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Total Synthesis and Antimalarial Activity of Symplostatin 4

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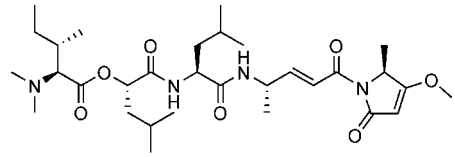
ABSTRACT



Symplostatin 4

The first total synthesis of symplostatin 4, a marine cyanobacterium-derived natural product, is described. Notable features of the route include the efficient preparation of three key fragments and final assembly to the natural product via sequential imide and amide couplings. Symplostatin 4 was also demonstrated to possess significant antimalarial activity (ED_{50} of 74 nM against *Plasmodium falciparum,* strain 3D7).

Symplostatin 4 (**1**, Figure 1) is a linear depsipeptide natural product of cyanobacterial origin, first isolated in 2009 from the genus *Symploca* sp. by Luesch and co-workers in Key Largo, Florida Keys.[1] Structurally, it comprises a peptidic backbone bearing a dimethylated *N*-terminal amino acid and *C*-terminal pyrrolinone moiety. Symplostatin 4 (**1**) has been shown to possess the same planar structure as gallinamide A (**2**) (Figure 1), isolated from the *Schizothrix* species of cyanobacteria collected off the Carribean coast of Panama in 2009,[2] but it has a diastereomeric configuration at the *N*-terminal amino acid (25*S*, 26*S*). In the case of gallinamide A, assignment of the absolute configurations of C25 and C26 has, to date, remained elusive.[1,2]

Both symplostatin 4 and gallinamide A also share structural features with dolastatin 15 (**3**),[3,4] dolastatin 10 (**4**),[5,6] and symplostatin 1 (**5**),[7] analogues of which have entered phase II clinical trials as anticancer agents (Figure 1).[8,9] Unique structural features of symplostatin 4 and gallinamide A include the depsipeptidic linkage, which in this instance is formed through a 2-hydroxyisocaproic acid residue and an unusual 4(*S*)-amino-2(*E*)-pentenoic acid unit. Initial biological screening of symplostatin 4 indicated only moderate cytotoxic activity (IC_{50} values of 12 and 53 μM against HeLa cervical carcinoma cells and HT-29 colon adenocarcinoma cells, respectively).[1] However, significantly, this natural product was also shown to synergize with another *Symploca*-derived natural product, largazole,[10] which may therefore present opportunities for the use of symplostatin 4 in the development of combination anticancer therapies. Gallinamide A has been reported to possess antimalarial

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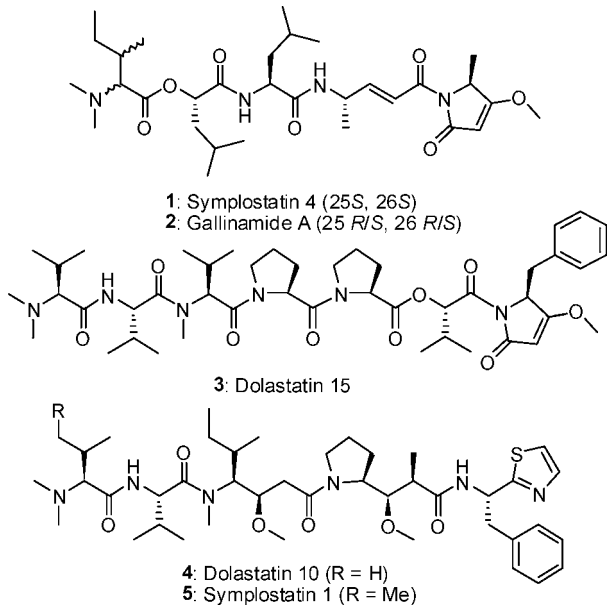


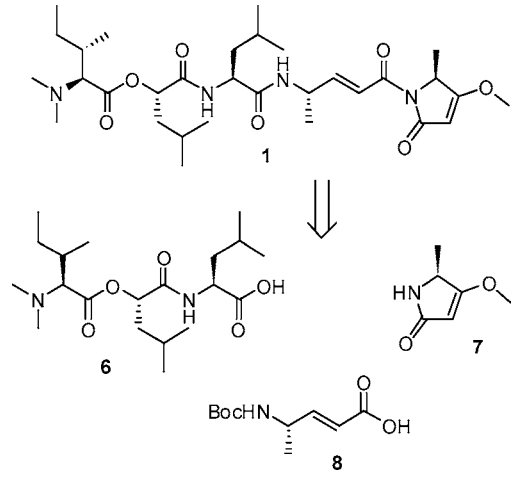
Figure 1. Symplostatin 4 (**1**) and structurally related marine natural products.

activity (IC_{50} of 8.4 μM against *Plasmodium falciparum*, W2 strain), moderate activity against mammalian Vero cells and, surprisingly, no detectable cytotoxicity to NCI-H460 human lung tumor or neuro-2a mouse neuroblastoma cell lines.[2] This is in stark contrast to dolastatin 10 and 15, which possess antimalarial activity but signficant cytotoxicity,[11] and suggests that symplostatin 4 and gallinamide A may serve as promising lead structures for the elucidation of antimalarial agents with low cytotoxicity.

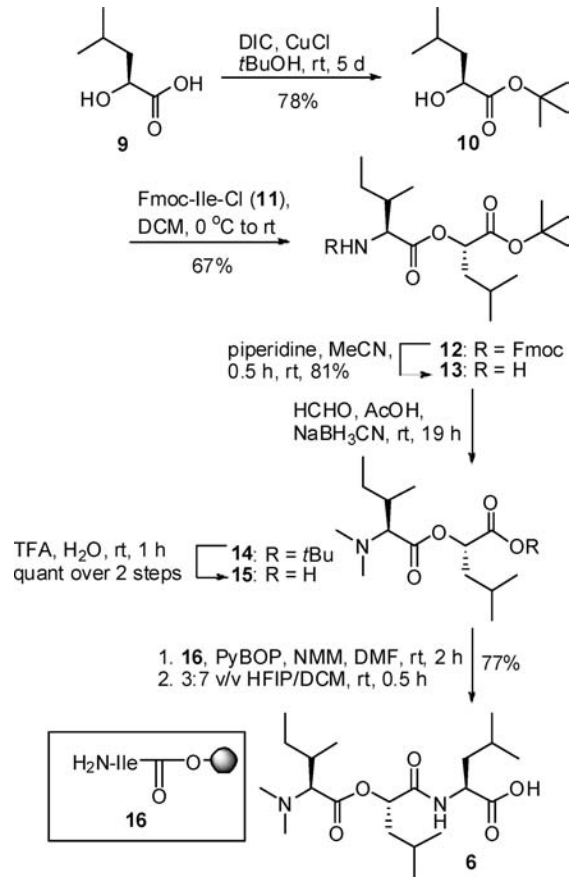
The interesting structural features of symplostatin 4, coupled with the intriguing pharmacological profile of this class of natural products,[12−14] prompted us to investigate the development of a succinct synthetic route. It was also anticipated that the approach described herein would prove amenable to the preparation of structurally related analogues, including gallinamide A (**2**). We envisaged that symplostatin 4 (**1**) could be assembled in a rapid and convergent manner by coupling three key fragment building blocks, namely depsipeptide **6**, pyrrolinone **7** and Boc-protected 4(*S*)-amino-2(*E*)-pentenoic acid **8** (Scheme 1).

The preparation of *N*-terminal ester **6** began from 2-hydroxy-L-isocaproic acid **9**, which upon treatment with DIC, CuCl, and *tert*-butyl alcohol, was converted to the corresponding *tert*-butyl ester **10** in good yield (78%, Scheme 2).[15] From here, reaction with the acid chloride of Fmoc-L-Ile-OH (**11**) proceeded smoothly and without epimerization (as determined by NMR spectroscopy) to give depsipeptide

Scheme 1. Retrosynthesis of Symplostatin 4



Scheme 2. Synthesis of Depsipeptide **6**



12 in 67% yield. Following esterification, removal of the *N*-terminal Fmoc carbamate (20% piperidine/MeCN) yielded the corresponding free amine **13** in 81% yield. At this stage, reductive amination of **13**, using formaldehyde and $NaBH_3CN$, afforded the desired dialkylated ester **14**. Facile acidolysis of the *C*-terminal *tert*-butyl ester of **14** with TFA gave the requisite amino acid **15** in essentially quantitative

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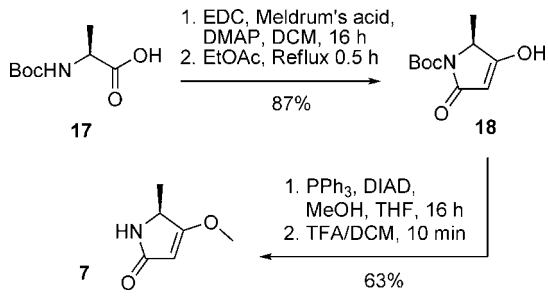
yield. Coupling of **15** to 2-chlorotrityl chloride resin preloaded with L-Leu **16** was readily achieved using PyBOP and NMM and was followed by mild acidolysis with hexafluoro-2-propanol (HFIP) to complete the desired key depsipeptide fragment **6** (77%).

With *N*-terminal depsipeptide fragment **6** now in hand, attention turned to the preparation of the required pyrrolinone fragment **7** (Scheme 3). Toward this end, Boc-L-alanine **17**

Scheme 3. Synthesis of Pyrrolinone Fragment **3**



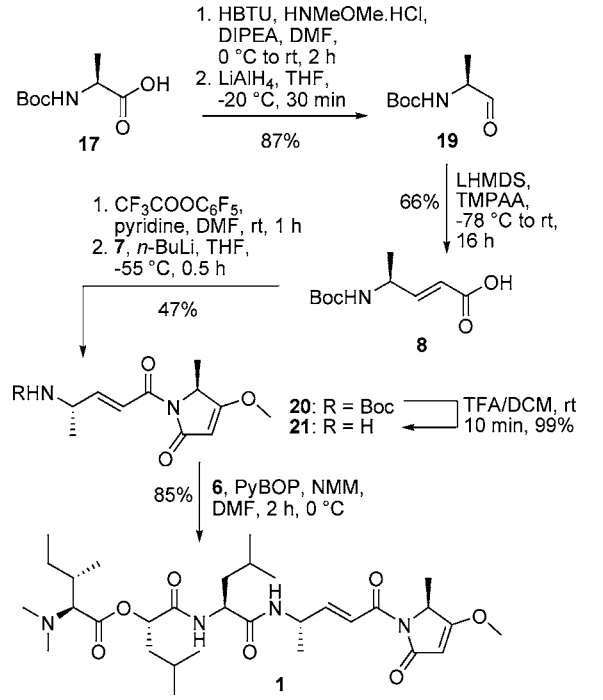
was condensed with Meldrum's acid using EDC and DMAP. Subsequent reflux in ethyl acetate facilitated the anticipated thermal cyclization[16] to the desired pyrrolinone **18** in 87% yield over two steps. Alkylation of the enol functionality under Mitsunobu conditions selectively generated the desired 4-methoxypyrrolinone and was followed by acidolysis of the Boc-carbamate with TFA to complete the preparation of **7** in good yield (63%).

Preparation of the unusual 4(*S*)-amino-2(*E*)-pentenoic acid subunit **8** commenced from Boc-L-Ala-OH (**17**) (Scheme 4). Conversion to the corresponding Weinreb amide, followed by reduction with $LiAlH_4$, provided Boc-L-alaninal **19** in 87% yield over the two steps.[17] From here, completion of the desired α,β unsaturated amino acid was neatly achieved using a Horner−Wadsworth−Emmons reaction[18] between **19** and trimethylphosphonoacetic acid (TMPAA) to give **8** in 66% yield and with complete selectivity for the desired *E*-isomer.

In order to facilitate acylation of the pyrrolinone moiety, fragment **8** was first activated as the corresponding pentafluorophenyl (Pfp) ester using pentafluorophenyl trifluoroacetate and pyridine (Scheme 4).[19] Deprotonation of **7**, followed by addition of the Pfp ester, successfully generated the desired coupled product **20** with no detectable epimerization as determined by NMR spectroscopy.[16] Removal of the residual Boc protecting group was then readily achieved by treatment with TFA to afford **21** in excellent yield (99%).

Our attention now turned to the key coupling between *C*-terminal fragment **21** and *N*-terminal fragment **6** (Scheme

Scheme 4. Completion of Symplostatin 4 (**1**) Synthesis



4). We chose to perform this coupling at 0 °C in a concentrated solution with PyBOP and NMM to reduce epimerization of the *C*-terminal L-isoleucine residue of **6**. Pleasingly, under these conditions, the desired product was formed with minimal epimerization (<2%). The small quantity of epimer was successfully removed during purification by preparative reversed-phase HPLC to afford symplostatin 4 (**1**) in 85% yield. Gratifyingly, the spectroscopic data obtained for synthetic symplostatin 4 (^{1}H and ^{13}C NMR, MS) and the measured specific rotation, $[\alpha]^{20}_D$ −47 (c = 0.06, MeOH) cf. −53 correlated fully with that provided for the isolated material,[1] providing convincing evidence that the relative and absolute configurations of **1** are identical to those reported by Luesch and co-workers (see the Supporting Information). Furthermore, the spectroscopic data for synthetic **1** were significantly different from those reported for gallinamide A (**2**).[1,2] Specifically, resonances corresponding to the *N*-terminal dimethylated isoleucine residue showed the greatest differences in chemical shift, providing convincing evidence for the diastereomeric relationship of this natural product.

With synthetic symplostatin 4 now in hand, and given the promising antimalarial activity exhibited by gallinamide A against *P. falciparum*, we were next interested in assessing the biological activity of our synthetic material. Toward this end, **1** was screened against the drug sensitive 3D7 strain of *P. falciparum* using a ^{3}H hypoxanthine incorporation assay (see the Supporting Information).[20,21] Gratifyingly, symplostatin 4 exhibited potent inhibition of *P. falciparum* growth, with an ED_{50} of 74 nM. Furthermore, it was demonstrated that symplostatin 4 did not cause lysis of red blood cells up to a concentration of 25 μM, suggesting

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that the antimalarial activity was due to targeting of the parasite (see the Supporting Information). Future work will involve a detailed investigation of the mode of action of symplostatin 4.

In summary, we have completed the first total synthesis of the cyanobacterium-derived depsipeptide natural product symplostatin 4 (seven steps in the longest linear sequence, 23% overall yield). It is anticipated that the synthetic route described should provide access to related members of this class of compounds, including gallinamide A and structural analogues thereof. This may prove to be extremely valuable for the development of selective antiparasitic agents against drug resistant *P. falciparum* and other related organisms with low cytotoxicity toward human cells. Studies toward this end will be the focus of future work in these laboratories.

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Supporting Information Available: Experimental procedures and characterization for all new compounds. Experimental details and raw data for biological assays and ^{1}H and ^{13}C NMR spectra for all novel compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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