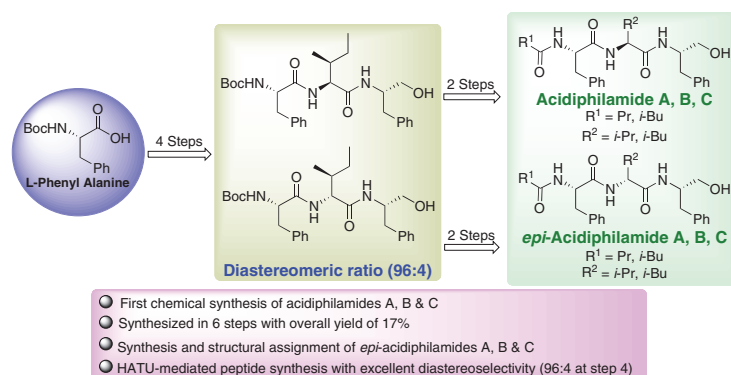


Synthesis of Acidiphilamide A–C: Secondary Metabolites from the Genus *Streptacidiphilus*

Poosa Mallesham^{a,b}
 Karri Raghavulu^a
 Venkatesh Miriyala^a
 Raju Doddipalla^a
 Satyanarayana Yennam^a
 Paul Douglas Sanasi^b
 Manoranjan Behera^{*a}

^a Chemistry Services, Aragen Life Sciences (Formerly known as GVK Biosciences), Survey No: 125 (part) & 126, IDA Mallapur, Hyderabad-500076, Telangana State, India
 Manoranjan.Behera@aragen.com

^b Department of Engineering Chemistry, Andhra University, Waltair Junction, Visakhapatnam, Andhra Pradesh, 530003, India



Received: 10.11.2022

Accepted after revision: 15.02.2023

Published online: 15.02.2023 (Accepted Manuscript),

28.03.2023 (Version of Record)

DOI: 10.1055/a-2035-9753; Art ID: SO-2022-11-0059-OP

License terms:

© 2023. The Author(s). This is an open access article published by Thieme under the terms of the Creative Commons Attribution License, permitting unrestricted use, distribution and reproduction, so long as the original work is properly cited. (<https://creativecommons.org/licenses/by/4.0/>)

Abstract We describe the efficient total syntheses of naturally occurring tripeptides acidiphilamides A–C and *epi*-acidiphilamides A–C, which were prepared from commercially available L-phenylalanine using hexafluorophosphate azabenzotriazole tetramethyl uronium (HATU) as peptide coupling reagent. The structures of the natural acidiphilamides A, B and C were characterized by NMR, MS and SOR data, which match those of natural products, whereas the structures of *epi*-acidiphilamides A, B and C were confirmed by 2D NMR studies.

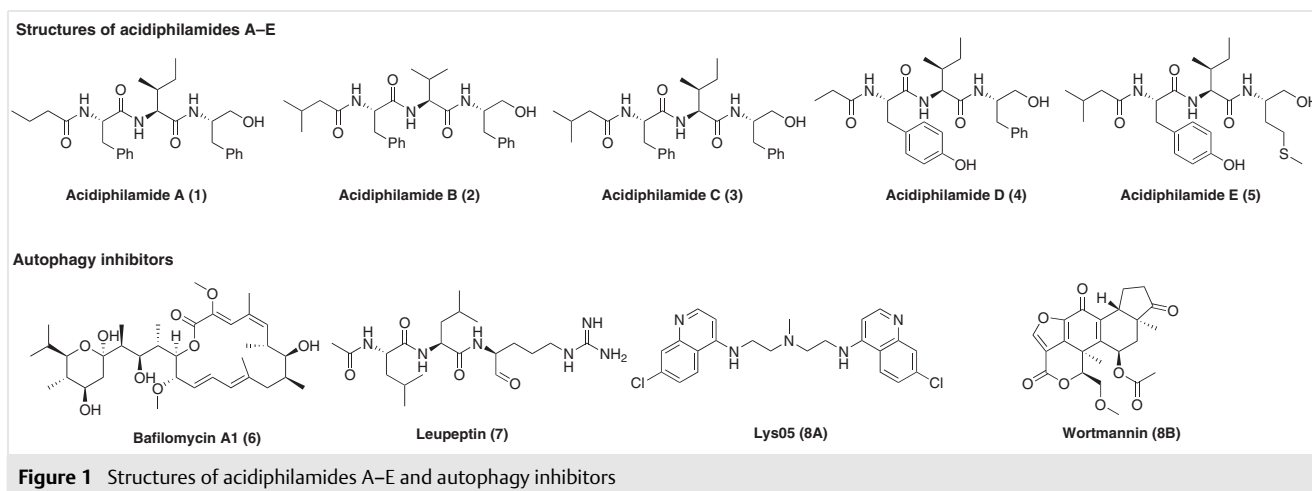
Key words total synthesis, natural products, acidiphilamides, *epi*-acidiphilamides, structural elucidation, amide coupling reagents

The role of natural products in the field of modern drug discovery cannot be denied, as about 35% of medicines have originated from natural products.¹ Many antimicrobial and anticancer agents were derived either directly or indirectly, from natural products. The advancement of genome sequencing uncovered a huge untapped potential in the bacterial phylum Actinobacteria as sources of new drug leads.² *Streptacidiphilus* are obligate *acidophilic Actinobacteria*, which can be isolated from acidic soils and debris. Based on their genotypic and phenotypic properties, the genus *Streptacidiphilus* is taxonomically positioned under the family *Streptomycetaceae*, which includes the genera *Streptomyces*, one of the major producers of antibiotics, and *Kitatospora*. Since their taxonomic characterization in 2003, research on *Streptacidiphilus* was mainly focused on the isolation and identification of new species. Even though the potential of acidophilic Actinobacteria as a source of an-

timicrobials was highlighted, less research was done to explore their natural products. One of the rare examples is the discovery of a group of peptides that modulate cellular autophagic flux in tumor cells.³

Autophagy is a proteolytic process that involves lysosomal degradation of a range of substrates.⁴ Autophagy inhibitors have been shown to affect cellular autophagic flux, boosting tumor cell death in areas where autophagy is frequently activated.^{5,6} For example, in rat pancreatitis models, wortmannin (Figure 1), a natural product from the fungus *Penicillium funiculosum*, inhibits the phosphatidylinositol 3-kinase (PI3K), suppressing early phases of autophagy, preventing reactive oxygen species (ROS) formation, and reducing thyroid tissue damage. Bafilomycin A1 (BafA1) (Figure 1), a family of macrolide antibiotics that prevents the union of the autophagosome and the lysosome, inhibited the proliferation of various tumor cell types. Actinobacteria are the bacteria that produce the most bioactive secondary metabolites.⁷ Previously, chemical studies of actinobacteria were mostly focused on the genus *Streptomyces*, which accounted for 85% of all actinobacterial metabolites described.⁸ Rare actinobacterial genera later accounted for a higher share of reported bioactive chemicals, accounting for 30% of total actinobacterial metabolites. The ongoing discovery of new, rare actinobacterial species has allowed researchers to investigate their various secondary metabolites, some of which have medicinal potential.⁹

In 2019, five novel tripeptides, acidiphilamides A–E (1–5; Figure 1), were identified in *Streptacidiphilus* acidic forest soil.¹⁰ Based on the NMR and mass spectroscopy data, the structures of natural products 1–5 were identified as modified tripeptides containing phenylalaninol or methioninol fragments. Advanced Marfey's technique and GITC (2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl isothiocyanate) derivatization followed by LC-MS analysis were used to determine the absolute configurations of the amine units. The first



secondary metabolites identified from the rare actinobacterial genus *Streptacidiphilus*, acidiphilamides A and B (**1** and **2**), dramatically decreased autophagic flux but not proteasome activity in HeLa cells. In the late stage of cellular autophagy, these compounds appeared to impede primarily the autophagosome lysosome fusion phase.¹¹

In the past decade, peptides and their derivatives have attracted great attention in the field of drug development.¹² Peptides modulate many physiological operations, performing at some sites as endocrine or paracrine signals and at others as neurotransmitters or improvement factors, for instance. Peptides have been used as drugs or starting points for drug discovery programs for many decades; the challenges lie in their redesign to retain the biological activity whilst overcoming DMPK issues. A good example of a tripeptide would be the combination of glycine, histidine and lysine, which is said to help improve skin condition as well as giving it a smoother appearance.¹³

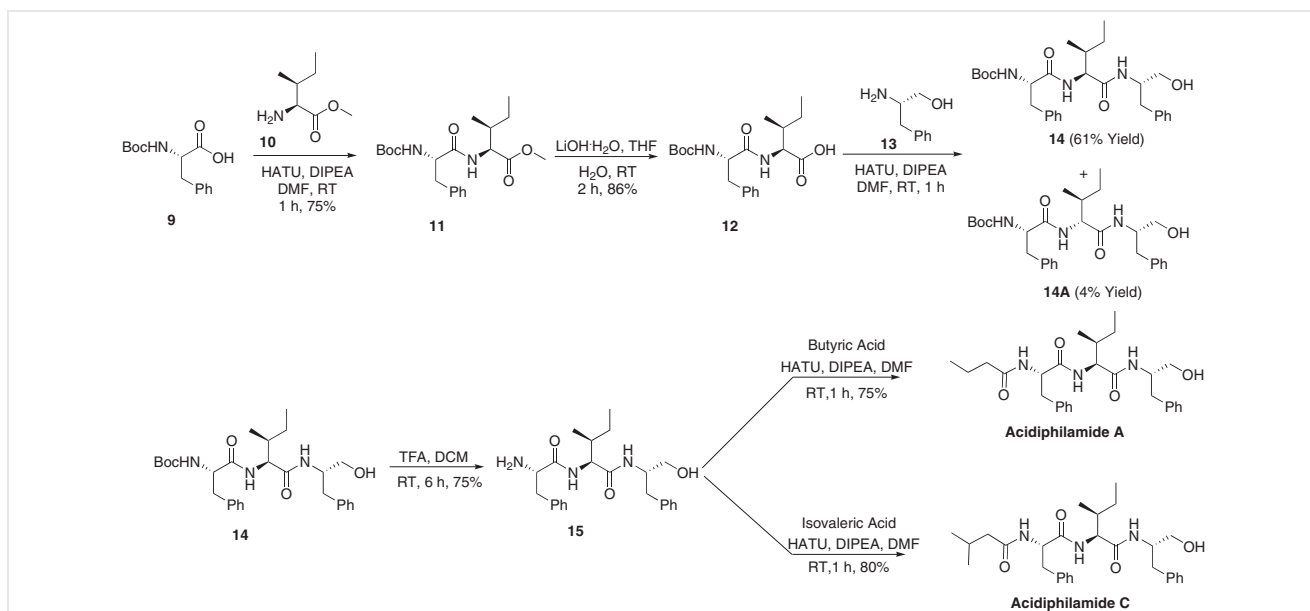
Recently, Malins *et al.*, reported the electrochemical synthesis of the natural product acidiphilamide A.¹⁴ But, to our knowledge, the chemical synthesis of the tripeptides acidiphilamides A–E has not been reported to date. In our continuing effort to make novel hybrid molecules,¹⁵ we were interested in preparing peptides for conformational studies and came across the interesting new acidiphilamides A–E. Herein, we report the first synthesis of acidiphilamides A–C starting from L-phenyl alanine.

Tripeptides are the smallest peptide sequence in which the impact of neighboring amino acid residues on the middle residue can be examined. As structural information of tripeptide segments is useful in the building of protein structures, comparing the structural characteristics of the middle residue in a tripeptide with that in proteins could help in the exploration of protein structures.¹⁶ The endogenous tripeptides clearly have important signaling roles in biology, lending credence to the contention that motifs as small as three amino acids are indeed important and capable of valuable function. However, contiguous tripeptide se-

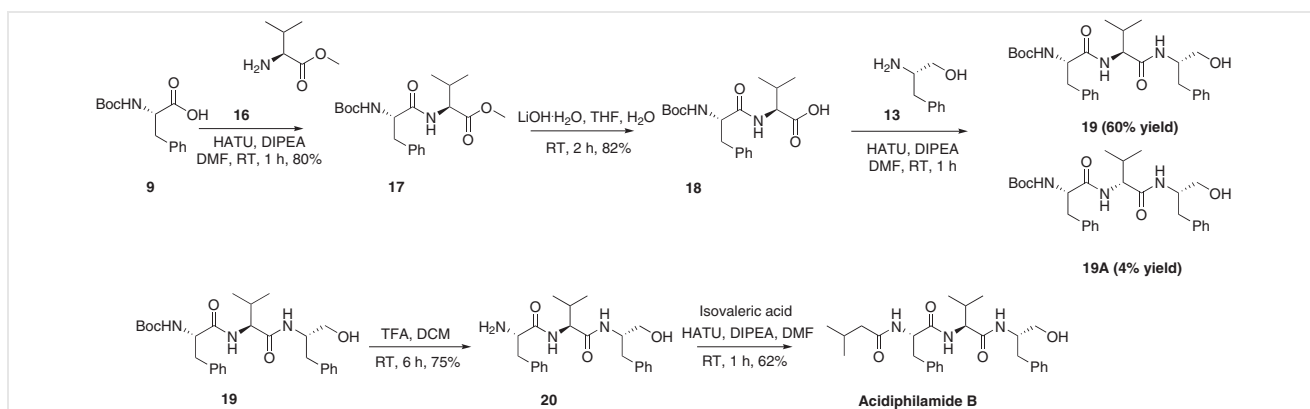
quence embedded in larger peptides and proteins also have useful signaling properties.¹⁷ The minimal useful length may be a product of the number of effective molecular interactions that is required between a ligand and protein receptor to have useful efficacy.¹⁸ The same approach has been used for the synthesis of acidiphilamides A–C, which will throw some light on the structural information from the analysis of these peptides.

The synthesis commenced from commercially available Boc-Phe-OH (**9**), which was coupled with L-isoleucine methyl ester **10** using HATU/DIPEA in DMF, to give dipeptide **11** in 75% yield (Scheme 1). This dipeptide **11** was subjected to hydrolysis using LiOH·H₂O in THF/H₂O to yield the corresponding acid **12** in 86% yield. The next step was the coupling of dipeptide **12** with L-phenyl alaninol (**13**)¹⁹ using HATU/DIPEA in DMF, which gave tripeptides **14** and **14A** as mixture of diastereomers in 96:4 ratio, based on LCMS and HPLC analysis, which were separated over silica gel chromatography with 61 and 4% isolated yields, respectively. The formation of the side product **14A** might be because of the epimerization²⁰ at the amidic NH chiral centre during the amidation reaction. Both isomers were well characterized by ¹H NMR and LCMS analysis. One of the tripeptides, compound **14**, was then subjected to Boc deprotection using TFA/DCM to afford compound **15** (Scheme 1). With key intermediate **15** in hand, we next carried out the amide coupling with butyric acid and isovaleric acid using HATU/DIPEA. The corresponding natural products acidiphilamides A and C were obtained in 75 and 80% yield, respectively. Their spectroscopic data were consistent with the reported data.¹⁰

Inspired by these results, we turned our consideration to the synthesis of acidiphilamide B by coupling compound **9** with L-valine methyl ester **16** using HATU/DIPEA in DMF, which gave dipeptide **17** in 80% yield (Scheme 2). This dipeptide was subjected to hydrolysis using LiOH·H₂O in THF/H₂O to give acid **18** in 82% yield.



Scheme 1 Synthesis of acidiphilamides A and C



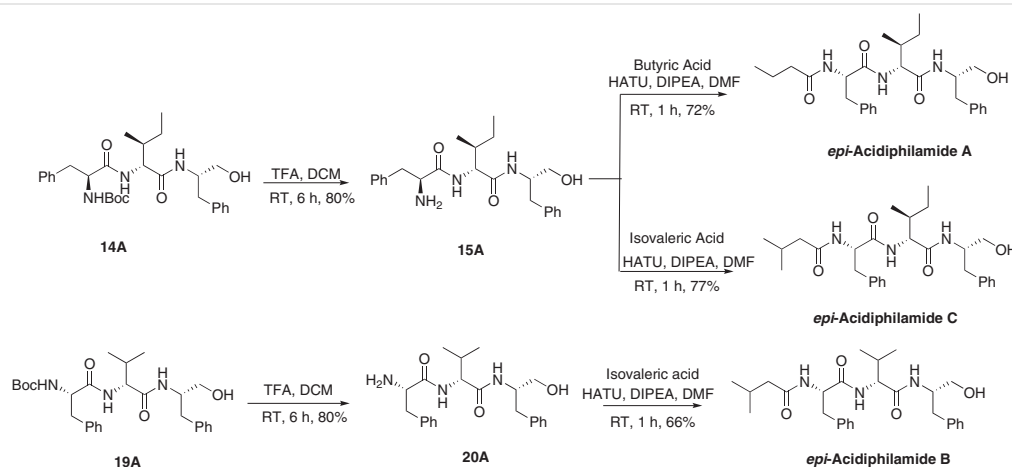
Scheme 2 Synthesis of acidiphilamide B

Next, L-phenyl alaninol **13** was coupled to furnish tripeptide **19** and **19A** in 96:4 ratio, respectively, as a mixture of diastereomers. Both the diastereomers were well characterized by NMR and LCMS analysis. As shown in Scheme 2, finally, the tripeptide **19** was subjected to Boc deprotection followed by amide coupling with isovaleric acid to accomplish the natural product acidiphilamide B, which was well characterized by NMR spectroscopy, LCMS and SOR data, all of which were consistent with those of the natural product.¹⁰

As we obtained the *epi*-diastereomer **14A** during the synthesis of the tripeptide **14**, we took this *epi*-isomer **14A** and focused on the synthesis of the corresponding *epi*-acidiphilamides A and C by following the same synthetic sequence as that of the natural counterparts, as shown in Scheme 3. The structures of both the *epi*-acidiphilamides A and C were confirmed by 2D NMR studies. Along similar

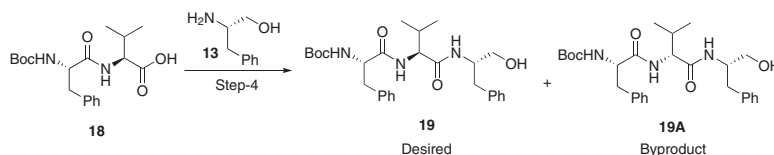
lines, tripeptide **19A** was subjected to Boc deprotection followed by amide coupling with isovaleric acid to yield *epi*-acidiphilamide B, as depicted in Scheme 3. The structural assignment of *epi*-acidiphilamides B was confirmed by 2D NMR studies.

As we obtained the mixture of isomers during the synthesis of key tripeptide fragment, we focused on examining the extent of racemization using different coupling reagents, as tabulated in Table 1. The coupling of dipeptide **18** with L-phenyl alaninol **13** using different coupling reagents such as HATU, PYBOP, T³P, DCC, EDC-HCl, were performed in DMF and DCM as the solvents. The experiments with additives like HOBT (entry 6–8) also gave mixtures of diastereomers in varying ratios. However, the use of HATU with DIPEA as base in DMF (entry 1) gave good results for the generation of the desired diastereomer **19** with excellent diastereomeric ratio (dr) (96:4).



Scheme 3 Synthesis of *epi*-acidiphilamides A, B and C

Table 1 Effect of Coupling Reagents on the Racemization^a



Entry	Coupling reagent	Solvent	19/19A (%) ^b
1	HATU	DMF	96:4
2	PyBOP	DMF	65:35
3	T ³ P	DMF	57:43
4	DCC	DMF	52:48
5	EDC-HC	DMF	76:24
6	EDC-HC HOBt	DMF	67:32
7	PyBOP HOBt	DMF	46:53
8	DCC HOBt	DMF	27:73
9	PyBOP	DCM	72:22
10	T ³ P	DCM	87:10
11	DCC	DCM	85:15
12	EDC-HC	DCM	29:68

^a Reaction conditions: **18**, coupling reagent, DIPEA, solvent, at RT for 1h.

^b Determined by chiral HPLC analysis.

To confirm the relative configuration, acidiphilamide A and its *epi* counterpart were submitted to 2D NMR experiments. In natural acidiphilamide A, the H7 proton has two vicinal proton couplings with amidic NH and another chiral proton of the isoleucine group, thus showing a triplet at 4.11 ppm with 8.2 Hz coupling constant, whereas for the *epi* isomer, the a chiral proton H7, shows a doublet of dou-

plet at 4.22ppm with 9.0 and 4.7 Hz coupling constants. In both compounds, strong coupling for H7 is concluded from amidic NH by gCOSY data.

The other varying coupling for H7 is from the adjacent chiral proton of the isoleucine group; i.e., 8.2 Hz in natural isomer and 4.7 Hz in the *epi* isomer. As per the Karplus equation curve, the difference in orientation of H7 would lead to the difference in the dihedral angle and thereby cou-

pling constant. As represented in Figure 2 with arrows along with expanded NMR peak region, the data suggests that the racemization occurred at the C7 chiral center.

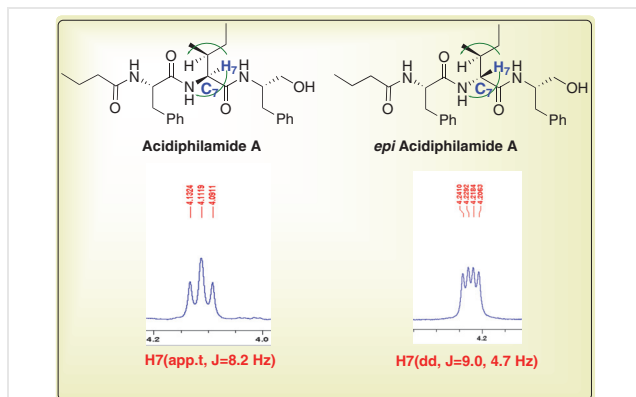


Figure 2 Differences in coupling constant in natural and *epi*-acidiphilamides

In conclusion, the first total syntheses of acidiphilamides A, B and C were accomplished, as were their *epi* diastereomers. Comparison of the spectroscopic data provides insight into the stereochemistry of the natural products.

All solvents were acquired from commercial sources and used as received unless otherwise stated. All other chemicals were acquired from Merck or Aldrich and used without further purification. The melting points were measured with a Stuart melting-point apparatus and are uncorrected. IR spectra were recorded with a Smart iTR, which is an ultra-high-performance, versatile Attenuated Total Reflectance (ATR) sampling accessory on the Nicolet iS10 FT-IR spectrophotometer. NMR spectra were recorded with a Bruker Avance III 400 MHz spectrophotometer (400 MHz for ^1H and 100 MHz for ^{13}C) using TMS as an internal standard and $\text{DMSO}-d_6$ as a solvent; chemical shifts are expressed as δ (ppm) units. Mass spectra were recorded with a GC-MS-QP1000EX spectrometer using an inlet type at 70 eV. Elemental analyses were carried out with a EuroVector instrument C, H, N analyzer EA3000 Series.

Methyl (*tert*-Butoxycarbonyl)-L-phenylalanyl-L-isoleucinate (**11**)

To a stirred solution of (*tert*-butoxycarbonyl)-L-phenylalanine (**9**) (2 g, 7.547 mmol) in *N,N*-dimethylformamide (20 mL) was added DIPEA (1.46 g, 11.32 mmol) and HATU (3.72 g, 9.811 mmol) at 0 °C and the solution was stirred for 10 min at 0 °C. Then methyl L-isoleucinate (**10**) (1.17 g, 8.301 mmol) was added and the resultant mixture was stirred at ambient temperature for 1 h. Reaction mass was quenched with cold water (50 mL) to get precipitation, the mixture was filtered, and the solid was dried to afford **11**.

Yield: 2.2 g (75%); off-white solid; mp 99–102 °C; $[\alpha]_D^{25}$ –10.344 (c 0.75, MeOH).

FT-IR (KBr): 3307, 3063, 2974, 2877, 1967, 1757, 1690, 1652, 1537, 1447, 1381, 1265, 1166, 1043, 1015, 992, 855, 777, 603, 504 cm^{-1} .

^1H NMR (500 MHz, $\text{DMSO}-d_6$): δ = 8.10 (d, J = 7.5 Hz, 1 H), 7.26–6.92 (m, 5 H), 6.93 (d, J = 8.0 Hz, 1 H), 4.27 (t, J = 9.0 Hz, 2 H), 3.62 (s, 3 H), 2.95 (dd, J = 3.2 Hz, J = 2.4 Hz, 1 H), 2.75 (t, J = 13.5 Hz, 1 H), 1.79–1.75 (m, 1 H), 1.41–1.37 (m, 1 H), 1.29 (s, 9 H), 0.84 (s, 6 H).

^{13}C NMR (125 MHz, $\text{DMSO}-d_6$): δ = 171.9, 171.8, 155.1, 138.0, 129.1, 127.9, 126.1, 77.9, 56.2, 55.3, 51.6, 37.1, 36.4, 28.0, 24.6, 15.3, 11.0.

MS (EI): m/z (%) = 393 (100) [M+1].

Synthesis of (*tert*-Butoxycarbonyl)-L-phenylalanyl-L-isoleucine (**12**)

To a cold solution of methyl (*tert*-butoxycarbonyl)-L-phenylalanyl-L-isoleucinate (**11**) (1.2 g, 3.061 mmol) in $\text{THF}/\text{H}_2\text{O}$ (1:1; 10 mL) was added $\text{LiOH}\cdot\text{H}_2\text{O}$ (110 mg, 4.591 mmol) and the resultant mixture was stirred at 25 °C for 2 h. The reaction mixture was evaporated to give the crude compound, which was acidified with sat. citric acid solution to adjust to ca. pH 6 and the aqueous layer was extracted with EtOAc (2 \times 50 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 , filtered, and evaporated to give a crude solid. The crude solid product was washed with *n*-pentane to afford compound **12**.

Yield: 1.0 g (86%); off-white solid; mp 69–72 °C; $[\alpha]_D^{25}$ –0.480 (c 0.5, MeOH).

FT-IR (KBr): 3329, 2974, 1725, 1535, 1377, 1253, 1165, 1022, 855, 698, 496 cm^{-1} .

^1H NMR ($\text{DMSO}-d_6$, 400 MHz): δ = 12.69 (br s, 1 H), 7.89 (d, J = 8.5 Hz, 1 H), 7.31–7.18 (m, 5 H), 6.96 (d, J = 9 Hz, 1 H), 4.25–4.20 (m, 2 H), 2.97 (dd, J = 4 Hz, J = 3.5 Hz, 1 H), 2.75 (t, J = 12.9 Hz, 1 H), 1.80–1.77 (m, 1 H), 1.45–1.39 (m, 1 H), 1.29 (s, 9 H), 0.87–0.84 (m, 6 H).

^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ = 172.8, 171.7, 155.2, 138.1, 129.1, 127.9, 126.1, 78.0, 56.1, 55.5, 37.1, 36.6, 30.4, 28.0, 27.8, 24.5, 15.4, 11.2.

MS (EI): m/z (%) = 379 (100) [M–1].

tert-Butyl ((*S*)-1-(((2*S*,3*S*)-1-(((*S*)-1-Hydroxy-3-phenylpropan-2-yl)amino)-3-methyl-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (**14** and **14A**)

To (*tert*-butoxycarbonyl)-L-phenylalanyl-L-isoleucine (**12**) (1.8 g, 4.761 mmol) in *N,N*-dimethylformamide (20 mL) was added DIPEA (0.92 g, 7.141 mmol) and HATU (2.35 g, 6.189 mmol) at 0 °C and the mixture was stirred at 0 °C for 10 min. (*S*)-2-Amino-3-phenylpropan-1-ol (**13**) (0.79 g, 5.238 mmol) was added and the solution was stirred for 1 h at 25 °C. The resulting mixture was added into cold water (50 mL) to obtain an off-white solid, which was filtered and dried to give 1.6 g of a mixture of diastereomers **14** and **14A** as 96:4 ratio (by LC-MS analysis). The diastereomers were separated by preparative HPLC purification to afford compound **14** (1.5 g, 61% isolated yield) and **14A** (0.1 g, 4% isolated yield) as off-white solids.

tert-Butyl ((*S*)-1-(((2*S*,3*S*)-1-(((*S*)-1-Hydroxy-3-phenylpropan-2-yl)amino)-3-methyl-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (**14**)

Yield: 1.5 g (61%); off-white solid; mp 188–191 °C; $[\alpha]_D^{25}$ –46.540 (c 0.2, MeOH).

FT-IR (KBr): 3309, 2961, 1645, 1536, 1383, 1170, 1038, 691, 523 cm^{-1} .

^1H NMR ($\text{DMSO}-d_6$, 400 MHz): δ = 7.82 (d, J = 8.0 Hz, 1 H), 7.66 (d, J = 8.8, 1 H), 7.25–7.11 (m, 10 H), 7.02 (d, J = 8.8 Hz, 1 H), 4.75 (t, J = 5.2 Hz, 1 H), 4.19–4.13 (m, 2 H), 3.92 (d, J = 5.6 Hz, 1 H), 3.26–3.31 (m, 1 H), 2.92–2.58 (m, 4 H), 1.66–1.64 (m, 1 H), 1.44–1.40 (m, 1 H), 1.29 (s, 9 H), 1.07–1.00 (m, 1 H), 0.80–0.77 (m, 6 H).

^{13}C NMR (100 MHz, DMSO- d_6): δ = 171.1, 170.3, 155.2, 138.9, 138.2, 129.1, 128.9, 128.0, 127.9, 126.0, 125.7, 78.0, 62.3, 56.7, 55.7, 52.1, 36.9, 36.2, 28.0, 24.0, 15.1, 10.9.

MS (EI): m/z (%) = 512.59 (100) [M + 1].

tert-Butyl ((S)-1-(((2R,3S)-1-(((S)-1-Hydroxy-3-phenylpropan-2-yl)amino)-3-methyl-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (14A)

Yield: 0.1 g (4%); mp 233–236 °C; $[\alpha]_D^{25}$ –28.3 (c 0.25, DMSO).

FT-IR (KBr): 3336, 3289, 3078, 2971, 2869, 1694, 1648, 1531, 1448, 1375, 1293, 1245, 1167, 1036, 754, 701 cm^{-1} .

^1H NMR (400 MHz, DMSO- d_6): δ = 7.80 (d, J = 8.0 Hz, 1 H), 7.70 (d, J = 9.6 Hz, 1 H), 7.26–7.15 (m, 10 H), 7.03 (d, J = 8.4 Hz, 1 H), 4.82 (t, J = 5.2 Hz, 1 H), 4.27–4.24 (m, 2 H), 3.96–3.95 (m, 1 H), 3.41–3.40 (m, 1 H), 2.89–2.86 (m, 2 H), 2.72–2.71 (m, 1 H), 2.60–2.58 (m, 1 H), 1.56–1.51 (m, 1 H), 1.29 (s, 9 H), 1.00–0.94 (m, 1 H), 0.77–0.76 (m, 1 H), 0.71 (d, J = 6.8 Hz, 3 H), 0.47 (d, J = 7.2 Hz, 3 H).

^{13}C NMR (100 MHz, DMSO- d_6): δ = 171.4, 170.3, 155.2, 139.1, 137.9, 129.1, 129.0, 127.9, 126.1, 125.7, 78.0, 63.0, 55.9, 55.1, 52.6, 37.5, 37.2, 36.7, 28.1, 25.5, 13.9, 11.5.

MS (EI): m/z (%) = 512.59 (100) [M + 1].

(2S,3S)-2-((S)-2-Amino-3-phenylpropanamido)-N-((S)-1-hydroxy-3-phenylpropan-2-yl)-3-methylpentanamide (15)

At 0 °C, TFA (0.223 g, 1.956 mmol) was added to a stirred solution of *tert*-butyl ((S)-1-((2S,3S)-1-(((S)-1-hydroxy-3-phenylpropan-2-yl)amino)-3-methyl-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl (14) (0.5 g, 0.978 mmol) in dichloromethane and the solution was stirred for 6 h at room temperature. The reaction mixture was concentrated under reduced pressure to get a semi-solid residue, which was put into sat. NaHCO_3 (50 mL) and agitated at room temperature for 30 minutes. The solid was filtered and dried in vacuum to yield compound 15.

Yield: 0.3 g (75%); off-white solid; mp 130–133 °C; $[\alpha]_D^{25}$ –34.6599 (c 0.75, CHCl_3).

FT-IR (KBr): 3476, 3291, 2966, 1951, 1675, 1639, 1547, 1448, 1378, 1202, 1135, 1038, 833, 748, 697, 681 cm^{-1} .

^1H NMR (400 MHz, DMSO- d_6): δ = 8.11 (d, J = 8.4 Hz, 1 H), 7.89 (d, J = 8.4 Hz, 1 H), 7.25–7.13 (m, 10 H), 4.80–4.75 (m, 1 H), 4.17 (t, J = 5.2 Hz, 1 H), 3.93 (d, J = 5.2 Hz, 1 H), 3.64–3.60 (m, 1 H), 3.29–3.26 (m, 1 H), 2.97–2.50 (m, 4 H), 1.63 (d, J = 6.0 Hz, 1 H), 1.37–1.35 (m, 1 H), 1.01–0.93 (m, 1 H), 0.81–0.76 (m, 6 H).

^{13}C NMR (100 MHz, DMSO- d_6): δ = 170.2, 139.0, 137.3, 129.3, 129.0, 128.2, 128.0, 126.3, 125.8, 62.4, 56.6, 54.96, 52.3, 37.1, 36.3, 24.1, 15.1, 11.0.

MS (EI): m/z (%) = 412.52 (100) [M + 1].

(2R,3S)-2-((S)-2-Amino-3-phenylpropanamido)-N-((S)-1-Hydroxy-3-phenylpropan-2-yl)-3-methylpentanamide (15A)

Compound 15A was made by following the procedure for the preparation of compound 15.

mp 163–166 °C; $[\alpha]_D^{25}$ +6.8514 (c 0.75, CHCl_3).

FT-IR (KBr): 3590, 3284, 2957, 2922, 1643, 1544, 1382, 1320, 1239, 1080, 1039, 754, 701 cm^{-1} .

^1H NMR (400 MHz, DMSO- d_6): δ = 7.86–7.80 (m, 2 H), 7.25–7.15 (m, 10 H), 4.83–4.80 (m, 1 H), 4.25–4.23 (m, 1 H), 3.94–3.93 (m, 1 H), 3.48–3.47 (m, 1 H), 3.38–3.37 (m, 1 H), 2.94–2.71 (m, 4 H), 2.60–2.57 (m, 2 H), 1.64–1.61 (m, 2 H), 1.49–1.46 (m, 1 H), 1.01–0.99 (m, 1 H), 0.78–0.76 (m, 6 H).

^{13}C NMR (400 MHz, DMSO- d_6): δ = 173.9, 170.5, 139.1, 138.8, 129.2, 129.0, 128.0, 127.9, 125.9, 125.7, 63.1, 55.9, 55.2, 52.5, 41.1, 38.8, 37.5, 36.6, 25.5, 13.9, 11.7, 11.5.

MS (EI): m/z (%) = 412.52 (100) [M + 1].

(2S,3S)-2-((S)-2-Butyramido-3-phenylpropanamido)-N-((S)-1-hydroxy-3-phenylpropan-2-yl)-3-methylpentanamide (Acidiphilamide A)

To a stirred solution of (2S,3S)-2-((S)-2-amino-3-phenylpropanamido)-N-((S)-1-hydroxy-3-phenyl propan-2-yl)-3-methylpentanamide (15) (0.13 g, 0.316 mmol) in *N,N*-dimethylformamide (10 mL) was added DIPEA (0.061 g, 0.474 mmol) and HATU (0.15 g, 0.410 mmol) at 0 °C and the reaction mixture was stirred at 0 °C for 10 min. Butyric acid (0.031 g, 0.347 mmol) was then added, and the reaction mixture was agitated for 1 h at room temperature. The reaction mixture was then put into crushed ice (25 mL) and extracted with EtOAc (2 × 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to yield the crude product, which was purified over 60–120 mesh silica gel mesh column chromatography, and the compound was eluted with EtOAc/Pet ether (30%) to get acidiphilamide A.

Yield: 114 mg (75%); off-white solid; mp 222–225 °C; $[\alpha]_D^{25}$ –33.40 (c 0.2, MeOH).

IR (KBr): 3285, 3074, 2962, 2868, 2737, 1638, 1542, 1448, 1381, 1279, 1219, 1034, 901, 696, 568, 496 cm^{-1} .

^1H NMR (500 MHz, DMSO- d_6): δ = 8.03 (d, J = 8.5 Hz, 1 H), 7.75 (q, J = 9.0 Hz, 2 H), 7.24–7.12 (m, 10 H), 4.75 (t, J = 5.3 Hz, 1 H), 4.57–4.51 (m, 1 H), 4.12 (t, J = 8.0 Hz, 1 H), 3.93–3.89 (m, 1 H), 3.27–3.21 (m, 1 H), 2.93 (dd, J = 4.3 Hz, J = 14.0 Hz, 1 H), 2.87 (dd, J = 5.3 Hz, J = 13.9 Hz, 1 H), 2.71 (dd, J = 10.5 Hz, J = 13.9 Hz, 1 H), 2.61 (dd, J = 8.4 Hz, J = 13.9 Hz, 1 H), 1.99 (t, J = 7.0 Hz, 2 H), 1.64–1.59 (m, 1 H), 1.39–1.34 (m, 3 H), 1.01–0.99 (m, 1 H), 0.88–0.78 (m, 3 H), 0.76 (d, J = 7.0 Hz, 3 H), 0.69 (t, J = 7.5 Hz, 3 H).

^{13}C NMR (125 MHz, DMSO- d_6): δ = 172.4, 171.5, 170.7, 139.4, 138.5, 129.59, 129.5, 128.5, 128.4, 126.5, 126.3, 62.8, 57.4, 54.0, 52.5, 37.6, 37.5, 37.3, 36.8, 24.6, 19.0, 15.6, 13.8.

HRMS (ESI): m/z [M + H]⁺ calcd for $\text{C}_{28}\text{H}_{40}\text{N}_3\text{O}_4$: 482.3019; found: 482.3101.

(2R,3S)-2-((S)-2-Butyramido-3-phenylpropanamido)-N-((S)-1-hydroxy-3-phenylpropan-2-yl)-3-methylpentanamide (epi-Acidiphilamide A)

To a stirred solution of (2R,3S)-2-((S)-2-amino-3-phenylpropanamido)-N-((S)-1-hydroxy-3-phenyl propan-2-yl)-3-methylpentanamide (15A) (0.15 g, 0.364 mmol) in *N,N*-dimethylformamide (10 mL) was added DIPEA (0.070 g, 0.546 mmol) and HATU (0.17 g, 0.473 mmol) at 0 °C and the reaction mixture was stirred at 0 °C for 10 min. After adding butyric acid (0.035 g, 0.400 mmol) the reaction mixture was then agitated at room temperature for 1 h. Ice-cold water (25 mL) was added to the reaction mixture, which was then extracted with EtOAc (2 × 50 mL). The organic layers were mixed, and the organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to yield crude product, which was purified over 60–120 mesh silica gel column chromatography and eluted with EtOAc/pet ether (30%) to obtain the pure *epi*-acidiphilamide A.

Yield: 130 mg (72%); off-white solid; mp 225–228 °C; $[\alpha]_D^{25} +42.86$ (c 0.2, MeOH).

IR (KBr): 3282, 3064, 2965, 2869, 1639, 1543, 1374, 1039, 755, 698 cm^{-1} .

$^1\text{H NMR}$ (400 MHz, $\text{DMSO-}d_6$): δ = 8.12 (d, J = 8.0 Hz, 1 H), 7.85 (d, J = 9.2 Hz, 2 H), 7.77 (d, J = 8.4 Hz, 1 H), 7.27–7.13 (m, 10 H), 4.79 (t, J = 5.6 Hz, 1 H), 4.66–4.59 (m, 1 H), 4.24 (dd, J = 4.9 Hz, J = 9.5 Hz, 1 H), 3.95–3.89 (m, 1 H), 3.41–3.32 (m, 3 H), 2.92 (dd, J = 6.6 Hz, J = 13.5 Hz, 1 H), 2.85 (dd, J = 5.8 Hz, J = 13.5 Hz, 1 H), 2.78 (dd, J = 9.2 Hz, J = 13.5 Hz, 1 H), 2.60 (dd, J = 9.5 Hz, J = 13.5 Hz, 1 H), 2.02 (t, J = 7.9 Hz, 2 H), 1.60–1.55 (m, 1 H), 1.42 (q, J = 7.2 Hz, 2 H), 0.88–0.80 (m, 1 H), 0.74 (t, J = 7.5 Hz, 3 H), 0.68 (d, J = 6.6 Hz, 1 H), 0.48 (d, J = 6.8 Hz, 3 H).

$^{13}\text{C NMR}$ (100 MHz, $\text{DMSO-}d_6$): δ = 172.1, 171.3, 170.4, 139.2, 137.6, 129.1, 129.0, 127.9, 127.8, 126.1, 125.7, 63.1, 55.1, 54.1, 52.6, 37.7, 36.9, 36.6, 36.5, 25.5, 18.4, 14.0.

HRMS (ESI): m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{28}\text{H}_{40}\text{N}_3\text{O}_4$: 482.3019; found: 482.2951.

(2S,3S)-N-((S)-1-Hydroxy-3-phenylpropan-2-yl)-3-methyl-2-((S)-2-(3-methylbutanamido)-3-phenylpropanamido)pentanamide (Acidiphilamide C)

To a stirred solution of (2S,3S)-2-((S)-2-amino-3-phenylpropanamido)-N-((S)-1-hydroxy-3-phenylpropan-2-yl)-3-methylpentanamide (**15**) (0.13 g, 0.316 mmol) in *N,N*-dimethylformamide (10 mL) was added DIPEA (0.061 g, 0.474 mmol) and HATU (0.15 g, 0.410 mmol) at 0 °C and the reaction mixture was stirred at 0 °C for 10 min. Isovaleric acid (0.031 g, 0.347 mmol) was added and the reaction mixture was stirred for 1 h at RT. The reaction mixture was poured into cold water (25 mL) and the aqueous layer was extracted with EtOAc (2 × 50 mL). The combined organic layers were dried over sodium sulfate and evaporated under vacuum to give the crude compound, which was purified by flash column chromatography by using 60–120 mesh silica gel and the compound was eluted with EtOAc/Pet ether (30%) to afford the pure acidiphilamide C.

Yield: 125 mg (80%); off-white solid; mp 230–233 °C; $[\alpha]_D^{25} -14.55$ (c 0.5, MeOH);

IR (KBr): 3282, 3072, 2954, 2861, 2747, 1638, 1545, 1452, 1375, 1219, 1044, 905, 697.

$^1\text{H NMR}$ (400 MHz, $\text{DMSO-}d_6$): δ = 8.03 (d, J = 8.8 Hz, 1 H), 7.75 (dd, J = 2.4 Hz, J = 3.2 Hz, 2 H), 7.25–7.12 (m, 10 H), 4.76 (t, J = 5.6 Hz, 1 H), 4.56–4.55 (m, 1 H), 4.13 (t, J = 8.0 Hz, 1 H), 3.93–3.91 (m, 1 H), 3.31–3.20 (m, 2 H), 2.95–2.84 (dd, J = 4.0 Hz, J = 13.8 Hz, 1 H), 2.87 (dd, J = 5.4 Hz, J = 13.9 Hz, 1 H), 2.70 (dd, J = 10.6 Hz, J = 13.9 Hz, 1 H), 2.61 (dd, J = 8.3 Hz, J = 13.8 Hz, 1 H), 1.89–1.81 (m, 3 H), 1.64 (d, J = 6.0 Hz, 1 H), 1.40–1.32 (m, 1 H), 1.05–0.95 (m, 1 H), 0.79 (t, J = 7.6 Hz, 3 H), 0.74 (d, J = 6.8 Hz, 3 H), 0.73 (d, J = 6.8 Hz, 3 H), 0.67 (d, J = 6.4 Hz, 3 H).

$^{13}\text{C NMR}$ (100 MHz, $\text{DMSO-}d_6$): δ = 171.4, 171.1, 170.2, 138.9, 138.0, 129.0, 129.0, 128.0, 127.9, 126.0, 125.8, 62.3, 56.9, 53.5, 52.1, 44.4, 37.1, 36.8, 36.3, 25.4, 24.1, 22.1, 22.0, 15.1, 11.0.

HRMS (ESI): m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{29}\text{H}_{42}\text{N}_3\text{O}_4$: 496.3175; found: 496.3099.

(2R,3S)-N-((S)-1-Hydroxy-3-phenylpropan-2-yl)-3-methyl-2-((S)-2-(3-methylbutanamido)-3-phenylpropanamido)pentanamide (epi-Acidiphilamide C)

To a stirred solution of (2R,3S)-2-((S)-2-amino-3-phenylpropanamido)-N-((S)-1-hydroxy-3-phenylpropan-2-yl)-3-methylpentanamide (**15A**) (0.15 g, 0.364 mmol) in *N,N*-dimethylformamide (10 mL) was added DIPEA (0.070 g, 0.546 mmol) and HATU (0.17 g, 0.473 mmol) at

0 °C and the reaction mixture was stirred at 0 °C for 10 min. Isovaleric acid (0.04 g, 0.4 mmol) was added and the reaction mixture was stirred at RT for 1 h. The reaction was partitioned between EtOAc and water then separated and the organic layer was taken, dried using Na_2SO_4 and concentrated under reduced pressure to give the crude compound, which was purified over 60–120 mesh silica gel column chromatography (eluted with EtOAc/Pet ether, 30%) to afford the pure *epi*-acidiphilamide C.

Yield: 140 mg (77%); off-white solid; mp 238–241 °C; $[\alpha]_D^{25} +37.05$ (c 0.55, MeOH).

IR (KBr): 3750, 3685, 3281, 3059, 2958, 2958, 2872, 1638, 1544, 1443, 1375, 1230, 1037, 911, 759, 694, 596, 498 cm^{-1} .

$^1\text{H NMR}$ (400 MHz, $\text{DMSO-}d_6$): δ = 8.12 (d, J = 7.6 Hz, 1 H), 7.84 (dd, J = 9.2 Hz, J = 8.8 Hz, 1 H), 7.78 (d, J = 8.4 Hz, 1 H), 7.27–7.13 (m, 10 H), 4.79 (t, J = 5.2 Hz, 1 H), 4.65–4.61 (m, 1 H), 4.24 (dd, J = 4.8 Hz, J = 9.0 Hz, 1 H), 3.94 (d, J = 4.3 Hz, 1 H), 3.40 (dd, J = 5.3 Hz, 10.3, 1 H), 2.92 (dd, J = 5.6 Hz, J = 13.4 Hz, 1 H), 2.86 (dd, J = 4.3 Hz, J = 13.8 Hz, 1 H), 2.77 (d, J = 9.5 Hz, 1 H), 2.73 (d, J = 9.8 Hz, 1 H), 2.79–2.72 (m, 1 H), 2.58 (dd, J = 9.3 Hz, J = 13.3 Hz, 1 H), 1.91–1.86 (m, 3 H), 1.58–1.55 (m, 1 H), 0.86–0.81 (m, 1 H), 0.76 (d, J = 5.8 Hz, 3 H), 0.72–0.66 (m, 6 H), 0.48 (d, J = 6.8 Hz, 3 H).

$^{13}\text{C NMR}$ (100 MHz, $\text{DMSO-}d_6$): δ = 171.6, 171.3, 170.4, 139.2, 137.6, 129.1, 129.0, 127.94, 127.90, 126.1, 125.7, 63.1, 55.1, 54.1, 52.6, 44.3, 10.1, 39.9, 39.7, 39.4, 39.2, 39.0, 38.8, 37.7, 36.7, 36.5, 25.5, 25.4, 22.2, 22.1, 14.0.

HRMS (ESI): m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{29}\text{H}_{42}\text{N}_3\text{O}_4$: 496.3175; found: 496.3095.

Methyl (tert-Butoxycarbonyl)-L-phenylalanyl-L-valinate (17)

DIPEA (1.46 g, 11.32 mmol) and HATU (3.72 g, 9.811 mmol) were added at 0 °C to a stirred solution of (tert-butoxycarbonyl)-L-phenylalanine (**9**) (2 g, 7.547 mmol) in *N,N*-dimethylformamide (20 mL) and the reaction mixture was agitated for 10 minutes at 0 °C. Methyl L-valinate (**16**) (1.08 g, 8.301 mmol) was added and the resultant mixture was stirred for 1 h at ambient temperature. To precipitate the solid, the reaction mixture was placed into 50 mL of crushed ice. The product was filtered, washed with water, and vacuum dried to yield compound **17**.

Yield: 2.3 g (80%); off-white solid; mp 114–117 °C; $[\alpha]_D^{25} -0.112$ (c 0.25, MeOH).

FT-IR (KBr): 3313, 3071, 2971, 1748, 1684, 1655, 1542, 1444, 1392, 1268, 1175, 1020, 859, 763, 648, 504.

$^1\text{H NMR}$ (400 MHz, $\text{DMSO-}d_6$): δ = 8.09 (d, J = 8.0 Hz, 1 H), 7.27–7.18 (m, 5 H), 6.94 (d, J = 8.8 Hz, 1 H), 4.29–4.20 (m, 2 H), 3.63 (s, 3 H), 2.96 (dd, J = 4.0 Hz, J = 4.0 Hz, 1 H), 2.76–2.69 (m, 1 H), 2.09–2.02 (m, 1 H), 1.29 (s, 9 H), 0.91–0.87 (m, 6 H).

$^{13}\text{C NMR}$ (100 MHz, $\text{DMSO-}d_6$): δ = 172.0, 171.8, 155.2, 138.0, 129.1, 127.9, 126.1, 77.9, 57.2, 55.4, 51.6, 37.1, 30.0, 28.0, 18.8, 18.1.

MS (EI): m/z (%) = 379.46 (100) $[\text{M} + 1]$.

(tert-Butoxycarbonyl)-L-phenylalanyl-L-valine (18)

$\text{LiOH}\cdot\text{H}_2\text{O}$ (110 mg, 5.554 mmol) was added to a stirred solution of methyl (tert-butoxycarbonyl)-L-phenylalanyl-L-valinate (**17**) (1.4 g, 3.703 mmol) in THF/ H_2O (1:1; 30 mL) at 0 °C and the reaction mixture was agitated at 25 °C for 2 h. The solvents were evaporated, leaving a residue that was acidified with sat. citric acid solution to adjust to pH 6 and extracted with EtOAc (2 × 50 mL). The organic layers were combined, dried over Na_2SO_4 , and concentrated under reduced pressure to yield the pure compound **18**.

Yield: 1.1 g (82%); off-white solid; mp 76–79 °C; $[\alpha]_D^{25}$ –0.0806 (c 0.25, MeOH).

FT-IR (KBr): 3650, 3414, 3331, 2971, 2930, 2878, 1724, 1533, 1373, 1266, 1158, 1050, 1024, 856, 745, 703 cm^{-1} .

^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ = 12.56 (s, 1 H), 7.87 (d, J = 8.8 Hz, 1 H), 7.27–7.18 (m, 5 H), 6.97 (d, J = 8.4 Hz, 1 H), 4.23–4.16 (m, 2 H), 2.98–2.70 (m, 2 H), 2.09–2.05 (m, 1 H), 1.29 (s, 9 H), 0.89 (d, J = 4.4 Hz, 6 H).

^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ = 172.8, 171.8, 155.2, 138.1, 129.1, 127.9, 126.1, 78.0, 57.0, 55.6, 37.1, 30.1, 28.0, 19.0, 17.8.

MS (EI): m/z (%) = 365.43 (100) [M–1].

***tert*-Butyl ((*S*)-1-(((*S*)-1-(((*S*)-1-Hydroxy-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (**19** and **19A**)**

DIPEA (1.16 g, 9.064 mmol) and HATU (2.98 g, 7.855 mmol) were added to a stirring solution of *tert*-butoxycarbonyl-L-phenylalanyl-L-valine (**18**) (2.2 g, 6.043 mmol) in *N,N*-dimethylformamide (22 mL) at 0 °C and the reaction mixture was stirred for 10 minutes. (*S*)-2-Amino-3-phenylpropan-1-ol (1.0 g, 6.647 mmol) was added and the reaction mixture was stirred at r.t. for 1 h. Crushed ice (50 mL) was added to the reaction mixture and the precipitated solid was filtered, washed with water, and dried under vacuum to yield **19** and **19A** as a mixture of diastereomers in 96:4 ratio (by LCMS Analysis). Prep HPLC purification yielded compound **19** (1.8 g, 61% isolated yield) and **19A** (0.2 g, 4% isolated yield) as an off-white solid.

***tert*-Butyl ((*S*)-1-(((*S*)-1-(((*S*)-1-Hydroxy-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (**19**)**

Yield: 1.8 g (61%); mp 159–162 °C; $[\alpha]_D^{25}$ +1.2897 (c 0.25, MeOH).

FT-IR (KBr): 3285, 2967, 2871, 1697, 1643, 1549, 1549, 1526, 1387, 1248, 1169, 1038, 746, 697.

^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ = 7.84 (d, J = 8.0 Hz, 1 H), 7.64 (d, J = 8.8 Hz, 1 H), 7.25–7.11 (m, 10 H), 6.55 (d, J = 7.6 Hz, 1 H), 4.75 (t, J = 5.2 Hz, 1 H), 4.19–4.11 (m, 2 H), 3.92–3.91 (m, 1 H), 3.27–3.16 (m, 2 H), 2.94–2.58 (m, 4 H), 1.91–1.86 (m, 1 H), 1.29 (s, 9 H), 0.82–0.79 (m, 6 H).

^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ = 171.2, 170.2, 155.2, 138.9, 138.2, 129.1, 128.9, 128.0, 127.9, 126.0, 125.8, 78.0, 62.3, 57.4, 55.8, 52.1, 37.1, 36.2, 31.0, 28.0, 19.1, 17.9.

MS (EI): m/z (%) = 498.60 (100) [M + 1].

***tert*-Butyl ((*S*)-1-(((*R*)-1-(((*S*)-1-Hydroxy-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (**19A**)**

Yield: 0.2 g (4%); mp 241–244 °C; $[\alpha]_D^{25}$ –0.7577 (c 0.25, DMSO).

FT-IR (KBr): 3326, 3290, 3069, 2966, 1691, 1649, 1533, 1369, 1298, 1171, 1041, 753, 698.

^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ = 7.86 (d, J = 8.4 Hz, 1 H), 7.73 (d, J = 8.8 Hz, 1 H), 7.31–7.13 (m, 10 H), 7.01 (d, J = 8.4 Hz, 1 H), 4.79–4.77 (m, 1 H), 4.26–4.12 (m, 2 H), 3.95–3.94 (m, 1 H), 3.42–3.27 (m, 2 H), 2.95–2.50 (m, 4 H), 1.78–1.74 (m, 1 H), 1.29 (s, 9 H), 0.57–0.51 (m, 6 H).

^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ = 17.4, 170.1, 155.2, 139.1, 129.2, 129.0, 128.0, 127.9, 126.1, 125.8, 78.0, 62.9, 57.0, 55.9, 52.6, 37.6, 36.6, 30.8, 28.1, 27.8, 19.0, 17.3.

MS (EI): m/z (%) = 498.6 (100) [M + 1].

(*S*)-2-(((*S*)-2-Amino-3-phenylpropanamido)-*N*-((*S*)-1-hydroxy-3-phenylpropan-2-yl)-3-methylbutanamide (20**)**

TFA (0.229 g, 2.012 mmol) was added to a cold solution of *tert*-butyl ((*S*)-1-(((*S*)-1-(((*S*)-1-hydroxy-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (**19**) (0.5 g, 1.006 mmol) in dichloromethane (10 mL) at 0 °C and the reaction mixture was agitated at room temperature for 6 hours. The volatiles were removed under reduced pressure to yield a residue, which was put into sat. NaHCO_3 (50 mL) and agitated at room temperature for 30 min to create a white precipitate. The solid was filtered, washed with water, and vacuum dried to provide the compound **20**.

Yield: 0.3 g (75%); off-white solid.

FT-IR (KBr): 3286, 2956, 2931, 1702, 1652, 1549, 1364, 1204, 1134, 1043, 838, 751, 699 cm^{-1} .

^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ = 8.45 (d, J = 8.8 Hz, 1 H), 7.97 (d, J = 8.0 Hz, 1 H), 7.84–7.77 (m, 2 H), 7.22–7.14 (m, 10 H), 4.78 (t, J = 5.2 Hz, 1 H), 4.19 (t, J = 8.0 Hz, 1 H), 4.07–3.9 (m, 1 H), 3.96–3.94 (m, 1 H), 3.39–2.60 (m, 6 H), 1.92 (q, J = 6.8 Hz, 1 H), 0.86 (d, J = 6.4 Hz, 6 H).

^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ = 169.7, 169.4, 139.0, 135.9, 129.4, 129.0, 128.3, 127.9, 126.8, 125.8, 63.0, 57.2, 53.9, 52.5, 36.6, 31.0, 18.9.

MS (EI): m/z (%) = 398.1 (100) [M + 1].

(*R*)-2-(((*S*)-2-Amino-3-phenylpropanamido)-*N*-((*S*)-1-hydroxy-3-phenylpropan-2-yl)-3-methylbutanamide (20A**)**

Synthesized by following the general procedure for preparing **20** to yield the title product.

Yield: 0.32 g (80%); off-white solid; mp 177–180 °C; $[\alpha]_D^{25}$ +84.9088 (c 0.75, CHCl_3).

FT-IR (KBr): 3286, 2956, 2931, 1702, 1652, 1549, 1364, 1204, 1134, 1043, 838, 751, 699.

^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ = 8.29 (d, J = 8.4 Hz, 1 H), 7.99 (d, J = 8.4 Hz, 1 H), 7.35–7.16 (m, 10 H), 7.14 (d, J = 5.0 Hz, 1 H), 4.19 (t, J = 6.4 Hz, 1 H), 4.16–3.95 (m, 2 H), 3.41–3.26 (m, 2 H), 3.03–2.50 (m, 4 H), 1.68–1.65 (m, 1 H), 0.50 (dd, J = 6.4 Hz, J = 6.8 Hz, 6 H).

^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ = 169.7, 169.4, 139.0, 135.9, 129.4, 129.0, 128.3, 127.9, 126.8, 125.8, 63.0, 57.2, 53.9, 52.5, 36.6, 31.0, 18.9.

MS (EI): m/z (%) = 398.49 (100) [M + 1].

(*S*)-*N*-((*S*)-1-Hydroxy-3-phenylpropan-2-yl)-3-methyl-2-(((*S*)-2-(3-methylbutanamido)-3-phenylpropanamido)butanamide (Acid-iphilamide B)

To a stirred solution of (*S*)-2-(((*S*)-2-amino-3-phenyl propanamido)-*N*-((*S*)-1-hydroxy-3-phenylpropan-2-yl)-3-methylbutanamide (**20**) (200 mg, 0.503 mmol) in *N,N*-dimethylformamide (5 mL) was added DIPEA (0.097 g, 0.754 mmol) and HATU (0.248 g, 0.653 mmol) at 0 °C and the reaction mixture was stirred at 0 °C for 10 min. Isovaleric acid (0.056 g, 0.553 mmol) was added and the reaction mixture was stirred at RT for 1 h. The reaction mixture was poured into crushed ice (50 mL) and stirred at RT for 30 min to form a white precipitate. The white precipitate was filtered and dried to give acidiphilamide B.

Yield: 0.15 g (62%); off-white solid; mp 226–229 °C; $[\alpha]_D^{25}$ –38.176 (c 0.25, MeOH).

FT-IR (KBr): 3287, 2959, 1636, 1549, 1222, 1040, 693, 572 cm^{-1} .

¹H NMR (400 MHz, DMSO-*d*₆): δ = 8.08 (d, *J* = 8.4 Hz, 1 H), 7.86 (t, *J* = 6.8 Hz, 2 H), 7.24–7.14 (m, 10 H), 4.80–4.78 (m, 1 H), 4.58 (t, *J* = 6.8 Hz, 1 H), 4.10 (t, *J* = 8.0 Hz, 1 H), 3.92–3.90 (m, 1 H), 3.27–3.25 (m, 2 H), 2.97 (dd, *J* = 3.8 Hz, *J* = 14.2 Hz, 1 H), 2.89 (dd, *J* = 5.8 Hz, *J* = 14.2 Hz, 1 H), 2.74 (dd, *J* = 10.4 Hz, *J* = 13.6 Hz, 1 H), 2.64 (dd, *J* = 7.8 Hz, *J* = 13.5 Hz, 1 H), 1.88–1.83 (m, 4 H), 0.78 (d, *J* = 6.4 Hz, 3 H), 0.74 (d, *J* = 6.4 Hz, 3 H), 0.67 (d, *J* = 6.4 Hz, 6 H).

¹³C NMR (100 MHz, DMSO-*d*₆): δ = 171.4, 171.1, 170.2, 139.0, 138.1, 129.09, 129.02, 128.0, 127.8, 126.0, 125.8, 62.3, 57.8, 53.7, 52.2, 44.4, 37.1, 36.3, 30.8, 25.4, 22.1, 22.0, 19.0, 18.0.

HRMS (ESI): *m/z* [M + H]⁺ calcd for C₂₈H₄₀N₃O₄: 482.3019; found: 482.3050.

(R)-N-((S)-1-Hydroxy-3-phenylpropan-2-yl)-3-methyl-2-((S)-2-(3-methylbutanamido)-3-phenylpropanamido)butanamide (epi-Acidiphilamide B)

epi-Acidiphilamide B was prepared by following the general procedure for synthesizing compound acidiphilamide B from compound **20A** (200 mg, 0.503 mmol) to yield epi-acidiphilamide B.

Yield: 0.16 g (66%); off-white solid; mp 245–248 °C; [α]_D²⁵ +28.6146 (c 0.25, MeOH).

FT-IR (KBr): 3278, 2957, 1641, 1541, 1374, 1243, 1042, 750, 700.

¹H NMR (400 MHz, DMSO-*d*₆): δ = 8.08 (d, *J* = 8.0 Hz, 1 H), 7.89 (d, *J* = 8.8 Hz, 1 H), 7.84 (d, *J* = 8.4 Hz, 1 H), 7.27–7.12 (m, 10 H), 4.80 (t, *J* = 5.6 Hz, 1 H), 4.67–4.57 (m, 1 H), 4.13 (dd, *J* = 6.5 Hz, *J* = 9.6 Hz, 1 H), 3.96–3.89 (m, 1 H), 3.42–3.27 (m, 2 H), 2.97 (dd, *J* = 5.5 Hz, *J* = 13.8 Hz, 1 H), 2.89 (dd, *J* = 5.0 Hz, *J* = 13.8 Hz, 1 H), 2.76 (dd, *J* = 10.0 Hz, *J* = 13.5 Hz, 1 H), 2.61 (dd, *J* = 9.6 Hz, *J* = 13.7 Hz, 1 H), 1.90–1.78 (m, 4 H), 0.75 (d, *J* = 6.4 Hz, 3 H), 0.68 (d, *J* = 6.4 Hz, 3 H), 0.53 (d, *J* = 6.7 Hz, 6 H).

¹³C NMR (100 MHz, DMSO-*d*₆): δ = 171.5, 171.2, 170.1, 139.1, 137.7, 129.1, 129.0, 127.9, 127.8, 126.1, 125.7, 63.0, 57.1, 54.0, 52.6, 44.4, 37.8, 36.5, 30.4, 25.4, 22.2, 22.0, 19.0, 17.2.

HRMS (ESI): *m/z* [M + H]⁺ calcd for C₂₈H₄₀N₃O₄: 482.3019; found: 482.3088.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgment

Poosa Mallesham is grateful to Aragen Life Sciences for allowing the Ph.D. work in accordance with the company's Higher Education Policy. The analytical department assistance is greatly appreciated. We thank Dr Somesh Sharma for his continuous support and encouragement.

Supporting Information

Supporting information for this article is available online at <https://doi.org/10.1055/a-2035-9753>.

References

- (1) Calixto, J. B. *An. Acad. Bras. Cienc.* **2019**, *91*, Suppl. 3.
- (2) Malik, A.; Kim, Y. R.; Kim, S. B. *Genes* **2020**, *11*, 1166.

- (3) Elsayed, S. S.; Genta-Jouve, G.; Carrión, V. J.; Nibbering, P. H.; Siegler, M. A.; de Boer, W.; Hankemeier, T.; van Wezel, G. P. *J. Org. Chem.* **2020**, *85*, 10648.
- (4) Galluzzi, L.; Baehrecke, E. H.; Ballabio, A.; Boya, P.; Bravo-San Pedro, J. M.; Cecconi, F.; Choi, A. M.; Chu, C. T.; Codogno, P.; Colombo, M. I.; Cuervo, A. M.; Debnath, J.; Deretic, V.; Dikic, I.; Eskelinen, E. L.; Fimia, G. M.; Fulda, S.; Gewirtz, D. A.; Green, D. R.; Hansen, M.; Harper, J. W.; Jaattela, M.; Johansen, T.; Juhasz, G.; Kimmelman, A. C.; Kraft, C.; Ktistakis, N. T.; Kumar, S.; Levine, B.; Lopez-Otin, C.; Madeo, F.; Martens, S.; Martinez, J.; Melendez, A.; Mizushima, N.; Munz, C.; Murphy, L. O.; Penninger, J. M.; Piacentini, M.; Reggiori, F.; Rubinsztein, D. C.; Ryan, K. M.; Santambrogio, L.; Scorrano, L.; Simon, A. K.; Simon, H. U.; Simonsen, A.; Tavernarakis, N.; Tooze, S. A.; Yoshimori, T.; Yuan, J.; Yue, Z.; Zhong, Q.; Kroemer, G. *EMBO J.* **2017**, *36*, 1811.
- (5) Cheng, Y.; Ren, X.; Hait, W. N.; Yang, J. M. *Pharmacol. Rev.* **2013**, *65*, 1162.
- (6) Choi, A. M.; Ryter, S. W.; Levine, B. N. *Engl. J. Med.* **2013**, *368*, 651.
- (7) Baltz, R. H. *Curr. Opin. Pharmacol.* **2008**, *8*, 557.
- (8) Bérdy, J. *J. Antibiot.* **2012**, *65*, 385.
- (9) Lazzarini, A.; Cavaletti, L.; Toppo, G.; Marinelli, F. *Antonie van Leeuwenhoek* **2000**, *78*, 399.
- (10) Hwang, S.; Yun, Y.; Choi, W. H.; Kim, S. B.; Shin, J.; Lee, M. J.; Oh, D. C. *J. Nat. Prod.* **2019**, *82*, 341.
- (11) Nakamura, S.; Yoshimori, T. *J. Cell Sci.* **2017**, *130*, 1209.
- (12) (a) Liu, J.; Cheng, X.; Tian, X.; Guan, D.; Ao, J.; Wu, Z.; Huang, W.; Le, Z. *Bioorg. Med. Chem. Lett.* **2019**, *29*, 896. (b) Yamada, K.; Matsumoto, R.; Suzuki, Y.; Mori, S.; Kitajima, S. *Bioorg. Med. Chem. Lett.* **2020**, *30*, 127000. (c) Park, S.; Park, Y.; Son, S. H.; Lee, K.; Jung, Y. W.; Lee, K. Y.; Jeon, Y. H.; Byun, Y. *Bioorg. Med. Chem. Lett.* **2017**, *27*, 4710. (d) Lsidro-Llobet, A.; Kenworthy, M. N.; Mukherjee, S.; Kopach, M. E.; Wegner, K.; Gallou, F.; Smith, A. G.; Roschangar, F. *J. Org. Chem.* **2019**, *84*, 4615. (e) Mijalis, A. J.; Thomas, D. A. III.; Simon, M. D.; Adamo, A.; Beaumont, R.; Jensen, K. F.; Pentelute, B. L. *Nat. Chem. Biol.* **2017**, *13*, 464. (f) Santos-Merino, M.; Singh, A. K.; Ducat, D. C. *Front. Bioeng. Biotechnol.* **2019**, *7*, 33. (g) Mueller, L. K.; Baumruck, A. C.; Zhdanova, H.; Tietze, A. A. *Front. Bioeng. Biotechnol.* **2020**, *8*, 162.
- (13) Santos, S.; Torcato, I.; Castanho, M. A. R. B. *Pept. Sci.* **2012**, *98*, 288.
- (14) Lin, Y.; Malins, L. R. *J. Am. Chem. Soc.* **2021**, *143*, 11811.
- (15) (a) Balakrishna, C.; Payili, N.; Yennam, S.; Devi, P. U.; Behera, M. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 4753. (b) Kandula, V.; Gudipati, R.; Chatterjee, A.; Kaliyaperumala, M.; Yennam, S.; Behera, M. *J. Chem. Sci.* **2017**, *129*, 1233. (c) Gudipati, R.; Kandula, V.; Raghavulu, K.; Basavaiah, K.; Yennam, S.; Behera, M. *ChemistrySelect* **2020**, *5*, 7093. (d) Kandula, V.; Thota, P. K.; Mallesham, P.; Raghavulu, K.; Chatterjee, A.; Yennam, S.; Behera, M. *Synlett* **2019**, *30*, 2295. (e) Balakrishna, C.; Kandula, V.; Gudipati, R.; Yennam, S.; Devi, P. U.; Behera, M. *Synlett* **2018**, *29*, 1087. (f) Sambaiah, M.; Raghavulu, K.; Kumar, K. S.; Yennam, S.; Behera, M. *New J. Chem.* **2017**, *41*, 10020. (g) Sambaiah, M.; Gudipati, R.; Kumar, K. S.; Yennam, S.; Behera, M. *Tetrahedron Lett.* **2016**, *57*, 403. (h) Raghavulu, K.; Sambaiah, M.; Gudipati, R.; Basavaiah, K.; Yennam, S.; Behera, M. *J. Saudi Chem. Soc.* **2019**, *23*, 1024.
- (16) Dayalan, S.; Gooneratne, N. D.; Bevinakoppa, S.; Schroder, H. *Bioinformatics* **2006**, *1*, 78.
- (17) Anishetty, S.; Pennathur, G.; Anishetty, R. *BMC Struct. Biol.* **2002**, *2*, 9.

- (18) (a) Ung, P.; Winkler, D. A. *J. Med. Chem.* **2011**, *54*, 1111.
(b) Reynolds, C. H.; Bembenek, S. D.; Tounge, B. A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4258. (c) Leach, A. R.; Bradshaw, J.; Green, D. V. S.; Hann, M. M.; Delany, J. J. *J. Chem. Inf. Comput. Sci.* **1999**, *39*, 1161.
- (19) Piel, I.; Steinmetz, M.; Hirano, K.; Frohlich, R.; Grimme, S.; Glorius, F. *Angew. Chem. Int. Ed.* **2011**, *50*, 4983.
- (20) Hiebl, J.; Alberts, D. P.; Banyard, A. F.; Baresch, K.; Baumgartner, H.; Bernwieser, I.; Bhatnagar, P. K.; Blanka, M.; Bodenteich, M.; Chen, T.; Esch, P. M.; Kollmann, H.; Lantos, I.; Leitner, K.; Mayrhofer, G.; Patel, R.; Rio, A.; Rovenszky, F.; Stevenson, D.; Tubman, K. D.; Undheim, K.; Weihtrager, H.; Welz, W.; Winkler, K. *J. Pept. Res.* **1999**, *54*, 54.