Pergamon

Tetrahedron Letters 43 (2002) 115–118

TETRAHEDRON LETTERS

Synthesis of all isomers of pulcherrimine, a bitter principle in the sea urchin ovary

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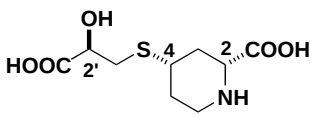
Received 3 September 2001; accepted 2 November 2001

Abstract—All eight isomers of pulcherrimine, a bitter principle of the sea urchin (*Hemicentrotus pulcherrimus*) ovary have been synthesized, which led to revision of the absolute stereochemistry of pulcherrimine. The synthetic pulcherrimine and the 2*S* isomer were highly bitter at a concentration of 1.0 mM. © 2001 Published by Elsevier Science Ltd.

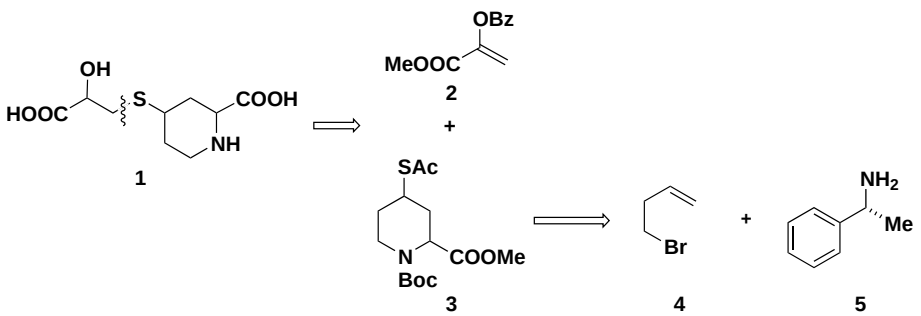
Recently, we reported the isolation and structure determination of pulcherrimine, a bitter principle of the sea urchin (*Hemicentrotus pulcherrimus*) ovary.[1] The structure of pulcherrimine was determined to be a novel sulfur-containing amino acid, 4*S*-(2′-carboxy-2′*R*-hydroxy-ethylthio)-2*R*-piperidinecarboxylic acid (**1**). Since pulcherrimine has three chiral centers, it is interesting to clarify the effect of their stereochemistry on the bitter taste of pulcherrimine. In this paper we describe the synthesis and bitterness of all eight stereoisomers of pulcherrimine.



Pulcherrimine (1)

Synthesis of pulcherrimine isomers. The retrosynthetic study readily resulted in the strategy outlined in Scheme 1. Michael addition of fully protected 4-thioacetyl-2-piperidinecarboxylic acid (**3**) to benzoyl enolate **2**, followed by deprotection will lead to all isomers of pulcherrimine. 4-Thioacetyl-2-piperidinecarboxylic acid methyl ester (**3**) can be derived from 4-bromo-1-butene (**4**) and (*R*)-(+)-α-methylbenzylamine (**5**).

Among a number of methods to prepare 4-hydroxy-2-piperidinecarboxylic acid,[2–4] those of Skiles[5] and Gillard[6] were adopted here. Starting from the commercially available (*R*)-(+)-α-methyl benzylamine (**5**) and 4-bromo-1-butene (**4**), *N*-Boc-4-hydroxy-2-piperidinecarboxylic acid methyl ester (**6a**) was prepared essentially according to the literature,[5–7] whereas **6b** was



Scheme 1.

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0040-4039/02/$ - see front matter © 2001 Published by Elsevier Science Ltd.
PII: S0040-4039(01)02095-0

prepared from (2*S*,4*R*)-lactone (Scheme 2). Overall yields of two esters, **6a** and **6b** were 99 and 97% from the corresponding lactones, respectively.

Compound **6a** was treated with MsCl to obtain the mesylate **7a** in 98% yield, which was reacted with potassium thioacetate to afford the (2*R*,4*R*)-thioacetate **3b**[8] in 42% yield as shown in Scheme 2. On the other hand, the mesylate **7a** was converted to the iodide which was treated with potassium thioacetate to give another diastereomer (2*R*,4*S*)-thioacetate **3a** in 49% yield. In the same manner, **3c** and **3d** were derived from **6b**.

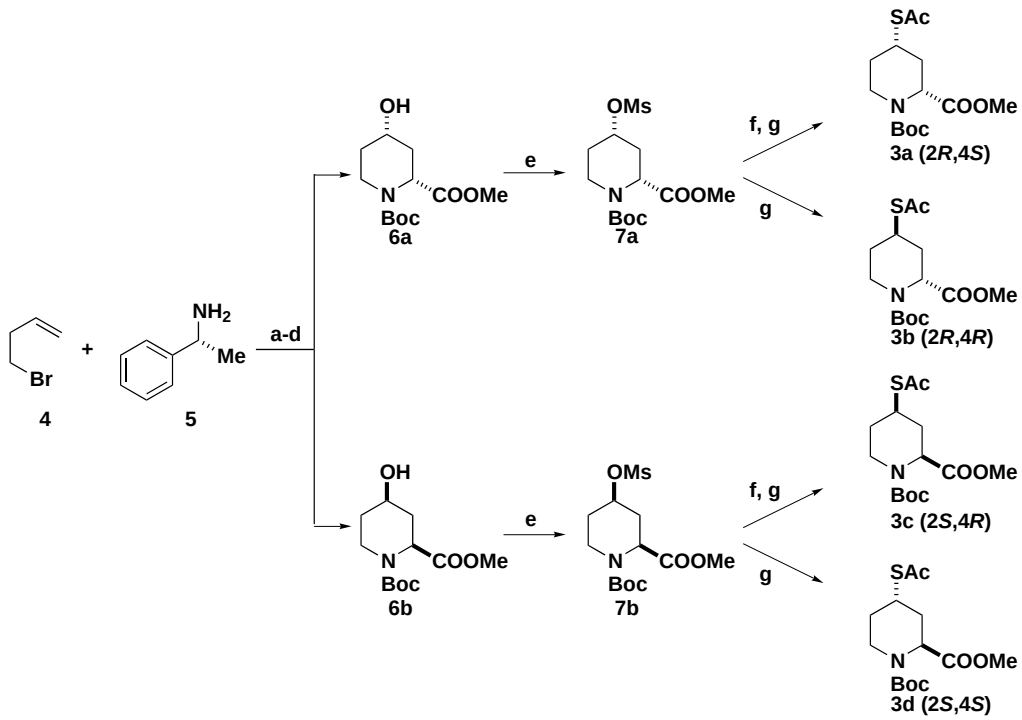
Michael addition of *N*-Boc-4-thioacetyl-piperidinecarboxylic acid methyl ester (**3a**) to the benzoyl enolate **2**[9] was effected with NaOMe in the presence of $NaBH_4$, which was accompanied by deprotection of acetate and benzoate to afford two diastereomers at the 2′-position [(2′*S*,2*R*,4*S*)-isomer **8a** and (2′*R*,2*R*,4*S*)-isomer **8b**] as shown in Scheme 3. These diastereomers were separated by repeated MPLC on silica gel with hexane/Et_2O (1:4), hexane/Et_2O/EtOH (20:80:0.1), or on ODS with MeCN/H_2O (30:70) to obtain pure **8a** and **8b** in 38 and 34% yields, respectively, which were deprotected with 1 M HCl at 100°C to give quantitative **1a** and **1b**, respectively. The other 6 isomers **1c**–**1h** were synthesized in the same fashion.

To determine the stereochemistry at C2′-position in all isomers obtained, **1a**–**1h**[10] were synthesized from chiral benzyl glycidates, instead of **2**, which were prepared from L- and D-serine in three steps (Scheme 4).[11–13] The ^1H NMR spectrum of the synthetic (2′*S*,2*R*,4*S*)-isomer **1a** was identical with that of the natural pulcherrimine. This result was contradictory to that obtained previously.[1] Moreover, chiral HPLC analysis using a SUMICHIRAL OA-5000 column led the same result.[14] Finally, modified Mosher analysis[15] applied to the *N*-Boc methyl ester derived from the natural pulcherrimine disclosed the 2′*S*-stereochemistry (Fig. 1). Thus, the stereochemistry of pulcherrimine was revised to 2′*S*, 2*R* and 4*S*.

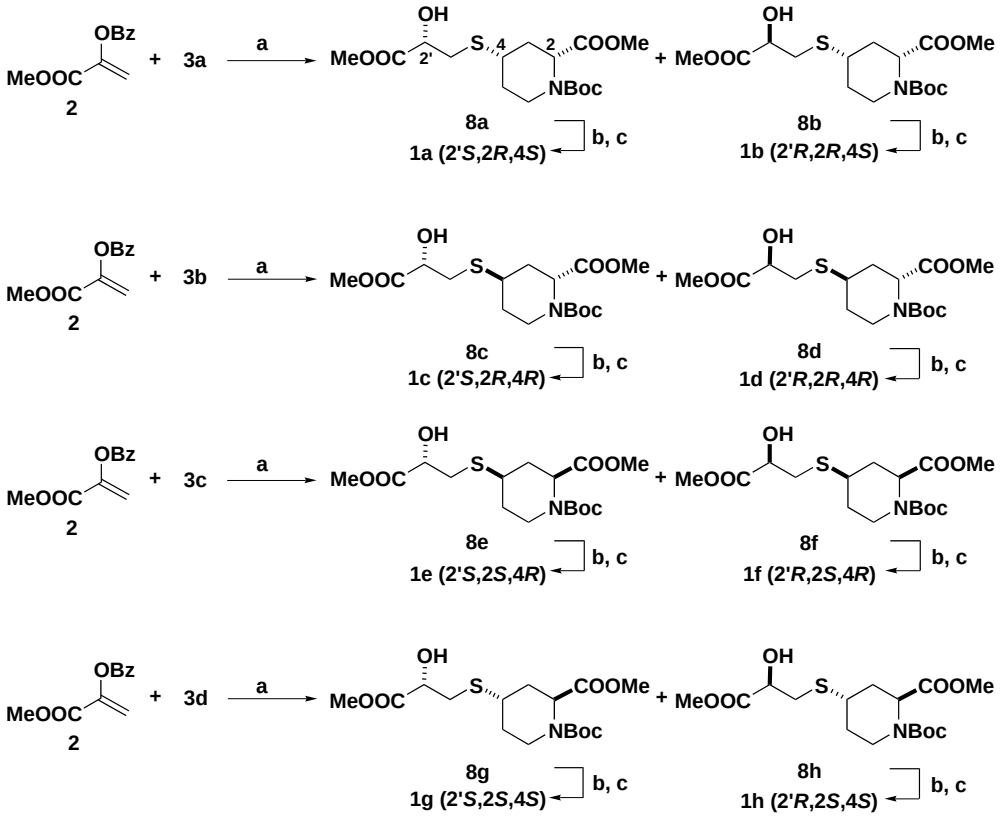
Bitterness of pulcherrimine isomers. A panel of three people evaluated bitterness of aqueous solutions of the natural pulcherrimine and synthetic eight isomers. Synthetic (2′*S*,2*R*,4*S*)-isomer **1a**, (2′*S*,2*S*,4*S*)-isomer **1g** and (2′*R*,2*S*,4*S*)-isomer **1h** were bitter, among which **1a** was most bitter, as bitter as the natural pulcherrimine. (2′*S*,2*S*,4*S*)-Isomer **1g** was also highly bitter, but tasted different from pulcherrimine. The detection and recognition thresholds for **1g** and **1h** were estimated to be 0.192/0.306 mM and 0.250/0.500 mM, respectively.[16,17]

Acknowledgements

We are grateful to Professor Nobuhiro Fusetani, University of Tokyo, for his critical reading of the manuscript. A Domestic Research Fellowship of Japan Science and Technology Corporation to N.U.S. is acknowledged.



Scheme 2. *Reagents and conditions*: (a) Et_3N, MeCN; (b) i. OHCCOOH, 4 Å MS, MeCN, ii. separation of diastereomers; (c) H_2/$Pd(OH)_2$, HCl–MeOH; (d) Boc_2O, Et_3N; (e) MsCl, CH_2Cl_2, pyr., DMAP; (f) NaI, acetone, reflux; (g) MeCOSK, MeCN, reflux.



Scheme 3. *Reagents and conditions*: (a) $NaBH_4$ (0.5 equiv.), NaOMe (2.5 equiv.), MeOH, 0°C; (b) 1 M aq. HCl, 100°C; (c) HPLC purification.

Scheme 4. *Reagents and conditions*: (a) $NaBH_4$ (0.5 equiv.), NaOMe (2.5 equiv.), MeOH, 0°C; (b) 1 M aq. HCl, 100°C; (c) HPLC purification.

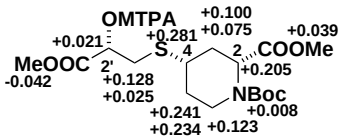


Figure 1. $\Delta\delta$ values obtained for MTPA esters of pulcherrimine derivative.

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10. Spectral data of eight isomers: **1a** (2′*S*,2*R*,4*S*) $[\alpha]_D^{22}$ −13.3° (*c* 0.10, H_2O) [lit. natural $[\alpha]_D^{24}$ −16.5° (*c* 0.20, H_2O)]; HRFABMS *m*/*z* 250.0736 $(M+H)^+$ $C_9H_{16}O_5NS$ (Δ +1.6 mmu). **1f** (2′*R*,2*S*,4*R*). $[\alpha]_D^{22}$ +12.4° (*c* 0.20, H_2O); HRFABMS *m*/*z* 250.0739 $(M+H)^+$ $C_9H_{16}O_5NS$ (Δ −1.1 mmu). Other spectral data are identical with those of natural pulcherrimine. **1b** (2′*R*,2*R*,4*S*). $[\alpha]_D^{23}$ −18.8° (*c* 0.20, H_2O); IR (film) ν_{max} 3423, 3080, 2970, 1727, 1631, 1400, 1286, 1102 cm^{-1}; ^1H NMR in D_2O–CD_3OD (40:1) δ 4.19 (1H, dd, $J=6.8$, 3.9 Hz), 3.64 (1H, dd, $J=12.7$, 3.4 Hz), 3.49 (1H, ddd, $J=13.2$, 4.4, 2.5 Hz), 3.10 (1H, m), 3.05 (1H, dd, $J=13.7$, 3.9 Hz), 3.04 (1H, m), 2.90 (1H, dd, $J=13.7$, 6.8 Hz), 2.59 (1H, m), 2.24 (1H, m), 1.64 (1H, m), 1.61 (1H, m); ^{13}C NMR in D_2O–CD_3OD (40:1) δ 180.0 s, 174.3 s, 72.5 d, 60.0 d, 44.1 t, 39.6 d, 35.3 t, 34.3 t, 29.8 t; HRFABMS *m*/*z* 250.0741 $(M+H)^+$ $C_9H_{16}O_5NS$ (Δ −0.8 mmu). **1e** (2′*S*,2*S*,4*R*). $[\alpha]_D^{23}$ +20.8° (*c*

0.20, H_2O); HRFABMS m/z 250.0736 $(M+H)^+$ $C_9H_{16}O_5NS$ (Δ −1.3 mmu). Other spectral data are identical with those of **1b**. **1c** (2′*S*,2*R*,4*R*). $[\alpha]_D^{23}$ +12.8° (*c* 0.20, H_2O); IR (film) ν_{max} 3419, 1715, 1633, 1395, 1315, 1090 cm^{-1}; ^1H NMR in D_2O–CD_3OD (40:1) δ 4.09 (1H, dd, $J=6.8$, 3.9 Hz), 3.86 (1H, dd, $J=10.3$, 4.4 Hz), 3.29 (1H, quint, $J=4.4$ Hz), 3.22 (1H, m), 3.21 (1H, m), 2.90 (1H, dd, $J=13.7$, 3.9 Hz), 2.79 (1H, dd, $J=13.7$, 6.8 Hz), 2.15 (1H, m), 2.10 (1H, m), 2.03 (1H, m), 1.83 (1H, m); ^{13}C NMR in D_2O–CD_3OD (40:1) δ 180.0 s, 174.7 s, 72.4 d, 55.6 d, 40.6 t, 38.5 d, 36.1 t, 32.2 t, 27.9 t; HRFABMS m/z 250.0739 $(M+H)^+$ $C_9H_{16}O_5NS$ (Δ −1.9 mmu). **1h** (2′*R*,2*S*,4*S*). $[\alpha]_D^{23}$ −13.3° (*c* 0.20, H_2O); HRFABMS m/z 250.0739 $(M+H)^+$ $C_9H_{16}O_5NS$ (Δ +0.3 mmu). Other spectral data are identical with those of **1c**. **1d** (2′*R*,2*R*,4*R*). $[\alpha]_D^{23}$ 0° (*c* 0.25, H_2O); IR (film) ν_{max} 3412, 3043, 1725, 1627, 1398, 1282, 1219, 1097 cm^{-1}; ^1H NMR in D_2O–CD_3OD (40:1) δ 4.12 (1H, dd, $J=6.8$, 3.9 Hz), 3.86 (1H, dd, $J=9.8$, 4.4 Hz), 3.30 (1H, quint, $J=4.4$ Hz), 3.22 (1H, m), 3.21 (1H, m), 2.96 (1H, dd, $J=13.7$, 3.9 Hz), 2.78 (1H, dd, $J=13.7$, 6.8 Hz), 2.16 (1H, m), 2.12 (1H, m), 2.03 (1H, m), 1.84 (1H, m); ^{13}C NMR in D_2O–CD_3OD (40:1) δ 180.0 s, 174.7 s, 72.3 d, 55.6 d, 40.5 t, 38.5 d, 36.2 t, 32.4 t, 27.7 t; HRFABMS m/z 250.0765 $(M+H)^+$ $C_9H_{16}O_5NS$ (Δ +1.6 mmu). **1g** (2′*S*,2*S*,4*S*). $[\alpha]_D^{23}$ 0° (*c* 0.25, H_2O); HRFABMS m/z 250.0728 $(M+H)^+$ $C_9H_{16}O_5NS$ (Δ −2.1 mmu). Other spectral data are identical with those of **1d**.

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