Synthesis of the Nonribosomal Peptide Phevalin and Analogs
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ABSTRACT: Phevalin, a cyclic nonribosomal peptide produced by Staphylococcus aureus, has intriguing biological properties. A synthetic route to access phevalin and similar pyrazinone natural products tyrvalin, leuvalin, phileucin, and a few synthetic analogs is described. The reaction sequence involves a one-pot carbamate deprotection/imine formation/aerobic oxidation to form the pyrazinone-containing products.

Phevalin 1a, also known as aureusimine B, was first isolated from a soil actinomycete (Streptomyces sp.) by Alvarez et al., who described it as a calpain inhibitor,1 although this activity was subsequently disproven.2 Related pyrazinone-containing natural products (tyrvalin (1b), leuvalin (1c), and phileucin (1i)) were later reported to be produced by nonribosomal peptide synthetases in S. aureus3,4 or E. salina.5 As a class, nonribosomal peptides are structurally diverse and exhibit a variety of biological activities.6 Since S. aureus is a major human pathogen, several groups have attempted to understand the biological significance of these natural products.7–10 Studies indicate that aureusimines do not display antibiotic activity on their own but are associated with host–pathogen interactions.9,10 The potential use of phevalin in quorum sensing studies has also been reported.11

Biosynthetically, aureusimines arise from a dipeptide assembled on the protein AusA. The protein reductively releases a dipeptidyl aldehyde, which undergoes condensation to form a cyclic imine. This imine is then oxidized to the aromatic natural product.3,4,12 In a previous synthesis of phevalin, we prepared the precursor amino aldehyde toward phevalin from the corresponding Boc-protected amine in situ and found that it underwent both cyclization and oxidation in trifluoroacetic acid upon heating (Figure 1c).2 Later, Aldrich and co-workers reconstituted the activity of AusA in vitro and confirmed that this oxidation is spontaneous.8 Interestingly, Fischbach and co-workers have reported that dipeptidyl aldehyde precursors to phevalin and related natural products have some stability in biological media and activity against cathepsins in cellular assays.13

In the above-noted synthesis, we prepared the aldehyde through a DIBAL-mediated reduction of the corresponding ester. We and others7,8,14 have since found that, although this step is reproducible, the ester reduction required careful monitoring and was often accompanied by alcohol resulting from over-reduction of the aldehyde. Given the continued interest in phevalin and other members of this class in biological studies, we now report full details of a modestly modified total synthesis of phevalin and nine additional members of the class.

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In modifying this route, we decided to replace the ester reduction step with an oxidation of the corresponding alcohol, readily prepared by coupling of Boc-Val with amino alcohol \(3\) (Scheme 1). Oxidation using Dess-Martin periodinane (DMP) reliably gave the aldehyde \(5a\) as a single diastereomer as expected.\(^{15}\) Deprotection using TFA gave phevalin in 46% yield over two steps from alcohol \(4a\). Although the reaction gave moderate yields, we did not isolate any other side products in this reaction.

This route was used to make other naturally occurring aureusimines and a few synthetic analogs (Scheme 2). The required dipeptide alcohols (\(4b\)–\(j\)) were synthesized from commercially available Boc-protected amino acids and amino alcohols. Oxidation of alcohol followed by deprotection of carbamate gave the natural products \(1b\), \(1c\), \(1i\), and six additional analogs in moderate yields (33–55%). In the case of tyrvalin (\(1b\)), \(\text{SO}_3\cdot\text{pyr}\) (Parikh–Doering oxidation) was employed for the oxidation of alcohol \(4b\), probably necessitated by the presence of the phenolic hydroxyl group. The aldehyde obtained was converted to tyrvalin (\(1b\)) using the procedure adopted for the synthesis of other compounds. All the compounds were fully characterized and prepared on ca. 30 mg scale.

The chemical mechanism of the spontaneous aerobic oxidation is currently unknown, although similar oxidations to form heterocycles have been documented.\(^{16–19}\) The reaction could possibly proceed via a radical pathway involving a captodative radical at the C-3 position of the pyrazinone.\(^{20}\) In such a case, a C-3 cyclopropyl group might be expected to undergo ring opening.\(^{21,22}\) However, upon submission to the standard conditions, pyrazinone \(1j\), which contains a cyclopropyl group at this position, was obtained, albeit in a slightly lower yield (33%), but without any observable byproduct resulting from ring opening. We also note that this sequence proceeds in very poor yield for substrates devoid of alkyl groups at C-3 or at C-6.

We briefly examined the use of hot hexafluoroisopropanol (HFIP) for the deprotection of \(5a\) (Scheme 3).\(^{23}\) As before, we observed complete consumption of starting material and phevalin (\(1a\)) was obtained in 15% yield, but under these circumstances a new product \(6\) was also observed. This product could be formed from the initially formed imine by a \([1,3]\)-hydride shift, indicating that there are two pathways possible for this intermediate. The product \(6\) did not convert to phevalin after stirring at room temperature for 2 days.

In summary, we have developed a convenient and reliable synthetic route to the pyrazinone natural products exemplified by phevalin, tyrvalin, leuvalin, and phileucin. In addition to these natural products, six synthetic analogs were also synthesized.

**EXPERIMENTAL SECTION**

**General Information.** Reactions were carried out in either round-bottom flasks or glass sample vials (TFE-lined cap). All reagents, starting materials, and solvents (including dry solvents) were obtained from commercial suppliers and used without further purification. Thin-layer chromatography (TLC) was performed using commercial silica gel 60 F254-coated aluminum-backed sheets. Visualization was accomplished with UV light, or by immersion in Seebach’s stain followed by heating with a heat gun for ca. 30 s. Purification was carried out by an automated flash chromatography/medium-pressure liquid chromatography (MPLC) system using normal phase silica gel.
flash columns. The infrared (IR) spectra were acquired as thin films using a universal ATR sampling accessory on an FT-IR spectrometer; the absorption frequencies are reported in cm$^{-1}$. All NMR spectra were recorded on a 400 MHz spectrometer with a dual carbon/proton cryoprobe. NMR samples were recorded in deuterated chloroform (CDCl$_3$) or deuterated methanol (CD$_2$OD). Chemical shifts are reported in parts per million (ppm) and referenced to the center line of residual solvent (CDCl$_3$, 7.26 ppm for $^1$H NMR and 77.16 for $^{13}$C NMR; CD$_2$OD: 3.31 ppm for $^1$H NMR and 49.00 for $^{13}$C NMR). Coupling constants are reported in hertz (Hz). HRMS data were collected with a time-of-flight mass spectrometer and an electrospray ion source (ESI). Melting points were determined in open capillary tubes using an automated melting point apparatus and are uncorrected. Optical rotations were recorded on an automatic polarimeter at 589 nm.

**General Procedure for Peptide Coupling.** To an oven-dried, round-bottomed flask equipped with a stir bar Boc-protected amino acid (200 mg, 1.0 equiv), amino alcohol (139 mg, 1.0 equiv), EDC (229 mg, 1.3 equiv), and HOBT (183 mg, 1.3 equiv) were added. Anhydrous DCM (20 mL) was then added, followed by DIPEA (0.74 mL, 3.0 equiv). The reaction mixture was stirred at rt under an inert atmosphere for 16 h. The reaction mixture was then diluted with DCM (10 mL), washed with brine (5 mL), and dried over anhydrous sodium sulfate and was concentrated under reduced pressure to give a crude residue, which was further purified on a semi-automated purification system using a normal phase silica flash column (100% hexanes to 100% EtOAc over 20 min) to give product.

**General Procedure for Conversion of Dipetide Alcohol to Pyrazinones.** To a glass vial equipped with a stir bar, the dipeptide alcohol (100 mg, 1.0 equiv), Dess-Martin periodinane (182 mg, 1.5 equiv) and DCM (5 mL) were added. The resulting cloudy solution was then allowed to stir at rt for 1.5 h. A solution of saturated Na$_2$SO$_4$ (2 mL) and saturated NaHCO$_3$ (2 mL) were added to the reaction mixture stirred vigorously until two clear layers separated. The organic layer was separated, and the aqueous layer was extracted with DCM (10 mL $\times$ 2). The combined organic layer was then dried over anhydrous sodium sulfate and filtered, and the solvent was removed under reduced pressure to give the crude residue, which was purified on a semi-automated purification system using a normal phase silica flash column (100% hexanes to 100% EtOAc over 20 min) to give product.

**Tyrrvalin (1b).** Parikh–Doering oxidation was used to prepare the aldehyde Sb. To a glass vial equipped with a stir bar was added tert-buty l-((S)-1-(((S)-1-Hydroxy-3-(4-hydroxyphenyl)propan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate (4b). To an oven-dried, round-bottomed flask equipped with a stir bar, the aldehyde and DCM (5 mL) were added. To this solution TFA (0.88 mL, 40 equiv) was added, and the reaction mixture was left to stir at rt for 16 h. The reaction mixture was diluted with DCM (10 mL), washed with brine (5 mL), and dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure to give the crude residue, which was further purified on a semi-automated purification system using a normal phase silica flash column (100% hexanes to 60% ethyl acetate in hexanes over 30 min) to give the product.

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**tert-Butyl-((S)-1-(((S)-1-Hydroxy-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate (4a).** Known compound; spectroscopic data matched those in the literature. Yield 87% (1.40 g) from L-phenylalaninol (300 mg) and N-Boc-D-valine (300 mg). Large-scale synthesis using a universal ATR sampling accessory on an FT-IR spectrometer; the absorption frequencies are reported in cm$^{-1}$. All NMR spectra were recorded on a 400 MHz spectrometer with a dual carbon/proton cryoprobe. NMR samples were recorded in deuterated chloroform (CDCl$_3$) or deuterated methanol (CD$_2$OD). Chemical shifts are reported in parts per million (ppm) and referenced to the center line of residual solvent (CDCl$_3$, 7.26 ppm for $^1$H NMR and 77.16 for $^{13}$C NMR; CD$_2$OD: 3.31 ppm for $^1$H NMR and 49.00 for $^{13}$C NMR). Coupling constants are reported in hertz (Hz). HRMS data were collected with a time-of-flight mass spectrometer and an electrospray ion source (ESI). Melting points were determined in open capillary tubes using an automated melting point apparatus and are uncorrected. Optical rotations were recorded on an automatic polarimeter at 589 nm.

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(complex, 4H); 13C{1H} NMR (100 MHz, CDCl3) δ 172.1, 156.1, 80.6, 66.0, 59.4, 50.4, 40.1, 28.4, 3.50, 23.2, 22.2, 13.9, 3.8, 3.3; HRMS (ESI-TOF) m/z: [M + H]+ calcd for C14H19N2O, 231.1528; found 231.1528.

6-Benzyl-3-isopropylpyrazin-2(1H)-one (Phevalin, 1). Yield 53% (35 mg) over two steps from 4h (100 mg). White solid, mp 179 °C; Rf = 0.35 (40% EtOAc/hexanes); IR 2951, 1646 cm−1; 1H NMR (400 MHz, CDCl3) δ 7.35–7.24 (complex, 3H), 7.18 (s, 1H), 3.47 (septet, J = 6.8 Hz, 1H), 2.84 (sec, J = 7.0 Hz, 2H), 2.28 (s, 3H), 1.22 (d, J = 6.8 Hz, 3H); 13C{1H} NMR (100 MHz, CDCl3) δ 161.1, 158.0, 134.4, 123.0, 30.0, 20.2; HRMS (ESI-TOF) m/z: [M + H]+ calcd for C14H19N2O, 231.1528; found 231.1528.

5-Benzyl-3-isopropylpyrazin-2(1H)-one (1f). Known compound. Yield 33% (20 mg) over two steps from 4d (100 mg). Yellow solid, mp 148–150 °C; [α]D 245.1 (c 0.70, CHCl3); Rf = 0.39 (40% EtOAc/hexanes); IR 2962, 1633 cm−1; 1H NMR (400 MHz, CDCl3) δ 12.35 (br s, 1H), 7.35–7.24 (complex, 3H), 6.38 (sh, 2H), 3.25 (septet, J = 6.9 Hz, 1H), 1.82 (m, 1H), 1.5S (m, 1H, partially obscured by water signal), 1.21 (d, J = 6.9 Hz, 3H), 0.92 (t, J = 7.4 Hz, 3H); 13C{1H} NMR (100 MHz, CDCl3) δ 161.5, 158.1, 137.0, 136.3, 129.3, 128.9, 127.4, 36.8, 27.6, 17.2, 12.2; HRMS (ESI-TOF) m/z: [M + H]+ calcd for C16H31N2O4, 315.2283; found 315.2283.

3-Cyclopropyl-6-isobutylpyrazin-2(1H)-one (1j). To a microwave vial equipped with a stir bar, the compound 5a (130 mg, 0.37 mmol) was added, followed by HFIP (3 mL) and sealed. This reaction mixture was then heated in a microwave reactor at 140 °C for 2h. The solvent was removed under reduced pressure and the crude was then purified on a semiautomatic purification system using a normal phase silica flash column (100% hexane to 60% ethyl acetate over 18 min). The product 6 was obtained as an oil (28 mg, 33% yield) along with 13 mg of 1a (15% yield). [α]D 113.0 (c 130, 4% EtOAc/hexanes); IR 2962, 1633 cm−1; 1H NMR (400 MHz, CDCl3) δ 1.0, 1.6; 1H, 3.27 (septet, J = 6.8 Hz, 1H), 2.94 (dd, J = 13.7, 5.0 Hz, 1H), 2.65 (dd, J = 13.7, 9.5 Hz, 1H), 1.14 (d, J = 6.8 Hz, 3H), 1.09 (d, J = 6.9 Hz, 3H); 13C{1H} NMR (100 MHz, CDCl3) δ 170.4, 157.7, 156.2, 123.3, 39.5, 28.4, 22.3, 11.4, 10.1; HRMS (ESI-TOF) m/z: [M + H]+ calcd for C29H31N2O4, 423.1571; found 423.1577.
REFERENCES


