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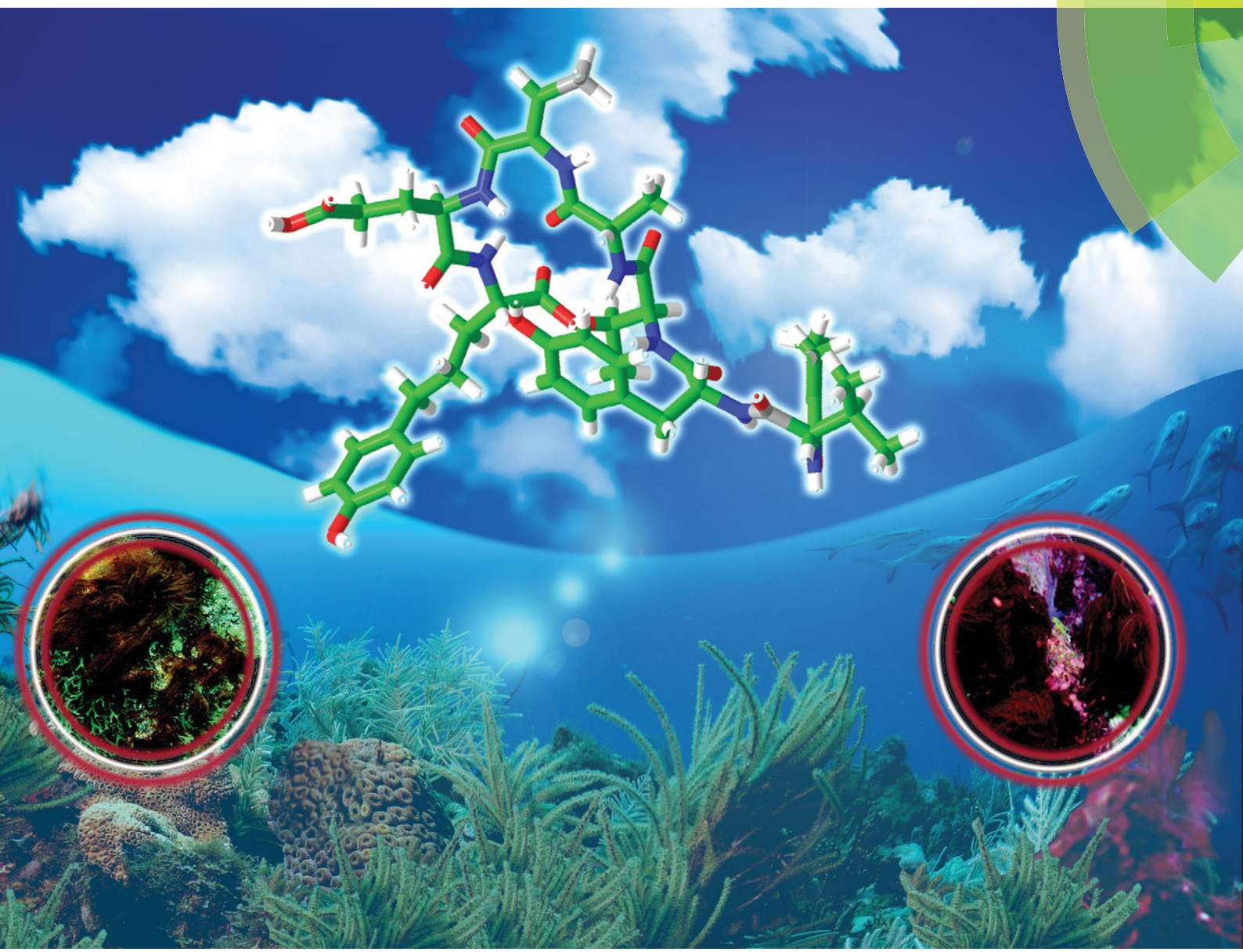

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Total synthesis of largamide B†

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Total synthesis of the cyanobacterial metabolite largamide B and the disproval of its originally assigned stereochemistry as well as confirmation of the revised stereochemistry are reported.

The cyclodepsipeptide largamide B (**1**) was isolated from marine cyanobacterium *Oscillatoria* sp. from the Florida Keys and structurally characterized by Plaza and Bewley in 2006.[1] Along with the related cyclodepsipeptide largamides A and C (Fig. 1), largamide B (**1**) possesses a 16-membered macrolactone composed of the unique non-proteinogenic amino acids 2-amino-5-(4′-hydroxyphenyl)pentanoic acid (Ahppa) and (*Z*)-2,3-dehydro-2-aminobutanoic acid, as well as D-Glu, Abu, L-Ala and L-Thr. The initially published structure of largamide B (**1a**)[1] revealed that a senecioic acid-containing side chain is appended to the N-terminus of the core depsipeptide. After extensive high-field NMR studies, Luesch and coworkers[2] suggested that the senecioic acid residue of largamides, originally assigned by Plaza and Bewley, should be revised to tiglic acid, as indicated in **1b** (Fig. 1). Largamides A–C exhibit inhibitory activity against the serine protease with selectivity for elastase over chymotrypsin and trypsin. As part of a program directed toward the synthesis, structural modification, and biological evaluation of marine natural products,[3] we have developed and report herein the first total synthesis of largamide B and unambiguously confirmed its structure. Since the only difference between **1a** and **1b** is the unsaturated acid moiety of the side chain, we planned a convergent synthetic approach (Fig. 1), which aimed for late-stage incorporation of the side chain, enabling facile divergence to both **1a** and **1b**. Our retrosynthetic analysis for largamide B (**1b**) is presented in Fig. 2. We chose to construct the macrocyclic core (**3**) *via* intramolecular coupling between D-Glu and L-Ahppa, on the basis of the literature precedent,[4] which suggests that macrolactamization between a D and an L-residue generally proceeds more efficiently than those employing L,L or D,D coupling partners. Thus, our target fragments for the assembly of largamide B were bis-protected L-Ahppa (**4**), dipeptide **5** and side-chain segment **2b**.

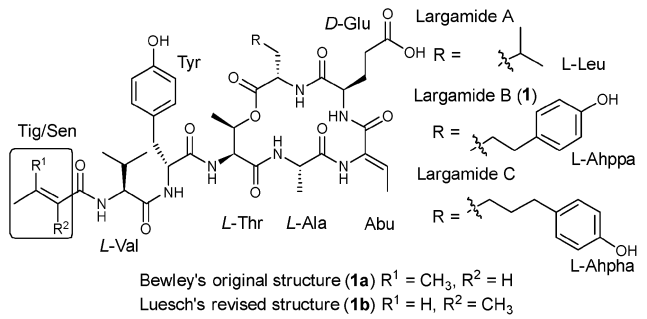

Fig. 1 Structure of largamides.

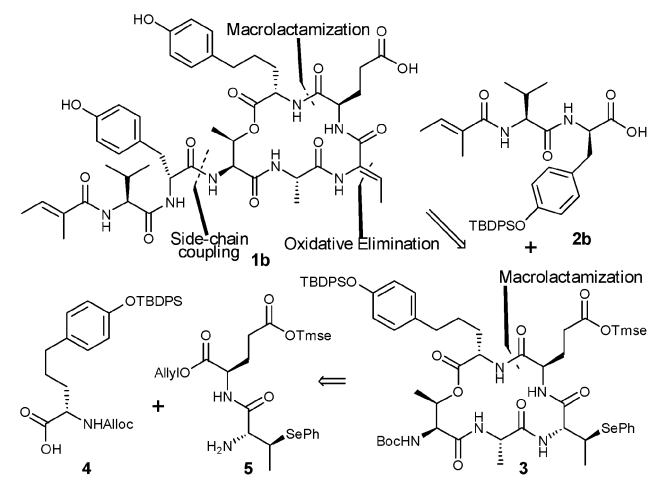

Fig. 2 Retrosynthetic analysis of **1b**.

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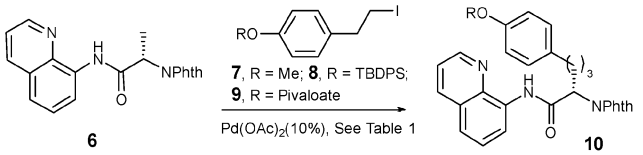
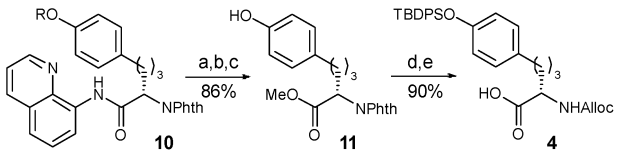
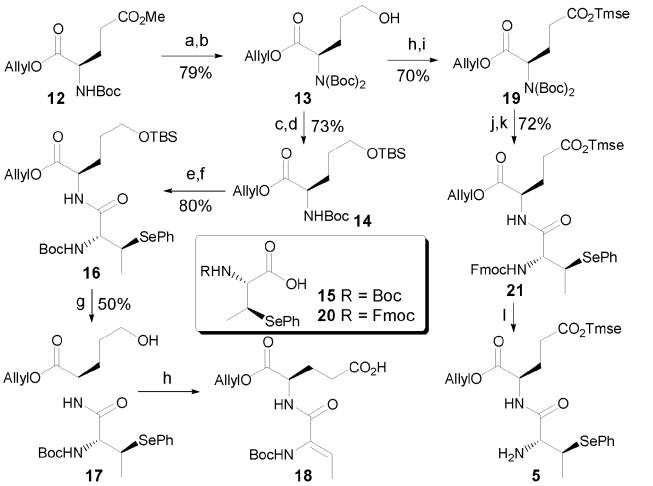


Scheme 1 Synthesis of **10** *via* C–H functionalization.



Scheme 2 *Reagents and conditions*: (a) 6 N HCl, reflux, 12 h; (b) MeOH, $SOCl_2$; (c) AllocCl, $NaHCO_3$, THF–H_2O, 86% from **10**; (d) TBDPSCl, TEA, DCM, 95%; (e) LiOH, THF–H_2O, 95%.



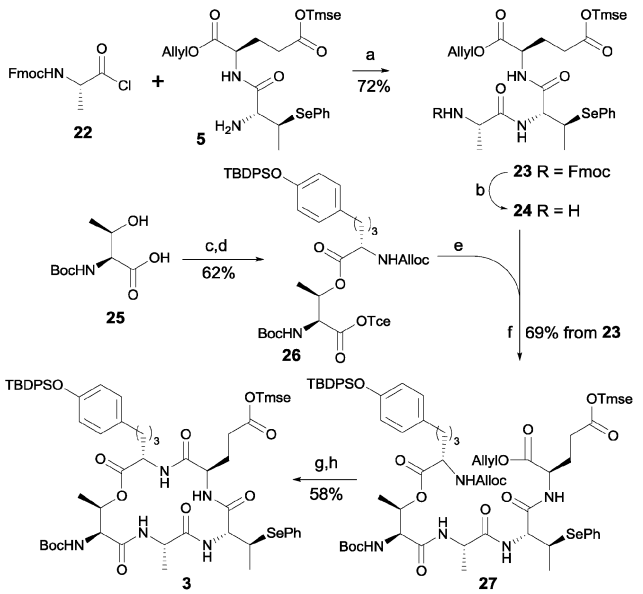
Scheme 3 *Reagents and conditions*: (a) Boc_2O, DMAP, MeCN; (b) DIBAL-H, THF; (c) LiBr, MeCN; (d) TBSCl, Imid., DCM; (e) TMSOTf, 2,6-lutidine, DCM; (f) **15**, HATU, HOAt, DIPEA, DCM; (g) aq. HCl, THF; (h) TEMPO, NaClO, $NaClO_2$, NaBr, MeCN-pH = 6.7 buffer; (i) TMSE-OH, EDCI, DMAP, DCM; (j) TMSI, DCM, TFA (20 eq.), DCM; (k) **20**, PyAOP, HOAt, DIPEA; (l) Et_2NH, MeCN.

The synthesis of macrocycle **3** commenced with the preparation of a bis-protected L-Ahppa (**4**) as described in Scheme 1. Inspired by the seminal work of Daugulis and co-workers describing the synthesis of substituted phenylalanine derivatives by using C–H bond functionalization,[5] we investigated the alkylation of alanine derivative **6** with alkyl iodides **7–9** under palladium-catalyzed conditions to give rise to L-Ahppa derivative **10** (Scheme 1). **10** was converted into the bis-protected L-Ahppa (**4**) in 78% overall yield by a sequence of protecting group manipulation including removal of the directing group 8-aminoquinoline, phthalimide and pivaloate under acidic conditions, followed by reprotection of the amino moiety as its *N*-alloc-carbamate and the phenol as a *tert*-butyldiphenylsilyl (TBDPS) ether, then saponification of the methyl ester affording bis-protected L-Ahppa (**4**) (Scheme 2).

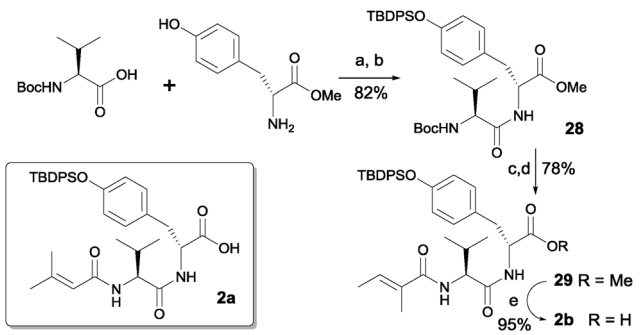
The synthesis of the dipeptide fragment **5** commenced from the fully protected D-glutamic acid **12**, which is readily available according to the literature procedure.[6] Attempts to directly convert the gamma-methyl ester into 2-trimethylsilylethyl ester (Tmse) turned out to be unsuccessful, and it was envisaged at first to reduce the methyl ester to the corresponding alcohol and then introduce the 2-trimethylsilylethyl ester at a later stage. Thus, the methyl ester **12** was converted to alcohol **13** in 79% yield *via* a two step sequence, including DIBAL reduction and introduced a second Boc group on the nitrogen.[7] Protection of the hydroxy group as its TBS ether followed by mono-deprotection of one Boc protecting group[8] to afford **14** in 73% yield. The remaining Boc group in **14** was removed using trimethylsilyl trifluoromethanesulfonate (TMSOTf) in the presence of 2,6-lutidine,[9] and the resulting free amine underwent a HATU-mediated coupling reaction with the known (2*R*,3*S*)-2-*N*-Boc-3-(phenylseleno)butanoic acid (**15**)[10] to afford dipeptide **16** in 80% yield. Removal of the TBS protecting group of **16** under acidic conditions afforded the corresponding primary alcohol **17**. We attempted to effect the oxidation of the primary alcohol of **17** to its carboxylic acid;[11] however, this oxidation process proved to be complicated by concomitant *syn* β-elimination of the phenylselenide group in **17**. Formation of **18** as the major product was observed as a significant and unavoidable side reaction. To circumvent this complication, we elected to introduce the trimethylsilylethyl ester prior to conducting the coupling reaction with (2*R*,3*S*)-2-amino-3-(phenylseleno)butanoic acid. Thus, oxidation of the primary hydroxyl group in **13** by the sequential action of TEMPO/NaClO and $NaClO_2$ afforded the corresponding acid,[11] which was then esterified with trimethylsilylethanol in the presence of EDCI and DMAP to produce compound **19** in 70% yield. Selective deprotection of the two Boc groups of **19** was achieved by the sequential action of trimethylsilyl iodide (TMSI) and trifluoroacetic acid, which left the TMSE esters intact. The resulting free amine underwent a PyAOP-mediated coupling reaction with (2*R*,3*S*)-2-*N*-Fmoc-3-(phenylseleno)butanoic acid (**20**)[12] to provide dipeptide **21** in 72% yield. Removal of the Fmoc protecting group of **21** was achieved by treatment with diethylamine in acetonitrile to afford **5** (Scheme 3).

With the two target fragments in hand, the stage was now set for their assembly and elaboration into macrocycle **3** (Scheme 4). Thus, treatment of the dipeptide fragment **5** with Fmoc-Ala-Cl (**22**) afforded tripeptide **23** in 72% yield. Deprotection of the Fmoc group in **23** with diethylamine in acetonitrile afforded the corresponding free amine **24**, which sets the stage for further fragment assembly. In parallel, *N*-Boc-threonine was converted into the corresponding trichloroethyl ester and subsequent esterification of the secondary hydroxy group with the bis-protected L-Ahppa (**4**) under Yamaguchi's protocol[13] gave rise to ester **26** in 62% yield over two steps. Reductive removal of the trichloroethyl ester in **26** under buffered conditions afforded the corresponding acid, which was coupled with amine **24** to furnish the protected linear precursor **27** in 69% yield. Deprotection of the allyl ester and alloc protecting groups by using $Pd(PPh_3)_4$ and $PhSiH_3$[14] and subsequent HATU-mediated macrolactamisation afforded macrocycle **3** in 58% over two steps.

The synthesis of the side chain fragment (**2b**) started with the coupling of *N*-Boc-L-valine with the methyl ester of D-tyrosine to afford the corresponding dipeptide, followed by protection of the phenolic OH as its TBDPS ether to provide **28** in 82% yield.



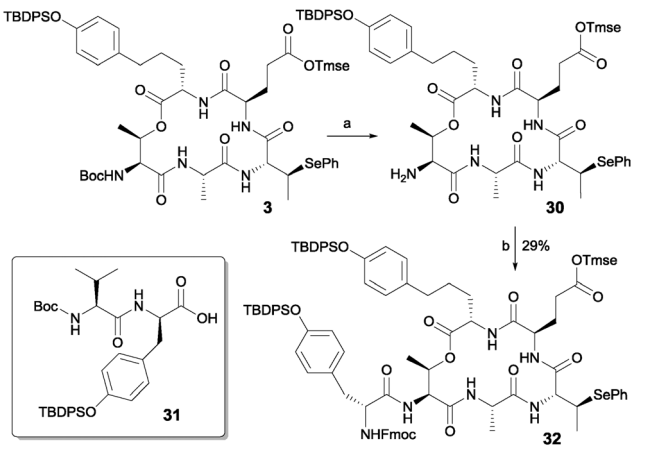
Scheme 4 *Reagents and conditions*: (a) NMM, DMAP; (b) Et_2NH, MeCN; (c) Cl_3CCH_2OH, EDCI, DMAP; (d) **4**, TCBC, DIPEA, then DMAP, PhMe; (e) Zn, THF, aq. KH_2PO_4, pH = 4.3; (f) HATU, HOAt, DIPEA, DMF; (g) $Pd(PPh_3)_4$, $PhSiH_3$, DCM; (h) HATU, HOAt, NMM, DMF (0.001 M).



Scheme 5 *Reagents and conditions*: (a) EDCI, HOAt, DIPEA; (b) TBDPSCl, TEA; (c) TFA, DCM; (d) tiglic acid, EDCI, HOAt, DIPEA, 78%; (e) LiOH, THF–H_2O.

The Boc group of **28** was selectively removed using TFA in dichloromethane at 0 °C and the resulting amine salt was coupled with tiglic acid in the presence of EDCI to give rise to intermediate **29** in 78% yield (Scheme 5). Saponification of the methyl ester of **29** provided the side chain fragment **2b**, which set the stage to append the side chain to the macrocycle **3**. Similarly, the side chain fragment **2a** was readily achieved by following the same synthetic procedure as for **2b**, but using senecioic acid instead of tiglic acid.

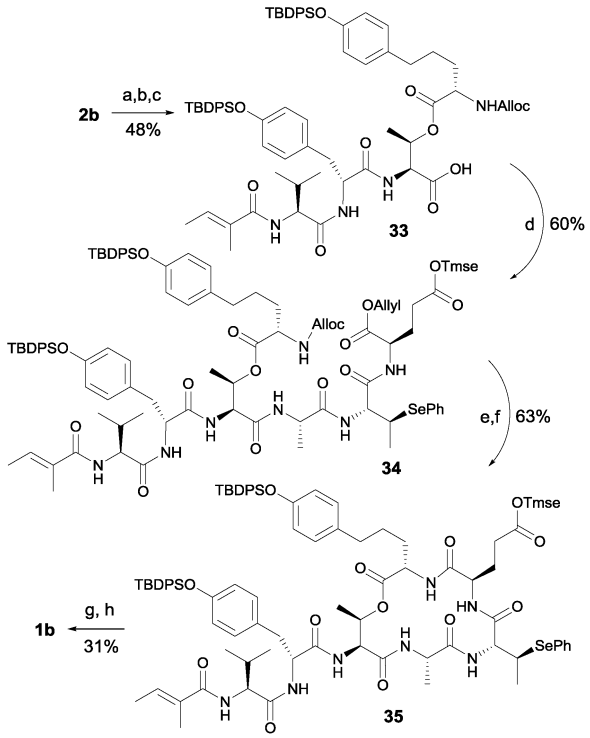
With the side chain fragments **2a** and **2b** in hand, efforts were focused on the key appendage of the side chain fragment to macrocycle **3**. As shown in Scheme 6, selective removal of the Boc carbamate from **3** could be achieved using trimethylsilyl iodide (TMSI) in acetonitrile to give the corresponding free amine **30**. Much to our disappointment, condensation of **30** with either side chain acid **2b** or dipeptide acid **31**, using various coupling agents, including EDCI, HATU, PyAOP and DEPBT, were unsuccessful, and no desired products could be detected in the complex mixture of products. We then attempted to



Scheme 6 *Reagents and conditions*: (a) TMSI, MeCN; (b) DMAP, NMM, toluene.

condense *O*-TBDPS-*N*-Fmoc-D-tyrosyl chloride with **30** in the presence of DMAP, NMM in toluene to afford the coupling product **32** in 29% yield. The formation of **32** confirmed that the inherent stability of the depsipeptide macrocycle is able to suppress the propensity for *O*,*N*-acyl migration of **30** which contains a free *N*-terminal amino group.[15] To circumvent the problems encountered in the above strategy toward the side chain attachment, we decided to revise our synthetic strategy which includes the incorporation of the side chain into an appropriate linear precursor prior to the formation of a macrocycle.

Thus, the side chain fragment **2b** was elongated to acid **33** *via* a three-step sequence including PyAOP promoted coupling of **2b** and L-threonine trichloroethyl ester, subsequent esterification of the secondary hydroxy group of L-threonine with the bis-protected L-Ahppa (**4**) under Yamaguchi conditions, and reductive cleavage of the trichloroethyl ester. Condensation of acid **33** with tripeptide **24** was achieved through carboxyl activation with 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one (DEPBT)[16] and provided fully protected linear precursor **34** in 60% yield. This compound was then submitted to our previously established protocol for the deprotection of the allyl ester and alloc protecting groups in **27**. Much to our surprise, attempts to remove the same protecting groups in **30** by using $Pd(PPh_3)_4$ and $PhSiH_3$ were unsuccessful, and led only to decomposition of the starting material. After screening a number of reagents and conditions, we eventually found that the combination of $Pd(PPh_3)_4$ and (NDMBA)[17] comprised a particularly effective means for the removal of both allyl ester and alloc protecting groups in **34**. The resulting amino acid was cyclised with HATU/HOAt in the presence of NMM to give rise to the corresponding macrolactam **35** in 63% yield over two steps. Treatment of **35** with *tert*-butyl hydroperoxide in dichloromethane, the phenylselenide group in **35** was converted into the corresponding selenoxides that underwent concomitant *syn* β-elimination to afford the corresponding alkene derivative. Subsequent global removal of the remaining silyl-protecting groups afforded **1b** in 31% yield based on **35**. With many building blocks already at hand, the next step was to prepare **1a**, and this was readily



Scheme 7 *Reagents and conditions*: (a) L-Thr-OTce, PyAOP, DIPEA, DCM, 85%; (b) **4**, TCBC, DIPEA, then DMAP, PhMe, 71%; (c) Zn, THF, aq. KH_2PO_4, pH = 4.3 buffer, 81%; (d) **24**, DEPBT, NMM, DMF, 60%; (e) $Pd(PPh_3)_4$ (0.2 eq.), 1,3-dimethylbarbituric acid, THF; (f) HATU, HOAt, NMM, DMF (0.001 M), 63% from **34**; (g) *t*-BuOOH, DCM, 62%; (h) HF, Pyr., THF; then TASF, DMF, 50%.

achieved by following the same synthetic procedure as for **1b**, but using **2a** as the starting material. A thorough examination of ^{1}H and ^{13}C NMR spectra of **1a** and **1b** and comparison of reported spectra of natural largamide B revealed that the true structure of natural largamide B was **1b**. Moreover, the optical rotation (in both sense and magnitude) of the synthetic material (**1b**), $[\alpha]_D^{20} = -72.6$ (*c* 0.12, MeOH), was in close agreement with those reported in the original isolation paper $[\alpha]_D^{20} = -71.5$ (*c* 0.3, MeOH) (Scheme 7).

In conclusion, we have resolved the structural ambiguities of the marine cyclodepsipeptide, largamide B, by completing the total synthesis of two previously assigned structures. The synthesis of largamide B proceeds in 14 steps (longest linear sequence) and 3.4% yield. Notable features include the construction of a key unnatural amino acid by using C–H bond functionalization, and the use of oxidative elimination processes to control the stereochemistry of a 2,3-dehydro-2-aminobutanoic acid unit present in the natural product.

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