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An Efficient Solid-Phase Strategy for Total Synthesis of Naturally Occurring Amphiphilic Marine Siderophores: Amphibactin-T and Moanachelin ala-B

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Abstract:

Microorganisms such as bacteria, fungi and some plants secrete an abundance of suites of low molecular weight, high-affinity iron(III)-chelating acylated siderophores. The peptide composition of a suite of amphiphilic siderophores generated by a *Vibrio* species, isolated from oligotrophic open ocean water, contained the same iron(III)-scavenging polar head group and is attached to a fatty acid. In the present study, we report the first total synthesis of the naturally obtainable marine siderophores amphibactin-T and moanachelin ala-B on solid-phase using standard Fmoc-chemistry. Furthermore, we have discussed the preparation of orthogonal protected Orn amino acid 'N^{α}-Fmoc-N^{δ}-(acetyl)-N^{δ}-(benzoyloxy)-ornithine' [Fmoc-Orn(Ac,OBz)-OH], which is most important constructive building block for amphibactin and moanachelin siderophores syntheses. The applications of this Orn unit on solid-phase have also been discussed.

Keywords: Iron, Marine siderophores, Amphiphilic siderophores, Peptide-based, Amphibactins, Moanachelins, Solid-Phase Peptide Synthesis.

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Introduction:

Iron is the fourth most abundant transition metal in the earth's crust. It is an indispensable element to growth for virtually all living microorganisms because it is involved in essential chemical transformations such as photosynthesis, respiration, nitrogen fixation, oxygen metabolism, electron transfer, DNA and RNA synthesis and other enzymatic processes.[1, 2] Iron is barely soluble under aerobic conditions, near neutral pH conditions needed for most bacterial growth and therefore severely limits the availability of this essential nutrient.[1, 3] To conquer this low solubility, most of the microorganisms evolve low molecular weight and highly specific strong binding ferric ion (Fe^{3+}) organic ligands, called as 'siderophores' (from the Greek: "iron carriers"). Almost all aerobic and facultative anaerobic microorganisms synthesize at least one siderophore. These are commonly generated under low conditions of iron accessibility and secreted into the neighbouring atmosphere where they can chelate ferric ion.[3]

Research in this field began about seven decades ago, and have established significant interest due to their exceptional strong binding and high selectivity towards ferric ion. In addition, they have applications in medicine as antibiotic and antitumor drugs and for iron and aluminium therapy in case of toxic overload.[3, 4] Barley and wheat are agricultural species that are also capable of efficient requisition of iron by liberating phytosiderophores *via* their root into the surrounding soil rhizosphere.[5] Additionally, siderophores display interesting non-iron chemistry with other hard Lewis-acids found among the lanthanides and actinides.[6]

Many structures have been determined for siderophores produced by terrestrial and enteric bacteria;[7, 8] however, few siderophores have been structurally characterized from marine bacteria and microorganisms.[9-12] The study of siderophores produced by bacteria of marine origin is relatively new and a modest number of structures have been elucidated (Fig. 1). Two interesting subjects have become evident from the known siderophore structures of marine bacteria in

contrast to known terrestrial bacteria: (i) the production of suites of amphiphilic siderophores composed of an acyl peptidic head group and one of a series of differing length, degree of (un)saturation, and degree of hydroxylated fatty acid attachments,[12, 13] and (ii) the presence of photo labile moieties that are reactive when bound to Fe(III), producing an oxidized siderophore ligand and Fe(II) under irradiation with natural sunlight or ultra-violet light.[14, 15] The common iron(III)-binding motif found amongst the known amphiphilic siderophore structures is the hydroxamate derivative, N^{δ}-hydroxy-N^{δ}-acetyl-ornithine (highlighted in Fig. 1),but other iron binding functional groups can also be present (Fig. 1).[16]

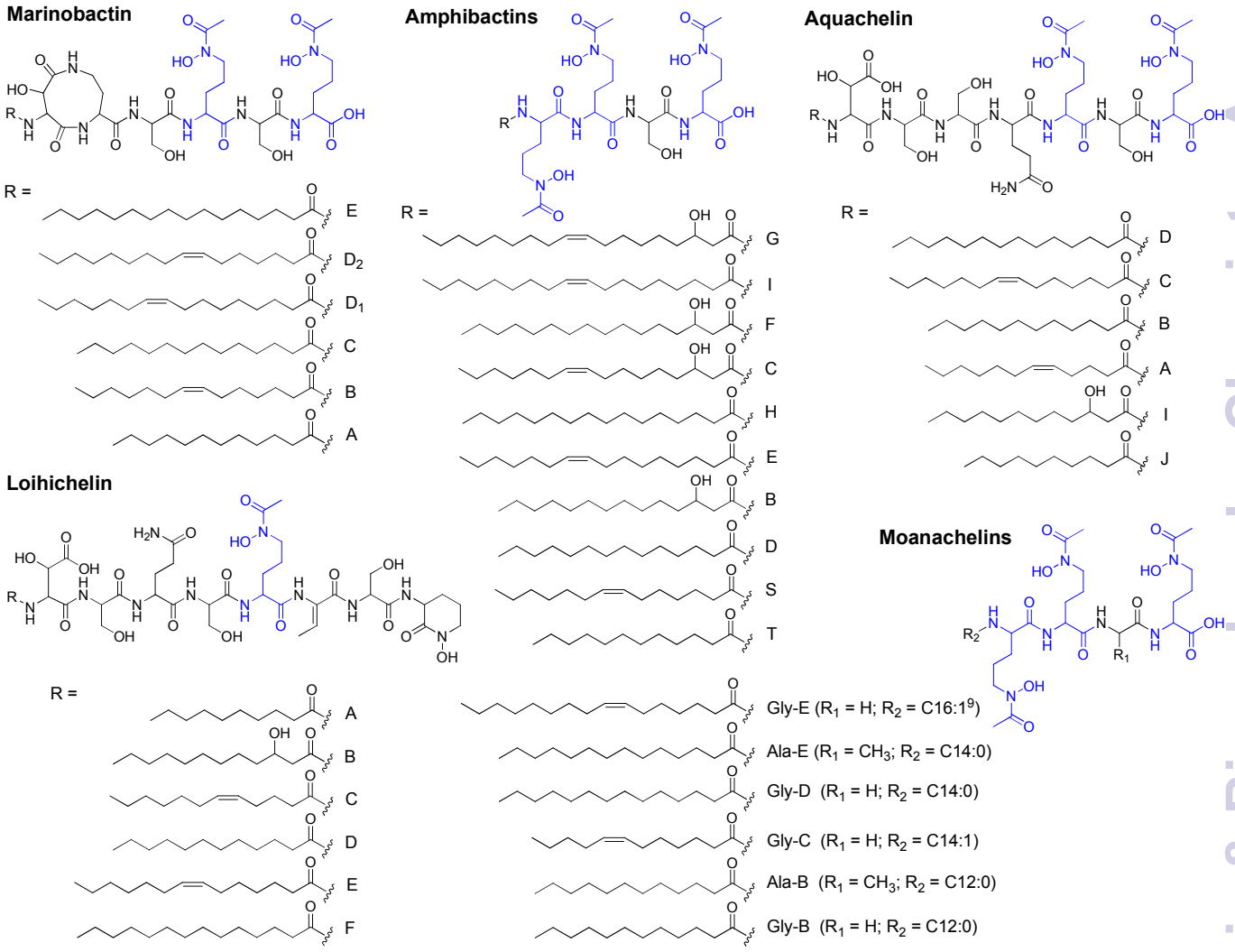


Fig. 1. Structures of selected amphiphilic siderophores: Suites of marine siderophores include the marinobactins, amphibactins, aquachelins, loihichelins, and moanachelins.

The suites of marine amphiphilic siderophores include the marinobactins produced by *Marinobacter* sp. DS40M6 and DS40M8,[12] the amphibactins produced by *Vibrio* sp. R-10,[13] the aquachelins produced by *Halomonas aquamarina* DS40M3,[12] the loihichelins produced by *Halomonas* LOB-5,[17] and the moanachelins produced by *Vibrio* sp. Nt1[18] isolated from oligotrophic open ocean water. Numerous siderophores are non-ribosomal peptides,[19, 20] while several are biosynthesised independently.[21] Only a few reports were found on the synthesis of analogs of amphiphilic marine siderophores and may be due to the pathway for elaboration to the uncommon hydroxamate-containing siderophores is not as obvious. To investigate this important family of natural peptides, the development of a suitable and friendly strategy is desirable. Herein, we are reporting the convenient method for synthesising fully protected N^{δ}-acetyl-N^{δ}-hydroxy-L-ornithine, which can be employed for the preparation of peptide siderophores by Fmoc-based solid-phase peptide synthesis (SPPS). Additionally, this strategy involves the solid-phase removal of the Bz group before the final global deprotection step. The use of this approach has allowed the first synthesis of marine siderophores amphibactin-T and moanachelin ala-B.

Results and Discussion:

The suites of amphibactin and moanachelin siderophores are the composition of the uncommon N^{δ}-hydroxy-N^{δ}-acetyl-ornithine amino acid group at first, third and fourth positions, including natural amino acids (Ser, Gly, or Ala) at the second position, and various lengths of (un)saturated aliphatic fatty acids at the *N*-terminus (Fig. 1). For the preparation of these siderophores on solid-phase using Fmoc/*t*Bu strategy, our approach was to synthesize the orthogonally protected Orn building block 'N^{α}-Fmoc-N^{δ}-(acetyl)-N^{δ}-(hydroxy-protected)-ornithine' (Fig. 2).

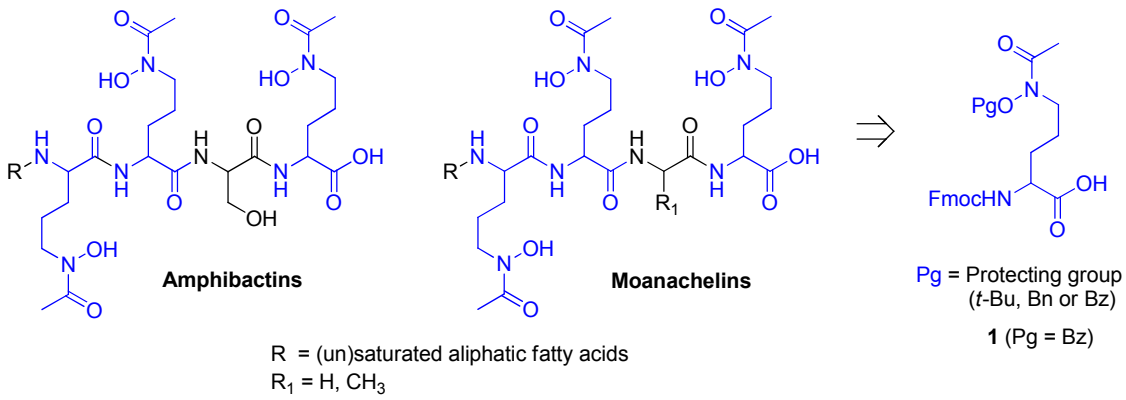
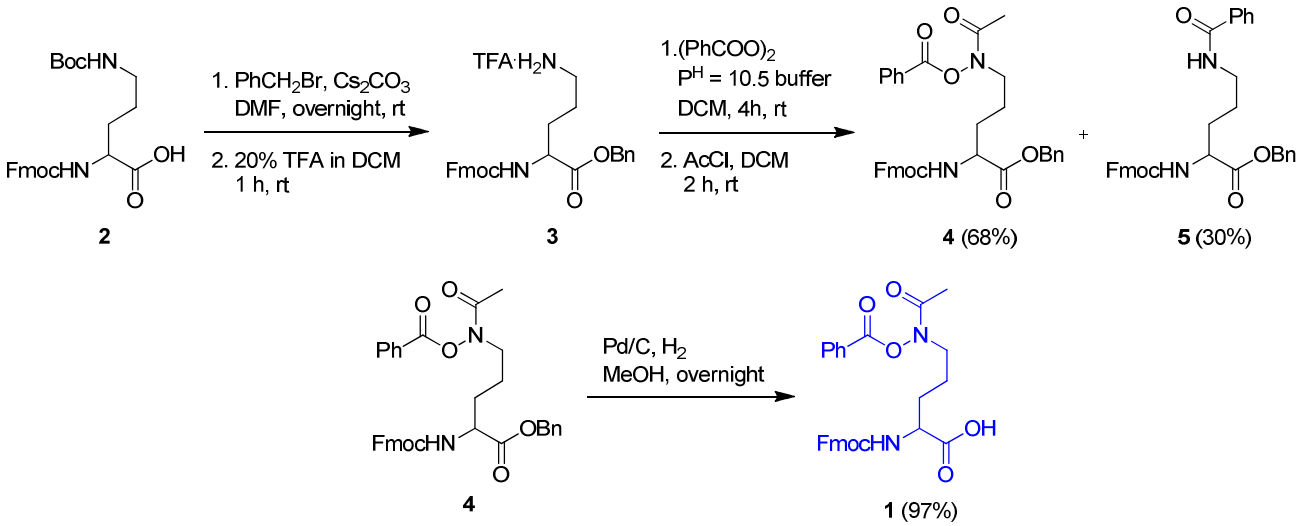


Fig. 2. The structure of Orn building block for synthesis of amphibactins and moanachelins.

Several methods have been reported for the synthesis of N^{δ}-(hydroxy)-ornithine amino acid, in which most of them started from glutamic acid and involved multi-step processes and ended with low yields.[22-26] The unprotected hydroxyl Orn derivative, N^{δ}-acetyl-N^{δ}-hydroxy-L-ornithine, has also been synthesized from Cbz-L-Glu-O*t*Bu in 23% overall yield.[27] For the solid-phase approach, it is fundamental to protect the orthogonal functional groups of amino acids to avoid the formation of side products. The synthesis of N^{δ}-(acetyl)-N^{δ}-(hydroxy-protected)-ornithine (**1**) amino acid involves hydroxylation on the side chain amino group, protection of the hydroxyl group followed by acetylation or *vice versa*. To evade these three synthetic steps Mileweska *et al.* developed a direct oxidation step on primary amines (Cbz-L-Orn-O*t*Bu) by benzoyl peroxide (BPO) in the presence of Na_2CO_3 as the base, followed by acetylation to give Cbz-L-Orn(Ac,OBz)-O*t*Bu and the Bz removal was easily achieved using NH_3/MeOH.[28] The same BPO oxidation method was used on N^{α}-Boc protected Orn derivative, Boc-L-Orn-O*t*Bu, for the synthesis of Boc-L-Orn(OBz)-O*t*Bu.[29] In recent years, progress in the synthetic strategy of BPO oxidation was achieved in the presence of buffer (0.75 M $NaHCO_3$–1.5 M NaOH, pH = 10.5)/DCM (1:1) on N^{α}-Cbz/Boc protected Lys and debenzoylation was easily accomplished under mild basic conditions.[30, 31] A recent article from Fujii *et al.* described the synthesis of N^{α}-Fmoc-protected N^{δ}-acetyl-N^{δ}-*tert*-butoxy-L-ornithine [Fmoc-L-Orn(Ac,O*t*Bu)-OH] for the Fmoc-based solid-phase path, in which the building block was obtained in an overall yield of 29% in eight steps starting from the commercially available Cbz-Glu(*t*Bu)-OH.[32] Another important recent article was from Meijler *et al.* that describes the synthesis of N^{α}-protected formyl

hydroxyl ornithine, N^{α}-Fmoc-N^{δ}-formyl-N^{δ}-benzyloxy-L-ornithine [Fmoc-L-Orn(formyl,Bn)-OH], from commercially available Boc-L-Orn-O*t*Bu with an overall yield of 23% (five steps).[33]

The earlier methods involved many synthetic steps and delivered the final compound in very low isolated yields. With these conserved features in mind, herein, we described an efficient methodology from the past to prepare a fully protected Orn derivative. This approach involves only three synthetic steps with an exceptional yield of the final building block (**1**). The synthesis of compound **1** began with commercially available N^{α}-Fmoc protected Orn amino acid, Fmoc-L-Orn(Boc)-OH (L-form or D-form of Orn derivatives could be used) **2**, which was protected with benzyl group to afford the ester. Removal of the side chain Boc group was easily carried out using 20% TFA in DCM resulting Fmoc-L-Orn-OBn▪trifluoro acetate **3**, in quantitative yield (Scheme 1). Compound **3** was oxidized by treatment with benzoyl peroxide, followed by acetylation in a one-pot method developed by Wang *et al.*,[34] to furnish the desired compound **4**, Fmoc-L-Orn(Ac,OBz)-OBn, in 68% yield and a benzamide by-product **5**, Fmoc-L-Orn(Bz)-OBn, was also isolated in 30% yield. Finally, the compound **4** on treatment with hydrogen in the presence of Pd/C catalyst resulted in hydrogenolysis of the benzyl protected ester to accomplish the final fragment **1**, N^{α}-Fmoc-N^{δ}-(acetyl)-N^{δ}-(benzoyloxy)-L-ornithine [Fmoc-Orn(Ac,OBz)-OH], in excellent isolated yield (97%). In our initial attempts, allyl and ethyl groups were used for acid protection in the first step, but the final deprotection of these groups did not provide good yields and led to mixtures of by-products.

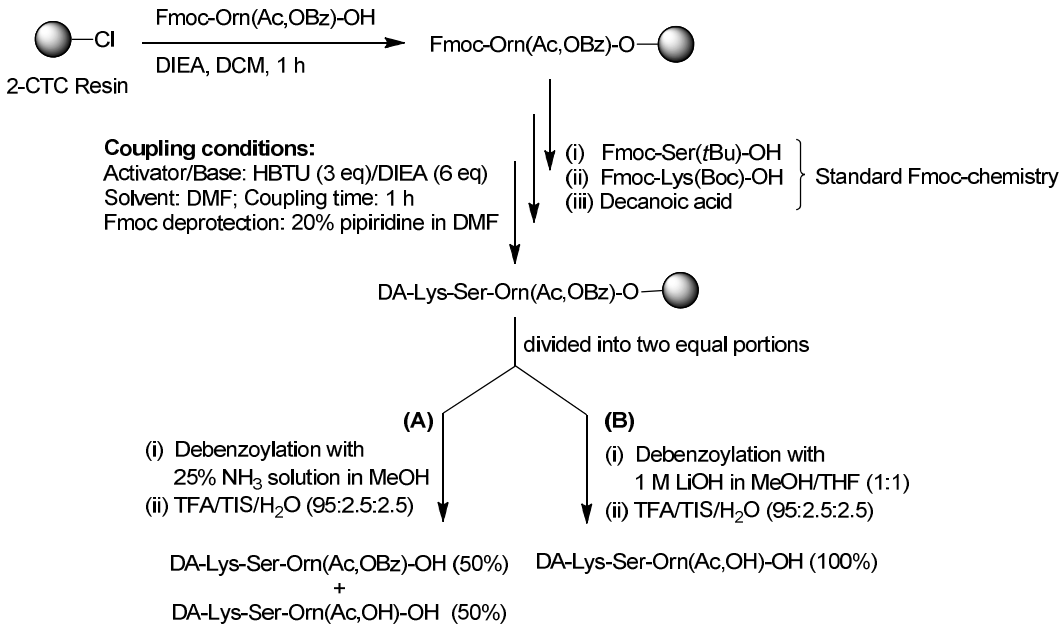


2 **3** **4** (68%) **5** (30%)

4 **1** (97%)

Scheme 1. Synthetic route for 'Fmoc-Orn(Ac,OBz)-OH' building block.

With the sufficient key compound 'Fmoc-Orn(Ac,OBz)-OH' (**1**) in hand, we have prepared a model peptide to observe its stability nature under solid-phase conditions. Peptide with the sequence of DA-Lys-Ser-Orn(OH,Ac)-OH was prepared on 2-CTC resin (83 mg, 0.05 mmol, 0.6 mmol/g) under standard Fmoc-chemistry protocols (Scheme 2).[35, 36] To optimize the debenzoylation process, the resin was divided into two equal portions; first portion of resin was treated with 25% NH_3 in MeOH (3× 30 min) where as other portion was treated with 3 M LiOH in THF/MeOH (1:1) (2 × 30 min). Global cleavage from the resin was carried out with TFA/TIS/H_2O (95:2.5:2.5) (2 × 30 min) and subjected for HPLC purity. HPLC analysis of crude peptides showed an excellent purity in both cases (Fig. 3). Remarkably, debenzoylation with LiOH was obtained in more than 95% purity with 100% debenzoylation, but in the case of NH_3 treatment only a 50% formation of product was observed (Fig. 3). We have found that even 1 M LiOH solution is sufficient for debenzoylation.



Scheme 2. The synthetic route involved for model peptide DA-Lys-Ser-Orn(Ac,OH)-OH.

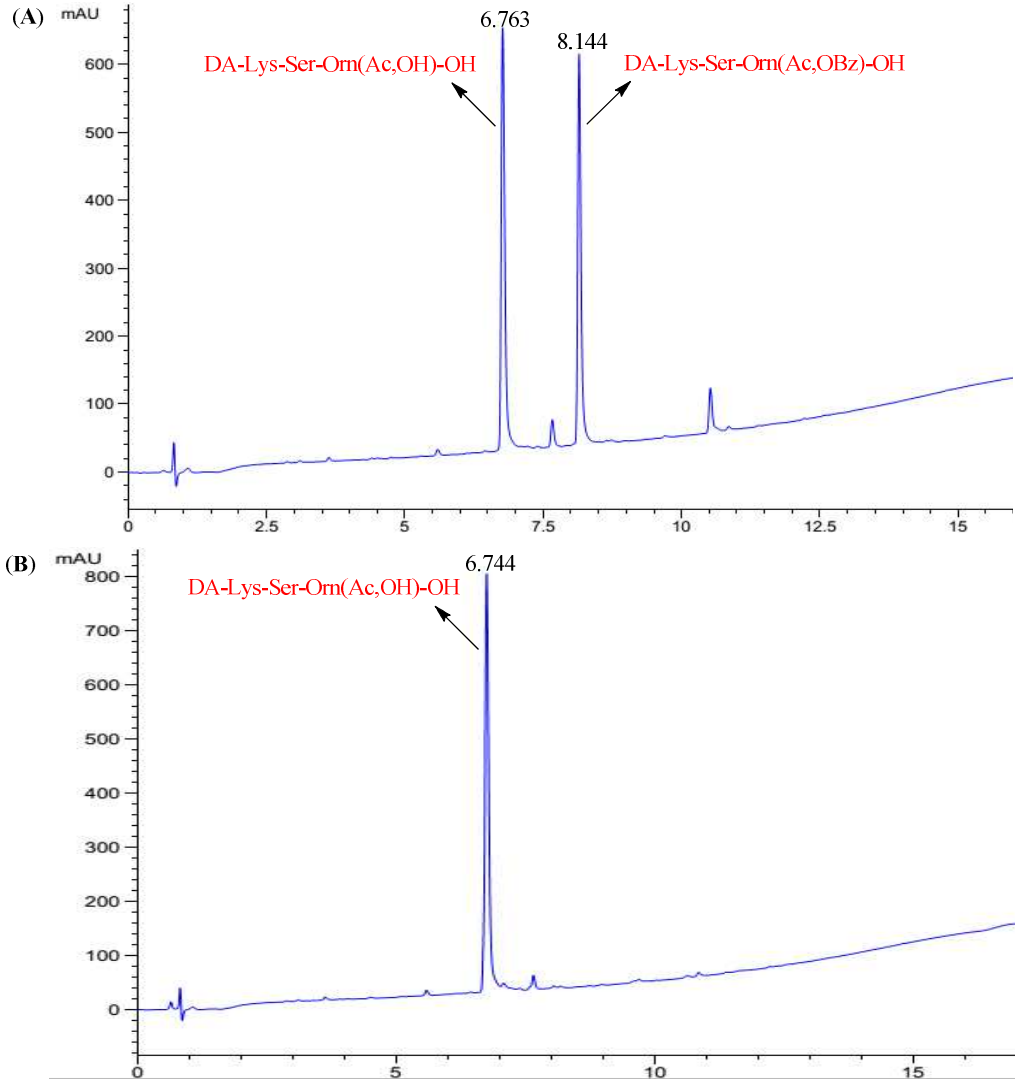
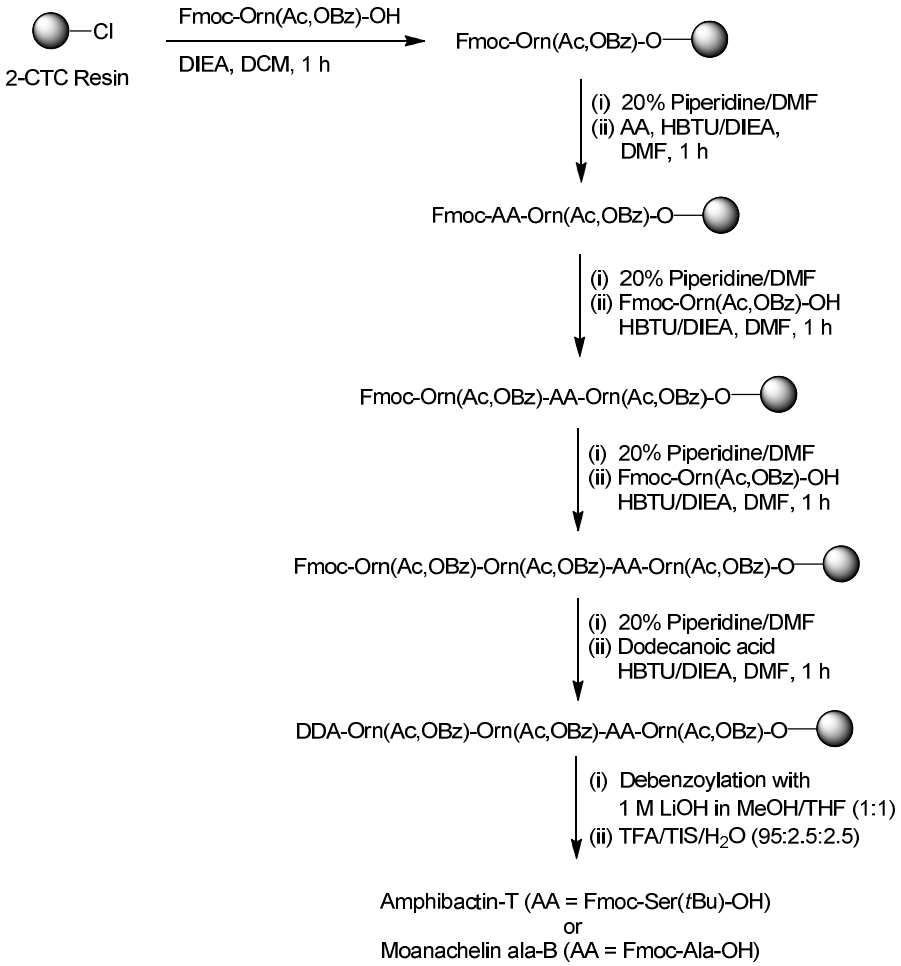


Fig. 3 HPLC Conditions: Column: Phenomenex column C18 (3 µm x 4.6 x 50 mm); Eluent: 5-95% CH_3CN/0.1% TFA in 15 min; Sol. A: 100% H_2O/0.1% TFA; Sol. B: 100% CH_3CN/0.1% TFA; Wavelength: 220 nm; Flow rate: 1 mL/min; HPLC chromatograms of crude peptides directly after precipitation of the cleavage cocktail. (**A**) Debenzoylation with 25% NH_3 in MeOH. (**B**) Debenzoylation with 1 M LiOH in THF/MeOH.

After successful synthesis of the model peptide, DA-Lys-Ser-Orn(Ac,OH)-OH, on solid-phase employing the Orn building block [Fmoc-Orn(Ac,OBz)-OH], we could conclude that the N^{δ}-hydroxy-N^{δ}-acetyl-ornithinyl residue protected with the Bz group is very much stable under these conditions. Furthermore and very importantly, removal of the Bz group can be done with excellent yields in rather mild conditions and totally compatible with a peptide scaffold.

We have used this key building block (**1**) for the synthesis of amphibactin-T and moanachelin ala-B siderophores. For this purpose, we anchored Fmoc-Orn(Ac,OBz)-OH to 2-CTC resin (50 mg, 0.6 mmol/g loading) to render the starting resin Fmoc-Orn(Ac,OBz)-O-CTC-resin (Scheme 3). Elongation of the peptide chain was carried out consecutively with Fmoc-Ser(*t*Bu)-OH (for amphibactin-T)/Fmoc-Ala-OH (for moanachelin ala-B), Fmoc-Orn(Ac,OBz)-OH, Fmoc-Orn(Ac,OBz)-OH, and dodecanoic acid (Scheme 3). No recouplings were done. At the end of the syntheses, debenzoylation has been carried out by treatment of the peptide resins with 1 M LiOH in THF/MeOH (1:1) (2 × 30 min), followed by global cleavage from resin with TFA/TIS/H_2O (95:2.5:2.5) (2 × 30 min) rendered both peptides with excellent yields 68% and 76%, respectively (Fig. 4).



Scheme 3. The synthetic route involved for amphibactin-T and moanachelin ala-B siderophores.

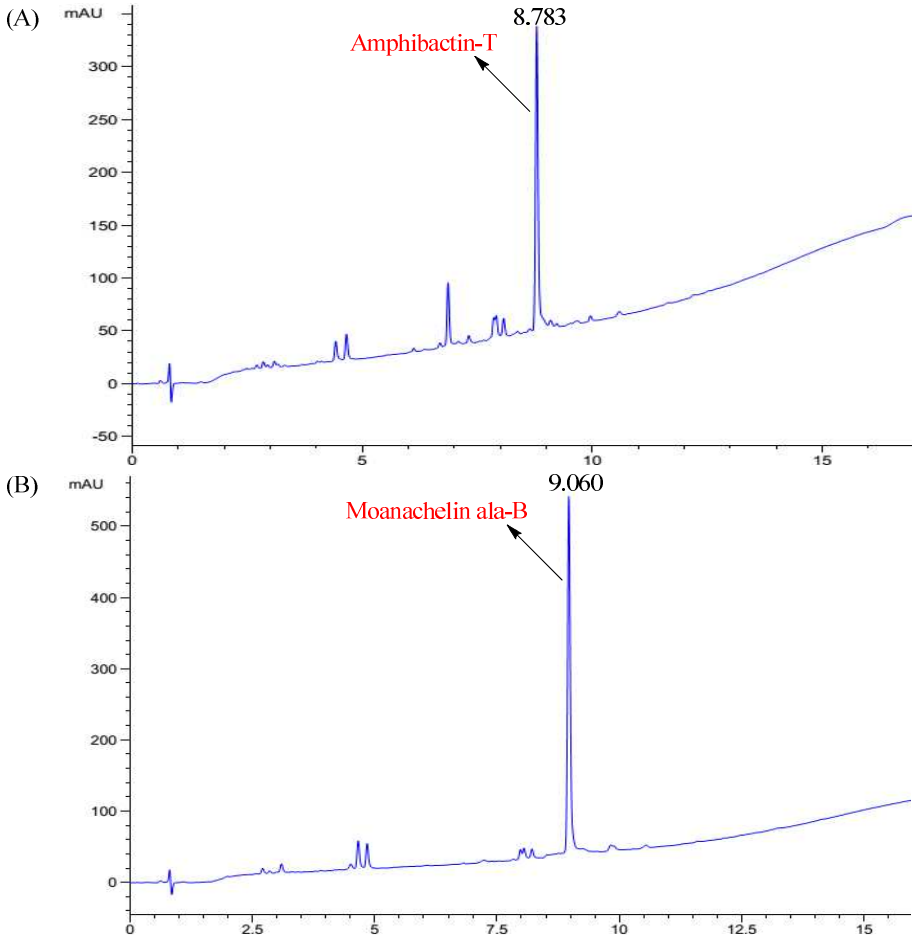


Fig. 4 HPLC Conditions: Column: Phenomenex column C18 (3 µm x 4.6 x 50 mm); Eluent: 5-95% CH_3CN/0.1% TFA in 15 min; Sol. A: 100% H_2O/0.1% TFA; Sol. B: 100% CH_3CN/0.1% TFA; Wavelength: 220 nm; Flow rate: 1 mL/min; HPLC chromatograms of crude peptides directly after precipitation of the cleavage cocktail. (**A**) HPLC chromatogram of crude amphibactin-T. (**B**) HPLC chromatogram of crude moanachelin ala-B.

Experimental Section:

General Information:

Chemicals were obtained from commercial sources and used as received. 2-CTC resin, Fmoc-amino acids, and HBTU were purchased from GL Biochem Pvt. Ltd., China. DIEA, piperidine, TFA, AcCl, benzyl bromide, Cs_2CO_3, Pd/C, benzoyl peroxide, LiOH, decanoic acid, dodecanoic acid, DMF, DCM, and MeOH were from Sigma-Aldrich, Germany/Merck, Germany/Fluka.

Agilent 1100 analytical HPLC system and phenomenex column C18 (3 μm x 4.6 x 50 mm) were used for reaction monitoring with wavelength at 220 nm at a flow rate of 1 mL/min using 0.1% TFA in H_2O as mobile phase A and 0.1% TFA in ACN as mobile phase B with a linear gradient of 5-95% of B into A over a period of 15 min. The intermediates were monitored by Shimadzu 2020 UFLC-MS using an YMC-Triart C18 (5 μm x 4.6 × 150 mm) column. Buffer A: 0.1% formic acid in H_2O; buffer B: 0.1% formic acid in ACN. Crude peptides were purified on Shimadzu semi-preparative HPLC using phenomenex C18 column (10 μm x 10 x 250 mm) with a wavelength at 220 nm at a flow rate of 7 mL/min over 30 min. Mobile phases used were same as those described for analytical HPLC. High resolution mass spectrometry (HRMS) was performed using a Bruker ESI-QTOF mass spectrometer in positive mode. Thin layer chromatography (TLC) was performed using DC-Fertigfolien ALUGRAM® Xtra Sil G/UV254 plates and TLC plates were visualized with ultraviolet light (254 nm) and/or in $KMnO_4$ staining solution. Purifications of products were performed by flash chromatography using silica gel 60 (40–63 μm particle size) from Sigma-Aldrich. Fractions were collected in test tubes and analyzed either using TLC. NMR spectra (^1H and ^{13}C) were measured on a Bruker 400 MHz or 600 MHz instruments at 25 ± 2 °C in DMSO-d_6.

Synthesis of Fmoc-L-Orn-OBn▪TFA (3):

Cs_2CO_3 (1.43 g, 4.4 mmol) was added in one portion to a solution of Fmoc-Lys(Boc)-OH (1 g, 2.2 mmol) and benzyl bromide (379 μL, 3.3 mmol) in DMF (10 mL) and then stirred overnight at room temperature. When TLC (10% MeOH in DCM) indicated the consumption of the starting material, distilled water (20 mL) was added followed by extraction with EtOAc (3 × 20 mL). The combined organic phase was washed with water (2 × 20 mL), brine (20 mL), dried over anhydrous $MgSO_4$ and filtered. The solvent was removed under reduced pressure to give pale yellow color oil that was further treated with 20% TFA in DCM (20 mL) at room temperature. After 1 h stirring the volatiles were removed under vaccum to give a light brown color oil, which further washings with Et_2O (3–4 times) delivered the product, Fmoc-L-Orn-OBn▪TFA (**3**), as a white solid in quantitative yield (>99% pure). ^1H NMR (DMSO-d_6, 400 MHz): δ 8.01–8.16 (m, 3H), 7.89 (d, 2H, J = 7.6 Hz), 7.72 (d, 2H, J = 7.6 Hz) 7.39–7.43 (m, 3H), 7.30–7.36 (m, 7H), 5.13 (s, 2H), 4.19–4.35 (m, 3H), 4.08–4.13 (m, 1H), 2.73–2.81 (m, 2H), 1.61–1.85 (m, 4H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 172.0, 156.2, 143.8, 140.8, 135.8, 128.4,

128.0, 127.8, 127.7, 127.1, 125.2, 120.2, 66.0, 65.8, 53.6, 46.6, 38.2, 27.5, 23.8; HRMS (ESI-QTOF) Calc. for $C_{27}H_{28}N_2O_4$ 445.2121, found 445. 2167.

Synthesis of Fmoc-L-Orn(Ac,OBz)-OBn (4) and Fmoc-L-Orn(Bz)-OBn (5):

Benzoyl peroxide (with 25% H_2O) (867 mg, 3.6 mmol) was added quickly to a stirring biphasic mixture of Fmoc-L-Orn-OBn▪TFA (**3**) (1.0 g, 1.8 mmol) in a 1:1 ratio of buffer solution (20 mL, 0.75 M $NaHCO_3$ and 1.5 M NaOH, pH = 10.5) and dichloromethane (DCM; 20 mL) at room temperature. After stirring for 4 h, TLC (5% NH_3 in MeOH) indicated the complete consumption of the starting material. A solution of the acetyl chloride (200 μL, 2.7 mmol) in 1 mL of DCM was added to the reaction mixture and stirred for 2 h. The layers were separated, and the aqueous portion was extracted with DCM (3 × 15 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated to get pale yellow oil. The crude product was purified by column chromatography over silica gel to isolate the *O*-benzoyl hydroxamate in 68% (726 mg). **Fmoc-L-Orn(Ac,OBz)-OBn (4)**: colorlessoil; R_f = 0.63 (50% EtOAc in hexane); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.04 (d, 2H, J = 7.6 Hz), 7.86–7.90 (m, 3H), 7.75 (t, 1H, J = 7.6 Hz), 7.68–7.70 (m, 2H), 7.57 (t, 2H, J = 7.6 Hz), 7.41 (t, 2H, J = 7.6 Hz), 7.28–7.34 (m, 7H), 5.12 (s, 2H), 4.17–4.32 (m, 3H), 4.10–4.16 (m, 1H), 3.71–3.80 (m, 2H), 2.01 (br s, 3H), 1.61–1.85 (m, 4H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 179.8, 172.0, 156.1, 143.8, 143.7, 140.7, 136.8, 135.9, 129.7, 129.1, 128.4, 128.0, 127.6, 127.0, 125.2, 121.3, 120.1, 120.0, 109.7, 65.9, 65.7, 53.6, 46.6, 27.7, 25.7, 20.2; HRMS (ESI-QTOF) Calc. for $C_{36}H_{34}N_2O_7$ 607.2438, found 607.2403; **Fmoc-L-Orn(Bz)-OBn (5):** 30% (326 mg), colorless solid, R_f = 0.54 (50% EtOAc in hexane); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.46 (t, 1H, J = 5.4 Hz), 7.84–7.90 (m, 5H), 7.72 (d, 2H, J = 7.6 Hz), 7.38–7.54 (m, 5H), 7.28–7.34 (m, 8H), 5.13 (s, 2H), 4.27–4.35 (m, 1H), 4.20–4.24 (m, 1H), 4.13–4.19 (m, 1H), 3.12–3.26 (m, 2H), 1.55–1.87 (m, 4H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 172.3, 166.2, 156.2, 143.8, 143.7, 140.7, 136.0, 134.7, 131.0, 128.4, 128.2, 128.0, 127.7, 127.2, 127.1, 125.2, 120.1, 65.9, 65.7, 53.9, 46.7, 38.6, 28.2, 25.8; HRMS (ESI-QTOF) Calc. for $C_{34}H_{32}N_2O_5$ 549.2383, found 549.2384.

Synthesis of Fmoc-L-Orn(Ac,OBz)-OH (1):

To a solution of **4** (726 mg, 1.2 mmol) in MeOH (15 mL), 10% Pd/C (25 mg, 1 mol%) was added, a balloon with hydrogen was attached and the reaction mixture was stirred under

hydrogen atmosphere for overnight at room temperature. The catalyst was filtered off through a Celite pad, the solid on the filter was washed with MeOH (20 mL), and the volatiles were removed under reduced pressure providing colorless oil. The crude material was chromatographed on silica gel (50% EtOAc in hexane) to afford the product as thick syrup, which upon titration with Et_2O (4–5 times) gave a colorless foam compound. Lyophilization delivered **1** as a white foam (602 mg, 97%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.6 (br s, 1H), 8.05 (d, 2H, J = 7.6 Hz), 7.88 (d, 2H, J = 7.6 Hz), 7.66–7.77 (m, 4H), 7.57 (t, 2H, J = 7.6 Hz), 7.41 (t, 2H, J = 7.6 Hz), 7.31 (t, 2H, J = 7.6 Hz), 4.19–4.27 (m, 3H), 3.94–4.02 (m, 1H), 3.72–3.81 (m, 2H), 2.01 (br s, 3H), 1.61–1.85 (m, 4H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 177.6, 173.8, 156.2, 143.8, 143.7, 140.7, 136.3, 129.7, 129.2, 127.6, 127.1, 125.3, 120.1, 120.0, 65.6, 53.4, 46.6, 27.9, 25.7, 20.2; HRMS (ESI-QTOF) Calc. for $C_{29}H_{28}N_2O_7$ 517.1969, found 517.1975.

Synthesis of amphibactin-T and moanachelin ala-B:

2-CTC resin (50 mg, 0.6 mmol/g loading) was placed in a 5 mL polypropylene syringe fitted with a polyethylene filter disc. The resin was washed with DMF (3 × 2 mL, 1 min) followed by DCM (3 × 2 mL, 1 min). The synthetic protocol involved was: (i) Coupling: Fmoc-Orn(Ac,OBz)-OH (16 mg, 1 equiv), DIEA (31 μL, 6 equiv) dissolved in 0.5 mL DCM, 1 h; then addition of MeOH (50 μL), 30 min (ii) Washings: DCM (3 × 2 mL), DMF (3 × 2 mL); (iii) Fmoc removal: 20% piperidine (2 × 10 min); (iii) Washings, coupling: Fmoc-Ser(*t*Bu)-OH (3 equiv, for amphibactin-T)/Fmoc-Ala-OH (3 equiv, for moanachelin ala-B), HBTU (3 equiv), DIEA (6 equiv) dissolved in 0.5 mL DMF, 1 h; (iv) Washings, Fmoc removal: 20% piperidine (2 × 10 min); (v) Washings, coupling: Fmoc-Orn(Ac,OBz)-OH (3 equiv), HBTU (3 equiv), DIEA (6 equiv) dissolved in 0.5 mL DMF, 1 h; (vi) Washings, Fmoc removal: 20% piperidine (2 × 10 min); (vii) Washings, coupling: Fmoc-Orn(Ac,OBz)-OH (3 equiv), HBTU (3 equiv), DIEA (6 equiv) dissolved in 0.5 mL DMF, 1 h; (viii) Washings, Fmoc removal: 20% piperidine (2 × 10 min); (ix) Washings, coupling: dodecanoic acid (3 equiv), HBTU (3 equiv), DIEA (6 equiv) dissolved in 0.5 mL DMF, 1 h; (x) Washings, debenzoylation with 1 M LiOH in THF/MeOH (1:1) (2 × 30 min). (xi) Washings, H_2O (3 × 2 mL), DMF (3 × 2 mL) and DCM (3 × 2 mL). In all cases the ninhydrin test was negative.[37] The protected peptide was cleaved from the resin by TFA/TIS/H_2O (95/2.5/2.5) (3 × 30 min), the filtrate was collected in 50 mL round bottom flask

and TFA evaporated under reduced pressure. Cold Et_2O was added to the flask to give the product as a white precipitate that was subsequently purified by semi-preparative HPLC. Amphibactin-T (16.5 mg, 68%, pale yellow solid): HRMS (ESI-QTOF) Calc. for $C_{36}H_{65}N_7O_{13}$ 804.4713, found 804.4707; Moanachelin ala-B (18.1 mg, 76%, pale yellow solid): HRMS (ESI-QTOF) Calc. for $C_{36}H_{65}N_7O_{12}$ 788.4763, found 788.4770.

Abbreviations: AcCl, acetyl chloride; ACN, acetonitrile; Bn, benzyl; Boc, *tert*-butyloxycarbonyl; *t*-Bu, *tert*-butyl; Bz, benzoyl; Cbz, carboxybenzyl; 2-CTC, 2-chlorotrityl chloride; DA, decanoic acid; DDA, dodecanoic acid; DIEA, diisopropylethylamine; DMF, dimethylformamide; Et_2O, diethyl ether; Fmoc, 9-fluorenylmethoxycarbonyl; HBTU, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; Pd/C, palladium on carbon; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TLC, thin-layer chromatography.

Conclusion:

In summary, efficient syntheses of marine siderophores amphibactin-T and moanachelin ala-B on solid-phase have been reported. Importantly, a convenient synthetic route has been developed for the preparation of 'N^{α}-Fmoc-N^{δ}-(acetyl)-N^{δ}-(benzoyloxy)-ornithine' [Fmoc-Orn(Ac,OBz)-OH], as well as a method for removal of the Bz group on solid-phase. This method has shown to be efficient for the removal of three Bz groups. This full strategy is being applied to the preparation of other members of this family of amphiphilic marine siderophores such as marinobactins, amphibactins, aquachelins, loihichelins, and moanachelins.

Associated Content:

Electronic supplementary material contains copies of ^{1}H and ^{13}C NMR, HPLC profiles and HRMS (ESI-QTOF) mass spectrum profiles of intermediates/products. This material is available free of charge *via* the internet at http://pubs.rsc.org/

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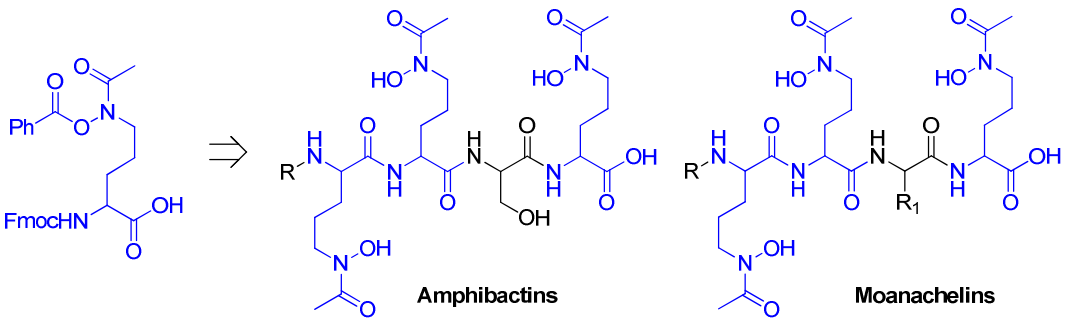
Graphical Abstract



R = (un)saturated aliphatic fatty acids
R_1 = H, CH_3