Natural Products

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A Bioinspired Synthesis of (±)-Rubrobramide, (±)-Flavipucine, and (±)-Isoflavipucine

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Abstract: *A biomimetic synthesis of naturally occurring lactams rubrobramide, flavipucine, and isoflavipucine is described. The key step is a regioselective Darzens reaction between isobutyl glyoxal and an α-bromo-β-ketoamide. The construction of the core tricyclic ring system of rubrobramide was achieved by a cascade reaction in a single step from an α,β-epoxy-γ-lactam. Furthermore, the absolute configuration of naturally occurring (+)-rubrobramide was determined by vibrational circular dichroism. (±)-Flavipucine and (±)-isoflavipucine were synthesized from an epoxyimide, which was prepared by reaction of isobutyl glyoxal with a protected α-bromo-β-ketoamide. Deprotection of the epoxyimide and formation of the pyridone ring gave (±)-flavipucine, which was converted into (±)-isoflavipucine by thermal isomerization.*

Rubrobramide (**1**), which has a highly oxidized ring system, was first isolated from the culture filtrate of *Cladobotryum rubrobrunnescens* (Figure 1).[1] Although rubrobramide is an optically active metabolite {$[\alpha]_D + 177$ (*c* 1.0, $CHCl_3$)}, its absolute configuration has not yet been determined. This compound is structurally related to flavipucine (**2**), which was isolated from the same fungus.[2] (−)-Flavipucine [(−)-**2**] has been isolated from *Aspergillus flavipes*,[3] as well as the fungus-caused *Macrophoma* fruit rot.[4] (+)-Flavipucine [(+)-**2**] has been isolated from the culture extract of *Phoma* sp.[5] The absolute configuration of (+)-**2** was determined to be *S*,*S* by comparison of the experimental and calculated circular dichroism (CD) spectra.[5] Interestingly, an optically inactive form of (±)-isoflavipucine [(±)-**3**] has been isolated from *Aspergillus flavipes*[6] and *Phoma* sp.,[5] produced by rearrangement of optically active (−)- or (+)-**2**.[5–7] It has been proposed that the natural products **1**–**3** are biogenetically produced by a hybrid polyketide synthase (PKS) and non-ribosomal peptide synthetase (NRPS) system (Scheme 1),

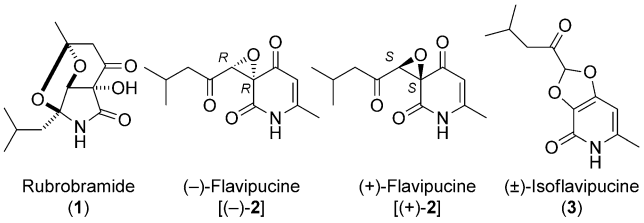
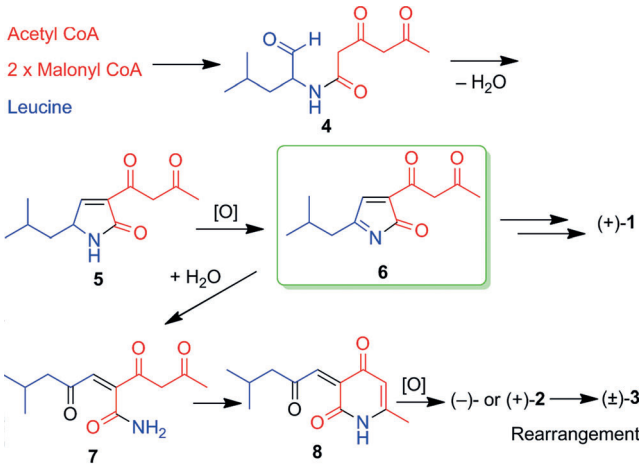


Figure 1. Structures of rubrobramide (**1**), (−)-flavipucine [(−)-**2**], (+)-flavipucine [(+)-**2**], and (±)-isoflavipucine [(±)-**3**].



Scheme 1. Proposed biosynthesis of **1**–**3**.

although the detailed biosynthetic pathways, especially for the synthesis of rubrobramide, remain unclear.[8] The amide **4** is formed from acetyl CoA, malonyl CoA, and leucine by PKS-NRPS. Dieckmann condensation of **4** affords **5**, which can be oxidized to yield the key intermediate **6**. Construction of the tricyclic ring from **6** then gives (+)-**1**. Hydrolysis of the key intermediate **6**, followed by transamidation of the resultant product **7** leads to the pyridone **8**. Epoxidation of **8** gives (−)- or (+)-**2**, which can rearrange to (±)-**3**. The interesting biosyntheses of **1**–**3** motivated us to start synthetic studies of these natural lactams. Herein, a bioinspired approach to the total syntheses of (±)-**1**–**3** is described. Furthermore, determination of the absolute configuration of naturally occurring (+)-**1** by the exciton chirality method

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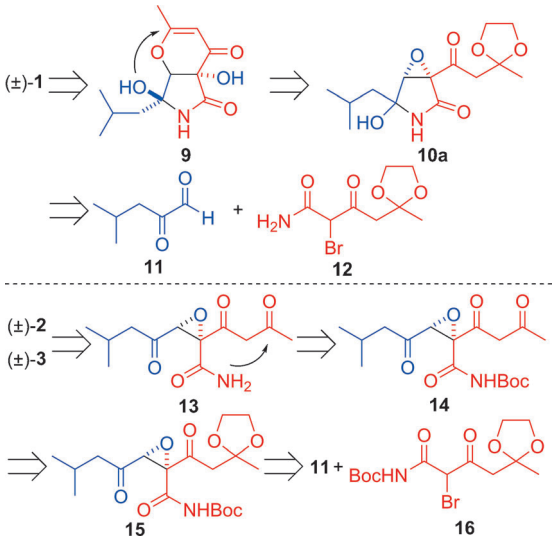
using vibrational circular dichroism (VCD)[9] is also described.

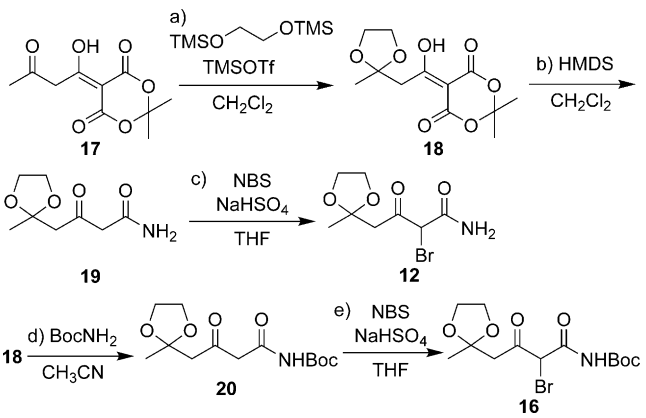
Our retrosynthetic analysis of (±)-**1**, (±)-**2**, and (±)-**3** is shown in Scheme 2. The compound (±)-**1** is prepared from γ-lactam **9** via an intramolecular oxy-Michael addition of the hydroxy group to the enone. The compound **9** is synthesized by deprotection of the ketal in **10a**, followed by an intramolecular epoxide-opening reaction. The compound **10a** would be prepared by Darzens reaction of isobutyl glyoxal (**11**)[10] with the α-bromo-β-ketoamide **12**.[11] The compounds (±)-**2** and (±)-**3** are prepared from the epoxyamide **13**, which is prepared by removal of the *tert*-butoxycarbonyl (Boc) group in **14**, which comes from deprotection of the ketal in **15**. The epoxyimide **15** is prepared by a Darzens reaction between **11** and **16**.



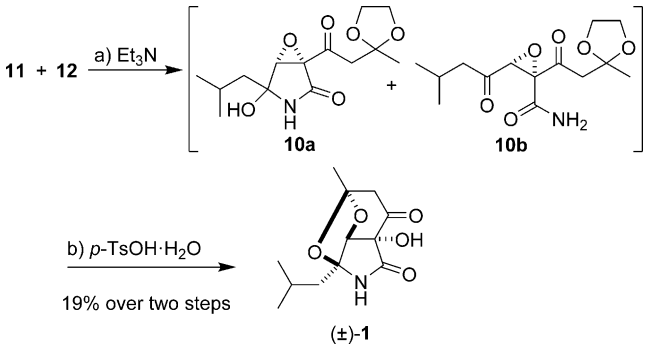
Scheme 2. Retrosynthetic analysis of (±)-**1**–**3**.

The syntheses of **12** and **16** are shown in Scheme 3. The ketone in **17**[12] was protected as a 1,3-dioxolane to give **18**.[13] Subsequent amidation with hexamethyldisilazane (HMDS), and bromination of **19** with *N*-bromosuccinimide (NBS) in the presence of sodium hydrogen sulfate[14] afforded **12**. Similarly, amidation of **18** with *tert*-butyl carbamate, followed by bromination of the resultant **20**, afforded **16**.

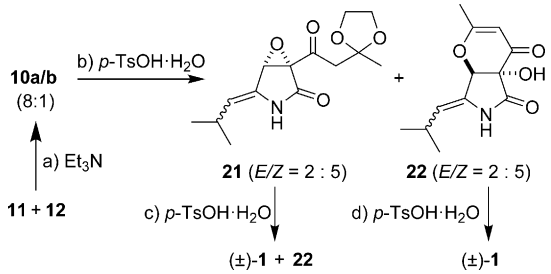
The synthesis of (±)-**1** is shown in Scheme 4. Darzens reaction between **11** and **12** in the presence of triethylamine gave the tautomers **10a/b**. The ^{1}H NMR spectrum of the crude reaction mixture indicates that it exists mainly as a mixture of cyclic hemiaminal diastereomers **10a** (d.r. = 4:1) with the open-chain tautomer **10b** as the minor component (**10a**/**10b** = 8:1). The compounds **10a/b** were unstable and decomposed during purification. Thus, the crude reaction mixture was used in the next reaction without purification. Treatment with *p*-toluenesulfonic acid monohydrate (*p*-TsOH·H_2O) in CH_2Cl_2 for 19 hours afforded (±)-**1** in 19% yield over the two steps. The ^{1}H and ^{13}C NMR spectra for synthetic (±)-**1** are in agreement with those reported for natural **1**.



Scheme 3. Synthesis of the α-bromo-β-ketoamides **12** and **16**. Reagents and conditions: a) 1,2-Bis(trimethylsiloxy)ethane (2.0 equiv), TMSOTf (1.0 equiv), CH_2Cl_2, −20 °C, 2 d, 93%; b) HMDS (1.0 equiv), CH_2Cl_2, reflux, 30 min, 77%; c) NBS (0.95 equiv), $NaHSO_4$ (0.42 equiv), THF, 0 °C, 15 min, 90%; d) $BocNH_2$ (1.0 equiv), CH_3CN, 30 min, 85%; e) NBS (0.95 equiv), $NaHSO_4$ (0.25 equiv), THF, 0 °C, 20 min, 81%. Boc = *tert*-butoxycarbonyl, HMDS = hexamethyldisilazane, NBS = *N*-bromosuccinimide, Tf = trifluoromethanesulfonyl, THF = tetrahydrofuran, TMS = trimethylsilyl.

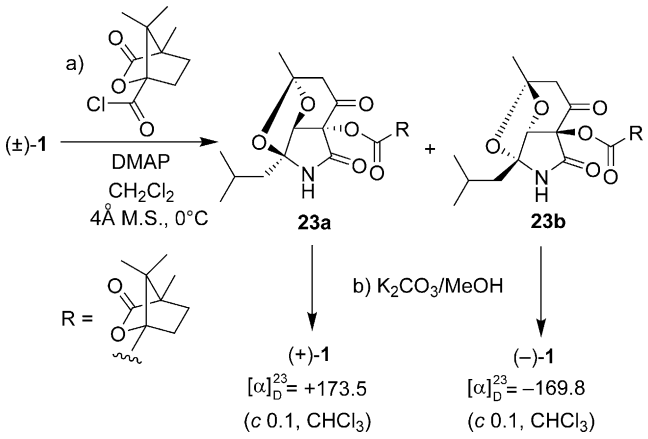


Scheme 4. Synthesis of (±)-rubrobramide [(±)-**1**]. Reagents and conditions: a) **11** (1.1 equiv), **12** (1.0 equiv), Et_3N (1.0 equiv), THF/H_2O (10:1), RT, 30 min, **10a**/**10b** (8:1), d.r. = 4:1 at the hemiaminal; b) *p*-TsOH·H_2O (0.3 equiv), CH_2Cl_2, RT, 19 h, 19% over two steps. Ts = 4-toluenesulfonyl, d.r. = diastereomeric ratio.



Scheme 5. Isolation of the key intermediates for the transformation of **10a** into (±)-**1**. Reagents and conditions: a) **11** (1.1 equiv), **12** (1.0 equiv), Et_3N (1.0 equiv), THF/H_2O (10:1), RT, 30 min, **10a**/**10b** (8:1), d.r. = 4:1 at the hemiaminal; b) *p*-TsOH·H_2O (0.3 equiv), CH_2Cl_2, RT, 30 min, **21** (18%), **22** (29%) over two steps.; c) *p*-TsOH·H_2O (1.0 equiv), acetone/H_2O (3:1), 70 °C, 30 h, (±)-**1** (22%), **22** (13%); d) *p*-TsOH·H_2O (1.0 equiv), acetone/H_2O (3:1), 70 °C, 6 d, 76%.

Transformation of **10a** into (±)-**1** involved multiple transformations. Key intermediates were isolated during examination of the reaction conditions (Scheme 5). When **10a/b** were treated with *p*-TsOH·H_2O for 0.5 hours, the compounds **21** and **22** were obtained in 18 and 29% yield, respectively. Both **21** and **22** were characterized as an inseparable mixture of *E* and *Z* isomers (2:5). Treatment of **21** with *p*-TsOH·H_2O in acetone/H_2O (3:1) at 70°C gave (±)-**1** and **22** in 22 and 13% yields, respectively. Treatment of **22** under the same reaction conditions afforded (±)-**1** in 76% yield. These results indicate that both **21** and **22** are intermediates in the conversion of **10a** into (±)-**1** [see Scheme S1 in the Supporting Information for a proposed mechanism for the transformation of **10a** into (±)-**1**].



Scheme 6. Optical resolution of [(±)-**1**]. Reagents and conditions: a) (−)-camphanic chloride (1.0 equiv), DMAP (2.0 equiv), CH_2Cl_2, 4Å M.S., 0°C, 15 min, **23a** (42%), **23b** (42%); b) K_2CO_3 (1.0 equiv), MeOH, 0°C, (+)-**1** (80%), (−)-**1** (78%). DMAP = *N*,*N*′-dimethyl-4-aminopyridine, M.S. = molecular sieves.

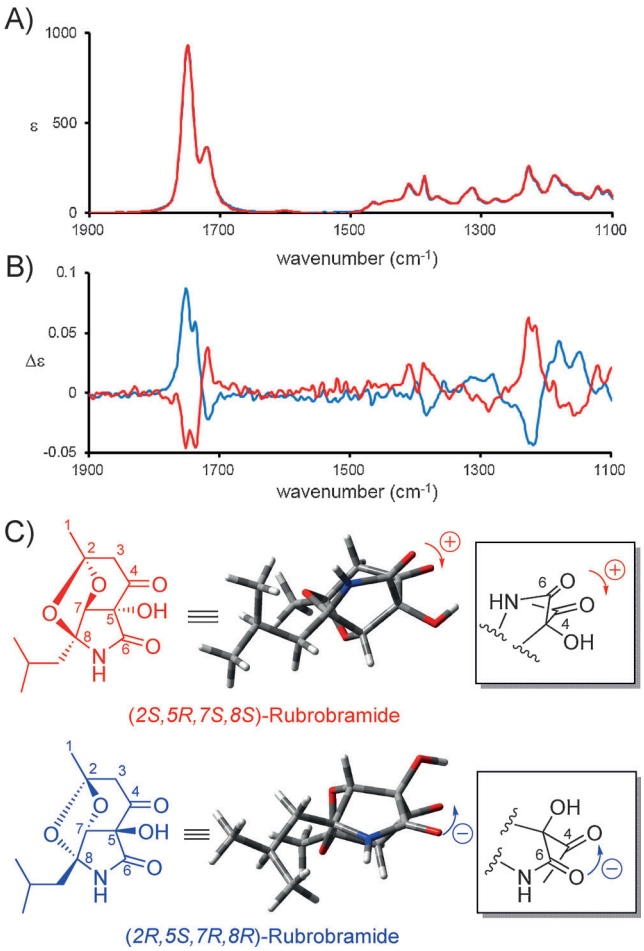
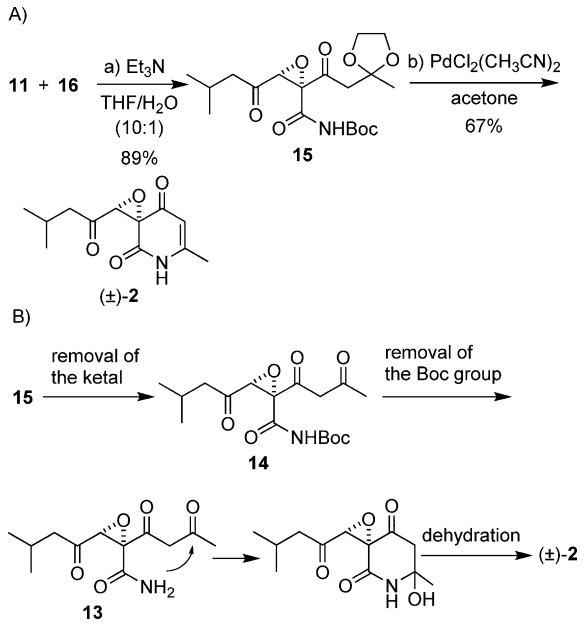


Figure 2. Determination of the absolute configuration of (+)-**1** and (−)-**1**. A) IR and B) VCD spectra of (+)-**1** (red) and (−)-**1** (blue). The IR and VCD spectra were measured in $CDCl_3$ (0.125 M for (+)-**1** and 0.140 M for (−)-**1**, *l* = 50 μm). C) Schematic structures for (+)-**1** and (−)-**1**. The structures were optimized using the DFT B3LYP/6–311G-(d,p) method as implemented in the Gaussian 09 program.[15]

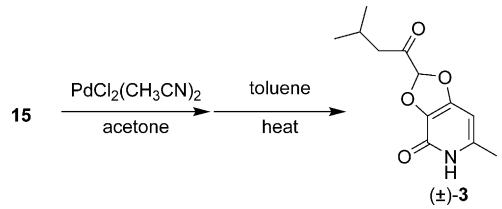
The optical resolution of (±)-**1** was carried out to determine the absolute configuration of both enantiomers of **1** (Scheme 6). Esterification of (±)-**1** with (−)-camphanic chloride afforded the diastereomers **23a** and **23b**, which were separated by column chromatography. Methanolysis of **23a** and **23b** under basic conditions gave (+)-**1** and (−)-**1**, respectively. The specific rotations of (+)-**1** and (−)-**1** were +173.5 and −169.8 (*c* 0.1, $CHCl_3$), respectively. The absolute configuration of (+)-**1** and (−)-**1** were determined by the VCD exciton chirality method[9,11] (Figure 2). The IR spectra of (+)-**1** and (−)-**1** showed strong absorptions at 1750 and 1721 cm^{-1}, representing the C=O stretching vibrations of the lactam at C6 and the ketone at C4, respectively (Figure 2a). The corresponding VCD signals in the C=O stretching region exhibited a strong bisignate pattern (Figure 2b). The VCD spectrum of (+)-**1** showed a positive-negative couplet from the lower to higher frequencies, thus indicating a clockwise orientation between the two adjacent carbonyl groups at C4 and C6 (Figure 2c). This result suggests that the absolute configuration of naturally occurring (+)-**1** is 2*S*,5*R*,7*S*,8*S*. Meanwhile, the negative-positive VCD couplet of (−)-**1** in the C=O stretching region is indicative of the counterclockwise orientation of the two carbonyl groups, by which the absolute configuration of (−)-**1** is determined to be 2*R*,5*S*,7*R*,8*R*. These assignments were further supported by a comparison of the experimental and theoretical VCD spectra (see Figure S1 in the Supporting Information).

The synthesis of (±)-**2** is shown in Scheme 7. A Darzens reaction between **11** and **16** gave the epoxyimide **15** in 89% yield with complete regioselectivity (Scheme 7A). Treatment of **15** with a catalytic amount of $PdCl_2(CH_3CN)_2$[16] in acetone afforded (±)-**2** as the sole product in 67% yield. Formation of (±)-**2** involved removal of the ketal in **15**, removal of the Boc group in the resultant **14**, and formation of the pyridone ring (Scheme 7B). Actually, the enol tautomer of **14** was obtained by treatment of **15** with [$PdCl_2(CH_3CN)_2$] in acetone/toluene (see Scheme S2 in the Supporting Information).

A one-pot synthesis of (±)-**3** from **15** was also accomplished (Scheme 8). After treatment of **15** with $PdCl_2(CH_3CN)_2$ in acetone for 4 days, toluene was added to the reaction mixture. The acetone was removed by heating at 70°C for 1 hour, and the resultant reaction mixture was



Scheme 7. Synthesis of (±)-flavipucine [(±)-**2**]. A) Synthesis of (±)-**2**. B) Proposed synthetic pathway from **15** to (±)-**2**. Reagents and conditions: a) **11** (1.2 equiv), **16** (1.0 equiv), Et_3N (1.0 equiv), THF/H_2O (10:1), RT, 2 h, 89%; b) $PdCl_2(CH_3CN)_2$ (0.3 equiv), acetone, 5 d, 67%.



Scheme 8. One-pot synthesis of (±)-isoflavipucine [(±)-**3**] from epoxyimide **15**. Reagents and conditions: $PdCl_2(CH_3CN)_2$ (0.3 equiv), acetone, 4 d, then toluene, 70 °C, 1 h, followed by 160 °C for 7 h, 50% over two steps.

heated at 160 °C under sealed conditions to give (±)-**3** in 50% yield.

In conclusion, a biomimetic synthesis of (±)-rubrobramide as well as structurally related (±)-flavipucine and (±)-isoflavipucine has been achieved. Detailed reaction pathways from **10a** to (±)-rubrobramide, and from **15** to (±)-flavipucine were elucidated. Furthermore, both enantiomers of rubrobramide were obtained by optical resolution of a synthetic racemic sample. The absolute configuration of natural rubrobramide was determined to be 2*S*,5*R*,7*S*,8*S* by using the VCD exciton chirality method. We believe that the results obtained in this study will help elucidate the biosynthetic pathways of these lactam natural products.

Acknowledgements

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Keywords: biomimetic synthesis · configuration determination · lactams · total synthesis · vibrational spectroscopy

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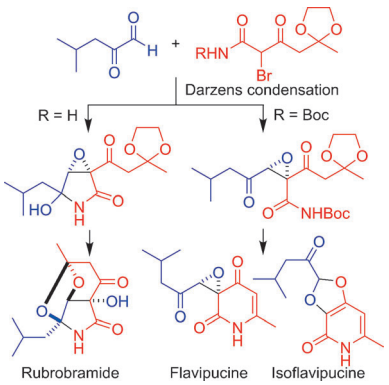
A Bioinspired Synthesis of (±)-Rubrobramide, (±)-Flavipucine, and (±)-Isoflavipucine



Three pieces: Total synthesis of (±)-rubrobramide, (±)-flavipucine, and (±)-isoflavipucine was achieved by a Darzens reaction between isobutyl glyoxal and α-bromo-β-ketoamides, removal of the protecting group(s), and ring formation. After optical resolution of synthetic (±)-rubrobramide, the absolute configuration of naturally occurring (+)-rubrobramide was determined by vibrational circular dichroism.