane (TESCl)[19a,b] in THF or THF/PhMe (1:1, v/v) at −78 °C. Under these conditions, the silylenol ether derived from **6** was only an obtainable product, whose geometrical stereochemistry was not determined.

21. We believe that the TES group in **14** migrated to the tertiary hydroxy group in the oxidation step. To suppress the formation of **16**, we examined a variety of oxidation conditions. However, the ratio of **15** to **16** was approximately 2:1 in all cases.

22. Azaspirene (**3**) was obtained as yellow crystals: mp 165.5−166.0 °C [EtOAc/hexane (1:2)]; TLC R_f 0.38 (EtOAc/hexane, 1:1); IR (KBr) 3250, 1735, 1715, 1675, 1610 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.07 (t, 3 H, *J* = 7.3 Hz), 1.76 (s, 3 H), 2.24 (dq, 2 H, *J* = 4.6, 7.3 Hz), 2.96, 3.27 (2 d, each 1 H, *J* = 13.9 Hz), 2.98 (d, 1 H, *J* = 10.0 Hz, O*H*), 4.50 (d, 1 H, *J* = 10.0 Hz), 6.02 (br s, 1 H, O*H*), 6.23−6.36 (m, 3 H), 6.56 (br s, 1 H, N*H*), 7.25−7.38 (m, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 5.6, 12.8, 26.3, 42.8, 74.7, 84.5, 93.2, 110.6, 114.6, 127.6, 128.4, 128.8 × 2, 130.4 × 2, 134.2, 142.1, 148.3, 165.0, 183.3, 198.4; HRMS calcd for $C_{21}H_{23}NO_5$ (M^+) *m/z* 369.1576, found 369.1572.