Organic & Biomolecular Chemistry

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

rsc.li/obc

View Article Online
DOI: 10.1039/C8OB00263K

Journal Name

ARTICLE

The First Total Synthesis of Gobichelin B: A Mixed-ligand Siderophore of *Streptomyces sp.* NRRL F-4415

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Perali Ramu Sridhar,* Bhumireddy Chinnachennaiahgari Venkatesh, Shaik Kalesha and Chalapala Sudharani

The first total synthesis of structurally diverse mixed ligand siderophore, Gobichelin B produced by Streptomyces sp. NRRL F-4415 is reported. The systematic assembly of the building blocks to synthesize gob-B 1st half and gob-B 2nd half and subsequent coupling of these two fragments followed by global deprotection using Pearlman's catalyst led to the isolation of gobichelin B in excellent yield and purity.

Introduction

Iron is a vital trace element for almost all the life processes of living systems.[1] However, its bioavailability is very low as most of the iron exists in the Fe^{+3} state in earth's crust. The solubility of Fe^{+3} is very low, ~10^{-18} M in water of biological pH, whereas >1 μM of ferric ion concentration is required for the growth of microbes.[2] This scarcity of soluble iron enforces the microbes to excrete siderophores, iron (Fe^{3+}) chelating molecules to acquire iron from the host.[3] Iron acquisition is one of the major challenges to the pathogenic bacteria during infection and post-infection.[4] Although hosts utilize effective Iron-transport systems such as iron-loaded lactoferrin, transferrin and heme proteins the bacterial siderophores have much higher affinity towards iron than the host proteins. The bacterial siderophores called "stealth siderophores" even can strip iron from host iron-binding proteins and restore the access to iron for bacteria.[5] The battle for this coveted transition metal at host and pathogen interface is a very important task for the survival during infections. Apart from this, recent studies revealed that siderophores play a major role in bacterial virulence by acting as signals leading to a robust host defence via inducing cytokine production, hypoxic responses and mitophagy (a selective process where cells degrade mitochondria). Bacterial siderophores are necessary components in biofilm formation (extracellular polysaccharide synthesis) which promotes the antibiotic resistance to the organism.[6,7] Thus, blocking of siderophore synthesis or its function is a promising alternative approach, even for multidrug-resistant, for the development of a new class of antibacterial drugs.[8] Fe^{+3}-siderophore complexes are usually taken up into bacterial cells by siderophore specific extracellular receptors.[9] Because of the strong iron binding properties of siderophores *viz.*, enterobactin, desferrioxamine B, mycobactins *etc.,* (Figure **1**), they have been using in metal chelation therapy,[10] treatment for iron poisoning and thalassemia.[11] Substantial work has been done on mycobactins to understand and develop the novel antibiotics for the world threat disease, tuberculosis.[12]Also, Miller's[13-15] and Raymond's[16-19] pioneered work in the understanding of siderophore-mediated iron transport in microbes led to the discovery of innovative antibiotics and novel iron chelators.

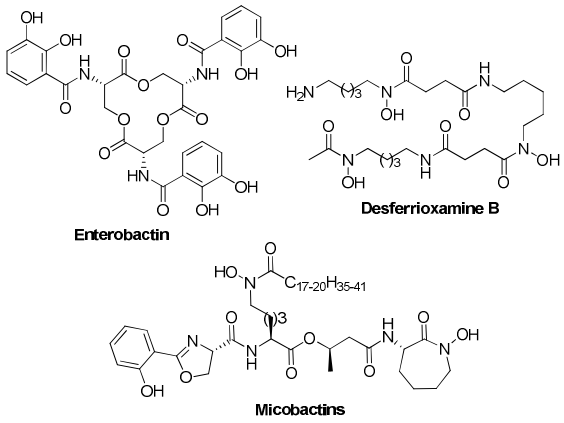


Figure 1. Structures of Enterobactin, Desferrioxamine B and Micobactins.

Typically, siderophores bind with ferric ions with three bidentate ligand groups to form a stable hexadentate, octahedral (or distorted octahedral) complexes. Though several types of siderophores are known to date, the constitutional iron binding functional groups are almost similar to hydroxamic acids, catechols, salicylate, and aryloxazoline units.[20]

Recently, *Kelleher et al.*, discovered two new mixed ligand siderophores from *Streptomyces* sp. NRRL F-4415 using proteomics-based approach "Proteomic Investigation of

School of Chemistry, University of Hyderabad, Gachibowli, Hyderabad – 500 046, INDIA
**Corresponding Author: p_ramu_sridhar@uohyd.ac.in*
*Electronic Supplementary Information (ESI) available: ^{1}H, ^{13}C, DEPT135 and HRMS. COSY and HSQC NMR for compounds **25** and gobichelin B. See*
DOI: 10.1039/x0xx00000x

View Article Online
DOI: 10.1039/C8OB00263K

Secondary Metabolism (PrISM)" and named as Gobichelin A & B (Figure **2**).[21] Distinct from most of the siderophores, gobichelins possess one hydroxamate, one salicylate / oxazoline unit and two basic amine groups (imidazole of histidine unit and a terminal amine of lysine unit) which make them interesting in iron chelation. Structurally, gobichelin A & B slightly resembles with oxachelin[22] and amychelin.[23] The structure of gobichelin A (Gob-A) has fully elucidated by spectroscopic techniques whereas the supporting data is not available for gobichelin B (Gob-B). The polar nature and the combination of three different iron chelating units involving hydroxamate, salicylate groups as well as infrequent basic amino acid residues - histidine and D-lysine, serve Gob-B as an inimitable mixed ligand iron-chelator. On the other hand, biological profile of gobichelins is also not fully established, which may be due to the distinctive minute quantities, difficult-to-reach, and often fleeting environments makes their total synthesis efficacious.

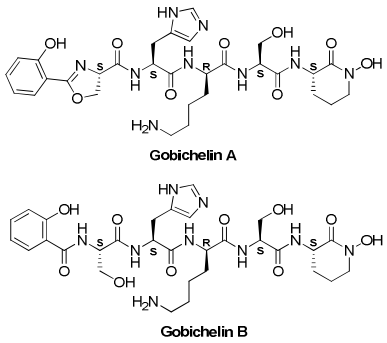
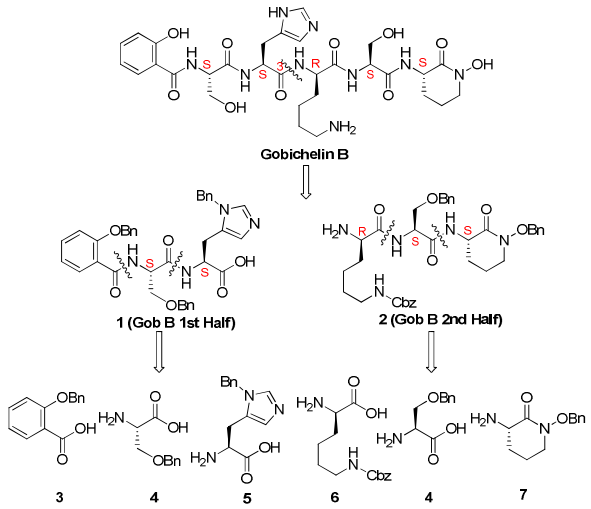


Figure 2. Structure of Gobichelin A & B.

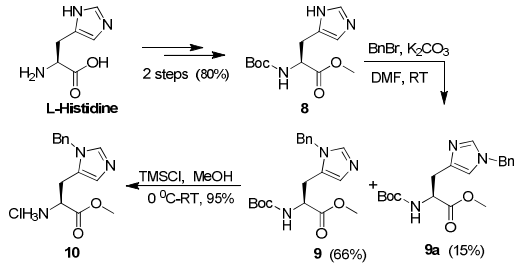
Results and Discussion

In our retro synthetic approach, the disconnection was made at 3rd peptide bond that leads to two fragments, Gob-B 1st half (**1**) and Gob-B 2nd half (**2**). Further disconnection of **1** lead to the three units, *O*-benzyl salicylic acid **3**, *O*-benzyl serine **4** and His(Bzl)-OH **5**. A similar type of disconnection of **2**also leads to *D*-Boc-Lys(Z)-OH **6**, *O*-benzyl serine **4** and cyclic ornithine derivative **7** as the required amino acid residues (**Scheme 1**).



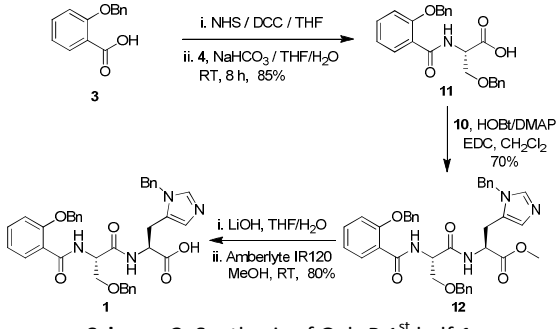
Scheme 1. Retrosynthetic approach of gobichelin B

The synthesis of Gob-B 1st half (**1**) was planned to initiate by procuring the required protected synthons. *O*-benzyl salicylic acid **3**[24] and *O*-benzyl serine **4**[25] have been synthesized by following the literature procedures. However, our initial attempts to synthesize His(Bzl)-OH **5** by following the reported method using Boc-His(Boc)-OMe under triflic anhydride conditions[26] was not successful in our hands. Therefore, an alternative protocol was designed starting from histidine. Thus, Boc-His-OMe **8** was prepared from histidine in 80% overall yield over 2 steps.[27] Ester **8** on further reaction with benzyl bromide in the presence of anhydrous K_2CO_3 in DMF afforded *N*α-boc-*N*^{1}(im)-benzyl histidine methyl ester **9** in 66% yield and 15% of the regioisomeric *N*α-boc-*N*^{3}(im)-benzyl histidine methyl ester **9a**. Subsequent treatment of **9** with TMSCl in methanol at room temperature produced His(Bzl)-OMe hydrochloride **10** in 95% yield (Scheme **2**).



Scheme 2. Synthesis of His(Bzl)-OMe hydrochloride **10**.

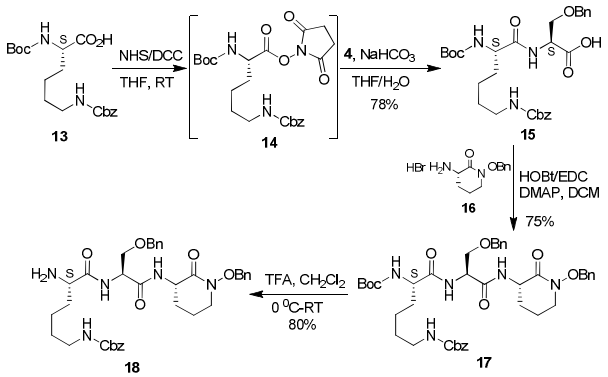
Having all the required synthons for the construction of Gob-B 1st half, we proceeded to couple the three fragments using the *N*-hydroxysuccinimide (NHS) activation protocol. Thus, activation of *O*-benzyl salicylic acid (**3**) using DCC/NHS, followed by addition of *O*-benzyl serine **4** afforded *N*-((2-benzyloxy)-benzoyl)-*O*-benzyl serine **11** in 85% yield. Acid **11** on further coupling with **10** produced Gob-B 1st half methyl ester **12** as a mixture of rotamers.[28] Subjecting **12** to LiOH mediated ester hydrolysis afforded the gob-B 1st half (**1**) (Scheme **3**).



Scheme 3. Synthesis of Gob-B 1st half **1**.

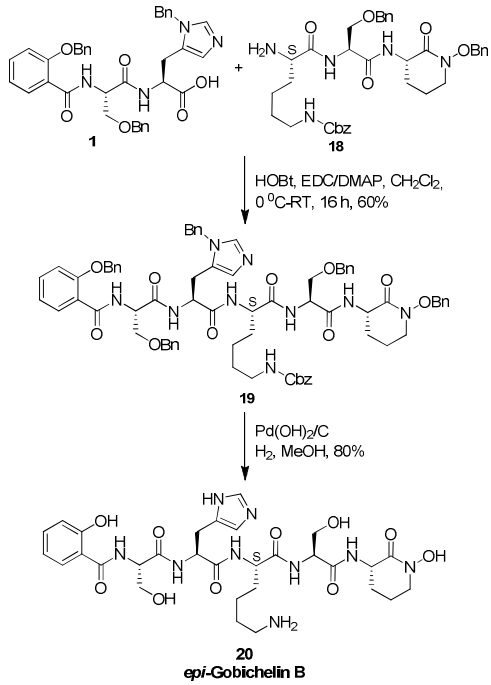
View Article Online
DOI: 10.1039/C8OB00263K

Considering the outlay of the D-lysine, initially, we decided to optimize the reaction conditions using L-lysine. Thus, *in situ* activation of L-Boc-Lys(Z)-OH (**13**) with DCC-NHS followed by addition of *O*-benzyl serine (**4**) produced lys-ser dipeptide **15** in good yield. Subsequent coupling of **15** with cyclic ornithine derivative **16**[29] afforded boc protected tripeptide **17** in 75% yield. A brief treatment of this tripeptide **17** with trifluoroacetic acid in dichloromethane resulted in Gob-B 2nd Half like peptide (epimeric at lysine) **18** (Scheme **4**).



Scheme 4. Synthesis of *epi*-Gob-B 2nd half tripeptide **18**.

Having both of the required tri-peptide units **1** and **18** in hand, we further proceeded to couple the two halves to obtain the fully protected *epi*-gobichelin B. To accomplish the coupling reaction of **1** and **18**, various coupling reagents like HOBt, EDC, DMAP, BOP, PyBOP, and DIPEA were chosen to screen in varying combinations. Finally, the combination of

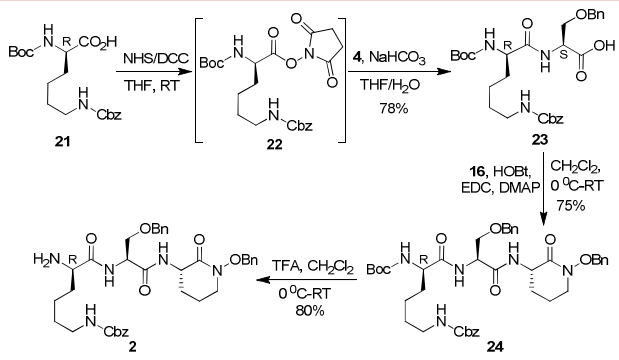


Scheme 5. Synthesis of epi-Gobichelin B (**20**).

HOBt/EDC and DMAP in dichloromethane proved to be the best conditions to achieve the pentapeptide **19** (Scheme **5**). Hence, equimolar amounts of **1** and **18** were coupled under the above-said conditions. After the disappearance of the starting materials and normal workup, the crude compound was directly purified by preparative HPLC using C18 reverse phase column eluting with 65% acetonitrile/water (isocratic) under non-acidic buffer conditions to get the protected *epi*-gobichelin B (**19**) in 60% yield. Besides the NMR spectra, in HRMS spectrum the base peak at m/z 1274.5926 corresponding to M+H of $C_{73}H_{80}N_9O_{12}$ (calculated for 1274.5926) confirmed the fully protected pentapeptide **19**.

The global deprotection of five benzyls and one Cbz group was planned to intend by employing hydrogenolysis conditions using Pd/C under hydrogen atmosphere. Regrettably, this method didn't work to afford the final compound, as the compound underwent partial degradation to form a mixture of products. To our fortunate, when the global deprotection was attempted using Pearlman's catalyst proved to be worthy of achieving the transformation. Hence, a suspension of an equimolar amount of Pearlman's catalyst and compound **19** was stirred in anhydrous methanol under hydrogen atmosphere (1 atm) at room temperature afforded *epi*-gobichelin B **20** in 80% yield (Scheme **5**). In the NMR spectra, appearance of six amide bond peaks at δ 173.6, 172.3, 172.2, 171.7, 171.0, 169.7 ppm and phenolic carbon (*C*-OH) peak at 157.0 ppm, in addition to the presence of appropriate peaks in ^{1}H and ^{13}C NMR spectra, confirms the structure of *epi*-gobichelin B. Additionally, this was supported by HRMS spectrum of **20** showing base peak at m/z 690.3216 pertaining to M+H of $C_{30}H_{44}N_9O_{10}$ (calc. 690.3211).

After accomplishing the synthesis of *epi*-gobichelin B (**20**), in which L-lysine was placed instead of the D-lysine unit, we applied the same protocol for the total synthesis of natural siderophore gobichelin B. Thus, D-lysine containing tripeptide **2** (the Gob-B 2nd half) was synthesized by NHS-DCC activation of D-Boc-Lys(Z)-OH (**21**), to give **22**, followed by addition of *O*-benzyl serine **4** to afford D-lys-L-ser dipeptide **23** in excellent yield. Further coupling with **16**, to give compound **24**, followed by Boc deprotection produced gob-B 2nd half (**2**) in good yield (Scheme **6**).

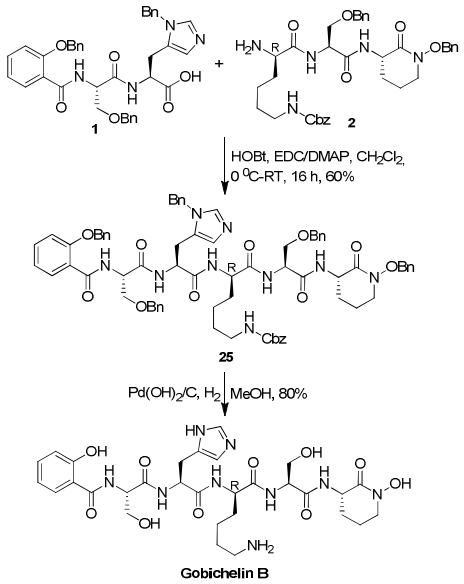


Scheme 6. Synthesis of gob-B 2nd half **2**.

Similar to the synthesis of *epi*-gobichelin B (**20**), gob-B 1st half (**1**) and Gob-B 2nd half (**2**) were coupled in the presence of

View Article Online
DOI: 10.1039/C8OB00263K

HOBt-EDC and DMAP in DCM afforded the fully protected gobichelin B **25**. Finally, the global deprotection using Pearlman's catalyst allowed us to achieve the first total synthesis of natural gobichelin B (Scheme **7**).



Scheme 7. Final steps towards the synthesis of Gobichelin B.

In addition to the other relevant peaks in ^{1}H, ^{13}C NMR spectrum of Gob-B, the presence of five peptide bond carbonyl peaks at 173.8, 172.5, 171.8, 171.1, 171.0, one cyclic ornithine amide peak at 169.7 and one salicylate *C*-OH peak at 156.9 ppm confirmed its structure. COSY, HSQC and HMBC NMR spectra analyses were in agreement with the structure of gobichelin B. Further, in HRMS, m/z peak at 690.3216 corresponding to M+H peak (calc. 690.3211 for $C_{30}H_{44}N_9O_{10}$ [M+H]) confirms the structure of gobichelin B.

To study the iron-binding properties, gobichelin B was treated with 2 fold excess of $FeCl_3$ in water at room temperature and kept at 4 ºC for 10 hr and purified by preparative HPLC to get the corresponding iron bound form of gobichelin B with *m/z* 743.2324 (calc. 743.2326 for $C_{30}H_{41}FeN_9O_{10}$ $[M-2H+Fe(III)]^+$).

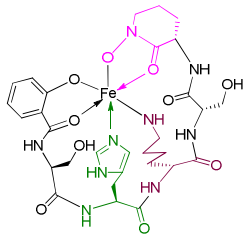


Figure 3. Possible Fe-Gobichelin B complex.

Conclusions

In conclusion, the first total synthesis of gobichelin B and *epi*-gobichelin B were achieved successfully. This is a robust and scalable synthetic route for the total synthesis of gobichelin B. The similar methodology can be adopted for the synthesis of congeners of gobichelin A and B. Amputation of five benzyls and -Cbz groups was achieved in an exceptional way using Pearlman's catalyst under hydrogen atmosphere to get the final compound in excellent yield and high purity. The biological importance of the synthesized intermediates and siderophores and their iron chelating properties are in progress.

Experimental Section

General Information

Chemicals and solvents were purchased from the local suppliers and Sigma-Aldrich®, Carbosynth chemical company. Solvents were used in the reactions after distilled over the dehydrating agents. All the reactions were carried out under appropriate conditions and monitored by the thin layer chromatography (TLC) using silica gel on aluminum plates (GF_{254}) by charring with 3% (w/v) ninhydrin in n-butanol or by 3% (w/v) phosphomolybdic acid (PMA) stain or by ultra violet (UV) detection. Silica-gel (100-200 or 230-400 mesh) was used for column chromatography to purify the compounds. Optical rotation was measured on Rudolph Autopol VI instrument. HPLC purifications were carried out on Shimadzu 2100 Series preparative HPLC system equipped with a UV detector (180-700 nm). ^{1}H, ^{13}C, DEPT spectra were recorded on Bruker® 400 and 500 Avance MHz spectrometer in duterated solvents ($CDCl_3$/ DMSO-d_6 / D_2O). ^{1}H NMR chemical shifts were reported in parts per million (ppm) (δ) with TMS as an internal standard (δ0.00), and ^{13}C NMR were reported in chemical shifts with solvent reference ($CDCl_3$, δ77.00, DMSO-$d_6$$\delta$39.52 ppm). High-resolution mass spectra (HRMS) were recorded on Bruker maXis ESI-TOF spectrometer.

Abbreviations:
RT: Room temperature; DCM: Dichloromethane; EDC·HCl: 3-Ethyl-1-(3-dimethylaminopropyl)carbodiimide hydrochloride; DMF: Dimethyl formamide; TFA: Trifluoroacetic acid; TMSCl: Trimethylsilyl chloride; NHS: N-hydroxysuccinimde; DCC: *N*,*N*'-Dicyclohexylcarbodiimide; DCU: *N*,*N*'-Dicyclohexylurea; HOBt: 1-Hydroxybenzotriazole; DMAP: 4-Dimethylaminopyridine; Boc: *tert*-Butoxycarbonyl; Cbz: Benzyloxycarbonyl.

Boc-His(Bzl)-OMe (9):
L-Boc-His-OMe (**8**) was prepared from *L*-histidine following the literature procedure.[27] To the solution of compound **8** (5.61 g, 20.85 mmol) in DMF (80 mL), anhydrous K_2CO_3 (1.98 g, 14.59 mmol) and benzyl bromide (3.56 g, 20.85 mmol) were added and stirred at room temperature for 12 hr. After completion of the reaction, DMF was removed under reduced pressure and the residue was taken up in Ethyl acetate (200 mL) and water (100 mL). The organic layer was separated and washed with water, brine and dried over anhydrous Na_2SO_4 and concentrated on rotavac followed by purification on column chromatography, produced regioisomers, **9** (4.94 g, 66%) and **9a** (1.12 g, 15%) as colourless gum which solidified slowly upon cooling. **9**: $[\alpha]_D^{25}$ = +3.8° (c 1.0 in $CHCl_3$); **δH (400 MHz; $CDCl_3$;**

View Article Online
DOI: 10.1039/C8OB00263K

Me_4Si) 7.47 (s, 1H), 7.39-7.33 (m, 3H), 7.13 (d, 2H, *J* = 6.5 Hz), 6.66 (s, 1H), 5.94 (d, 1H, *J* = 8.2 Hz), 5.05 (s, 2H), 4.57-4.53 (m, 1H), 3.65 (s, 3H), 3.09 (dd, 1H, *J* = 5.4, 14.7 Hz), 3.00 (dd, 1H, *J* = 4.7, 14.6 Hz), 1.44 (s, 9H); **δC (100 MHz; $CDCl_3$; Me_4Si)** 172.5, 155.6, 138.0, 137.2, 136.0, 128.9, 128.2, 127.2, 116.9, 79.5, 53.6, 52.0, 50.7, 30.3, 28.3; **HRMS (ESI)** calcd. for $C_{19}H_{26}N_3O_4$ [M+H] 360.1923, found 360.1922.

9a: **δH (400 MHz; $CDCl_3$; Me_4Si)** 7.34-7.15 (m, 4H), 6.94 (bs, 2H), 6.73 (bs, 1H), 5.75 (bs, 1H), 5.02-4.93 (m, 2H), 4.36 (bs, 1H), 3.57 (d, 3H, *J* = 5.2 Hz), 2.91-2.80 (m, 2H), 1.29 (s, 9H); **δC (100 MHz; $CDCl_3$; Me_4Si)** 171.7, 155.2, 138.0, 136.0, 128.9, 128.3, 128.0, 126.7, 126.6, 79.8, 53.0, 52.3, 48.4, 28.2, 26.7; **HRMS (ESI)** calcd. for $C_{19}H_{26}N_3O_4$ [M+H] 360.1923, found 360.1922.

***L*-His(Bzl)-OMe hydrochloride (10):**

To a solution of *L*-Histidine derivative **9** (1.60 g, 4.45 mmol) in anhydrous MeOH (10 mL), TMSCl (2.82 mL, 22.25 mmol) was added dropwise at 0 °C and stirred at room temperature for 4 hr. After completion of the reaction, solvent was removed, triturated with distilled hexanes and dried *in vacuo* to get compound **10** (1.25 g, 95%). The solid compound was used in the next step without further purification.

***L*-*O*-benzyl *N*-(2-Benzyloxybenzoyl)serine (11):**

O-Benzyl salicylic acid **3** (1.00 g, 4.38 mmol) and NHS (0.53 g, 4.64 mmol) were dissolved in dry THF (10 mL) and cooled to 0 °C. To this reaction mixture, a solution of DCC (0.92 g, 4.46 mmol) in dry THF (10 mL) was added and the reaction was stirred at room temperature for 6 hr. After complete conversion of acid to active ester, the resulting DCU was filtered off and washed with THF. The THF filtrate was used as the active ester solution in the following reaction.

To a solution of *L*-*O*-benzyl serine **4** (1.00 g, 5.16 mmol) and $NaHCO_3$ (0.95 g, 11.40 mmol) in THF/H_2O (40 mL, 4:3), the active ester obtained from above was added at room temperature and the reaction mixture was stirred for 8 h. The volatiles were evaporated, and the aqueous residue was diluted with EtOAc (50 mL) and acidified to pH 2 with 10% aqueous citric acid. The organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined extracts were washed with brine, dried with anhydrousNa_2SO_4, filtered and concentrated and purified by flash column chromatography on silica gel (5% MeOH/$CHCl_3$) to give **11** (1.50 g, 85%) as a colourless gummy compound which solidified slowly after several hours. $[\alpha]_D^{25}$ = -18.2° (c 1.0 in $CHCl_3$); **δH (400 MHz; $CDCl_3$; Me_4Si)** 10.51 (bs, 1H), 9.04 (d, 1H, *J* = 7.5 Hz), 8.31 (dd, 1H, *J* = 1.6, 7.7 Hz), 7.48-7.44 (m, 3H), 7.36-7.25 (m, 8H), 7.12-7.05 (m, 2H), 5.23 (d, 1H, *J* = 11.6 Hz), 5.18 (d, 1H, *J* = 11.8 Hz), 5.10-5.07 (m, 1H), 4.41 (s, 2H), 3.98 (dd, 1H, *J* = 3.7, 9.6 Hz), 3.82 (dd, 1H, *J* = 3.5, 9.7 Hz); **δC (100 MHz; $CDCl_3$; Me_4Si)** 174.0, 165.8, 157.1, 137.6, 135.5, 133.3, 132.4, 128.7, 128.4, 128.3, 127.8, 127.6, 127.5, 121.5, 120.9, 113.0, 73.1, 71.3, 69.3, 53.4; **HRMS (ESI)** calcd. for $C_{24}H_{24}NO_5$ [M+H] 406.1654, found 406.1650.

Gobichelin B 1st Half Methyl Ester:
[(*S*)-methyl 3-(1-benzyl-1*H*-imidazol-5-yl)-2-((*S*)-3-(benzyloxy)-2-(2-(benzyloxy)benzamido)propanamido)propanoate] (12):

O-Benzyl *N*-((2-benzyloxy)benzoyl)serine **11** (1.00 g, 2.46 mmol) and compound **10** (0.80 g, 2.71 mmol) were suspended in 20 mL of anhydrous CH_2Cl_2 and cooled to 0 °C. To this, HOBt (0.34 g, 2.46 mmol), DMAP (0.90 g, 7.38 mmol) were added and stirred for 10 min. After that, EDC·HCl (0.71 g, 3.69 mmol) was added and stirred at 0 °C for 15 min and at room temperature overnight.After completion of the reaction, the reaction solutionwas concentrated and treated with EtOAc (100 mL) and H_2O (50 mL). After separation of the layers, the organic layer was washed with 5% $NaHCO_3$ (2 x 50 mL), 10% aqueous citric acid (2 x 50 mL), and brine (100 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude compound was purified by column chromatography (50% EtOAc/Hexanes) to give **12** as white foam (1.11 g, 70%). $[\alpha]_D^{25}$ = +17.0° (c 1.0 in $CHCl_3$); **δH (400 MHz; $CDCl_3$; Me_4Si) (Rotamers)** 8.90 (d, 1H, *J* = 7.4 Hz), 8.87 (d, 0.4 H, *J* = 7.4 Hz), 8.23-8.20 (m, 0.6 H), 8.15-8.11 (m, 1H), 7.43-7.36 (m, 3H), 7.30-7.20 (m, 11H), 7.04-6.99 (m, 4H), 6.93 (s, 0.6 H), 6.84 (s, 0.4 H), 6.62 (s, 0.4 H), 6.57 (s, 0.6 H), 5.21-5.10 (m, 2H), 4.95-4.88 (m, 1H), 4.84-4.75 (m, 3H), 4.44-4.29 (m, 2H), 3.93-3.90 (m, 1H), 3.63-3.60 (m, 4H), 3.10-2.95 (m, 2H); **δC (100 MHz; $CDCl_3$; Me_4Si) (Rotamers)** 171.7, 171.6, 170.0, 169.9, 165.3, 165.1, 157.0, 156.9, 137.9, 137.9, 137.5, 137.4, 136.9, 136.7, 136.1, 136.1, 135.8, 135.7, 133.0, 132.9, 132.3, 128.9, 128.8, 128.7, 128.7, 128.3, 128.2, 128.1, 128.1, 127.6, 127.5, 127.5, 127.4, 127.2, 127.1, 121.7, 121.6, 121.3, 117.2, 117.0, 113.1, 113.0, 73.1, 72.9, 71.1, 71.0, 69.5, 69.3, 53.8, 53.3, 52.5, 52.3, 52.1, 50.5, 29.9, 29.8; **HRMS (ESI)** calcd. for $C_{38}H_{39}N_4O_6$ [M+H] 647.2870, found 647.2864.

Gobichelin B 1st Half:
[(*S*)-3-(1-benzyl-1*H*-imidazol-5-yl)-2-((*S*)-3-(benzyloxy)-2-(2-(benzyloxy)benzamido)propanamido)propanoic acid] (1):

To a compound **12** (0.50 g, 0.77 mmol) in THF/H_2O (10 mL, 9:1), LiOH·H_2O (40 mg, 1.00 mmol) was added and stirred at room temperature for 2 hr. After completion of the reaction, solvent was removed and the residue was dissolved in MeOH (15 mL) and Amberlite IR120 (hydrogen form, 300 mg) was added and stirred at room temperature for 2 hr and filtered and concentrated to get acid compound **1** (0.389 g, 80%). HRMS (ESI) calcd. for $C_{37}H_{37}N_4O_6$ [M+H] 633.2713, found 633.2712.

(*L*,*L*)-*O*-Benzyl (*Nα*-Boc-*Nε*-Cbz-lysinyl)serine (15):

L-Boc-Lys(Z)-OH **13** (1.70 g, 4.47 mmol) and NHS (0.54 g, 4.74 mmol) were dissolved in dry THF (10 mL) and cooled to 0 °C. To this reaction mixture, a solution of DCC (0.97 g, 4.74 mmol) in dry THF (10 mL) was added and the reaction was stirred at room temperature for 8 h. The resulting DCU was filtered off and washed with THF. The THF filtrate was used as the active ester solution in the following reaction.

To the solution of *O*-benzyl serine **4** (0.95 g, 4.91 mmol) and $NaHCO_3$ (0.97 g, 11.60 mmol) in THF/H_2O (40 mL/30 mL), the active ester obtained from above was added at room

View Article Online
DOI: 10.1039/C8OB00263K

temperature and the reaction mixture was stirred at room temperature for 6 h. The volatiles were evaporated, and the aqueous residue was diluted with EtOAc (50 mL) and acidified to pH 2 with 10% aqueous citric acid. The organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined extracts were washed with brine, dried over anhydrousNa_2SO_4, filtered and concentrated and purified by flash column chromatography on silica gel using 10% MeOH/$CHCl_3$ to give **15** (1.94 g, 78%) as a colourless gummy compound. $[\alpha]_D^{25}$= +8.7° (*c* 2.0 in $CHCl_3$); **δH (500 MHz; $CDCl_3$; Me_4Si)** 8.54 (bs, 1H), 7.60 (br d, 1H), 7.34-7.24 (m, 10H), 5.74-5.72 (bs, 1H), 5.23-5.08 (m, 3H), 4.77 (bs, 1H), 4.49 (s, 2H), 4.31 (bs, 1H), 3.90 (dd, 1H, *J* = 3.4, 9.6 Hz), 3.70 (dd, 1H, *J* = 2.6, 9.3 Hz), 3.10 (bs, 2H), 1.78-1.73(bs, 1H), 1.63-1.58 (bs, 1H), 1.43-1.34 (m, 13H); **δC (125 MHz; $CDCl_3$; Me_4Si)** 172.8, 172.5, 156.7, 156.1, 137.5, 136.6, 128.5, 128.4, 128.1, 128.0, 127.7, 127.6, 80.2, 73.2, 69.6, 66.6, 54.1, 52.6, 40.5, 32.2, 29.2, 28.3, 22.4; **HRMS (ESI)** calcd for $C_{29}H_{40}N_3O_8$ [M+H] 558.2815, found 558.2820.

(*S*)-3-Benzyloxycarbonylamino-1-benzyloxy-2-piperidinone (A):

Compound **A** was prepared from *L*-ornithine hydrochloride by following literature procedure.[2] $[\alpha]_D^{25}$= +55.3° (c 1.0 in CH_2Cl_2; lit:[3] $[\alpha]_D^{25}$= +52.0° (c 1.45 in CH_2Cl_2); mp 87-89 °C; **δH (400 MHz; $CDCl_3$; Me_4Si)** 7.45-7.33 (m, 10H), 5.73 (bs, 1H), 5.14 (s, 2H), 4.98 (d, 1H, *J* = 10.6 Hz), 4.90 (d, 1H, *J* = 10.6 Hz), 4.21-4.16 (m, 1H), 3.47-3.40 (m, 1H), 3.36-3.33 (m, 1H), 2.43 (bs, 1H), 1.91-1.82 (m, 2H), 1.56 (qd, 1H, *J* = 4.0, 12.5, 24.9 Hz); **δC (100 MHz; $CDCl_3$; Me_4Si)** 167.8, 156.4, 136.3, 135.1, 129.5, 128.8, 128.5, 128.1, 75.9, 66.8, 52.7, 51.2, 28.1, 20.7; **HRMS (ESI)** calcd for $C_{20}H_{23}N_2O_4$ [M+H] 355.1658, found 355.1658.

(*S*)-3-Amino-1-benzyloxypiperidin-2-one hydrobromide (16):

Following the literature procedure,[2] brieftreatment of A with 33% HBr in acetic acid afforded cyclic ornithine fragment **16** in 95% yield.

Protected Gob-B 2nd Half:

Benzyl *tert*-butyl ((S)-6-(((S)-3-(benzyloxy)-1-(((S)-1-(benzyloxy)-2-oxopiperidin-3-yl)-amino)-1-oxopropan-2-yl)-amino)-6-oxohexane-1,5-diyl)dicarbamate (17):

(*L,L*)-*O*-Benzyl (*Nα*-Boc-*Nε*-Cbz-lysinyl)serine **15** (1.86 g, 3.34 mmol) and compound **16** (0.95 g, 3.17 mmol) were suspended in 20 mL of anhydrous $CH_2Cl_2$and cooled to 0 °C. To this, HOBt (0.53 g, 3.40 mmol), DMAP (1.10 g, 9.02 mmol) were added and stirred for 10 min. After that, EDC·HCl (0.96 g, 5.01 mmol) was added. After being stirred at 0 °C for 15 min and room temperature overnight, the reaction mixture was concentrated and treated with EtOAc (100 mL) and H_2O (50 mL). After separation of the layers, the organic layer was washed with 5% aqueous $NaHCO_3$ (2 x 50 mL), 10% aqueous citric acid (2 x 50 mL), and brine (100 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude compound was purified by column chromatography (50% EtOAc/Hexanes) to give **17** (1.90 g, 75%) as a white foam. $[\alpha]_D^{25}$ = +41.9° (c 1.0 in $CHCl_3$); **δH (400 MHz; $CDCl_3$; Me_4Si) (Rotamers)** 7.41-7.29 (m, 16H), 7.18-6.91 (br d,1H), 5.26 (br d, 1H), 5.09 (d, 2H, *J* = 5.7 Hz), 4.95 (dd, 1H, *J* = 5.0, 10.5 Hz), 4.87 (t, 1H, *J* = 10.0 Hz), 4.70-4.55 (m, 3H), 4.43-4.95 (m, 1H), 4.13-4.07 (m, 1H), 3.96 (d, 1H, *J* = 6.2 Hz), 3.64-3.59 (m, 1H), 3.43-3.36 (m, 1H), 3.31 (bs, 1H), 3.24-3.16 (m, 2H), 2.35 (bs, 1H), 1.86 (bs, 4H), 1.2-1.64 (m, 1H), 1.55-1.36 (m, 14H); **δC (100 MHz; $CDCl_3$; Me_4Si) (Rotamers)** 172.2, 170.0, 169.8, 167.4, 167.2, 156.7, 156.5, 155.8, 137.6, 137.5, 136.7, 136.7, 135.2, 135.1, 129.5, 128.7, 128.4, 128.4, 128.0, 128.0, 127.8, 127.8, 127.7, 80.0, 75.9, 75.8, 73.4, 73.2,69.5, 69.3, 66.4, 54.8, 54.5, 52.8, 51.5, 51.1, 51.0, 40.4, 40.3, 31.9, 29.3, 28.3, 27.4, 27.4, 22.4, 20.8; **HRMS (ESI)** calcd for $C_{41}H_{53}N_5NaO_9$ [M+Na] 782.3741, found 782.3744.

Benzyl ((*S*)-5-amino-6-(((S)-3-(benzyloxy)-1-(((S)-1-(benzyloxy)-2-oxopiperidin-3-yl)amino)-1-oxopropan-2-yl)-amino)-6-oxohexyl)carbamate (18):

Tripeptide **17** (1.00 g, 1.32 mmol) was dissolved in 50 mL of 10% TFA/$CH_2Cl_2$and 0.2 mL of anisole was added and stirred for 1 hr at 0 °C. After completion of the reaction, volatiles were removed and theresidue was then dissolved in ethyl acetate and washed twice with 5% aqueous $NaHCO_3$ solution followed by water and brine solution. The organic layer was dried with anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was then taken up in a small amount of $CH_2Cl_2$and applied to a silica plug. The deprotected tripeptide **18** (0.87 g, 80%) was then eluted in 5% MeOH/$CHCl_3$ as a colorless gum. $[\alpha]_D^{25}$= +10.0° (c 0.5 in $CHCl_3$); **δH (400 MHz; $CDCl_3$; Me_4Si)** 7.98 (d, 1H, *J* = 7.2 Hz), 7.53 (d, 1H, *J* = 5.5 Hz), 7.39-7.30 (m, 15H), 5.16 (bs, 1H), 5.08 (s, 2H), 4.94 (d, 1H, *J* = 10.4 Hz), 4.86 (d, 1H, *J* = 10.4 Hz), 4.68-4.62 (m, 1H), 4.58 (s, 2H), 4.38-4.32 (m, 1H), 3.91 (dd, 1H, *J* = 4.2, 9.2 Hz), 3.63 (dd, 1H, *J* = 6.4, 8.9 Hz), 3.4 (t, 2H, *J* = 4.5 Hz), 3.30 (bs, 1H), 3.17 (d, 2H, *J* = 5.9 Hz), 2.35-2.33 (m, 1H), 2.08 (bs, 2H), 1.80 (bs, 3H), 1.50 (bs, 4H), 1.42-1.38 (m, 2H); **δC (100 MHz; $CDCl_3$; Me_4Si)** 175.5, 170.3, 167.5, 156.5, 137.6, 136.7, 135.1, 129.5, 128.8, 128.5, 128.5, 128.4, 128.1, 128.0, 127.8, 127.8, 127.7, 76.0, 73.3, 69.6, 66.5, 55.0, 52.5, 51.5, 51.2, 40.6, 34.4, 29.6, 27.4, 22.7, 20.8; **HRMS (ESI)** calcd for $C_{36}H_{46}N_5O_7$ [M+H] 660.3397, found 660.3397.

[Benzyl ((*S*)-5-((*S*)-3-(1-benzyl-1*H*-imidazol-5-yl)-2-((*S*)-3-(benzyloxy)-2-(2-(benzyloxy)-benzamido)propanamido)-propanamido)-6-(((*S*)-3-(benzyloxy)-1-(((*S*)-1-(benzyloxy)-2-oxopiperidin-3-yl)amino)-1-oxopropan-2-yl)amino)-6-oxohexyl)carbamate] (Protected *epi*-Gobichelin B) (19):

Gob-B 1st Half acid **1** (400 mg, 0.63 mmol) and amine **18** (420 mg, 0.63 mmol) were suspended in 10 mL of anhydrous $CH_2Cl_2$and cooled to 0°C. To this, HOBt (86 mg, 0.63 mmol), DMAP (200 mg, 1.63 mmol) were added and stirred for 10 min. After that, EDC·HCl (181 mg, 0.95 mmol) were added and stirred at 0 °C for 15 min and at room temperature for 16 h.After completion of the reaction, the reaction mixture was concentrated and treated with EtOAc (70 mL) and H_2O (20 mL). After separation of the layers, aqueous layer was discarded and the organic layer was washed with 5% aqueous $NaHCO_3$ (2 x 15 mL), 10% aqueous citric acid (2 x 15 mL), and brine, dried

View Article Online
DOI: 10.1039/C8OB00263K

over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude compound was purified onpreparative HPLC using Phenomenex Luna C18 column (5 µm, 21.2 x250 mm)eluting with an isocratic 65% acetonitrile/water (1% NH_4CO_3) gave **19** (484 mg, 60%) as a white foam. **δH (500 MHz; $CDCl_3$; Me_4Si)** 9.45 (d, 1H, *J* = 6.3 Hz), 9.13 (d, 1H, *J* = 4.6 Hz), 8.0 (dd, 1H, *J* = 1.6, 7.8 Hz), 7.59 (t, 2H, *J* = 9.0 Hz), 7.48-7.46 (m, 2H), 7.43-7.41 (m, 2H), 7.35-7.30 (m, 18H), 7.24-7.20 (m, 5H), 7.18 (d, 1H, *J* = 7.2 Hz), 7.14-7.12 (m, 2H), 7.08 (d, 1H, *J* = 8.4 Hz), 7.02 (t, 1H, *J* = 7.4 Hz), 6.96 (bs, 2H), 6.58 (s, 1H), 5.97 (s, 1H), 5.18 (d, 2H, *J* = 2.3 Hz), 5.16-5.14 (m, 1H), 5.05 (s, 2H), 4.92 (d, 2H, *J* = 2.1 Hz), 4.76-4.71 (m, 3H), 4.57-4.51 (m, 4H), 4.35-4.26 (m, 2H), 4.21 (d, 1H, *J* = 12.1 Hz), 4.17 (d, 1H, *J* = 12.1 Hz), 3.95 (dd, 1H, *J* = 7.0, 10.0 Hz), 3.88 (dd, 1H, *J* = 4.7, 10.0 Hz), 3.55 (dd, 1H, *J* = 3.7, 9.7 Hz), 3.40 (td, 1H, *J* = 4.4, 11.1 Hz), 3.28-3.26 (m, 1H), 3.16 (dd, 1H, J *J* = 3.1, 14.8 Hz), 3.06 (q, 2H, 6.3 Hz), 2.82 (dd, 1H, *J* = 4.7, 14.7 Hz), 2.11 (bs, 1H), 1.88-1.80 (m, 3H), 1.76-1.64 (m, 4H), 1.44 (m, 2H); **δC (125 MHz; $CDCl_3$; Me_4Si)** 172.1, 171.6, 171.2, 169.8, 166.8, 166.7, 157.6, 156.3, 138.3, 137.8, 137.5, 136.7, 136.2, 135.7, 135.6, 135.4, 133.6, 132.1, 129.5, 128.9, 128.7, 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.6, 127.3, 127.2, 121.2, 120.9, 117.2, 113.2, 75.6, 72.8, 72.8, 71.5, 69.5, 68.4, 66.4, 56.3, 54.8, 53.8, 53.4, 51.6, 50.9, 50.5, 40.6, 30.6, 29.0, 27.6, 27.2, 22.2, 21.1; **HRMS (ESI)** calcd for $C_{73}H_{80}N_9O_{12}$ [M+H] 1274.5926, found 1274.5926.

***Epi*-Gobichelin B (20):**

Protected *Epi*-Gobichelin B **19** (100 mg, 0.078 mmol) was dissolved in MeOH (8 mL) and $Pd(OH)_2/C$ (100 mg) was added and stirred under hydrogen atmosphere (1 atm) for 6 hr. The progress of the reaction was monitored by HRMS. After observing single peak at 690.3220 [M+H], the reaction was filtered through a pad of celite and concentrated to get the *epi*-Gobichelin B **20** (43.30 mg, 80%) as white solid. **δH (500 MHz; D_2O)** 8.28 (s, 1H), 7.65 (d, 1H, *J* = 7.1 Hz), 7.36 (bs, 1H), 7.12 (s, 1H), 6.90 (bs, 2H), 4.62 (bs, 2H), 4.45 (s, 1H), 4.31 (s, 1H), 4.24 (s, 1H), 4.17 (s, 1H), 3.81 (s, 2H), 3.75 (bs, 2H), 3.21 (s, 1H), 3.16 (bs, 2H), 3.05 (dd, 1H, *J* = 8.4, 14.5 Hz), 2.78 (t, 2H, *J* = 7.1 Hz), 1.94 (bs, 1H), 1.77-1.59 (m, 5H), 1.50 (t, 2H, *J* = 6.8 Hz), 1.24 (bs, 2H); **δC (125 MHz; D_2O)** 173.6, 172.3, 172.2, 171.7, 171.0, 169.7, 157.0, 134.6, 133.7, 129.4, 129.2, 129.0, 120.3, 117.2, 116.5, 61.2, 60.9, 56.0, 55.6, 53.7, 52.6, 49.7, 48.9, 41.6, 39.2, 30.4, 26.8, 26.7, 22.0, 20.3; **HRMS (ESI)** calcd for $C_{30}H_{44}N_9O_{10}$ [M+H] 690.3211, found 690.3216.

(*D*,*L*)-*O*-Benzyl (*N*α-Boc-*N*ε-Cbz-lysinyl)serine (23):

D-Lys+*L*-Ser dipeptide **23** was prepared from *D*-Boc-Lys(Z)-OH **21** following the similar procedure used for the synthesis of *L*-Lys+*L*-Ser dipeptide **15**. Compound **23** was obtained as a colourless gum. $[\alpha]_D^{25}$= +19.3° (c 2.0 in $CHCl_3$). **δH (500 MHz; $CDCl_3$; Me_4Si)** 7.44-7.25 (m, 12H), 5.55 (d, 1H, *J* = 7.3 Hz), 5.14-4.99 (m, 3H), 4.77 (bs, 1H), 4.51 (s, 2H), 4.34 (bs, 1H), 3.91 (dd, 1H, *J* = 3.0, 9.5 Hz), 3.71 (dd, 1H, *J* = 2.5, 8.6 Hz), 3.12 (bs, 2H), 1.76-1.74 (m, 1H), 1.65-1.60 (m, 1H), 1.49-1.35 (m, 13H); **δC (100 MHz; $CDCl_3$; Me_4Si)** 172.6, 172.4, 156.5, 155.9, 137.3, 136.4, 128.3, 128.2, 127.9, 127.7, 127.6, 127.4, 80.0, 73.0, 69.4, 66.5, 53.9, 52.4, 40.4, 32.1, 29.0, 28.1, 22.3; **HRMS (ESI)** calcd for $C_{29}H_{39}N_3O_8$ [M+H] 558.2815, found 558.2816.

Benzyl tert-butyl ((R)-6-(((S)-3-(benzyloxy)-1-(((S)-1-(benzyloxy)-2-oxopiperidin-3-yl)-amino)-1-oxopropan-2-yl)-amino)-6-oxohexane-1,5-diyl)dicarbamate (24):

Tripeptide **24** was obtained from (*D,L*)-*O*-Benzyl (*D*-*N*α-Boc-*N*ε-Cbz-lysinyl)serine **23** following the similar procedure used for the synthesis of tripeptide **17**. Compound **24** was obtained as a white foam. $[\alpha]_D^{25}$ = +54.52° (c 1.0 in $CHCl_3$). **δH (400 MHz; $CDCl_3$; Me_4Si)** 7.48 (bs, 1H), 7.40-7.29 (m, 15H), 7.14 (bs,1H), 5.39 (br d, 1H), 5.17 (br d, 1H), 5.09 (s, 2H), 4.95 (d, 1H, *J* = 10.3 Hz), 4.87 (d, 1H, *J* = 9.9 Hz), 4.67-4.65 (m, 1H), 4.59-4.55 (m, 2H), 4.35-4.32 (m, 1H), 4.14 (bs, 1H), 3.95 (bd, 1H, *J* = 8.7 Hz), 3.65-3.61 (m, 1H), 3.42-3.38 (m, 1H), 3.31 (bs, 1H), 3.17 (bd, 2H), 2.31 (s, 2H), 1.83 (bs, 3H), 1.73-69 (m, 1H), 1.52 (bs, 3H), 1.45-1.42 (m, 10H); **δC (100 MHz; $CDCl_3$; Me_4Si)** 172.3, 170.0, 167.3, 156.6, 155.8, 137.5, 136.6, 135.2, 129.5, 128.8, 128.5, 128.4, 128.1, 128.0, 127.9, 127.8, 80.2, 75.9, 73.4, 69.4, 66.5, 54.7, 52.6, 51.6, 51.2, 40.5, 31.8, 29.4, 28.3, 28.3, 27.4, 22.4, 20.9; **HRMS (ESI)** calcd for $C_{41}H_{54}N_5O_9$ [M+H] 760.3922, found 760.3913.

Gob-B 2nd Half: Benzyl ((*R*)-5-amino-6-(((S)-3-(benzyloxy)-1-(((S)-1-(benzyloxy)-2-oxopiperidin-3-yl)amino)-1-oxopropan-2-yl)amino)-6-oxohexyl)carbamate (2):

Gob-B 2nd half **2** was prepared following the procedure described for the synthesis of tripeptide **18**. Compound **2** was obtained as a colourless gum (0.87 g, 80%). $[\alpha]_D^{25}$= +23.8° (c 0.5 in $CHCl_3$). **δH (500 MHz; $CDCl_3$; Me_4Si)** 8.41 (d, 1H, *J* = 5.8 Hz), 8.09 (d, 1H, *J* = 5.2 Hz), 7.36-7.24 (m, 15H), 5.66 (t, 1H, *J* = 5.6 Hz), 5.40 (bs, 2H), 5.04 (s, 2H), 4.89 (d, 1H, *J* = 10.6 Hz), 4.82 (d, 1H, *J* = 10.6 Hz), 4.75-4.72 (m, 1H), 4.50 (s, 2H), 4.40-4.35 (m, 1H), 3.89 (dd, 1H, *J* = 5.2, 9.5 Hz), 3.82 (t, 1H, *J* = 6.0 Hz), 3.67 (dd, 1H, *J* = 4.6, 9.5 Hz), 3.4 (bs, 1H), 3.20 (d, 1H, *J* = 8.0 Hz), 3.10 (d, 2H, *J* = 5.1 Hz), 2.35-2.33 (m, 1H), 2.09 (bs, 1H), 1.76-1.69 (m, 5H), 1.43-1.38 (m, 4H); **δC (100 MHz; $CDCl_3$; Me_4Si)** 175.4, 170.3, 167.5, 156.4, 137.5, 136.6, 134.9, 129.4, 128.7, 128.4, 128.3, 128.0, 127.9, 127.7, 127.7, 127.6, 75.9, 73.2, 69.5, 66.4, 54.9, 52.4, 51.4, 51.1, 40.5, 34.4, 29.5, 27.4, 22.6, 20.7; **HRMS (ESI)** calcd for $C_{36}H_{46}N_5O_7$ [M+H] 660.3397, found 660.3386.

[Benzyl ((*S*)-5-((*S*)-3-(1-benzyl-1*H*-imidazol-5-yl)-2-((*S*)-3-(benzyloxy)-2-(2-(benzyloxy)-benzamido)propanamido)propanamido)-6-(((*S*)-3-(benzyloxy)-1-(((*S*)-1-(benzyloxy)-2-oxopiperidin-3-yl)amino)-1-oxopropan-2-yl)amino)-6-oxohexyl)carbamate] (Protected Gobichelin B) (25):

Protected gob-B **25** was synthesized following the similar procedure depicted for the synthesis of pentapeptide **23**, and was obtained as a white foam in 60% yield. **δH (500 MHz; $CDCl_3$; Me_4Si)** 8.99 (d, 1H, *J* = 4.7 Hz), 8.79 (d, 1H, *J* = 7.4 Hz), 8.08 (dd, 1H, *J* = 1.3, 7.7 Hz), 7.62 (d, 1H, *J* = 7.7 Hz), 7.40-7.36 (m, 5H), 7.34-7.30 (m, 10H), 7.28-7.26 (m, 5H), 7.24 (s, 4H), 7.21-7.20 (m, 3H), 7.11 (bs, 2H), 7.04-7.01 (m, 3H), 6.95 (t, 1H, *J* = 7.4 Hz), 6.77 (s, 1H), 6.15 (s, 1H), 5.99 (t, 2H, *J* = 5.1 Hz),

View Article Online
DOI: 10.1039/C8OB00263K

5.14 (s, 2H), 5.02 (d, 1H, *J* = 12.5 Hz), 4.99 (d, 1H, *J* = 12.1 Hz), 4.92 (d, 1H, *J* = 10.5 Hz), 4.86 (d, 1H, *J* = 10.5 Hz), 4.76 (d, 1H, *J* = 15.1 Hz), 4.64 (d, 1H, *J* = 15.1 Hz), 4.58 (d, 1H, *J* = 11.8 Hz), 4.53 (d, 1H, *J* = 11.8 Hz), 4.50-4.45 (m, 3H), 4.16 (d, 1H, *J* = 12.1 Hz), 4.11 (d, 1H, *J* = 12.1 Hz), 4.06-4.02 (m, 2H), 3.73-3.69 (m, 2H), 3.45 (td, 1H, *J* = 4.6, 10.6 Hz), 3.36 (dd, 1H, *J* = 3.7, 9.8 Hz), 3.32-3.29 (m, 1H), 3.28-3.25 (m, 1H), 3.22-3.14 (m, 2H), 2.75 (dd, 1H, *J* = 4.9, 14.7 Hz), 2.54 (bs, 2H), 2.10-2.08 (m, 1H), 2.01-1.95 (m, 1H), 1.90-1.88 (m, 1H), 1.80-1.64 (m, 5H), 1.48-1.43 (m, 2H); **δC (125 MHz; $CDCl_3$; Me_4Si)** 172.8, 172.0, 170.0, 169.7, 167.0, 166.7, 157.3, 156.6, 137.9, 137.5, 137.0, 136.6, 136.5, 136.2, 135.4, 135.4, 133.4, 132.6, 129.4, 128.7, 128.7, 128.6, 128.5, 128.4, 128.3, 128.3, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 127.3, 121.6, 120.9, 118.7, 113.0, 75.7, 73.3, 72.8, 71.4, 69.2, 68.4, 66.1, 56.1, 55.3, 53.4, 53.0, 51.0, 50.9, 50.4, 40.0, 29.7, 28.6, 28.3, 27.6, 22.8, 20.9; **HRMS (ESI)** calcd for $C_{73}H_{80}N_9O_{12}$ [M+H] 1274.5926, found 1274.5926.

Gobichelin B:

Adopting the procedure described for the synthesisof of *epi*-gobichelin B **20**, equal amounts of protected Gobichelin B **25** and 10% (w/w) $Pd(OH)_2/C$ were suspended in MeOH and stirred under hydrogen atmosphere (1 atm) at room temperature. The reaction was monitored by HRMS. After observing single molecular ion peak [M+H] at 690.3209, the reaction mass was filtered through a pad of celite and concentrated to get the Gobichelin B in 80% yield as colourless fluffy compound. **δH (500 MHz; D_2O)** 8.46 (s, 1H), 7.66 (d, 1H, *J* = 7.6 Hz), 7.36 (t, 1H, *J* = 7.3 Hz), 7.19 (s, 1H), 6.91-6.87 (m, 2H), 4.67 (bs merged in D_2O, 2H), 4.44 (q, 1H, *J* = 5.13 Hz), 4.31-4.29 (m, 1H), 4.25-4.18 (m, 1H), 3.87-3.69 (m, 4H), 3.52 (bs, 1H), 3.22 (dd, 1H, *J* = 5.5, 15.4 Hz), 3.14-3.12 (m, 1H), 3.09-3.04 (m, 1H), 2.85 (t, 2H, *J* = 7.2 Hz), 1.90-1.61 (m, 6H), 1.56 (quin, 2H, *J* = 7.1 Hz), 1.28-1.21 (m, 2H); **δC (125 MHz; D_2O)** 173.8, 172.5, 171.8, 171.1, 171.0, 169.7, 156.9, 134.6, 133.5, 129.4, 128.5, 120.3, 117.3, 117.1, 116.4, 61.1, 60.9, 56.1, 55.7, 54.0, 52.6, 49.7, 41.4, 39.2, 30.1, 26.7,26.3, 25.9, 22.0, 20.3; **HRMS (ESI)** calcd for $C_{30}H_{44}N_9O_{10}$ [M+H] 690.3211, found 690.3216.

Gob-B–Fe(III) Complex:

To a solution of gobichelin B (10 mg) in distilled water (4 mL), aqueous solution of $FeCl_3$ (2 fold excess) was added dropwise and stirred for 10 min at room temperature and then kept at 4 °C for 10 h. The resulting solution was purified on a preparative Phenomenex Luna C18 column (5 µm, 21.2 x250 mm) operating at 15 mL/min using 5 x1 mL injections. Fe(III)-Gobichelin B complex was eluted with a gradient of5-10% of CH_3CN/H_2O. Elution of the metal complex was observed using MLCT band at 435 nm and collected at RT 5.4 min. HRMS (ESI) calcd for $C_{30}H_{41}N_9O_{10}$ [M-2H+Fe^{+3}] 743.2326, found 743.2326.

Conflicts of interest

The authors declare no competing financial interest.

Acknowledgements

This work was supported by UPE and PURSE grants. B.C.V acknowledges the University Grants Commission (UGC), India for Dr. D. S. Kothari Post-Doctoral Fellowship (award No. F.4-2/2006(BSR)/CH/14-15/0074).

Notes and references

1 M. P. Soares and G. Weiss, *EMBO Rep.,* 2015, **16**, 1482.
2 E. D. Weinberg, *Microbiol. Rev.,* 1978, **42**, 45.
3 V. I. Holder and M. A. Bachman, *Metallomics,* 2015, **7**, 986.
4 M. Luo, E. A. Fadeev and J. T. Goves, *Nat. Chem. Biol.,* 2005, **1**, 149.
5 M. A. Fischbach, H. Lin, D. R. Liu and C. T. Walsh, *Nat. Chem. Biol.,* 2006, **2**, 132.
6 F. Harrison and A. Buckling, *ISME J.,* 2009, **3**, 632.
7 J. A. Gaddy, B. A. Arivett, M. J. McConnell, R. L. Rojas, J. Pachón and L. A. Actis, *Infect. Immun.,* 2012, **80**, 1015.
8 B. R. Wilson, A. R. Bogdan, M. Miyazawa, K. Hashimoto and Y. Tsuji, *Trends Mol. Med.,* 2016, **22**, 1077.
9 J. A. Morrissey, A. Cockayne, P. J. Hill and P. Williams, *Infect. Immun.,* 2000, **68**, 6281.
10 K. N. Raymond, E. A. Dertz and S. S. Kim, *Proc. Natl. Acad. Sci. USA,* 2003, **100**, 3584.
11 T. Zhou, Y. Ma, X. Kong and R. C. Hider, *Dalton Trans.,* 2012, **41**, 6371.
12 B. R. Byers, *Iron acquisition by the genus Mycobacterium. History, Mechanisms, Role of Siderocalin, Anti-Tuberculosis Drug Development*. Springer publications. ISSN 2212-9901.
13 M. J. Miller, *Chem. Rev.,* 1989, **89**, 1563.
14 C. Ji, P. A. Miller and M. J. Miller, *J. Am. Chem. Soc.,* 2012, **134**, 9898.
15 M. J. Miller, A. J. Walz, H. Zhu, C. Wu, G. Moraski, U. Mollmann, E. M. Tristani, A. L. Crumbliss, M. T. Ferdig, L. Checkley, R. L. Edwards and H. I. Boshof, *J. Am. Chem. Soc.,* 2011, **133**, 2076.
16 R. J. Abergel, A. M. Zawadzka and K. N. Raymond, *J. Am. Chem. Soc.,* 2008, **130**, 2124.
17 R. J. Abergel, J. A. Warner, D. K. Shuh and K. N. Raymond, *J. Am. Chem. Soc.,* 2006, **128**, 8920.
18 A. Stintzi, C. Barnes, J. Xu and K. N. Raymond, *Proc. Natl. Acad. Sci. USA,* 2000, **97**, 10691.
19 R. J. Abergel and K. N. Raymond, *J. Biol. Inorg. Chem.,* 2008, **13**, 229.
20 R. C. Hider and X. Kong, *Nat. Prod. Rep.*, 2010, **27**, 637.
21 Y. Chen, M. Unger, I. Ntai, R. A. McClure, J. C. Albright, R. J. Thomson and N. L. Kelleher, *Med. Chem. Commun.,* 2013, **4**, 233.
22 B. Sontag, M. Gerlitz, T. Paululat, H. F. Rasser, I. G. Wollny and F. G. Hansske, *J. Antibiot.,* 2006, **59**, 659.
23 M. R. Seyedsayamdost, M. F. Traxler, S. L. Zheng, R. Kolter and J. Clardy, *J. Am. Chem. Soc.,* 2011, **133**, 11434.
24 K. Raghavendra, R. A. Yellin, H. Traboulsi, G. Melman, N. Humbert, M. Elhabiri, E. G. Kontecka, A. M. Albrecht-Gary and A. Shanzer, *Inorg. Chem.,* 2007, **46**, 2485.
25 S. Yamada, A. Sudo, M. Goto and T. Endo, *RSC Adv.,* 2014, **4**, 29890.
26 J. C. Hodges, *Synthesis,* 1987, 20.
27 J. M. Chalker, S. B. Gunnoo, O. Boutureira, S. C. Gerstberger, M. F. González, G. J. L. Bernardes, L. H. GriffinHailu, C. J. Schofield and B. G. Davis, *Chem. Sci.,* 2011, **2**, 1666.
28 The formation of the rotamers was confirmed by the EXSY NMR. D. X. Hu, P. Grice and S. V. Lee, *J. Org. Chem.,* 2012, **77**, 5198.
29 L. Dong and M. J. Miller, *J. Org. Chem.,* 2002, **67**, 4759.

View Article Online
DOI: 10.1039/C8OB00263K

Table of content entry:

The first total synthesis of structurally diverse mixed ligand siderophore, gobichelin B and an *epi*-gobichelin B is reported.