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Total Synthesis of the Highly *N*-Methylated Acetylene-Containing Anticancer Peptide Jahanyne

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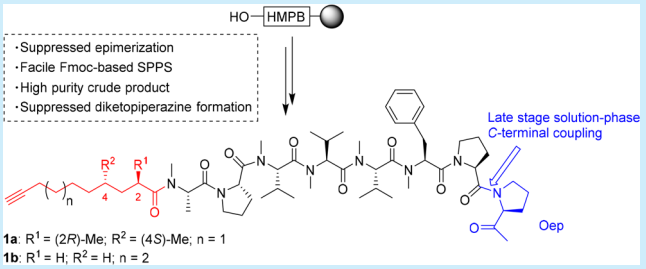
Supporting Information

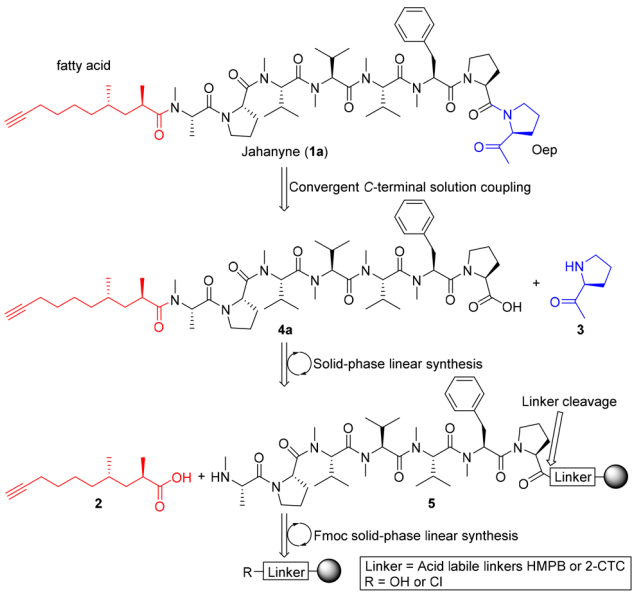
ABSTRACT: The first total synthesis of the highly *N*-methylated acetylene-containing lipopeptide jahanyne, an apoptosis-inducing natural product from marine cyanobacteria, is reported. A late-stage solution-phase coupling enabled introduction of the *C*-terminal ketone pyrrolidine moiety. A modified Fmoc solid-phase synthesis strategy was adopted to effectively couple multiple sterically hindered *N*-methylated amino acids while suppressing epimerization. The total synthesis has enabled confirmation of the proposed absolute configuration of natural jahanyne.



Marine cyanobacteria are a well-known source of structurally unique bioactive molecules.[1,2] The biological activity of these intriguing natural products ranges from anticancer to antiviral activity, which makes these secondary metabolites attractive leads for biomedical research and drug discovery.[2] Several of these compounds have been isolated, such as dolastatin-10. Synthetic analogues of dolastatin-10 are clinically used as payloads in antibody−drug conjugates for cancer therapy.[3] A small subset of these secondary metabolites share an unusual terminal alkynyl fatty acid motif, including dragonamide, kurahyne, viridamides, carmabin, almiramides, and jahanyne.[4−9] These heavily *N*-methylated acetylene-containing lipopeptides exhibit a range of antimicrobial, antifungal, and antitumor activities; hence, further studies enabled by the chemical synthesis of this subfamily of lipopeptides may afford new leads for drug discovery.

In early 2015, Suenaga et al.[9] reported the isolation and characterization of a novel acetylene-containing lipopeptide jahanyne (**1a**) from the marine cyanobacterium *Lyngbya* sp. (Scheme 1). Jahanyne (**1a**) contains the rare *N*-terminal acetylene-containing fatty acid (2*R*,4*S*)-2,4-dimethyldec-9-ynoic acid (**2**), a *C*-terminal (*S*)-1-(pyrrolidin-2-yl)ethan-1-one (Oep) (**3**), and multiple *N*-methylated amino acids. The substitution of canonical amino acids for *N*-methylated amino acids has been shown to alter biological functions of polypeptides such as improved stability, binding, and oral bioavailability.[10−12] This makes efficient incorporation of *N*-methyl amino acids of paramount importance for peptide-based drug discovery programs. However, the lack of efficient methods to synthesize highly *N*-methylated and acetylene-containing lipopeptides hampers detailed investigation of the properties and the biomedical potential of this family of secondary metabolites. To the best of our knowledge, no member of this family of acetylene-containing *N*-methylated lipopeptides has been synthesized using a 9-fluorenylmethoxycarbonyl (Fmoc)-solid-phase approach. Currently, the only methodology used to synthesize this class of lipopeptides is a solution-based approach.[4,5]

Scheme 1. Retrosynthetic Analysis of Jahanyne (1a)



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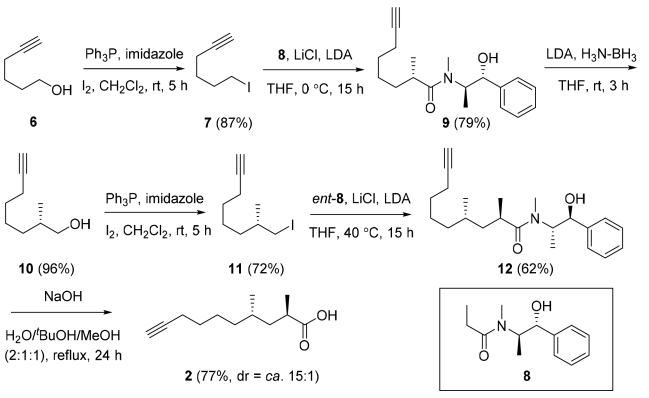
Herein, we describe the first total synthesis of jahanyne (**1a**) using a combination of solid-phase peptide synthesis and a late stage solution-phase coupling. It was envisaged that the total synthesis of **1a** would be achieved by a late stage convergent solution-phase coupling of Oep **3** with the *C*-terminal fragment **4a** (Scheme 1). Conceivably, intermediate **4a** would be synthesized by the solid-phase coupling of **5** with the *N*-terminal building block **2**, followed by mild linker cleavage. Assembly of the peptide chain **5** would be accomplished by a linear Fmoc SPPS approach on resin.

We envisaged that the required fatty acid **2** would be prepared in six steps from commercially available 5-hexyn-1-ol (**6**) (Scheme 2). Hence, the synthesis began by converting

Scheme 2. Synthesis of Fatty Acid Fragment 2



alcohol **6** to iodide **7** via an Appel reaction.[13] Asymmetric alkylation of the enolate generated from **8** with iodide **7**, under Myers conditions,[14] proceeded smoothly to afford amide **9**. Reductive cleavage of the chiral auxiliary in **9** afforded alcohol **10** which was then converted to **11**. Iodide **11** was employed in asymmetric alkylation of the enolate generated from *ent*-**8** to afford **12** thus installing the required 2,4-*anti*-dimethyl stereochemistry in the alkyl chain of amide **12**. Finally, basic hydrolysis of pseudoephedrine amide **12** afforded the target carboxylic acid **2** (dr = *ca.* 15:1). To confirm the absolute stereochemistry, the (*S*)-phenylglycine amide of **2**, prepared in the original isolation paper from **2** obtained by degradation of the isolated natural product sample, was also synthesized (see Supporting Information). The spectral data of both samples were in excellent agreement, confirming that the fatty acid moiety of the natural product possessed the 2,4-*anti*, (2*R*,4*S*) configuration.[9]

The requisite *C*-terminal Oep **3** was synthesized following a literature protocol.[15]

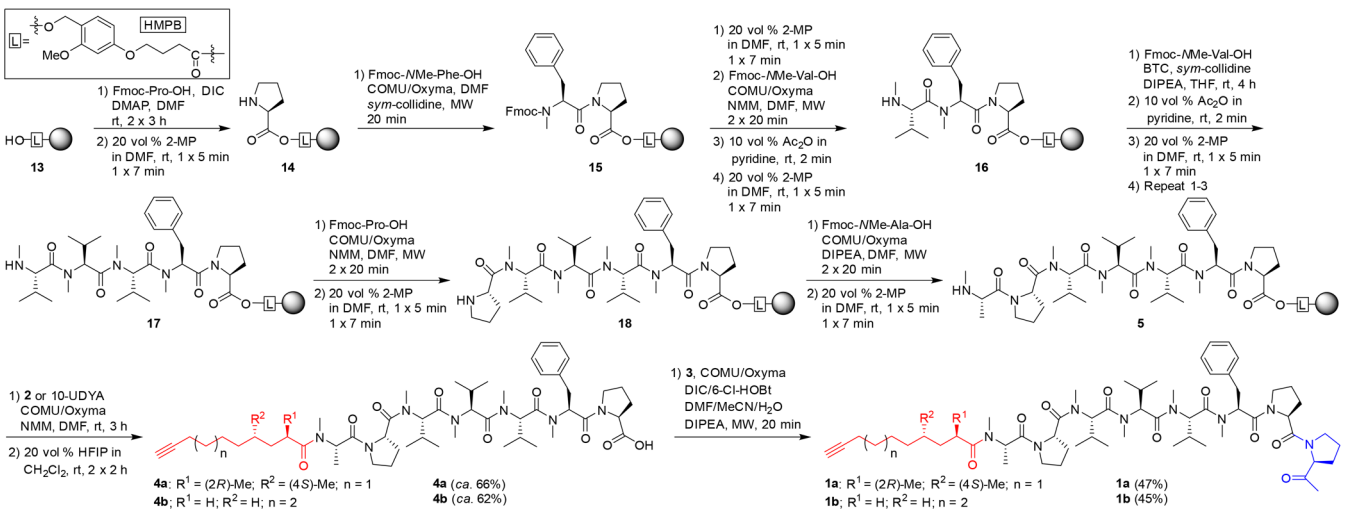
With the necessary building blocks in hand, we then focused on elaboration of the *N*-methylated peptide sequence. The particular challenge of synthesizing *N*-methylated peptides is their poor reactivity in SPPS due to steric hindrance imposed by the additional methyl group.[11,16] Yields are additionally compromised by diketopiperazine (DKP) formation which is prevalent in both the *N*-methyl amino acid coupling and Fmoc-removal steps.[16,17] Furthermore, epimerization at the α-*C* position of activated *N*-methyl amino acids during the coupling step is also problematic.[11,16]

We avoided using the *tert*-butyloxycarbonyl (Boc) protecting group strategy for SPPS, due to the documented acid lability of *N*-methylated amino acids, which are prone to fragment as well as undergo DKP formation.[17,18] We therefore examined linkers suitable for Fmoc SPPS that can be cleaved under mild conditions and have sufficient steric hindrance on the solid support to help suppress DKP formation.

Our first synthetic approach focused on using the 2-chlorotrityl-functionalized polystyrene resin (2-CTC) pre-loaded with Pro (0.62 mmol/g) at elevated temperatures to promote coupling of hindered amino acids. Initial results indicated that 2-CTC was unstable to microwave (MW) conditions and the high temperature used in MW assisted couplings led to hydrolysis of the chlorotrityl ester bond resulting in premature cleavage of the peptide from the *C*-terminus (see pp S16−S19 in Supporting Information).[19]

Given our findings using 2-CTC, we next investigated the use of the mild acid labile 4-(4-hydroxymethyl-3-methoxy-phenoxy)butyric acid (HMPB) linker (Scheme 3). We attached Fmoc-Pro-OH to preformed HMPB-aminomethyl polystyrene resin **13** and obtained an acceptable loading of 0.4 mmol/g (66%) based on the Fmoc-release assay (see p S8 in the Supporting Information). The resulting HMPB handle of **14** is cleavable under mild conditions using hexafluoroisopropanol (HFIP) facilitating the ability to monitor the reaction by

Scheme 3. Total Synthesis of Jahanyne (1a) and Analogue 1b



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HPLC/MS analysis. Removal of the Fmoc group from the Fmoc-*N*-methylated amino acids was carried out using the hindered Fmoc-deblocking reagent 2-methylpiperidine (2-MP).[20−22] This was used as a precautionary measure to prevent the risk of DKP formation or epimerization of sensitive amino acids.[23,24] Pleasingly, 2-MP quantitatively removed the Fmoc-protecting group, in an acceptable reaction time (1 × 5 min, 1 × 7 min) with no detectable side reactions, and was used for all subsequent Fmoc-removals.

Attention next turned to the optimal choice of coupling reagents to efficiently couple sequential sterically hindered Fmoc-protected *N*-methylated amino acids. For the majority of the jahanyne sequence, a mixture of (1-cyano-2-ethoxy-2-oxoethylidenaminooxy) dimethylaminomorpholinocarbenium hexafluorophosphate (COMU) and ethyl 2-cyano-2-(hydroxyimino)acetate (Oxyma) was used as previously reported to facilitate hindered amide bond formation.[25,26] Epimerization of **15** at the Phe residue from the COMU/Oxyma mediated coupling between Fmoc-*N*Me-Phe-OH and resin bound **14** could be negated by using 2,4,6-trimethylpyridine (*sym*-collidine) as a weaker base (see Figure S7 in the Supporting Information). However, use of MW irradiation was critical to expedite the amidation of **14** to **15** and was used for the following coupling steps involving Fmoc-protected *N*-methylated amino acids.[27] Fmoc-removal of **15** resulted in a mixture of HPLC separable peaks with identical mass, which we attributed to *cis*/*trans* amide conformers.[17,27,28] This isomerism could be controlled by heating the HPLC column to 77 °C resulting in a single peak due to accelerated *cis*/*trans* interconversion, thereby enabling accurate monitoring of reactions (see Figures S8−S9 in the Supporting Information).[27]

Next, we focused on the synthesis of the adjacent sterically demanding *N*Me-Val sequence. Attempts to couple the first Fmoc-*N*Me-Val-OH to deprotected **15** using COMU/Oxyma with *sym*-collidine under MW irradiation resulted in low coupling efficiency. Use of *N*,*N*-diisopropylethylamine (DIPEA) not only quantitatively coupled Fmoc-*N*Me-Val-OH but also promoted epimerization. Employing *N*-methylmorpholine (NMM) and COMU/Oxyma cleanly yielded the desired Fmoc-protected peptide **16** (see Figures S11−S13 in the Supporting Information).

Disappointingly, despite extensive screening of optimal coupling reagents, coupling of the second and third bulky Fmoc-*N*Me-Val-OH to peptidyl resin **16** was low yielding (see Table S1 in the Supporting Information). Pleasingly, the H*N*Me-Val coupling steps were solved by generating the Fmoc-amino acyl chlorides *in situ* using bis(trichloromethyl)carbonate (BTC) as reported by Falb et al.[29] Fmoc-*N*Me-Val-OH in dry THF was activated with BTC, and *sym*-collidine was then added to neutralize the liberated HCl. DIPEA was also added to resin bound **16** to prevent premature acidic cleavage from the resin as well as promote the ensuing reaction. The activated Fmoc-*N*Me-Val-Cl was added to the peptidyl resin pretreated with DIPEA and stirred at room temperature for 4 h, resulting in quantitative conversion to **17** with no detectable epimerization.[16,29] The remaining amino acid couplings to afford resin bound **5** were effected using COMU/Oxyma and appropriate base under MW irradiation.

Having established a reliable synthetic method to form the linear backbone of jahanyne, we next investigated coupling of the acetylene-containing fatty acid moiety **2** to the *N*-terminus of **5**. For model studies, we utilized commercially available 10-undecynoic acid (10-UDYA) to couple with peptidyl resin **5** using COMU/Oxyma and NMM, with a prolonged reaction time of 3 h at room temperature, followed by HFIP cleavage to afford **4b**. We then successfully appended synthetic fatty acid **2** to peptidyl resin **5** using similar coupling conditions with that of **4b**. Treatment of the fully assembled resin-bound peptide with 20 vol % HFIP in CH_2Cl_2, and analysis by HRMS, confirmed the presence of the desired peptide **4a** as the major product (*ca.* 66% as judged by peak area integration of RP-HPLC, λ = 210 nm, t_R = 59.6 min) (Figure 1). Fragmentation

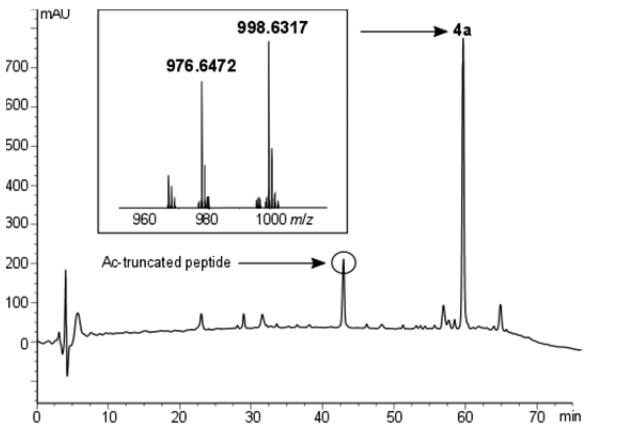

Figure 1. RP-HPLC analysis of crude **4a** using a Gemini C-18 column (5 μm, 10 mm × 250 mm). HRMS (EI): *m*/*z* $[M + H]^+$ calculated for $C_{54}H_{86}N_7O_9^+$: 976.6481, observed: 976.6472; $[M + Na]^+$ calculated for $C_{54}H_{85}N_7O_9Na^+$: 998.6301, observed: 998.6317.

of peptide **4a** was observed during HRMS, as a consequence of the labile *N*Me-Phe-Pro and *N*Me-Val-*N*Me-Phe amide bonds (see p S30 in the Supporting Information). The resulting crude peptide **4a** was then directly subjected to the final convergent solution-phase installation of the *C*-terminal Oep moiety.

We anticipated poor reactivity for the final solution-phase coupling of Oep **3** to linear peptide **4a**/**4b**, due to the hydrophobic properties of our heavily *N*-methylated peptide fragment and the need to effect formation of a tertiary amide bond. We therefore conducted model studies using Fmoc-Pro-OH to identify suitable coupling conditions. Initial studies using *N*,*N*′-dicyclohexylcarbodiimide (DCC) or *N*-(3-(dimethylamino)propyl)-*N*′-ethylcarbodiimide hydrochloride (EDC·HCl) to effect the coupling of Oep **3** to Fmoc-Pro-OH were disappointing. However, condensation using *N*,*N*′-diisopropylcarbodiimide (DIC) with 6-Cl-HOBt as an additive using DIPEA as a base was successful.

Unfortunately, extension of these model conditions to effect the desired coupling of Oep with **4a**/**4b** were unsuccessful. It was eventually determined that use of a modified cocktail of DIC/6-Cl-HOBt/DIPEA and COMU/Oxyma with DIPEA activation and MW irradiation successfully effected the hindered condensation of **4a**/**4b** with **3** (see Figures S26−S27 in the Supporting Information). Subsequent purification of the crude reaction mixture by RP-HPLC afforded jahanyne (**1a**, 47%, > 98% purity, t_R = 54.2 min) (Figure 2). Pleasingly, the spectral data for synthetic **1a** were observed to correspond well with those originally reported for the natural product **1a** (see pp S43−S62 in the Supporting Information).[9]

In summary, we have developed the first synthesis of enantiopure jahanyne (**1a**) and an analogue **1b** containing an achiral alkynoic acid, via Fmoc SPPS. The synthesis described herein not only confirmed the correct structure for the natural

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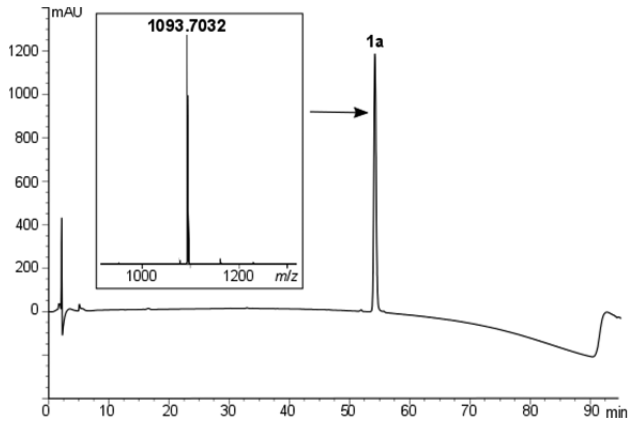

Figure 2. RP-HPLC analysis of purified **1a** using an XTerra MS C-18 column (5 μm; 4.6 mm × 150 mm). HRMS (EI): m/z [M + Na]$^+$ calculated for $C_{60}H_{94}N_8O_9Na^+$: 1093.7036, observed: 1093.7032.

product jahanyne (**1a**), but should also be applicable to the synthesis of other acetylene-containing lipopeptides which contain contiguous *N*-methylated amino acids.[4−8] Moreover, the methodology can be applied more generally to the synthesis of *N*-methylated peptides using an SPPS-based approach.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b03925.

Experimental procedures and spectral data (PDF)

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Notes

The authors declare no competing financial interest.

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