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Structure and Absolute Configuration of Auriculamide, a Natural Product from the Predatory Bacterium *Herpetosiphon aurantiacus*

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The genome of the filamentous, predatory bacterium *Herpetosiphon aurantiacus* harbors a plethora of genes that are predicted to be involved in natural product biosynthesis. Until now, however, no secondary metabolites have been described from this microorganism. Analysis of *H. aurantiacus* culture extracts by ^{1}H NMR spectroscopy now led to the discovery of a chlorinated amide, which we termed auriculamide. The configuration of the three chiral centers in auriculamide was solved by chromatographic comparison with stereospecifically prepared reference compounds following chemical degradation. Furthermore, a putative gene cluster for the biosynthesis of auriculamide was identified by genome mining.

Introduction

Predatory bacteria encompass a heterogeneous group of prokaryotes that share the ability to consume living microorganisms.[1] The feeding strategy of predatory bacteria typically involves the secretion of lytic enzymes and small bioactive molecules.[2,3] Antibiotics, which are produced by predatory bacteria, such as roimatacene,[4] corallopyronin,[5] or the recently described gulmirecins[6] and disciformycins,[7] are assumed to contribute to the killing of prey organisms. A deficiency in the biosynthesis of these compounds was shown to severely affect the predatory performance.[8] From a pharmaceutical perspective, predatory bacteria are hence a promising source for the discovery of antimicrobial compounds. Except for the predatory myxobacteria, however, this resource has barely been exploited.[9,10]

Herpetosiphon aurantiacus represents an illustrative example of a predatory bacterium with a little explored secondary metabolism. Analysis of its complete genome sequence revealed an abundance of biosynthesis genes, of which many are unique to this micropredator.[11] Moreover, *H. aurantiacus* appears to possess pathways to specific natural product building blocks, including the nonproteinogenic amino acid 4-hydroxyphenylglycine, which is known as a constituent of glycopeptide antibiotics.[12] Although the genus *Herpetosiphon* already demonstrated its potential for natural product biosynthesis,[13] the secondary metabolites of the sequenced type strain 114–95^T have remained elusive to date. Here, we report the discovery of the chlorinated amide auriculamide (**1**, Figure 1), its structure elucidation and stereochemical assignment. Furthermore, we propose a gene cluster for the biosynthesis of auriculamide from the genome of *H. aurantiacus* 114–95^T.

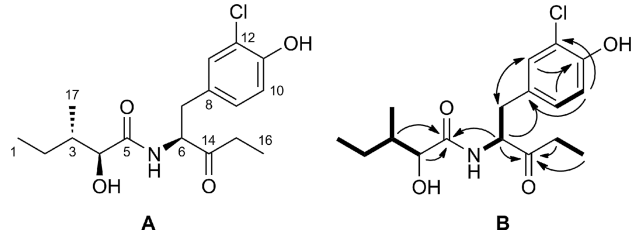

Figure 1. Structure of (A) auriculamide (**1**) and (B) selected COSY (bold lines) and HMBC (arrows) correlations in **1** (B).

Results and Discussion

For the production of auriculamide, *H. aurantiacus* 114–95^T was grown in a 30 L bioreactor in modified HP74 broth. After ten days of cultivation, the biomass was removed by centrifugation. Metabolites that had been secreted during the fermentation were recovered from the su-

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pernatant by adsorption onto XAD-2 resin. Elution of the resin with methanol and subsequent concentration yielded a brownish residue, which was dissolved in 60% aqueous methanol (MeOH) and exhaustively extracted with dichloromethane (CH_2Cl_2). The combined organic layers were fractionated by ODS flash chromatography using a water/methanol gradient. Proton NMR analysis indicated the 60% MeOH fraction to be of further interest due to the presence of several signals in the aromatic region that were absent in extracts originating from the pure growth medium. Subsequent purification by reverse-phase HPLC led to the isolation of 0.6 mg of auriculamide (**1**).

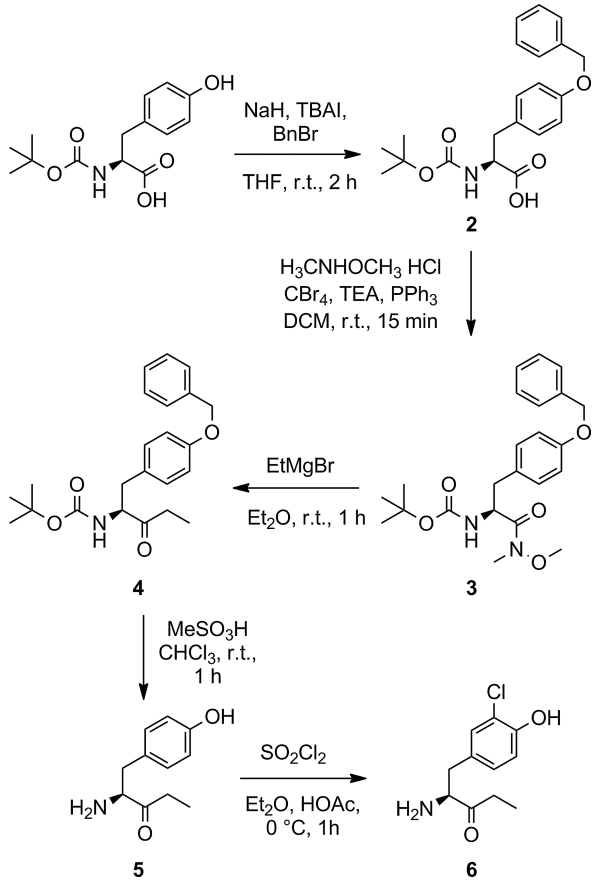
Compound **1** exhibits a pseudomolecular mass ion peak at *m*/*z* 340.1323 $[M - H]^-$, which is consistent with a sum formula of $C_{17}H_{24}ClNO_4$ and accounts for six double bond equivalents. The ^{13}C NMR spectrum of **1** indicated the presence of a ketone (C-14) and an amide (C-5), as well as six additional sp^2-hybridized carbon atoms (C-8 to C-13), corresponding to three C–C double bonds (Table 1). It was hence clear that **1** must feature a single ring structure to comply with the required degrees of unsaturation. Analysis of the ^1H NMR spectrum revealed 15 nonexchangeable signals, accounting for a total of 21 protons. These protons were attributed to their directly attached carbon atoms by heteronuclear single quantum coherence (HSQC). Proton-proton correlation spectroscopy (COSY) together with first-order multiplet analysis established four independent spin systems, including a 1,2,4-trisubstituted benzene and a 1-hydroxy-2-methylbutyl moiety. The secondary alcohol function of the latter was concluded from the chemical shift of C-4. All spin systems could be connected on the basis of $^1H,^{13}C$-long-range interactions (Figure 1). Heteronuclear multiple bond correlation (HMBC) data also enabled the placement of the amide and the ketone function. Finally, the positions of the chlorine and hydroxy substituents on the benzene ring were deduced from an analysis of ^{13}C chemical shifts.

Table 1. NMR spectroscopic data of auriculamide (**1**) in $CDCl_3$.

Pos.	δ_C [ppm]	δ_H [ppm], M (*J* inHz)	COSY	HMBC
1	11.8	0.91, t (7.5)	2a, 2b	2, 3
2	26.2	a: 1.41 m	1, 2b, 3	1, 3, 4, 17
		b: 1.26 m	1, 2a, 3	1, 3, 4, 17
3	38.3	1.83 m	2a, 2b, 4, 17	1, 2, 4, 5, 17
4	74.5	4.09 d (2.8)	3	2, 3, 5, 17
5	173.1			
6	58.1	4.84 dt (7.9, 6.8)	7a, 7b, NH	5, 7, 8, 14
7	36.7	a: 3.01 dd (14.2, 6.8)	6, 7b	6, 8, 9, 13, 14
		b: 2.88 dd (14.2, 6.8)	6, 7a	6, 8, 9, 13, 14
8	129.2			
9	129.1	6.94 dd (8.3, 2.0)	10, 13	7, 11, 13
10	116.4	6.91 d (8.3)	9	8, 11, 12
11	150.5			
12	119.9			
13	129.5	7.10 d (2.0)	9	7, 9, 11, 12
14	209.0			
15	34.4	a: 2.44 dq (18.4, 7.2)	15b, 16	14, 16
		b: 2.41 dq (18.4, 7.2)	15a, 16	14, 16
16	7.3	1.02 t (7.2)	15a, 15b	14, 15
17	12.6	0.69 d (6.9)	3	2, 3, 4
NH		6.98 d (7.9)	6	7

The structure of **1** includes three chiral centers at C-3, C-4, and C-6. For the stereochemical analysis, the amide bond of **1** was hydrolyzed with HCl and the two resulting fragments were analyzed by chromatographic comparison with synthetic standards. The configuration of the 2-hydroxy-3-methylvaleric acid (HMVA) residue was determined by chiral thin-layer chromatography (TLC). To this end, the four possible HMVA stereoisomers were prepared and individually spotted on a chiral TLC plate together with the hydrolysate of **1**.[14,15] This approach revealed that **1** contains (2*S*,3*S*)-HMVA (Figure S1).

To assign the stereochemistry of the 2-amino-1-(3-chloro-4-hydroxyphenyl)pentan-3-one (CHPO) fragment, the HCl hydrolysate was subjected to chiral HPLC. Reference compounds included the (*S*)-enantiomer of CHPO (**6**) as well as a racemic mixture of this building block (**9**). The stereospecific preparation of **6** started with Boc-protected L-tyrosine (Scheme 1), which was initially converted into Boc-L-Tyr(Cbz)-OH (**2**). Subsequent conversion into Weinreb amide **3**,[16] and Grignard reaction with ethylmagnesium bromide generated the desired pentan-3-one side-chain in **4**. The cleavage of both protective groups with methanesulfonic acid yielded enantiopure (*S*)-2-amino-1-(4-



Scheme 1. Preparation of (*S*)-2-amino-1-(4-hydroxyphenyl)pentan-3-one (**6**).

hydroxyphenyl)pentan-3-one (**5**). Chlorination of the aromatic ring in the *meta*-position was achieved by addition of SO_2Cl_2 in a mixture of Et_2O and HOAc. Racemic 2-amino-1-(4-hydroxyphenyl)pentan-3-one (**8**) was prepared from L-tyrosine by a Dakin–West reaction with propionic anhydride and subsequent hydrolysis.[17] Chlorination of **8** was achieved as described for the enantiopure compound **5**. Chromatographic comparison of the two reference compounds **6** and **9** with the hydrolysis product of **1** indicated C-6 to be *S*-configured (Figure S2).

Auriculamide is the first secondary metabolite to be reported from the predatory bacterium *H. aurantiacus* 114–95^T. A retrobiosynthetic analysis was carried out to clarify whether the structure of **1** could be associated with the previously annotated biosynthesis genes.[11] Auriculamide features an α-hydroxy acid moiety in its structure. Such monomers are known as constituents of various peptide natural products and their incorporation typically involves nonribosomal peptide synthetase (NRPS)-based chemistry.[18,19] In brief, the adenylation (A) domain of an NRPS activates an α-keto acid[20] in an ATP-driven process and tethers the resulting acyl adenylate as a pantetheinyl thioester to a peptidyl carrier protein (PCP) domain. The necessary reduction is subsequently carried out by a ketoreductase (KR) domain that is integrated into the same NRPS.[21,22] In case of **1**, an amide bond links the HMVA moiety to a tyrosine-like residue. Whereas the extension of HMVA with an amino acid can be rationalized by an NRPS-based mechanism,[18] the origin of the ethyl group next to the ketone function in **1** was not clear. A possible scenario involves the incorporation of a methylmalonyl-CoA-derived C_3 unit, mediated by a polyketide synthase (PKS). After hydrolytic release from the NRPS/PKS complex, the resulting carboxylic acid could be shortened by a decarboxylation reaction. Precedence for such a post-assembly-line modification comes from tautomycetin biosynthesis.[23]

Taken together, a hypothetical auriculamide assembly line should feature two discrete NRPS modules for the incorporation of HMVA and (3-chloro)tyrosine as well as an additional PKS module for the final carbon extension. Analysis of the annotated biosynthetic loci from the *H. aurantiacus* genome led to the identification of a putative auriculamide gene cluster (Figure 2). The domain architecture of the proteins encoded by haur_2414 – haur_2416 is consistent with the proposed biosynthetic model. Furthermore, 2-keto-3-methylvaleric acid and tyrosine were predicted as the most likely substrates of Haur_2414 and Haur_2415, thereby supporting an involvement of these proteins in the assembly of **1** (Table S1).[24–26] It must be noted that Haur_2414 is also the only NRPS-type enzyme of *H. aurantiacus* 114–95^T featuring a KR domain. The chlorination is expected to occur after the tyrosine has been tethered to the PCP domain of Haur_2415, thus paralleling the incorporation of chlorine atoms in the biosyntheses of balhimycin, C-1027, and chondrochloren.[27–29] Homology searches against the genome of *H. aurantiacus* 114–95^T did not reveal a clear candidate gene for the halogenation. The product of haur_2404 was annotated as a decarboxylase and could be involved in the final processing of the proposed carboxylic acid intermediate (Figure 2).

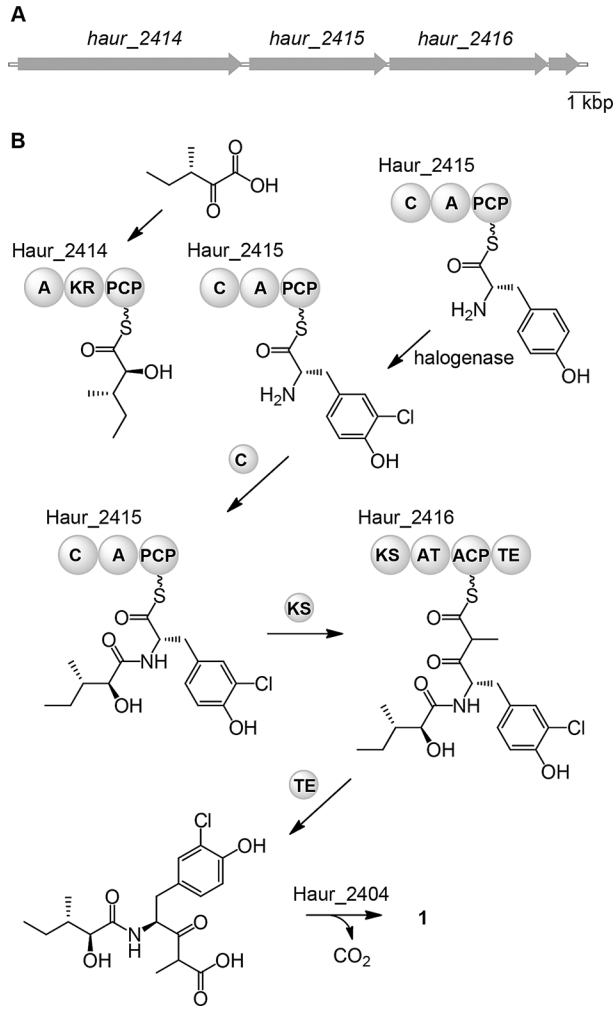


Figure 2. (A) Proposed auriculamide gene cluster and (B) assembly-line biosynthesis of **1**. The chlorination reaction might also occur at a later biosynthetic stage.[34] Domain notation: A, adenylation; KR, keto reductase; PCP, peptidyl carrier protein; C, condensation; KS, β-ketoacyl synthase; AT, acyl transferase; ACP, acyl carrier protein; TE, thioesterase. The gene haur_2417 was annotated as a type-II thioesterase.

According to a database search,[30] **1** shows only modest structural similarity to other natural products. The closest relatives are the depsipeptides ulongamide E and guineamide C, which were isolated from cyanobacteria.[31,32] Both compounds feature a HMVA moiety that is amidated with tyrosine in their structures, but lack the distinctive chlorination and the terminal ethyl group of **1**. This finding substantiates the potential of unexplored bacterial sources to produce new chemical scaffolds.[33]

Conclusion

The discovery of auriculamide provides the first evidence for the previously assumed biosynthetic potential of *H. aurantiacus* 114–95^T.[11] Although a possible gene cluster for

the assembly of the structural scaffold of **1** could be identified in this study, the genetic basis for specific tailoring reactions await further clarification. Since the lack of a genetic system for *H. aurantiacus* impedes inactivation and complementation studies, efforts are now underway to reconstitute the biosynthesis of **1** in a heterologous host.

Experimental Section

General Experimental Procedures: High-resolution mass analyses were performed with an Exactive Mass Spectrometer (ThermoScientific). NMR spectra were recorded at 300 K with Bruker spectrometers and [D_4]MeOH (δ_H = 3.31 ppm; δ_C = 49.0 ppm) or $CDCl_3$ (δ_H = 7.24 ppm; δ_C = 77.0 ppm) as solvents and internal standards. Preparative and analytical HPLC was conducted with a Shimadzu HPLC system (LC-20AT, SPD-M20A).

Production and Isolation of 1: *H. aurantiacus* strain 114–95^T was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ). The bacterium was routinely cultured at 30 °C in modified HP74 medium [sodium glutamate 1%, yeast extract 0.2%, $MgSO_4 \cdot 7H_2O$ 0.2%, D-(+)-glucose 1%, 50 mM phosphate buffer, pH 6.6]. For the production of **1**, fermentation was carried out in a 30 L bioreactor (20 L working volume). Aeration was set to 10 L min^{-1} and the culture broth was continuously stirred (200 rpm). The glucose concentration was maintained at ca. 3 g L^{-1} by continuous feeding. The pH was adjusted to values between 6.8 and 7.2. After ten days of growth, the cells were removed by centrifugation and the residual supernatant was mixed with 1.5 kg Amberlite XAD-2 (Supelco). After two washing steps with distilled water, the adsorbed metabolites were eluted from the resin with 10 L MeOH. The eluate was concentrated under vacuum to dryness prior to resuspension in 200 mL of 60% aqueous MeOH and five successive extractions with 200 mL of CH_2Cl_2. The organic layers were combined and residual water was removed by the addition of anhydrous Na_2SO_4. After filtration, the organic solvent was evaporated under reduced pressure. Initial fractionation of the CH_2Cl_2 extract was accomplished by flash chromatography over 50 g of Polygoprep 60–50 C_{18} (Macherey–Nagel) by using increasing concentrations of MeOH in H_2O. Proton NMR spectroscopic analysis indicated the 60% MeOH fraction to be of further interest. Final purification of **1** was achieved by HPLC with a Zorbax-Eclipse XDB C_8 column (Agilent) with a linear gradient of MeOH in H_2O + 0.1% trifluoroacetic acid and a flow rate of 2 mL min^{-1}.

Hydrolysis of 1: The amide bond in **1** was hydrolyzed by addition of 500 μL 4 N HCl and subsequent heating to 120 °C for 24 h. The acid was removed by evaporation and the residue was used directly for the chromatographic analyses described below.

Configurational Assignment of the HMVA Moiety in 1: The four possible stereoisomers of HMVA were prepared according to an established protocol.[14] In brief, the four stereoisomers of isoleucine (0.2 mmol) were each dissolved in 1 M H_2SO_4 (10 mL) in an ice bath. $NaNO_2$ (2 mmol) was added and the solutions were stirred at 0 °C for 10 h. Stirring was continued for two days at room temperature, then the aqueous solutions were saturated with NaCl and extracted with EtOAc (3 × 10 mL). The combined EtOAc extracts were dried under reduced pressure. Product formation was confirmed by LC-HR-ESI-MS analyses. Subsequently, the HMVA stereoisomers were subjected to TLC together with hydrolyzed **1** using ChiralPlate (Macherey–Nagel) as the stationary phase.[15] The TLC plate was developed in a mixture of 90% CH_2Cl_2 and 10% MeOH. Analytes were detected with vanadium(V) oxide spray reagent.

(*S*)-3-[4-(Benzyloxy)phenyl]-2-(*tert*-butoxycarbonylamino)propanoic Acid (2): Boc-protected L-tyrosine (5 mmol) and sodium hydride (5 mmol) were dissolved in anhydrous THF (5 mL) under an argon atmosphere and stirred for 15 min at room temperature. Tetrabutylammonium iodide (0.05 mmol) and benzyl bromide (5 mmol) were then added and the solution was stirred for 3 h at room temperature before brine (15 mL) was added and the solution was exhaustively extracted with diethyl ether (Et_2O). The organic layers were combined, the solvent was evaporated, and the crude product was purified by reversed-phase HPLC to give **2** (1690.3 mg, 91%).

(*S*)-3-[4-(Benzyloxy)phenyl]-2-(*tert*-butoxycarbonylamino)propanoic Acid (2): ^{1}H NMR (300 MHz, [D_4]MeOH, 300 K): δ = 7.29–7.45 (m, 5 H, 12–14-CH), 7.14 (d, $^3J_{H,H}$ = 8.5 Hz, 2 H, 7-CH), 6.91 (d, $^3J_{H,H}$ = 8.5 Hz, 2 H, 8-CH), 5.05 (s, 2 H, 10-CH_2), 4.29 (dd, $^3J_{H,H}$ = 9.0, 5.0 Hz, 4-CH), 3.09 (dd, $^3J_{H,H}$ = 14.0, 5.0 Hz, 1 H, 5b-CH), 2.84 (dd, $^3J_{H,H}$ = 14.0, 9.0 Hz, 1 H, 5a-CH), 1.37 (s, 9 H, 1-CH_3) ppm. ^{13}C NMR (75 MHz, [D_4]MeOH, 300 K): δ = 175.5 (C-15), 159.1 (C-9), 157.8 (C-3), 138.9 (C-11), 131.3 (C-13), 130.9 (C-14), 129.5 (C-7), 128.8 (C-6), 128.5 (C-12), 115.9 (C-8), 80.5 (C-2), 71.0 (C-10), 56.5 (C-4), 37.9 (C-5), 28.7 (C-1) ppm. HRMS (ESI): *m*/*z* calcd. for $C_{21}H_{25}NO_5$ [M – H]$^-$ 370.1660; found 370.1665.

***tert*-Butyl (*S*)-{3-[4-(Benzyloxy)phenyl]-1-[methoxy(methyl)amino]-1-oxopropan-2-yl}carbamate (3):** Tetrabromomethane, *N*,*O*-dimethylhydroxylamine, triethylamine, and **2** (3 mmol each) were dissolved in anhydrous CH_2Cl_2 (10 mL) under an argon atmosphere at room temperature. Triphenylphosphine (3 mmol) was slowly added and the solution was stirred for 10 min. Upon completion, the CH_2Cl_2 solution was washed with brine (10 mL), dried under reduced pressure, and purified by reversed-phase HPLC to give **3** (1056.2 mg, 85%).

***tert*-Butyl (*S*)-{3-[4-(Benzyloxy)phenyl]-1-[methoxy(methyl)amino]-1-oxo-propan-2-yl}carbamate (3):** ^{1}H NMR (600 MHz, [D_4]MeOH, 300 K): δ = 7.41 (d, $^3J_{H,H}$ = 7.3 Hz, 2 H, 12-CH), 7.35 (t, $^3J_{H,H}$ = 7.3 Hz, 2 H, 13-CH), 7.28 (t, $^3J_{H,H}$ = 7.3 Hz, 1 H, 14-CH), 7.12 (d, $^3J_{H,H}$ = 8.2 Hz, 2 H, 7-CH), 6.90 (d, $^3J_{H,H}$ = 8.2 Hz, 2 H, 8-CH), 5.05 (s, 3 H, 10-CH_3), 4.78 (m, 1 H, 4-CH), 3.70 (s, 3 H, 17-CH_3), 3.14 (s, 3 H, 16-CH_3), 2.92 (dd, $^3J_{H,H}$ = 13.5, 5.4 Hz, 1 H, 5b-CH), 2.72 (m, 1 H, 5a-CH), 1.37 (s, 9 H, 1-CH_3) ppm. ^{13}C NMR (150 MHz, [D_4]MeOH, 300 K): δ = 174.5 (C-15), 159.1 (C-9), 157.6 (C-3), 138.8 (C-11), 131.4 (C-14), 130.8 (C-6), 129.5 (C-7), 128.8 (C-13), 128.5 (C-12), 115.9 (C-8), 80.5 (C-2), 70.9 (C-10), 62.0 (C-17), 53.7 (C-4), 38.0 (C-5), 32.4 (C-16), 28.7 (C-1) ppm. HRMS (ESI): *m*/*z* calcd. for $C_{23}H_{30}N_2O_5$ [M + H]$^+$ 415.2227; found 415.2231.

***tert*-Butyl (*S*)-{1-[4-(Benzyloxy)phenyl]-3-oxopentan-2-yl}carbamate (4):** Compound **3** (2 mmol) was dissolved in anhydrous Et_2O under an argon atmosphere. Ethylmagnesium bromide (1.5 equiv.) was added and the solution was stirred at room temperature for 1 h. The reaction was then quenched by the addition of H_2O (500 μL), the solvent was evaporated under reduced pressure, and the residue was extracted with MeOH and purified by reversed-phase HPLC to give **4** (620.8 mg, 81%). ^{1}H NMR (500 MHz, [D_4]MeOH, 300 K): δ = 7.41 (d, $^3J_{H,H}$ = 7.3 Hz, 2 H, 12-CH), 7.35 (t, $^3J_{H,H}$ = 7.3 Hz, 2 H, 13-CH), 7.29 (t, $^3J_{H,H}$ = 7.3 Hz, 1 H, 14-CH), 7.11 (d, $^3J_{H,H}$ = 8.5 Hz, 2 H, 7-CH), 6.90 (d, $^3J_{H,H}$ = 8.5 Hz, 2 H, 8-CH), 5.05 (s, 2 H, 10-CH_2), 4.26 (dd, $^3J_{H,H}$ = 8.9, 5.8 Hz, 1 H, 4-CH), 2.98 (dd, $^3J_{H,H}$ = 14.0, 5.8 Hz, 1 H, 5b-CH), 2.72 (dd, $^3J_{H,H}$ = 14.0, 8.9 Hz, 1 H, 5a-CH), 2.52 (dq, $^3J_{H,H}$ = 14.1, 7.5 Hz, 1 H, 16b-CH), 2.40 (dq, $^3J_{H,H}$ = 14.1, 7.2 Hz, 1 H, 16a-CH), 1.37 (s, 9 H, 1-CH_3), 0.97 (t, $^3J_{H,H}$ = 7.2 Hz, 3 H, 17-CH_3) ppm. ^{13}CNMR (125 MHz, [D_4]-

MeOH, 300 K): δ = 212.6 (C-15), 159.1 (C-9), 157.9 (C-3), 138.8 (C-11), 131.4 (C-14), 131.0 (C-6), 129.5 (C-7), 128.8 (C-13), 128.5 (C-12), 116.0 (C-8), 80.6 (C-2), 71.0 (C-10), 62.2 (C-4), 37.0 (C-5), 34.0 (C-16), 28.7 (C-1), 7.7 (C-17) ppm. HRMS (ESI): *m*/*z* calcd. for $C_{23}H_{29}NO_4$ $[M + H]^+$ 384.2169; found 384.2173.

(*S*)-2-Amino-1-(4-hydroxyphenyl)pentan-3-one (5): Compound **4** (1.5 mmol) was dissolved in $CHCl_3$ (5 mL) and methanesulfonic acid (2 mL) was added dropwise. After 30 min of stirring the reaction was complete and H_2O (10 mL) was added. The acid was neutralized by the addition of solid $NaHCO_3$ and the solvents were removed under reduced pressure. The residue was extracted with MeOH and the extracted salt that precipitated at 4 °C was removed by filtration. The filtrate was purified by reversed-phase HPLC to give **5** (139.1 mg, 48%). ^{1}H NMR (500 MHz, [D$_4$]MeOH, 300 K): δ = 7.10 (dd, $^3J_{H,H}$ = 8.4 Hz, 2 H, 7-CH), 6.80 (d, $^3J_{H,H}$ = 8.4 Hz, 2 H, 8-CH), 4.31 (dd, $^3J_{H,H}$ = 8.1, 6.3 Hz, 1 H, 4-CH), 3.17 (dd, $^3J_{H,H}$ = 14.4, 6.3 Hz, 1 H, 5b-CH), 2.92 (dd, $^3J_{H,H}$ = 14.4, 8.1 Hz, 1 H, 5a-CH), 2.51 (q, $^3J_{H,H}$ = 7.1 Hz, 2 H, 2-CH$_2$), 1.04 (t, $^3J_{H,H}$ = 7.12 Hz, 3 H, 1-CH$_3$) ppm. ^{13}C NMR (125 MHz, [D$_4$]MeOH, 300 K): δ = 207.6 (C-3), 158.5 (C-9), 131.4 (C-7), 125.8 (C-6), 117.0 (C-8), 60.9 (C-4), 36.6 (C-5), 34.4 (C-2), 7.4 (C-1) ppm. HRMS (ESI): *m*/*z* calcd. for $C_{11}H_{15}NO_2$ $[M + H]^+$ 194.1176; found 194.1167.

(*S*)-2-Amino-1-(3-chloro-4-hydroxyphenyl)pentan-3-one (6): Compound **5** (0.5 mmol) was dissolved in a mixture of Et_2O and HOAc (1:1, 2 mL). The solution was cooled to 0 °C and SO_2Cl_2 (0.5 mmol) was added slowly. The mixture was stirred for 2 h at 0 °C, then the solvent was evaporated and the crude product was purified by reversed-phase HPLC to give **6** (66.0 mg, 58%). ^{1}H NMR (600 MHz, [D$_4$]MeOH, 300 K): δ = 7.27 (d, $^3J_{H,H}$ = 2.1 Hz, 1 H, 7-CH), 7.04 (dd, $^3J_{H,H}$ = 8.3, 2.1 Hz, 1 H, 11-CH), 6.92 (d, $^3J_{H,H}$ = 8.3 Hz, 1 H, 10-CH), 4.34 (dd, $^3J_{H,H}$ = 8.6, 6.1 Hz, 1 H, 4-CH), 3.21 (dd, $^3J_{H,H}$ = 14.4, 6.1 Hz, 1 H, 5b-CH), 2.89 (dd, $^3J_{H,H}$ = 14.4, 8.6 Hz, 1 H, 5a-CH), 2.54 (q, $^3J_{H,H}$ = 7.2 Hz, 2 H, 2-CH$_2$), 1.06 (t, $^3J_{H,H}$ = 7.2 H, 3 H, 1-CH$_3$) ppm. ^{13}C NMR (150 MHz, [D$_4$]MeOH, 300 K): δ = 207.3 (C-3), 154.2 (C-9), 131.8 (C-7), 129.9 (C-11), 127.4 (C-6), 122.3 (C-8), 118.2 (C-10), 60.7 (C-4), 36.0 (C-5), 34.3 (C-2), 7.4 (C-1) ppm. HRME (ESI): *m*/*z* calcd. for $C_{11}H_{14}ClNO_2$ $[M + H]^+$ 228.0786; found 228.0788.

4-(3-Oxo-2-propionamidopentyl)phenyl Propionate (7): L-Tyrosine (5 mmol) was dissolved in anhydrous pyridine (10 mL), propionic anhydride (5 equiv.) was added and the solution was heated to reflux at 120 °C for 8 h. The solvent was evaporated and the crude product was purified by reversed-phase HPLC to give **7** (1313.2 mg, 86%). ^{1}H NMR (500 MHz, $CDCl_3$, 300 K): δ = 7.09 (d, $^3J_{H,H}$ = 8.5 Hz, 2 H, 8-CH), 6.99 (d, $^3J_{H,H}$ = 8.5 Hz, 2 H, 7-CH), 6.01 (d, $^3J_{H,H}$ = 7.5 H, 1 H, 13-CH), 4.84 (ddd, $^3J_{H,H}$ = 7.5 Hz, 7.1 Hz, 5.8 Hz, 1 H, 4-CH), 3.06 (dd, $^3J_{H,H}$ = 14.0 Hz, 7.1 Hz, 1 H, 5b-CH), 3.00 (dd, $^3J_{H,H}$ = 14.0 Hz, 5.8 Hz, 1 H, 5a-CH), 2.55 (q, $^3J_{H,H}$ = 7.3 Hz, 2 H, CH$_2$–11), 2.40 (q, $^3J_{H,H}$ = 7.3 Hz, 2 H, 15-CH$_2$), 2.18 (q, $^3J_{H,H}$ = 7.6, 2 H, 2-CH$_2$), 1.23 (t, $^3J_{H,H}$ = 7.5 Hz, 3 H, 12-CH$_3$), 1.10 (t, $^3J_{H,H}$ = 7.6, 3 H, H-1), 1.00 (t, $^3J_{H,H}$ = 7.3 Hz, 3 H, 16-CH$_3$) ppm. ^{13}CNMR (125 MHz, $CDCl_3$, 300 K): δ = 209.3 (C-3), 173.3 (C-14), 172.9 (C-10), 149.8 (C-9), 133.4 (C-6), 130.1 (C-7), 121.7 (C-8), 58.4 (C-4), 37.1 (C-5), 34.3 (C-2), 29.5 (C-15), 27.7 (C-11), 9.6 (C-16), 9.0 (C-12), 7.4 (C-1) ppm. HRMS (ESI): *m*/*z* calcd. for $C_{17}H_{23}NO_4$ $[M + H]^+$ 306.1700; found 306.1704.

(*R*,*S*)-2-Amino-1-(4-hydroxyphenyl)pentan-3-one (8): 4-(3-Oxo-2-propionamidopentyl)phenyl propionate (2.5 mmol) was dissolved in 4 N HCl (10 mL) and heated to reflux at 100 °C for 8 h. Removal of HCl by evaporation gave **8** (449.2 mg, 93%). ^{1}H NMR (300 MHz, [D$_4$]MeOH, 300 K): δ = 7.10 (d, $^3J_{H,H}$ = 8.5 Hz, 2 H, 7-CH), 6.79 (d, $^3J_{H,H}$ = 8.5 Hz, 2 H, 8-CH), 4.13 (dd, $^3J_{H,H}$ = 7.9, 6.7 Hz, 1 H, 4-CH), 3.15 (dd, $^3J_{H,H}$ = 14.4, 6.7 Hz, 1 H, 5b-CH), 2.95 (dd, $^3J_{H,H}$ = 14.4, 7.9 Hz, 1 H, 5a-CH), 2.48 (q, $^3J_{H,H}$ = 7.3 Hz, 2 H, 2-CH$_2$), 1.02 (t, $^3J_{H,H}$ = 7.3 Hz, 3 H, 1-CH$_3$) ppm. ^{13}C NMR (75 MHz, [D$_4$]MeOH, 300 K): δ = 207.7 (C-3), 158.4 (C-9), 131.4 (C-7), 125.9 (C-6), 117.0 (C-8), 60.8 (C-4), 36.6 (C-5), 34.5 (C-2), 7.3 (C-1) ppm. HRMS (ESI): *m*/*z* calcd. for $C_{11}H_{15}NO_2$ $[M + H]^+$ 194.1176; found 194.1178.

(*R*,*S*)-2-Amino-1-(3-chloro-4-hydroxyphenyl)pentan-3-one (9): Compound **8** (1 mmol) was dissolved in a mixture of HOAc and Et_2O (1:1, 5 mL). The solution was cooled to 0 °C and SO_2Cl_2 (1 equiv.) was added slowly. The reaction mixture was stirred for 2 h at 0 °C, then the solvent was evaporated and the crude 2-amino-1-(3-chloro-4-hydroxyphenyl)pentan-3-one (**9**, racemic CHPO) was purified by reversed-phase HPLC to give **9** (141.2 mg, 62%). ^{1}H NMR (600 MHz, [D$_4$]MeOH, 300 K): δ = 7.27 (d, $^3J_{H,H}$ = 2.1 Hz, 1 H, 7-CH), 7.04 (dd, $^3J_{H,H}$ = 8.3, 2.1 Hz, 1 H, 11-CH), 6.92 (d, $^3J_{H,H}$ = 8.3 Hz, 1 H, 10-CH), 4.34 (dd, $^3J_{H,H}$ = 8.6, 6.1 Hz, 1 H, 4-CH), 3.21 (dd, $^3J_{H,H}$ = 14.6, 6.1 Hz, 1 H, 5b-CH), 2.89 (dd, $^3J_{H,H}$ = 14.6, 8.6 Hz, 1 H, 5a-CH), 2.55 (qd, $^3J_{H,H}$ = 7.1, 2.5 Hz, 2 H, 2-CH$_2$), 1.06 (t, $^3J_{H,H}$ = 7.1 Hz, 3 H, 1-CH$_3$) ppm. ^{13}C NMR (150 MHz, [D$_4$]MeOH, 300 K): δ = 207.3 (C-3), 154.2 (C-9), 131.8 (C-7), 129.9 (C-11), 127.4 (C-6), 122.3 (C-8), 118.2 (C-10), 60.7 (C-4), 36.0 (C-5), 34.3 (C-2), 7.4 (C-1) ppm. HRMS (ESI): *m*/*z* calcd. for $C_{11}H_{14}ClNO_2$ $[M + H]^+$ 228.0786; found 228.0787.

Configurational Assignment of the CHPO Moiety in 1: Chiral HPLC analysis was carried out with a Nucleodur delta RP 5 column (Macherey–Nagel). The column was operated at a flow rate of 0.5 mL min^{-1} using a linear gradient of MeOH in H_2O + 0.1% trifluoroacetic acid. Analytes were detected with a diode array detector.

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