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A total synthesis of mycalisine A

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ABSTRACT

In this paper, we report a total synthesis of a naturally occurring pyrrolo[2,3-*d*]pyrimidine nucleoside, mycalisine A. Our synthetic strategy uses D-xylose as the starting material and Vorbrüggen glycosylation as the key step. Mycalisine A was synthesized in 11 steps with a 15% overall yield.

1. Introduction

In 1985, two novel nucleosides, mycalisines A and B (Fig. 1), were isolated from a marine sponge *Mycale* sp., collected in the Gulf of Sagami, Japan [1]. They have the characteristic pyrrolo[2,3-*d*]pyrimidine base moiety, which has been found in many naturally occurring and biologically significant nucleosides, such as cadeguomycin [2], sangivamycin [3], and toyocamicin [4]. Both mycalisines A and B showed inhibitive activity in cell division of fertilized starfish eggs. However, mycalisine A was found to be 400 times more active than mycalisine B [1]. As part of our continuing effort in the synthesis of bioactive marine nucleosides [5–8], we report an efficient total synthesis mycalisine A in the present paper.

Until now, only one synthesis of mycalisine A has been reported, which used regioselective methylation of toyocamicin **2** as the key step (Fig. 1 route a) [9]. This poor regioselective methylation of the extraordinarily expensive toyocamicin makes this strategy impracticable. Our retrosynthetic analysis of mycalisine A is shown in Fig. 1 (route b). We reasoned that ribose acetate **3** would be a logical precursor for the late-stage Vorbrüggen glycosylation with nucleobase **4**. This intermediate **3** could be ultimately derived from commercially available D-xylose **5**.

2. Experimental

All reagents and catalysts were purchased from commercial sources and used without purification. MeCN, pyridine and DCM were dried with CaH_2 and distilled prior to use. THF was dried with $LiAlH_4$ and distilled prior to use. Thin layer chromatography was performed using silica gel GF-254 plates (Qingdao Chemical Company, China) detected by UV (254 nm) or charting with 10% sulfuric acid in ethanol. Column chromatography was performed on silica gel (200–300 mesh, Qingdao Chemical Company, China). NMR spectra were recorded on a Bruker AV400 spectrometer, and chemical shifts (δ) are reported in ppm. ^{1}H NMR and ^{13}C NMR spectra were calibrated with TMS as an internal standard, and coupling constants (J) are reported in Hz. The ESI-MS were obtained on a Bruker Dalton microTOFQ II spectrometer in the positive ion mode.

2.1. 5-*O*-Benzoyl-1,2-*O*-diacetyl-3-*O*-methyl-D-ribofuranose 9 (**3**)

5-*O*-Benzoyl-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-ribofuranose (**10**) (500 mg, 1.62 mmol) was dissolved in HOAc (15 mL) and Ac_2O (1.5 mL). Concentrated H_2SO_4 (0.75 mL) was added to the above mixture slowly. Then, the obtained solution was stirred at room temperature overnight and poured into ice water (100 mL). After extraction with DCM (50 mL × 3), the organic layer was washed with saturated $NaHCO_3$ and dried with anhydrous Na_2SO_4. After filtration and removal of solvent in vacuo, the obtained residue was purified by flash column to give compound **3** as a mixture (α:β = 2:3, 500 mg, 88%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06 (m, 2H), 7.56 (m, 1H), 7.43 (m, 2H), 6.14 (s, 1H), 5.32 (d, 1H,

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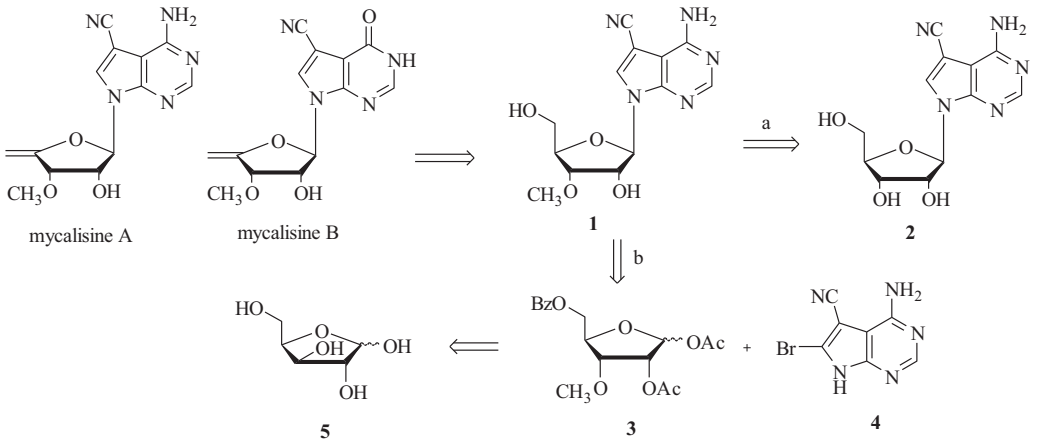


Fig. 1. Structures of mycalisines A and B and their retrosynthetic analysis.

J = 4.4 Hz), 4.65 (m, 1H), 4.40 (m, 1H), 4.30 (m, 1H), 4.06 (m, 1H), 3.40 (s, 3H), 2.14 (s, 3H), 1.91 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.7, 168.9, 166.1, 133.2, 129.8, 128.7, 128.4, 98.4, 79.7, 78.9, 73.0, 63.6, 59.3, 20.8, 20.6; ESI-MS: *m*/*z* 353.2 $[M+H]^+$.

*2.2. 4-Amino-6-bromo-5-cyano-7-(2-O-acetyl-3-O-methyl-5-O-benzoyl-β-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidine (**11**)*

4-Amino-6-bromo-5-cyano-7*H*-pyrrolo[2,3-*d*]pyrimidine (**4**) (525 mg, 2.20 mmol) was suspended in freshly distilled CH_3CN (5 mL). *N*,*O*-Bis(trimethylsilyl)acetamide (2.2 mL, 8.80 mmol) was added and the mixture was stirred for 15 min. Then 5-*O*-benzoyl-1,2-*O*-diacetyl-3-*O*-methyl-D-ribofuranose (**3**) (704 mg, 2.0 mmol) and trimethylsilyl trifluoromethanesulfonate (2.20 mL, 3.3 mmol) were added. After 10 min at room temperature, the reaction flask was placed in a preheated (80 °C) oil bath for 3 h. The reaction mixture was cooled to room temperature. Ice water (10 mL) was added carefully to quench the reaction. After extraction with EtOAc (50 mL × 3), the combined organic extracts were washed sequentially with saturated aqueous $NaHCO_3$, H_2O and brine. The obtained organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to dryness in vacuo. The residue was chromatographed on silica gel column developed with DCM/MeOH (50:1) to give compound **11** (790 mg, 58%) as a white solid. R_f 0.42 (CH_2Cl_2: EtOAc = 8:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.45 (s, 1H), 8.06 (m, 2H), 7.57 (m, 1H), 7.45 (m, 2H), 6.58 (m, 1H), 5.40 (m, 1H), 4.50 (m, 2H), 4.45 (m, 1H), 4.11 (m, 1H), 3.53 (s, 3H), 2.23 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1, 166.2, 154.1, 151.9, 150.2, 133.3, 129.7, 129.5, 128.5, 118.0, 114.0, 104.6, 86.4, 80.2, 79.5, 79.3, 70.9, 64.5, 59.1, 21.5; ESI-MS: *m*/*z* 530.3 $[M+H]^+$.

*2.3. 4-Amino-5-cyano-7-(2-O-acetyl-3-O-methyl-5-O-benzoyl-β-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidine (**12**)*

Compound **11** (200 mg, 0.377 mmol) was dissolved in THF (10 mL) and CH_3OH (5 mL). $Pd(OH)_2$ (50 mg) and Et_3N (0.1 mL) were added to the above solution. The resulted solution was hydrogenated for 5 h. After filtration through celite and evaporation of the mother liquor, the residue was chromatographed on a silica gel column to give compound **12** (148 mg, 93%) as a white solid. R_f 0.17 (CH_2Cl_2: EtOAc = 8:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.28 (s, 1H), 8.03 (m, 2H), 7.67 (m, 1H), 7.63 (m, 1H), 7.49 (m, 2H), 6.22 (d, 1H, *J* = 3.2 Hz), 6.07 (s, 1H), 5.79 (m, 1H), 4.74–4.71 (m, 2H), 4.41 (m, 1H), 4.28 (m, 1H), 3.44 (s, 3H), 2.18 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.7, 166.1, 156.5, 153.9, 149.9, 133.6, 130.2, 129.5, 129.3, 128.7, 114.9, 102.8, 88.3, 84.7, 80.1, 78.1, 73.8, 63.0, 59.4, 20.7; ESI-MS: *m*/*z* 452.3 $[M+H]^+$.

*2.4. 4-Amino-5-cyano-7-(3-O-methyl-β-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidine (**1**)*

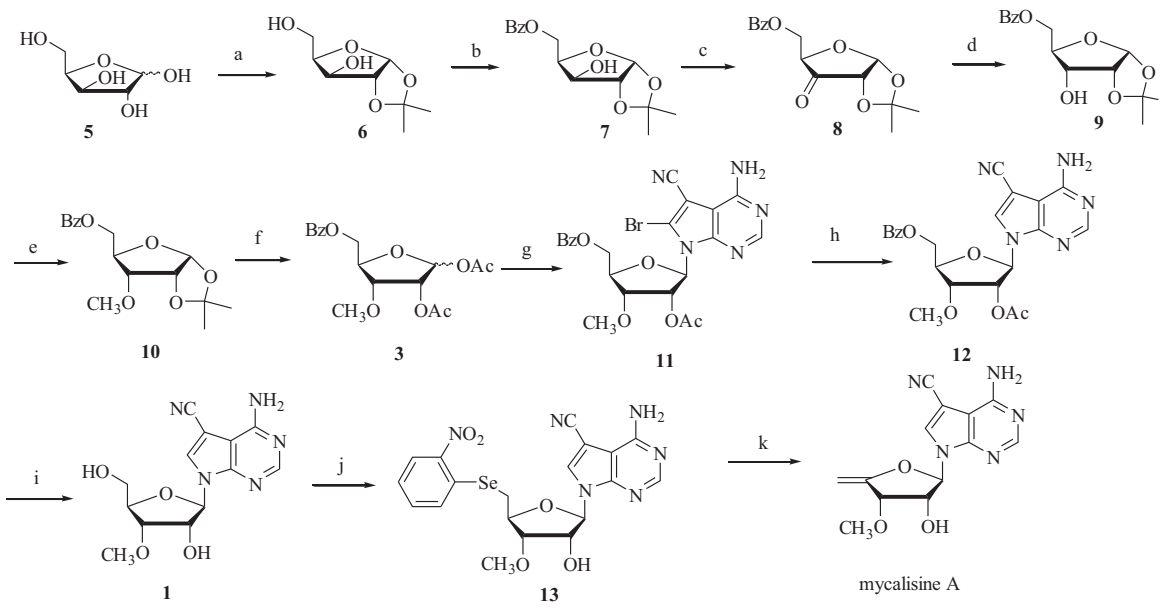
Compound **12** (200 mg, 0.443 mmol) was suspended in freshly prepared saturated methanolic ammonia (20 mL). After stirred at room temperature for 12 h, the solvent was removed in vacuo. The residue was purified using silica gel chromatography to give compound **1** (146 mg, 95%) as a white solid. R_f = 0.17 (DCM: MeOH = 30:1); ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.45 (s, 1H), 8.22 (s, 1H), 6.04 (d, 1H, *J* = 4.4 Hz), 5.57 (d, 1H, *J* = 5.6 Hz), 5.29 (m, 1H), 4.53 (m, 1H), 4.02 (s, 1H), 3.83 (s, 1H), 3.66 (m, 1H), 3.57 (m, 1H), 3.39 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 157.5, 154.0, 150.5, 132.7, 115.7, 101.7, 88.4, 83.4, 79.7, 73.8, 61.5, 58.0; ESI-MS: *m*/*z* 306.1 $[M+H]^+$, 304.1 $[M-H]^-$.

*2.5. 4-Amino-5-cyano-7-(3-O-methyl-5-(O-nitrophenylselenide)-β-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidine (**13**)*

O-Nitrophenylselenocyanate (204 mg, 0.9 mmol) and Bu_3P (0.225 mL, 0.9 mmol) were added to compound **1** (100 mg, 0.32 mmol) in freshly distilled pyridine (5 mL). The obtained mixture was stirred at room temperature for 4 h. After the solvent was removed in vacuo, the residue was purified using silica gel to give selenide 1 (143 mg, 87%) as a white solid. R_f = 0.42 (DCM: MeOH = 20:1); ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.46 (s, 1H), 8.26–8.22 (m, 2H), 7.78 (d, 1H, *J* = 8.0 Hz), 7.64 (t, 1H, *J* = 7.6 Hz), 7.44 (t, 1H, *J* = 7.6 Hz), 6.09 (d, 1H, *J* = 5.2 Hz), 5.72 (d, 1H, *J* = 6.4 Hz), 4.77–4.72 (m, 1H), 4.25–4.21 (m, 1H), 3.93–3.91 (m, 1H), 3.45 (d, 2H, *J* = 6.8 Hz), 3.39 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 157.5, 154.1, 150.8, 147.0, 134.7, 132.9, 131.9, 130.5, 126.8, 126.7, 115.6, 101.6, 88.4, 84.0, 82.9, 80.8, 72.9, 58.1, 23.9; ESI-MS: *m*/*z* 491.1 $[M+H]^+$, 489.4 $[M-H]^-$.

2.6. Mycalisine A

Selenide **13** (100 mg, 0.20 mmol) was dissolved in THF (5 mL). 30% H_2O_2 (0.172 mL, 2 mmol) was added and the resulted solution was stirred for 2 h at room temperature. Pyridine (2 mL) and Et_3N (0.4 mL, 0.3 mmol) were added. The obtained mixture was heated at 50 °C overnight. After the removal of the solvent in vacuo, the residue was purified using silica gel to give mycalisine A (52 mg, 87%) as a white solid. R_f = 0.14 (DCM: MeOH = 50:1); ^{1}H NMR



Scheme 1. Reagents and conditions: (a) Acetone, H_2SO_4, Na_2CO_3, r.t., 3 h, 73%; (b) BzCl, Py, r.t., 4 h, 75%; (c) Dess-Marton, DCM, r.t., 5 h, 97%; (d) $NaBH_4$, CH_3OH, r.t., 3 h, 97%; (e) NaH, DMF, CH_3I, 0 °C, 92%; (f) AcOH, Ac_2O, H_2SO_4, 24 h, 88%; (g) i. **4**, BSA, CH_3CN; ii. **3**, TMSOTf, CH_3CN, 58%; (h) 5% Pd/C, Et_3N, THF, CH_3OH, r.t., 4 h, 93%; (i) sat. NH_3 in MeOH, r.t., 3 h, 95%; (j) *O*-nitrophenylselenocyanate, Bu_3P, Py, r.t., 4 h, 87%; (k) i. H_2O_2, THF, r.t., 2 h; ii. Et_3N, THF, 50 °C, overnight, 87%.

(400 MHz, DMSO-d_6): δ 8.53 (s, 1H), 8.25 (s, 1H), 7.01 (brs, 2H), 6.32 (d, 1H, J = 6.8 Hz), 5.83 (d, 1H, J = 6.8 Hz), 4.92–4.89 (m, 1H), 4.44 (s, 1H), 4.30 (s, 1H), 4.22 (d, 1H, J = 4.8 Hz), 3.39 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 157.8, 157.5, 154.4, 151.3, 133.0, 115.4, 101.6, 87.8, 87.7, 84.6, 78.9, 72.5, 56.5; ESI-MS: m/z 288.2 $[M+H]^+$, 286.5 $[M-H]^-$.

3. Results and discussion

In the forward direction (Scheme 1), crystalline 1,2-*O*-isopropylidene-α-D-xylofuranose **6** was prepared in 73% yield with a modification of a reported procedure on 100 g scale [10]. Then 5-OH in **6** was selectively protected as the corresponding benzoate to give **7** in 75% yield. Dess-Martin oxidation of the 3-OH in **7** afforded ketone **8** in 97% yield [11]. Subsequently stereoselective reduction with $NaBH_4$ gave ribose **9** exclusively in 97% yield [12]. Next, compound **9** was subjected to methylation with CH_3I and NaH in DMF to provide **10** in excellent yield. Final cleavage of the acetonide with acetic acid/acetic anhydride/H_2SO_4 provided the key intermediate **3** in 88% yield as a mixture (α:β = 2:3) [13].

With compound **3** in hand, we proceeded to investigate the crucial late-stage Vorbrüggen glycosylation with nucleobase **4**, which was synthesized from tetracyanoethylene in two steps [14,15]. Much to our satisfaction, this transformation proved to be efficient to give nucleoside **11** stereo- and chemo selectively in 56% yield. The correct stereo- and regiochemical outcome of the glycosylation was confirmed by NMR analysis. Then debromination was conducted under hydrogenation conditions using 5% Pd/C as the catalyst to give **12** in 93% yield. Global deprotection under the Zemplén condition gave nucleoside **1** in 95% yield. At the final stage, treatment nucleoside **1** with *O*-nitrophenylselenocyanate and tributyl phosphine afforded selenide **13** in 87% yield. The selenide **13** was then oxidized with an excess of H_2O_2 in THF to the selenoxide, which was not stable. Without separation, the reaction mixture was treated with Et_3N and heated at 50 °C for 5 h. After removal of the solvent and column chromatography purification of the residue, mycalisine A was obtained in 87% yield. All spectral data were in accordance with those of the reported natural product.

4. Conclusion

In summary, we have achieved an efficient total synthesis of mycalisine A from D-xylose in 11 steps with a 15% overall yield. Our strategy integrates a stereo- and regioselective Vorbrüggen glycosylation and a new versatile nucleoside donor **3** for the preparation of the related nucleoside derivatives. Investigations in this transformation are well underway and will be reported in due course.

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