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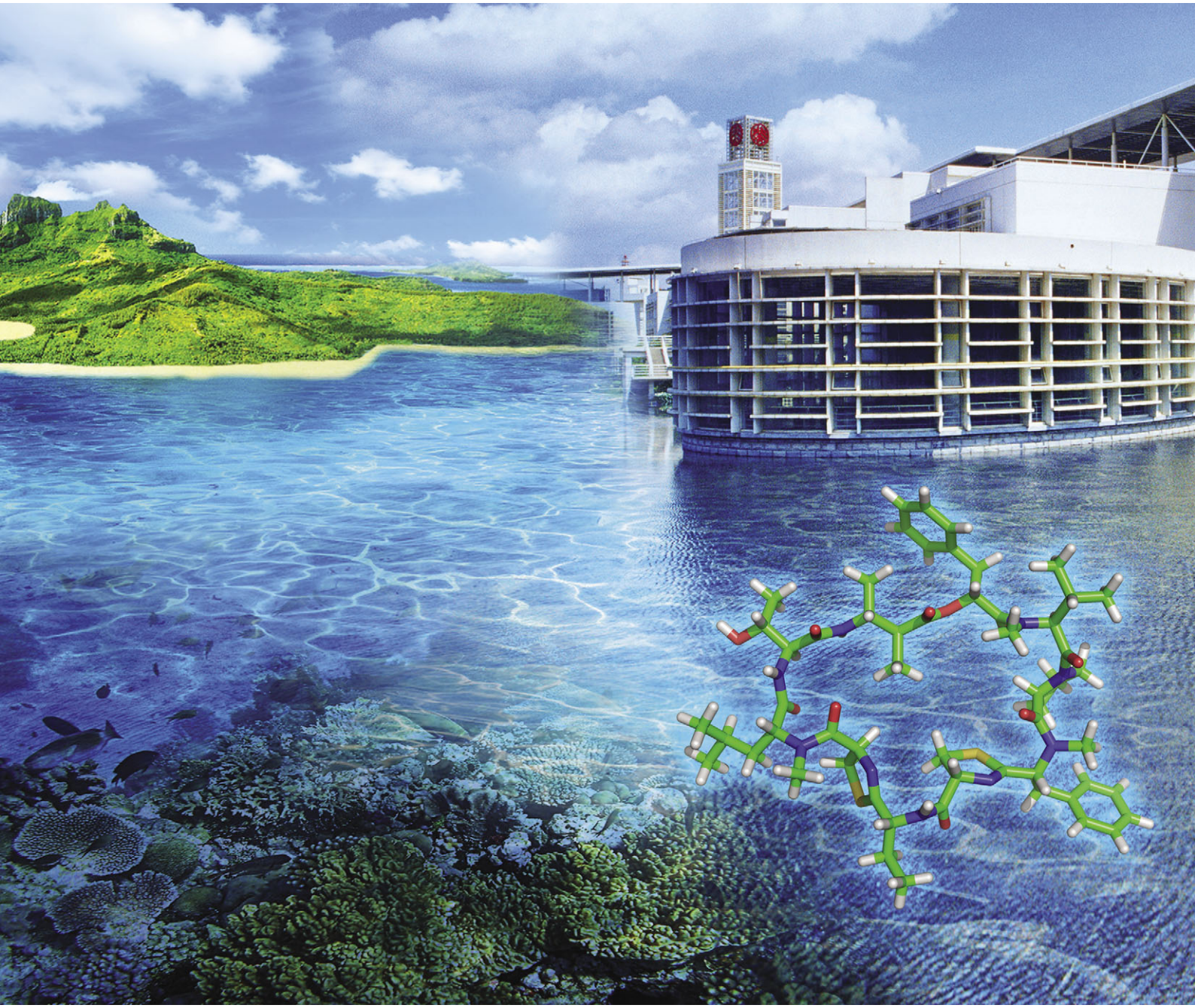

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Total synthesis of grassypeptolide

FEATURE ARTICLE
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Catalytic enantioselective synthesis of P-stereogenic compounds

Total synthesis of grassypeptolide†

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The first total synthesis of grassypeptolide, an anticancer cyclodepsipeptide isolated from marine cyanobacteria, has been achieved in 17 steps and an overall 11.3% yield.

Grassypeptolide (**1**), isolated from an extract of *Lyngbya confervoides* collected off Grassy Key in Florida, is a potential anticancer cyclodepsipeptide.[1] Grassypeptolide inhibited cancer cell growth with IC_{50} values from 1.0 to 4.2 μM. The gross chemical structure of grassypeptolide was established using a combination of chemical and spectral techniques. The absolute configuration of the thiazolines was elucidated through X-ray analysis. Grassypeptolide possesses a 31-membered macrolactone which is composed of unique, non-proteinogenic amino acid residues such as 2-methyl-3-aminobutyric acid (Maba), 2-aminobutyric acid (Aba), several D- and *N*-methylated amino acids and a tandem thiazoline-ring moiety.[1] We have been interested for some time in marine cyclopeptides and cyclodepsipeptides,[2] and view their syntheses as a key route to structural confirmation, structural modification and subsequent activity control. Here we report the first total synthesis of grassypeptolide.

The principal synthetic challenges associated with the preparation of grassypeptolide are the efficient formation of the delicate macrocyclic depsipeptide and assembly of the sensitive thiazoline[3] heterocycles in the presence of a secondary hydroxyl group. In our retrosynthetic strategy, we envisioned macrocyclization and late-stage introduction of the labile tandem thiazoline-ring moiety (Fig. 1). Thus, grassypeptolide would be derived from the macrocycle **2** by cyclodehydration of *β*-hydroxy thioamides. It was decided to close the macrocycle at the proline (C37–41)/*N*-Me-Phe-thn-ca (C24–36) peptide bond, despite the known problem of slower acylation of secondary amines. Further retrosynthetic analysis reveals that the linear precursor **3** may be most conveniently constructed by the assembly of the two fragments **4** and **5** with approximately equal complexity.

The synthesis of fragment **4** commenced with the coupling of L-proline *tert*-butyl ester (**6**) with *N*-Cbz-*N*-methyl-L-valine (**7**) to afford dipeptide **8** in 84% yield (Scheme 1). Hydrogenolysis removal of the *N*-Cbz protecting group in **8** followed by a coupling with hydroxy acid **9**[4] using bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOPCl)[5] in the presence of triethylamine furnished the corresponding tripeptide which was deacetylated to afford **10** in 95% yield. 2-Methyl-3-aminobutyric acid (**11**) was prepared from *N*-Cbz-D-alanine by a published route involving diazoketone formation,[6] Wolff rearrangement[6,7] and a diastereoselective methylation.[2m,8] Condensation of acid **11** with alcohol **10**, using various coupling agents including EDCI–DMAP,[9] DCC–DMAP,[9] and Mukaiyama reagent[10] always resulted in incomplete conversion and low yield presumably due to the congested steric environment and the reduced reactivity of the α-hydroxy amide. After extensive experimentation, we found that the acyl chloride, derived from acid **11**, was superior for this difficult coupling, and the key coupling product **4** was obtained in 90% yield.

With the key intermediate **4** in hand, we next turned our attention to the synthesis of the lower fragment **5**, which contains two thioamide bonds (Scheme 2). Thus, D-allo-threonine methyl ester (**12**) was reacted with *N*-Boc-*N*-methyl-D-leucine (**13**) using EDCI[9] with HOBt[9] to afford dipeptide **14** in 93% yield. The Boc group in **14** was removed with trimethylsilyl trifluoromethanesulfonate (TMSOTf) in the presence of 2,6-lutidine,[11] and the resulting free amine underwent a BOPCl-mediated coupling reaction[5] with *N*-Cbz-*O*-TBS-L-Ser-OH to afford tripeptide **15** in 89% yield.[12] Hydrogenolysis of the Cbz group of tripeptide **15** provided the corresponding free amine, which was coupled with the thioacylating agent **16**, prepared using the method described by Rapoport,[13] to afford the thioamide **17** in 88% yield. To continue the linear chain elongation, Boc group was selectively removed with TMSOTf/2,6-lutidine[11] to afford the corresponding amine, which was

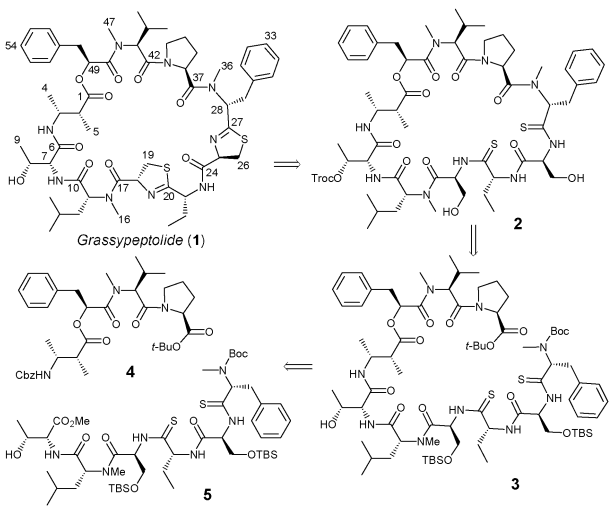

Fig. 1 Retrosynthetic analysis of grassypeptolide.

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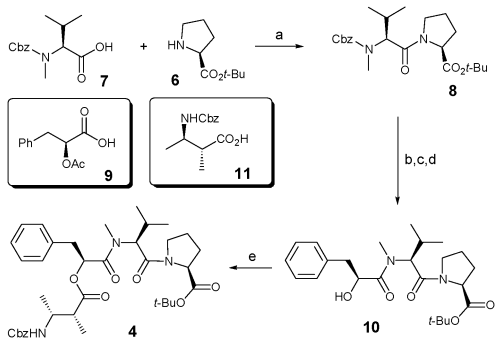
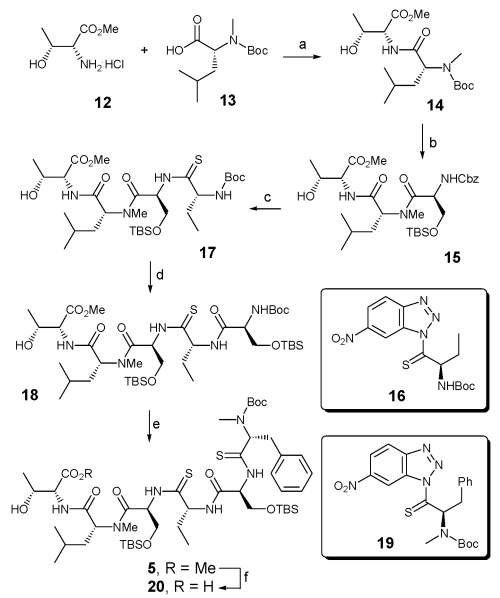
† Electronic supplementary information (ESI) available: Full details for experimental procedures, ^{1}H and ^{13}C NMR spectra for compounds **1**, **4**, **5**, **21**. See DOI: 10.1039/c0cc01200a



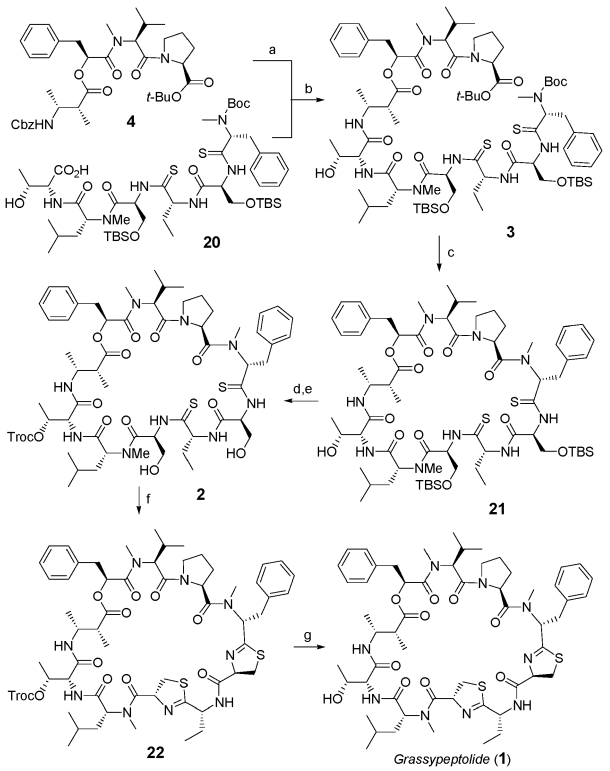
Scheme 1 (a) EDCI, HOBt, DIPEA, CH_2Cl_2, 0 °C to RT, 84%; (b) 10% Pd/C, H_2, MeOH–EtOAc; (c) **9**, BOPCl, Et_3N, CH_2Cl_2, 0 °C to RT, 92% from **8**; (d) K_2CO_3, MeOH, 0 °C, 95%; (e) **11**, $(COCl)_2$, cat. DMF, CH_2Cl_2, 0 °C to RT, then **10**, cat. DMAP, NMM, CH_2Cl_2, 0 °C to RT, 90%.

condensed with *N*-Boc-*O*-TBS-L-Ser-OH employing various coupling reagents. Only when PyAOP[14] was employed as a coupling agent, the reaction afforded amide **18** in high yield. To our surprise, the condensation resulted generally in sluggish reactions and low yields when other coupling reagents such as EDCI–HOAt,[9] HATU,[9] BOPCl,[5] and Mukaiyama reagent[10] were used. Intermediate **18** was then elaborated to the key fragment **5** in 82% yield[12] by an identical strategy as described for **17**, including Boc deprotection and thiolation of the resulting free amine with thioacylating agent **19**. Hydrolysis of the methyl ester was then carried out with lithium hydroxide to give free acid **20**.

With the two key fragments in hand, the stage was now set for their assembly and elaboration into grassypeptolide (Scheme 3). Thus, hydrogenolysis of the Cbz protecting group



Scheme 2 (a) EDCI, HOBt, Et_3N, CH_2Cl_2, 0 °C to RT, 93%; (b) 1. TMSOTf, 2,6-lutidine, CH_2Cl_2, RT; 2. *N*-Cbz-*O*-TBS-L-Ser-OH, BOPCl, DIPEA, CH_2Cl_2, 0 °C to RT, 89%; (c) 1. Pd/C, H_2, MeOH, RT; 2. **16**, THF, 0 °C, 88%; (d) 1. TMSOTf, 2,6-lutidine, CH_2Cl_2, RT; 2. *N*-Boc-*O*-TBS-L-Ser-OH, PyAOP, DIPEA, CH_2Cl_2, 0 °C to RT, 88%; (e) 1. TMSOTf, 2,6-lutidine, CH_2Cl_2, RT; 2. **19**, THF, 0 °C, then acidic work-up, 82%; (f) 1 N LiOH, *tert*-BuOH/THF, 0 °C.



Scheme 3 (a) 10% Pd/C, H_2, EtOAc, RT; (b) PyAOP, DIPEA, CH_2Cl_2, 0 °C to RT, 81%; (c) 1. TMSOTf, 2,6-lutidine, CH_2Cl_2, RT; 2. BOPCl, 2,6-lutidine, CH_2Cl_2 (0.001 M), 0 °C to RT, 72%; (d) TrocCl, Py, CH_2Cl_2, 0 °C; (e) TBAF/HOAc, THF, 0 °C to RT; (f) DAST, CH_2Cl_2, −78 °C to −50 °C; (g) Zn, 1 M NH_4OAc, THF, 0 °C, 37% from **21**.

in **4** afforded the corresponding amine, which underwent a PyAOP-mediated coupling reaction[14] with acid **20** to provide the linear precursor **3** in 81% yield. Simultaneous removal of the *tert*-butyl ester[15] and Boc-protecting group was achieved by treatment of **3** with TMSOTf/2,6-lutidine at room temperature to produce the desired amino acid which was immediately activated by BOPCl[5] in the presence of 2,6-lutidine to afford cyclodepsipeptide **21** in 72% yield[16] and without noticeable epimerization evident by NMR analysis. The secondary hydroxyl group of **21** was protected as Troc ester,[17] and the existing two primary *tert*-butyldimethylsilyl protecting groups were selectively removed using acetic acid buffered tetrabutylammonium fluoride to give the corresponding *β*-hydroxy thioamide **2**. Activation of the primary hydroxyl groups of thioamide **2** with diethylaminosulfur trifluoride (DAST)[18] in CH_2Cl_2 at −78 °C led to the cyclized product **22** which was then treated with activated zinc and aqueous NH_4OAc in THF to afford the fully synthetic grassypeptolide (**1**), in 37% yield (Scheme 3).

The spectral data for the synthetic material (^{1}H NMR, ^{13}C NMR, and HRMS) were identical to those published for the natural product and the optical rotation $[\alpha]_D^{20}$ +87 (*c* 0.12, CH_2Cl_2) was in close agreement with values reported in the literature for natural grassypeptolide, $[\alpha]_D^{20}$ +76 (*c* 0.1, CH_2Cl_2). Furthermore, biological evaluation using cell proliferation assay confirmed that the inhibitory effect of synthetic grassypeptolide (**1**) on cancer cell proliferation is

similar to that of naturally occurring grassypeptolide. The average IC_{50} values of Hela and of HT29 were 1.9 and 2.3 μM, respectively. The detailed biological studies, including the biological mode of action, are currently under way and will be reported in due course.

In summary, we have developed a convergent synthesis of grassypeptolide. Key strategic elements of the approach include the late stage introduction of the sensitive tandem thiazoline heterocycles which are potentially highly sensitive and prone to epimerization at no less than four of its stereogenic sites, and 31-membered macrocyclization conducted at the sterically congested secondary amide site in superb conversion (72% yield). Pre-organization of the cyclization precursor resulting in more favorable cyclization kinetics could be attributed to the efficiency of this transformation.

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9 Abbreviations: EDC = 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; HOBt = 1-hydroxybenzotriazole; HOAt = 1-hydroxy-7-azabenzotriazole; HATU = 2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; PyAOP = (7-azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate); BEP = 2-bromo-1-ethylpyridinium tetrafluoroborate.

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