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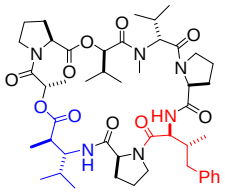
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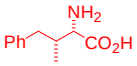
Synthetic Study on Dolastatin 16: Concise and Scalable Synthesis of Two Unusual Amino Acid Units

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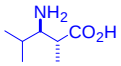
Taiki Umezawa,* Akinori Sato, Yasuto Ameda, Loida O. Casalme, Fuyuhiko Matsuda*



Dolastatin 16



Dolaphenvaline



Dolamethylleuine

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Synthetic Study on Dolastatin 16: Concise and Scalable Synthesis of Two Unusual Amino Acid Units

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ABSTRACT

A convenient and scalable synthesis of two unusual amino acid units found in dolastatin 16, dolaphenvaline and dolamethylleuine, is described. Dolastatin 16, which was first isolated from the sea hare *Dolabella auricularia* by Pettit, exhibits not only strong inhibition of growth for a variety of human cancer cell lines but also potent antifouling activity against the larvae of the barnacle *Balanus amphitrite*. The key element of the synthesis is an organocatalytic Mannich reaction to construct two contiguous stereocenters in the amino acid units with almost complete enantio- and diastereoselectivity.

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Dolastatin 16 (**1**), a macrocyclic depsipeptide, was first isolated by Pettit as a potential antineoplastic metabolite in 1997 from the sea hare *Dolabella auricularia*, collected in Papua New Guinea (Fig. 1).[1] This unique depsipeptide proved to be a strong growth inhibitor for a variety of human cancer cell lines and a candidate for further development. Five years after the original report, the isolation of **1** from a Madagascan cyanobacterium, *Lyngbya majuscule*, was described by Gerwick.[2] With regard to structural features, **1** contains the new and unusual amino acid units dolaphenvaline (**2**) and dolamethylleuine (**3**). Although the stereostructures of **2** and **3** were not assigned in these publications, their absolute configurations were determined to be (2*S*, 3*R*) and (2*R*, 3*R*), respectively, through *X*-ray crystallographic analysis of **1** performed by Pettit in 2011.[3]

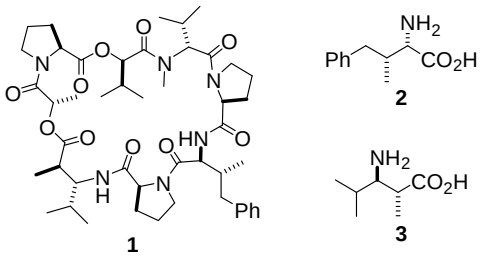


Figure 1. Dolastatin 16 (**1**) and the unusual amino acid units **2** and **3**

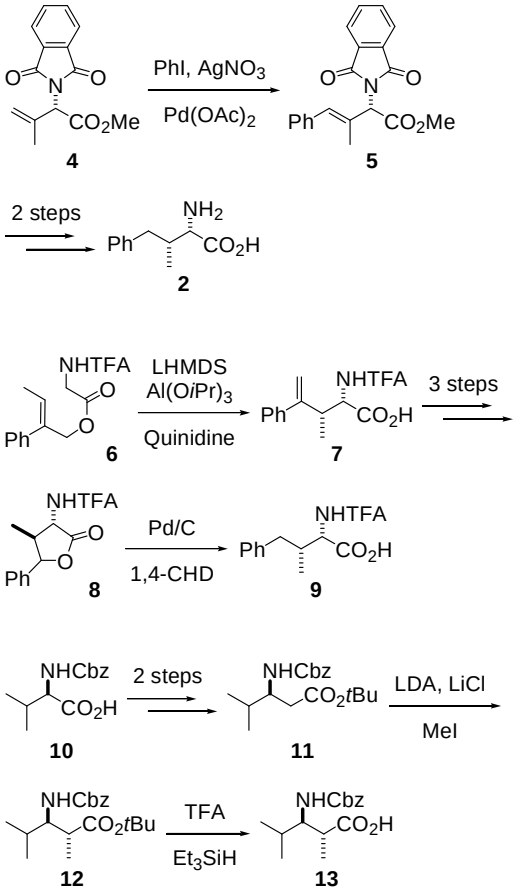
In 2010, Tan reported that **1** showed strong antifouling activity (EC_{50} 0.003 μg/mL) against the larvae of the barnacle *Balanus amphitrite*, as well as low toxicity (LC_{50} 20 μg/mL).[4] Biofouling – that is, adverse growth of marine organisms on manmade submerged structures – results in significant economic and environmental problems. Tributyltin (TBT),[5] which inhibits the settlement of larvae, has been widely used all over the world for this purpose since the early 1960s. However, the deleterious effects of TBT on marine ecosystems prompted the International Maritime Organization (IMO) to call in 2008 for a ban on the use of TBT-based antifouling paint on ships.[6] Since marine organisms prevent fouling of their outer surfaces through the use of natural substances with antifouling properties without causing serious environmental problems, natural antifouling products, especially those with good settlement-inhibiting properties but without biocidal properties, have attracted considerable attention.[7] Among these, **1** shows promise as a lead compound for the development of new environmentally friendly antifouling agents due to its potent antifouling activity and low toxicity.[8]

Because of its intriguing and unprecedented structure, **1** is an attractive target for total synthesis. For the total synthesis of **1**, synthetic methods for the optically active amino acid units **2** and **3** must be developed. Syntheses of these unusual amino acid units have been carried out previously. Scheuer synthesized all four stereoisomers of **2** from both enantiomers of *N*-phthaloyl-3,4-dehydrovaline (**4**) during structure elucidation of kulokekahilide-1, a cytotoxic depsipeptide from the cephalaspidean mollusk *Philinopsis speciosa*.[9] The synthesis involved a Mizorogi-Heck reaction of **4** with iodobenzene and non-diastereoselective hydrogenation of olefin **5** (Scheme 1).

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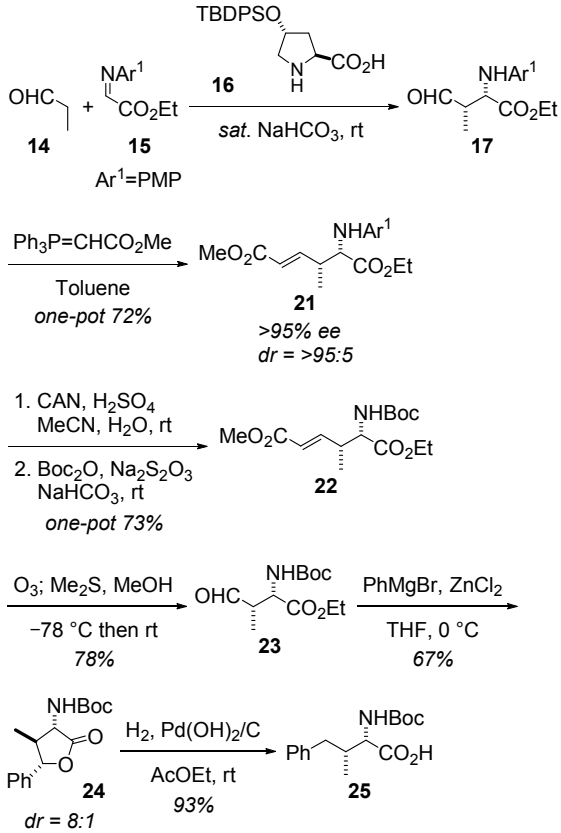
Pettit prepared *N*-TFA-dolaphenvaline (**9**) from allyl ester **6** through asymmetric Claisen rearrangement of **6** to give *syn*-carboxylic acid **7** and hydrogenolysis of lactone **8** derived from **7**.[3] Pettit also achieved a synthesis of *N*-Cbz-dolamethylleuine (**13**) through diastereoselective alkylation[10] of the β-amino acid ester **11** prepared from *N*-Cbz-L-valine (**10**) and cleavage of *t*-butyl ester of *anti*-ester **12**.[3] Although the two amino acid units have been prepared, total synthesis of **1** has not yet been reported.

In conjunction with our program directed toward a practical total synthesis of **1**, we developed a concise and scalable synthetic procedure for the unusual amino acid units **2** and **3** by using highly enantio- and diastereoselective Mannich reactions promoted by chiral organocatalysts.[11,12] This method provides flexible access to a wide variety of congeners of **2** and **3**, such as diastereomers and enantiomers, by simply changing the catalyst or starting material. The synthetic plan for both amino acid units (**2** and **3**) is shown in Scheme 2. We envisioned the derivation of **2** or **3** from *syn*-β-amino aldehyde **17** or *anti*-β-amino aldehyde **20**, which were prepared by Hayashi through enantio- and diastereoselective Mannich reactions with chiral organocatalysts **16** or **19**.[13,14] Herein, we report the asymmetric synthesis of these unusual amino acid units.

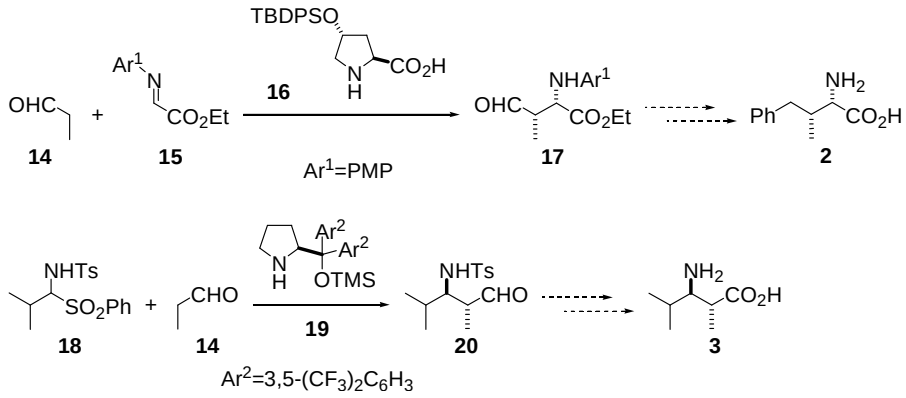


Scheme 1. Previous syntheses of **2** and **3**.

First, we synthesized *N*-Boc-dolaphenvaline (**25**) as illustrated in Scheme 3. As reported by Hayashi,[13] a *syn*-Mannich reaction between propanal (**14**) and ethyl α-imino glyoxylate **15** promoted by the chiral organocatalyst **16** afforded *syn*-adduct **17**, which was directly treated with Wittig reagent in a one-pot operation to isolate the *syn*-α,β-unsaturated ester **21** in 72% yield (2 steps) with excellent enantio- and diastereoselectivity (>95% ee, dr = >95:5).[15,16] Conversion of the aldehyde into the α,β-unsaturated ester was essential for further transformation because the aldehyde moiety of **17** was labile under the reaction conditions for addition of a phenyl group or removal of the *N*-*p*-methoxyphenyl (*N*-PMP) group. One-pot protecting group manipulation followed by ozonolysis produced aldehyde **23** in 57% yield (3 steps). While attempted nucleophilic addition of PhMgBr, PhLi, or $PhCeCl_2$[17] to the aldehyde part of **23** failed, we eventually found that the addition of PhMgBr took place cleanly in the presence of $ZnCl_2$,[18] and lactone **24** was obtained in 67% yield (dr = 8:1).[19] Reductive cleavage of the benzylic C-O bond of **24** under hydrogenolysis conditions provided **25** in 93% yield.

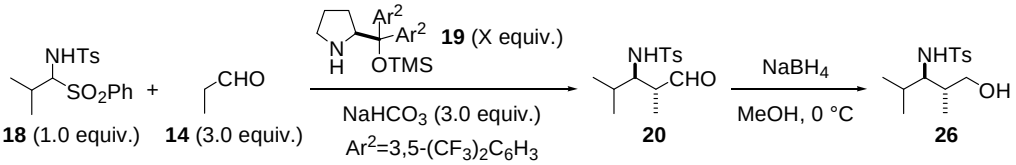


Scheme 3. Stereoselective synthesis of **25**.



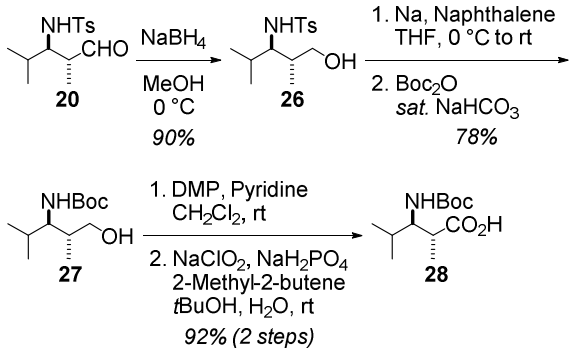
Scheme 2. Synthetic plan for **2** and **3**.

Table 1. Optimization of *anti*-Mannich reaction catalyzed by **19**.[a]



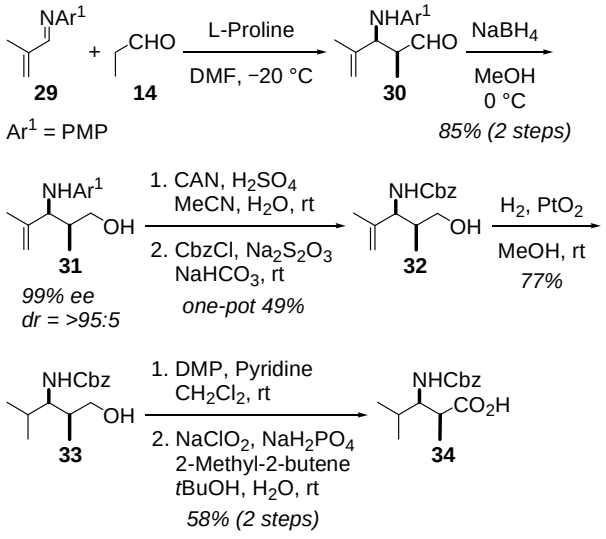
entry	X	Solvent	Temperature	Yield (%) of **20**[b]	dr (*anti*:*syn*)[c]	%ee[d]
1	0.2	1,4-dioxane	rt	75	68:32	95
2	0.2	1,4-dioxane	0 °C	31	73:27	94
3	0.2	DMF	0 °C	9	93:7	81
4	0.2	DMSO	rt	cm	nd	nd
5	0.2	THF	rt	59	94:6	98
6	0.2	THF	0 °C	44	>95:5	>99
7	0.2	THF	rt	92	92:8	98
8	0.2	THF	0 °C	74	>95:5	>99
9[e]	0.4	THF	0 °C	76	>95:5	98
10[f]	0.4	THF	0 °C	75	>95:5	98

[a] For optimizations, the reaction conducted with 30 μL (0.433 mmol) of **14** and 33.2 mg (0.087 mmol) of **18**; cm = complex mixture; nd = not determined. [b] Isolated yield of **20** after column chromatography. [c] Determined by ^{1}H-NMR with alcohol **26** obtained by $NaBH_4$-reduction of **20**. [d] Determined by chiral HPLC analysis with **26**. [e] Partial removal of TMS group of **19** was observed. Recovered **19** was used after silylation with TMSOTf and Et_3N. [f] For gram scale synthesis, 1.0 g (2.6 mmol) of **18** was used.



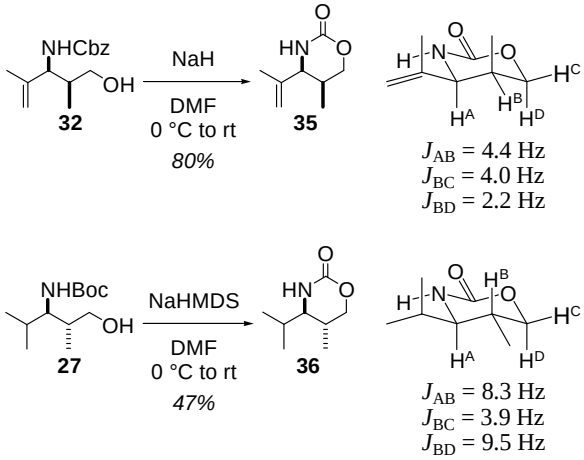
Scheme 4. Stereoselective synthesis of **28**.

We then turned our attention to the synthesis of *N*-Boc-dolamethylleuine (**28**). Hayashi reported that an *anti*-Mannich reaction between aminosulfone **18** and propanal (**14**) with chiral catalyst **19** (0.1 equiv.) afforded *anti*-adduct **20** with high stereoselectivity (98% ee, dr = 88:12) by performing the reaction in 1,4-dioxane at 10 °C.[14] For convenient gram-scale preparation of **20**, we attempted to optimize the reaction conditions for the *anti*-Mannich reaction. The results of the optimization aresummarized in Table 1. When the reaction was carried out at ambient temperature following the protocol described by Hayashi, excellent enantioselectivity (95% ee) was obtained but diastereoselectivity was low (dr = 68:32) (Entry 1). Lowering the reaction temperature to 0 °C caused a significant decrease in chemical yield (Entry 2). We then performed a solvent screen under the same reaction conditions. Although the Mannich reaction proceeded sluggishly in DMF or DMSO (Entries 3 and 4), THF was found to be superior to 1,4-dioxane in achieving the desired stereoselectivity (Entries 5, 6). In particular, **20** was exclusively formed as a single stereoisomer (>99% ee, dr = >95:5) at 0 °C. However, the chemical yield in THF was moderate at room temperature. A twofold increase in the catalyst loading led to a dramatic increase in chemical yield (Entries 7, 8). The chiral organocatalyst **19** was easily recovered in a yield of 76% by chromatographic separation of the reaction mixture, and was reused for the same Mannich reaction between **18** and **14** (Entry 9). Under the optimal conditions (with 0.4 equiv. of **19** in THF at 0 °C), the organocatalytic Mannich reaction was successfully used for a gram-scale synthesis of **20** to afford a comparable yield (75%) and stereoselectivity (98% ee, dr = >95:5) (Entry 10) to those obtained in a milligram-scale experiment (Entry 8). The resulting **20** was converted to **28** as shown in Scheme 4. After reduction of **20** with $NaBH_4$ to alcohol **26**, successive removal of the *N*-Ts group and Boc-protection in a one-pot operation gave alcohol **27** in 70% yield (3 steps). Stepwise oxidation of the primary alcohol to the carboxylic acid gave **28** in 84% yield (2 steps).



Scheme 5. Selective synthesis of **34**.

We also achieved the synthesis of *N*-Cbz-2-*epi*-dolamethyl-leuine (**34**). A highly stereoselective *syn*-Mannich reaction of aromatic *N*-PMP-aldimine with propanal (**14**), catalyzed by L-proline, was reported by Hayashi.[20] The α,β-unsaturated *N*-PMP-aldimine **29**, generated from methacrolein and *p*-anisidine, was subjected to an asymmetric *syn*-Mannich reaction with **14**. The 1,2-addition reaction occurred cleanly in a highly stereoselective manner (99% ee, dr = >95:5)[21] to give *syn*-adduct **30** almost exclusively. After $NaBH_4$-reduction of **30**, alcohol **31** was isolated in 85% yield (2 steps). One-pot protecting group transformation gave alcohol **32** (49% yield), which led to **34** via



Scheme 6. Confirmation of relative configuration of **34**.

hydrogenation of the olefin (77% yield) and oxidation of primary alcohol **33** to carboxylic acid (58% yield). The relative configuration of **34** was determined based on coupling constant analysis of tetrahydro-1,3-oxazin-2-ones **35** and **36**, prepared through base-induced cyclization of *N*-protected 1,3-amino alcohols **32** and **27**, respectively (Scheme 6). Since **27** was derived from the known *anti*-1,3-amino alcohol **26**[14] (Scheme 4), both of the vicinal methine protons (H^A and H^B) of **36**, prepared from **27**, were expected to adopt an axial orientation in the chair conformation of the 1,3-oxazine ring. Actually, a larger vicinal coupling constant, J_{AB} = 8.3 Hz (axial/axial relationship), was obtained in ^{1}H-NMR spectrum of **36**. The smaller vicinal coupling constant J_{AB} = 4.4 Hz (axial/equatorial relationship) for **35** reveals a *cis* relationship between the vicinal methine protons H^A and H^B and the *syn*-relative stereochemistry of **34**. In contrast, in the L-proline-catalyzed asymmetric Mannich reaction, L-proline always mediates a *si*-facial attack of the aldimine through an enamine intermediate generated from an aldehyde.[22] Therefore, the absolute configuration of **34** is expected to be (2*S*, 3*R*).

In summary, scalable and concise syntheses of *N*-Boc-dolaphenvaline (**25**) and *N*-Boc-dolamethylleuine (**28**), *N*-Boc-protected amino acid units of dolastatin 16 (**1**), were achieved by employing enantio- and diastereoselective organocatalytic Mannich reactions. The synthetic sequence provided subgram amounts of the amino acid units **25** and **28** with overall yields of 26% (5 steps) and 48% (5 steps), respectively. Furthermore, this synthetic approach is expected to be applicable to gram-scale preparation of various derivatives of these unusual amino acid units through the selection of appropriate chiral catalysts or starting materials. Indeed, *N*-Cbz-2-*epi*-dolamethylleuine (**34**) was prepared according to a similar scheme to that used for **28**. Further studies with the aim of achieving a practical and scalable total synthesis of dolastatin 16 (**1**) using **25** and **28** are ongoing.

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Supplementary Material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.tetlet.

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