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A concise synthesis of (+)-preussin

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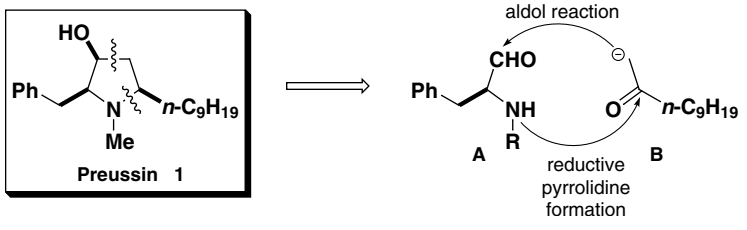
Abstract—A concise synthesis of (+)-preussin (**1**), an antifungal agent and a growth-inhibitor of fission yeast and human cancer cells, was accomplished employing a stereoselective aldol reaction between the zinc enolate of 2-undecanone and *N*-protected-L-phenylalaninal followed by reductive pyrrolidine formation as key steps.

1. Introduction

Preussin (L-657,398) (**1**), a naturally occurring pyrrolidine alkaloid, has been isolated from fermentation broths of *Aspergillus ochraceus* ACTT22947 and *Preussia* sp.[1] and exhibits antifungal and antibacterial activities.[2] Following the first asymmetric synthesis of **1** by Pak et al. in 1991,[3] several approaches to preussin itself and its stereoisomers have been reported.[4] In 1997, Yoshida, Horinouchi et al. have rediscovered preussin as a selective inhibitor for cell growth of the fission yeast *ts* mutants defective on *cdc2*-regulatory genes[5] and more recently, its activities in apoptosis-induction and as a potent inhibitor of cyclin E kinase in human tumor cells were reported by Müller et al.[6] We were interested in these bioactivities and investigated a concise synthetic route to **1** and its analogs. Herein, we report a short-step synthesis of preussin (**1**) with a stereoselective aldol reaction to *N*-protected-L-phenylalaninal and reductive pyrrolidine formation as key steps (**A**+**B** in Scheme 1).

2. Results and discussion

The substrate for the aldol reaction (**2b**) was prepared by $LiAlH_4$ reduction of Weinreb amide (**2a**)[7] in 41% yield after recrystallization (Scheme 2). As reported previously,[7b] DIBAL reduction of the corresponding methyl ester caused significant racemization of the product **2b** reducing the e.e. to 13%. We first used the lithium enolate of 2-undecanone for the aldol reaction with **2b** in THF at −78°C, but the reaction was found to be non-stereoselective (76% combined yield, **3a**/**3b**=1:1.7) as already known.[8,9] However, the chelation-controlled aldol reaction could be achieved by using the zinc enolate of 2-undecanone in CH_2Cl_2 at −78°C to provide *syn*-alcohol **3a** and its *anti*-isomer **3b** in a ratio of **3a**/**3b**=10:1 (72% combined yield). Each isomer could be isolated by silica gel column chromatography and their stereochemistries were confirmed by elaborating the major isomer **3a** to preussin as described below.



Scheme 1. Synthetic approach to preussin (**1**).

Keywords: concise synthesis; antifungal; antibacterial; cell growth inhibitor; apoptosis; preussin.

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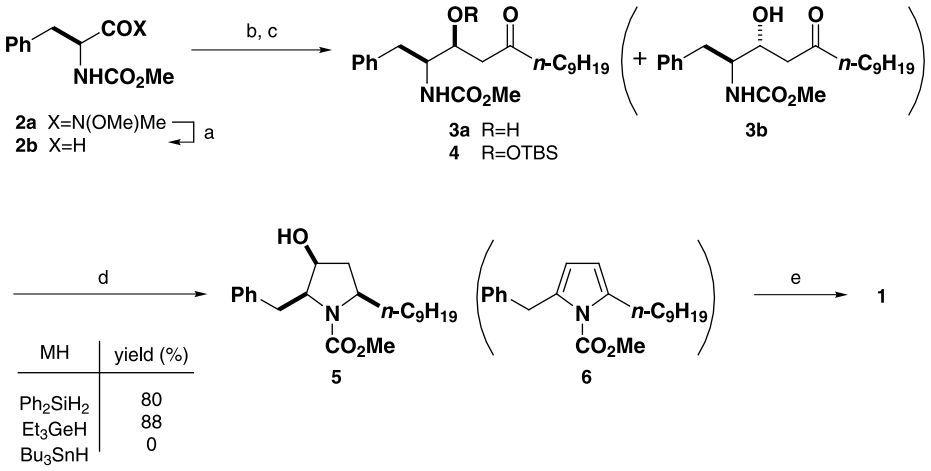

a) $LiAlH_4$, Et_2O, –10→–5°C, (41%). b) 2-undecanone, LiHMDS, $ZnCl_2$, CH_2Cl_2, (72%, **3a**:**3b**=10:1). c) TBSCl, Im., DMF, r.t. (83%). d) MH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, –78°C→r.t. e) $LiAlH_4$, THF, reflux (83%).

Scheme 2. Synthesis of preussin (**1**).

The hydroxyl group of **3a** was protected as the TBS ether **4**, which was then treated with Ph_2SiH_2 or Et_3GeH in the presence of $BF_3{\cdot}OEt_2$ (CH_2Cl_2, −78°C→rt).[10] Under these conditions, not only reductive cyclization but also desilylation took place to provide free alcohol **5** as a single stereoisomer[4e,11] (~80–88% yield). For this reaction, TBS protection was essential and when the nonprotected alcohol **3a** was reduced directly under the same conditions, aromatized pyrrole derivative **6** was obtained as the major product. Employing Bu_3SnH instead of Ph_2SiH_2 or Et_3GeH caused only 1,2-reduction of the ketone to give an alcohol. Finally, reduction of the methyl carbamate of **5** with $LiAlH_4$ gave preussin (**1**) as a single stereoisomer {83%, $[\alpha]_D^{25}=+29.3°$ (c 1.17, $CHCl_3$), [natural **1**, $[\alpha]_D^{25}=+22.0°$ (c 1.0, $CHCl_3$)]}. The ^1H and ^{13}C NMR spectral data of the synthetic material were identical with those of natural **1**.[2]

In conclusion, a short-step and stereoselective synthesis of preussin (**1**) was accomplished by using a stereoselective aldol reaction followed by reductive pyrrolidine formation of the resulting keto amide. The overall yield was 16% over five steps.

3. Experimental

Melting point was measured using a Büchi 535 melting point apparatus and is uncorrected. Infrared spectra were recorded on a Jasco FT/IR-620 spectrometer. ^1H NMR spectra were recorded on a JEOL JNM-A500 spectrometer (500 MHz). ^{13}C NMR spectrum was recorded on a Bruker AM300 spectrometer (75 MHz). Chemical shifts are reported in δ ppm and referenced to the residual proton signal for $CDCl_3$ (7.24 ppm) or DMSO-d_6 (2.49 ppm) and to internal $CDCl_3$ (^{13}C, 77.0 ppm). Specific rotations were measured with a Jasco DIP-1000 polarimeter. Mass spectra were obtained with a JEOL JMS-AX505WA instrument. Column chromatography was performed with MERCK silica gel 60 (0.063–0.200 mm) or FUJI SILYSIA CHEMICAL LTD. Chromatorex® NH (100–200 mesh).

3.1. (*S*)-2-Methoxycarbonylamino-3-phenylpropanal (*N*-methoxycarbonyl-L-phenylalaninal) (2b)

To a solution of (*S*)-[1-(methoxy)methylcarbamoyl-2-phenylethyl]carbamic acid methylester[7] (**2a**, 6.1 g, 22.9 mmol) in Et_2O (230 mL) was added $LiAlH_4$ powder (1.1 g, 29.0 mmol) at −10 to −5°C. The reaction mixture was stirred for 2 h, and then quenched by a solution of $KHSO_4$ (3.2 g) in water (110 mL). The organic layer was separated, and the aqueous layer was extracted with Et_2O. The combined organic layers were washed successively with 3N HCl, H_2O, saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4 and concentrated. The residue was recrystallized from Et_2O-hexane to give **2b** as a colourless powder (2.7 g, 41%). mp 78–80°C; $[\alpha]_D^{25}=-42.3°$ (c 1.01, MeOH); IR (KBr) 3336, 3032, 2951, 1741, 1693, 1542, 1271, 1071, 703 cm^{-1}; ^1H NMR ($CDCl_3$, at 55°C) δ 3.10 (br. d, 2H, J=6.1Hz), 3.67 (s, 3H), 4.46 (br. m, 1H), 5.12 (br. m, 1H), 7.15–7.31 (m, 5H), 9.62 (s, 1H); EIMS *m/z* 207 (M^+); HRMS (EI) *m/z* calc. for $C_{22}H_{13}NO_3$ 207.0895, found 207.0851.

3.2. (2*S*,3*S*)-3-Hydroxy-2-methoxycarbonylamino-1-phenyltetradecan-5-one (3a)

A solution of lithium bis(trimethylsilyl)amide (1.0 M in hexane, 1.1 mL, 1.10 mmol) in dry CH_2Cl_2 (3.0 mL) was cooled to −78°C, and a solution of 2-undecanone (210 μL, 1.02 mmol) in dry CH_2Cl_2 (3.0 mL) was added dropwise over 20 min. The reaction was stirred at −78°C for 50 min, and a solution of $ZnCl_2$ (1.0 M in Et_2O, 1.0 mL, 1.00 mmol) was introduced dropwise over 15 min. After 15 min, a solution of **2b** (99 mg, 0.349 mmol) in dry CH_2Cl_2 (2.0 mL) was added. After the mixture was stirred at −78°C for 15 min, the reaction was quenched by a dropwise addition of a solution of acetic acid (90 μL) in Et_2O (2.0 mL). The mixture was allowed to warm to room temperature, and was diluted with EtOAc. The organic layer was washed successively with 1N HCl, water, saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$ and

concentrated. The residue was purified by chromatography (Chromatorex® NH, hexane:CH_2Cl_2:Et_2O=2:2:1) to give **3a** as a colourless oil (87 mg, 66%) and **3b** (9.0 mg, 6.8%). **3a**: $[\alpha]_D^{25}=-44.3°$ (c 0.62, $CHCl_3$); IR (neat) 3349, 2922, 2852, 1698, 1533, 1250, 1051, 705 cm^{-1}; ^1H NMR ($CDCl_3$, at 55°C) δ 0.87 (t, 3H, J=7.3 Hz), 1.24 (m, 12H), 1.52 (m, 2H), 2.35 (dd, 1H, J=7.3, 7.3 Hz), 2.50 (br. d, 1H, J=17.7 Hz), 2.60 (dd, 1H, J=17.7, 9.8 Hz), 2.90 (m, 2H), 3.42 (m, 1H), 3.63 (s, 3H), 3.72 (m, 1H), 4.01 (dd, 1H, J=9.8, 1.8 Hz), 5.03 (br. d, 1H, J=10.0 Hz), 7.17–7.28 (m, 5H); FABMS m/z 378 (M+1); HRMS (FAB) m/z calcd for $C_{22}H_{36}NO_4$ 378.2644, found 378.2648. **3b**: $[\alpha]_D^{25}=+16.3°$ (c 0.57, $CHCl_3$); IR (neat) 3323, 2921, 2851, 1697, 1543, 1267, 1038, 704 cm^{-1}; ^1H NMR ($CDCl_3$, 55°C) δ 0.87 (t, 3H, J=6.7 Hz), 1.26–1.29 (m, 12H), 1.54 (m, 2H), 2.38 (m, 2H), 2.55 (dd, 1H, J=17.7, 8.5 Hz), 2.63 (dd, 1H, J=17.7, 3.1 Hz), 2.84 (dd, 1H, J=14.0, 7.9 Hz), 2.96 (dd, 1H, J=14.0, 4.9 Hz), 3.42 (m, 1H), 3.57 (s, 3H), 3.84 (m, 1H), 3.98 (m, 1H), 4.63 (m, 1H), 7.19–7.29 (m, 5H); FABMS m/z 378 (M+1); HRMS (FAB) m/z calcd for $C_{22}H_{36}NO_4$ 378.2644, found 378.2648.

3.3. (2*S*,3*S*)-3-*tert*-Butyldimethylsilyloxy-2-methoxycarbonylamino-1-phenyltetradecan-5-one (4)

Imidazole (20 mg, 0.294 mmol) and TBDMSCl (35 mg, 0.232 mmol) were added to a solution of **3a** (55 mg, 0.146 mmol) in DMF (1.0 mL) at 0°C. After the solution was stirred at room temperature for 1 day, water was added and the resulting mixture was concentrated in vacuo. The residue was diluted with EtOAc. The organic layer was washed successively with dilute HCl, water, saturated $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated. The residue was purified by chromatography on silica gel (hexane:Et_2O=3:1) to give **4** as a colourless oil (59 mg, 83%). $[\alpha]_D^{25}=-12.3°$ (c 1.24, $CHCl_3$); IR (neat) 3341, 2927, 2855, 1715, 1508, 1253, 1069, 836, 778, 699 cm^{-1}; ^1H NMR ($CDCl_3$, at 55°C) δ 0.00 (s, 3H), 0.10 (s, 3H), 0.84 (t, 3H, J=6.7 Hz), 0.90 (s, 9H), 1.23–1.28 (m, 12H), 1.49 (m, 2H), 2.29 (m, 2H), 2.48 (dd, 1H, J=17.1, 5.5 Hz), 2.62 (m, 1H), 2.68 (dd, 1H, J=14.0, 8.5 Hz), 2.82 (dd, 1H, J=14.0, 6.1 Hz), 3.52 (br. s, 3H), 3.85 (br. m, 1H), 4.28 (br. m, 1H), 4.73 (br. m, 1H), 7.13–7.24 (m, 5H); FABMS m/z 492 (M+1); HRMS (FAB) m/z calcd for $C_{28}H_{50}NO_4Si$ 492.3509, found 492.3516.

3.4. (2*S*,3*S*,5*R*)-2-Benzyl-3-hydroxy-1-methoxycarbonyl-5-nonylazolidine (5)

A solution of **4** (24 mg, 0.0488 mmol) in CH_2Cl_2 (1.0 mL) was cooled to −78°C, and Et_3GeH (25 μL, 0.155 mmol) and $BF_3{\cdot}OEt_2$ (25 μL, 0.197 mmol) were added. The resulting solution was allowed to warm to room temperature. After stirring for 15 h, saturated $NaHCO_3$ was added, and the reaction mixture was diluted with EtOAc. The organic layer was washed successively with water and brine, dried over $MgSO_4$ and concentrated. The residue was purified by chromatography on silica gel (hexane:Et_2O=1:2) to give **5** as a colourless oil (16 mg, 88%). $[\alpha]_D^{25}=-44.5°$ (c 0.70, $CHCl_3$); IR (neat) 3432, 2925, 2854, 1673, 1455, 1388, 1125, 1085, 773, 743, 699 cm^{-1}; ^1H NMR (DMSO-d_6, at 90°C) δ 0.87 (t, 3H, J=7.3 Hz), 1.24–1.39 (m, 15H), 1.57 (ddd, 1H, J=12.2, 7.9, 7.9 Hz), 1.92 (m, 1H), 2.16 (ddd, 1H, J=12.2, 6.7, 6.7 Hz), 2.66 (dd, 1H, J=14.0, 6.7 Hz), 3.02 (dd, 1H, J=14.0, 6.1 Hz), 3.36 (s, 3H), 3.62 (dddd, 1H, J=9.2, 7.3, 7.3, 4.3 Hz), 3.98 (dd, 1H, J=13.4, 6.7 Hz), 4.15 (m, 1H), 4.84 (d, 1H, J=4.3 Hz), 7.13–7.23 (m, 5H); FABMS m/z 362 (M+1); HRMS (FAB) m/z calcd for $C_{22}H_{36}NO_3$ 362.2695, found 362.2681.

3.5. (2*S*,3*S*,5*R*)-2-Benzyl-3-hydroxy-1-methyl-5-nonylazolidine [(+)-Preussin (1)]

To a solution of **5** (394 mg, 1.09 mmol) in THF (10 mL) was added $LiAlH_4$ powder (60 mg, 1.58 mmol) at room temperature. After the reaction mixture was heated and refluxed for 2 h, it was cooled to 0°C and quenched by addition of saturated NH_4Cl solution. The resulting mixture was diluted with EtOAc. The organic layer was washed successively with 2N NaOH, water and brine, dried over Na_2SO_4 and concentrated. The residue was purified by chromatography (Chromatorex® NH, hexane:EtOAc=3:1) to give **1** as a colourless oil (288 mg, 83%). $[\alpha]_D^{25}=+29.3°$ (c 1.17, $CHCl_3$); IR (neat) 3429, 2925, 2853, 2785, 1496, 1455, 1348, 1133, 1030, 929, 743, 966 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.83 (t, 3H, J=7.0 Hz), 1.21 (m, 14H), 1.35 (ddd, 1H, J=14.0, 6.1, 1.2 Hz), 1.66 (m, 2H), 1.78 (m, 1H), 2.06 (m, 1H), 2.13 (ddd, 1H, J=14.0, 9.2, 6.1 Hz), 2.21 (ddd, 1H, J=9.2, 5.5, 3.7 Hz), 2.28 (s, 3H), 2.79 (dd, 1H, J=13.4, 5.5 Hz), 2.81 (dd, 1H, J=13.4, 9.2 Hz), 3.74 (m, 1H), 7.13–7.25 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 26.3, 29.3, 29.5, 29.6, 29.9, 31.9, 33.6, 34.9, 38.6, 39.3, 65.8, 70.4, 73.6, 126.0, 128.3, 129.3, 139.4; FABMS m/z 318 (M+1); HRMS (FAB) m/z calcd for $C_{21}H_{35}NO$ 318.2797, found 318.2802.

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