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Cyclic Azole-Homologated Peptides from Marine Sponges

Tadeusz F. Molinski[a]

[a]Department of Chemistry and Biochemistry, and Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, 9500 Gilman Dr 0358., La Jolla, California, 92093, USA.

Abstract

This review discusses the chemistry of cyclic azole-homologated peptides (AHPs) from the marine sponges, *Theonella swinhoei*, other *Theonella* species, *Calyx* spp. and *Plakina jamaicensis*. The origin, distribution of AHPs and molecular structure elucidations of AHPs are described followed by their biosynthesis, bioactivity, and synthetic efforts towards their total synthesis. Reports of partial and total synthesis of AHPs extend beyond peptide coupling reactions and include creative construction of the non-proteinogenic amino acid components, mainly the homologated heteroaromatic and α-keto-β-amino acids. A useful conclusion is drawn regarding AHPs: despite their rarity, exotic structures and the potent protease inhibitory properties of some members, their synthesis is under-developed and beckons solutions for outstanding problems towards their efficient assembly.

Introduction

During the 1970's and 1980's – two decades that embraced the 'log phase' of discovery of marine natural products – attention was mainly given to non-polar, lipophilic compounds including terpenoids, polyketides, lipids – particularly polyhalogenated compounds from red algae. Relatively few peptides were described from marine organisms in this period. For example, although in 1985 the tally of marine natural products, listed by the late D. John Faulkner in the first three of his comprehensive reviews on the subject, was about 1,700[1] and, of these, fewer than 200 were peptides and amino acid (aa) acylates.[2] By the end of 2015, the *RSC* database MarinLit showed the inventory had grown to 1,450 peptides among a total of 28,255 marine natural products.‡

A. Structural Diversity of Marine-Derived Peptides

The structures of most peptides from marine organisms are cyclic; consequently they lack exposed N- and C- termini and are relatively non-polar; a property that allows their facile

Conflicts of interest

There are no conflicts to declare.

† Footnotes relating to the title and/or authors should appear here.

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‡I am happy to thank John Blunt for extracting this data from MarinLit.

recovery and purification beginning with solvent partitioning.[§] A notable characteristic of marine-derived peptides is the frequent presence of highly modified aa residues, many of them in the non-proteinogenic D- configuration. For example, two unusual metabolites from *Dysidea herbacea*, (–)-dysidenin (**1a**)[4a] and diketopiperazine **1b** (Figure 1) reported in the late 1970s by Kazlauskas and coworkers from an Australian specimen, are the first examples of L-5,5,5-trichloroleucine (TCL) containing peptide natural products. The structure of (+)-dysidin (**1c**), elucidated by Hofheinz and Obserhänsli, revealed a trichloro-metabolite suggestive of biosynthetic catabolism of TCL to (*S*)-4,4,4-trichloroisovaleric acid and its homologation by one malonate unit.[4c] Peptide **1a** also displays the cysteine-derived D-2-(2-thiazolyl)alanine. Subsequent reports have expanded the list of oxazole- and thiazole-containing peptides from marine organisms, including a special subclass: the azole-homologated peptides (AHPs) containing azole-homologated amino acid residues (AHAs). The chemistry of AHPs is the subject of this review.

B. Origin and Classification of Azole-Homologated Peptides

Cyclic AHPs are biosynthesised by non-ribosomal synthase/polyketide synthase (NRPS-PKS) and appear to be limited to a few genera of marine sponges. Structurally, they can be classified as Type I or Type 2, based on the presence of conserved tetrads of aa residues within the macrocyclic ring. Peptides of Type 1 contain an oxazole-homologated Ala, Aba, or *O*-Me-Ser (for the single exception, vide infra), ring-closed by a tetrad of conserved aa residues: in sequence, an α-keto-β-amino-alkanoic acid (mostly originating from Leu or Ile), Pro, Orn, and Trp (or 5-HO-Trp or a variant, thereof). In Type 2 peptides, the first two residues are retained, but Pro and Orn are replaced consistently by Ala and 2,3-diaminopropanoic acid (Dap), respectively. In all AHPs, the α-amino group of the Orn or Dap residue is extended through an amide bond to one or two additional aa residues, and the terminal aa is *N*-acylated by a short-chain carboxylic acid.

The structures of keramamides B-D (**2**-**4**) [5] and orbiculamide A (**5**)[6] (Figure 2) from the marine sponge *Theonella swinhoei*, representing the first Type 1 AHPs, were published ‘back-to-back’ in 1991 by the groups of Kobayashi and Fusetani, respectively. In the structures of **2**–**5**, the heteroaromatic unit is oxazole-homologated L-Ala – so-called ‘theonalanine’ (**6a**) [6]) or its homolog **6b** – and an α-keto-β-amino acid derived from isoleucine: so-called ‘theoleucine’.[6] All other AHPs display one of the thiazole-containing residues **7a**-**c**. Most AHPs described to date were isolated from *T. swinhoei* (which is also a prolific producer of polyketides, e.g. swinholide A [7]), but a few were found in *Calyx* spp. and one from *Discodermia jamaicensis*.

Subsequent to the reports of **2**–**5**, additional AHPs in the keramamide series from *T. swinhoei* were disclosed: (in approximate chronological order) keramamides E (**8**), G (**9**), H (**10**) and J (**11**),[8], F (**12**),[9] (E, F, G, H and J contain the rare D-isoserine residue), K (**13**), [10] and M (**14**) and N (**15**), the *O*-sulphate esters of **4** and **8**, respectively.[11][§§]

[§]An alternative interpretation reads, “marine natural product peptides are peptides successfully isolated by chemists through solvent extraction.”!

[§§]Keramamides A [30] and L [10], also from *T. swinhoei*,[10] are not discussed in this review and their structures are absent from Figure 2: both are cyclic peptides of a different class – a Type 3, if you will – the first members of which, mozamides A and B, were

The structure of oriamide (**16a**), from a South African *Theonella* aff. *swinhoei*, is closest to a Type 2 AHP with exceptions to the rule: the Trp-derived residue is replaced by cysteic acid, the Ala-derived AHA is replaced by one derived from Tyr, and the *N*-terminal group is a 2,5-dihydroxybenzamide. [13] The homologs discobahamins A and B (**17a**,**b**)[14] and diastereomeric calyxamides A and B (**18a**,**b**)[15] were isolated from Atlantic and Pacific *Discodermia* species, respectively. Most recently, jamaicensamide A (**19**) – so far, the only thiazole-homologated Type 1 AHP – was discovered in the rare Bahamian sponge *Plakina jamaicensis*.[16] Cycloneothellazoles A-C (**16b**-**d**) are *p*-hydroxybenzamide analogs of **16a** from the same sponge that delivered the latter.[17] Finally, of related interest, the depsipeptide scleritodermin A (**20a**, Figure 3) from the sponge *Scleritoderma nodosum*,[12] mostly resembles AHPs **16a**–**d**: **20a** contains a thiazole-double homologated Tyr ('ACT') linked to a diastereomeric α-keto-β-amino aa (L-keto-*allo*-isoleucine, or L-*allo*-Thi).

C. Biological Activity

Compounds **2**, **5**, **8**-**18** have been reported along with biological activity, mostly modest levels of cytotoxicity against cultured human cancer cells (e.g. P388, L1210). Keramamides B-D (**2**–**4**) inhibit superoxide generation (~30–50 nM) by human neutrophils stimulated with a chemotactic peptide.[5] Of outstanding interest is the potent protease activities of cyclotheonellazoles A-C (**16b**-**d**): all were shown to be nanomolar inhibitors of chymotrypsin and subnanomolar inhibitors of elastase.[17] The thrombin inhibitory activity of another peptide, cyclotheonamide A (**20b**, IC_{50} ~100 nM) from *T. swinhoei* has been more extensively studied,[20] and suggests a common link between the four compounds and protease activity: the α-keto-β-amino acid residue appears to be a transition state mimic of the hydrolytic active site of the enzymes. The protease activity of other AHPs – all of which bear the same or homologous aa residue – is under-investigated, but a worthy subject and opportunity for future investigations.

D. Biosynthesis

Comprehensive studies of molecular genetics and biochemistry of the prolific sponge *T. swinhoei* by Piel and coworkers using late-generation genetic tools,[18] revealed the origin of many of its peptide metabolites.[19] A single symbiotic γ-proteobacterium, '*Entotheonella* sp.' that lives within the tissue of *T. swinhoei* is the 'talented producer' responsible for biosynthesis of virtually all peptides associated with this sponge, including the keramamides [5,8–12] and other unrelated peptides.[19][§§§] For example, genetic evidence shows the CthA2 and KerA5 genes of *Entotheonella* sp. shows high specificity for adenylation of diaminopropanoic acid (Dap) in cyclotheonamide (and also **16a**-**d**, **18a**,**b**) and Leu, respectively, which is transformed to theoleucine in about half the keramamides (**9**-**13**).[19] The 13*R* configuration of some AHPs (e.g. **9** and **18b**) is unexpected as the gene cluster lacks an epimerase domain,[19] however, it is known this centre is prone to spontaneous epimerisation (see below)

reported by Faulkner and coworkers.[31] See Supporting Information of Reference 16. 'Keramamide I' seems to be an ordinal omission: a compound of this name is absent from the literature.

[§§§]Interestingly, sequencing of the *Entotheonella* spp. genomes has revealed the gene clusters responsible for a range of other *Theonella* NRPS-PKS natural products, such as konbamides, onnamides, polytheonamides, nazuamides and psymberin [ref. 18,19].

E. Synthetic Studies

Remarkably, few synthetic studies on AHPs have been reported and only one total synthesis, that of a stereoisomer of the keramamide J (**11**) that questions the configuration of the natural product. The lion's share of the work is design and synthesis of the non-proteinogenic aa residues; particularly the α-keto-β-amino acids and the AHA residues. Amide bond couplings follow standard protocols but strategic choices of which amide bond to close during macrolactamization is critical to any macrocyclic peptide synthesis.

E1. Approach to Keramamide B (2): Shioiri and Hughes

The partial synthesis of keramamide B comprising the preparation of the oligopeptide fragments **21**, **22** and **23** was described by Shioiri and Hughes (Scheme 1).[20] The protected oxazole-homologated aa residue **21** was derived from L-*N*-Boc-Ser and *N*-Boc-2-aminobutanoic acid (*N*-Boc-Aba), while the protected α-keto-β-amino acid residue (carried as its reduced α-acetoxy acid analog, **22**) was elaborated from a 2-substituted furan derived from L-*N*-Boc-Leu. The last fragment prepared was the differentially *N*-protected L-Orn-Pro-OMe dipeptide **23**.

In the forward direction (Scheme 2), L-*N*-Boc-Leu was converted to the Weinreb amide **24** under standard conditions, then transformed to the 2-furylketone **25** (2-lithiofuran, –78 ˚C). Reduction of **25** ($NaBH_4$) followed by *O*-acetylation gave an inconsequential mixture of α-acetoxy esters (**26**; the stereocentre would be removed after hydrolysis-oxidation). Perruthenate oxidation of **26** gave the free carboxylic acid **22**.

Reduction of the ester group of **29** ($LiBH_4$) to primary alcohol **30**, followed by oxidation with activated MnO_2, delivered aldehyde **31** which was subjected to Horner-Wadsworth-Emmons olefination under Masamune-Roush conditions to provide the protected ethyl ester **32** in good yield: Saponification of the latter delivered **21**.

Preparation of the oxazole-homologated aa residue **21** (Scheme 3) started with amide bond formation between L-*N*-Boc-Aba and L-Ser methyl ester followed by cyclodehydration of the dipeptide product **27** with the Burgess reagent to oxazoline **28** which was subsequently oxidized to afford oxazole **29** in low yield (27%).

The remaining peptide fragments were assembled from common L-amino acids and condensed, mostly, using the coupling reagent diethylcyanophosphoridate (DEPC; Schemes 4 and 5). The *N,N,O*-protected 2-bromo-5-hydroxytryptophan (**33**) was elaborated as shown in Scheme 4. L-HO-Trp was converted, through intermediates **34** and **35**, and coupled [$(PhO)_2P(O)Cl$ with oxazole-containing aa **21**] to dipeptide **36**. Bromination of the latter at C-2 of Trp (NBS) gave **37** in low yield (22%).

Condensation of L-*N*-Boc-Ile with L-methyl 2-aminopentanoate (Scheme 5) gave dipeptide **38** which – after removal of the Boc group – was coupled to L-isoleucic acid (**39**) to deliver dipeptide acylate **40**. *O*-Protection (TBSOTf, *sym*-collidine) gave methyl ester **41**: saponification of the latter delivered protected dipeptide **42**. Finally, dipeptide L-(N^{α}-Boc-

N$^{\varepsilon}$-Cbz)-Orn-L-Pro-OMe (**23**) was accessed through DEPC coupling of the respective Orn and Pro precursors.

E2. 13-*epi*-Keramamide J (43): Sowinsky and Toogood

The total synthesis of **43**, the C-13 epimer of keramamide J (**11**), was achieved by Toogood and coworkers using a different approach to both the AHP and the α-keto-β-amino aa residues and order of assembly of the cyclic peptide (Scheme 6) from the defined fragments **44**-**47**.[23] In the event, it was found the product, although bearing the constitution and stereochemistry assigned by Kobayashi to keramamide J[8], was not identical with the natural product raising questions about the configuration of C-13 and drawing to the conclusion that reassignment of the latter is necessary (vide infra).

Preparation of the thiazole-homologated aa (Scheme 7) began with the known thiazole-2-carboxylate ethyl ester **48**, derived earlier by the authors in high enantiomeric purity from *N*-Tr-*O*-methylserine thioamide via a modified Hantsch synthesis. [24] After saponification, coupling of the liberated carboxylic acid (MeNHOMe, BOP, Et_3N) gave Weinreb amide which was reduced to the corresponding aldehyde ($LiAlH_4$) and extended by Wittig olefination to conjugated thiazole **49**. The latter was saponified and coupled to L-Trp-OAllyl [$Ph_2P(O)Cl$] to provide dipeptide **45** (68%, 2 steps).

The key strategy for carrying forward the sensitive α-keto-β-amino aa residue of **11** was masking the latter as an SEM protected α-hydroxyester **44** (Scheme 7), and unmasking by late stage hydrolysis-oxidation. Vinyl ketone **50**, prepared from *N*-Cbz-L-Ile by vinylation of the corresponding Weinreb amide, was reduced under Luche conditions ($NaBH_4$, $CeCl_3$) to a 4:1 mixture of allylic alcohols, one of which, **51**, was separated and carried forward through five steps: SEM protection, ozonolysis to the aldehyde, Pinnick oxidation, and *N*-protecting group interchange (NHCbz to NHFmoc) to deliver **44**.

The *N*-formyl peptide side chain (Scheme 8) was assembled in a straightforward manner starting with commercially available (*S*)-glycidol (Scheme 8). Perruthenate oxidation of the primary alcohol, followed by amide bond coupling with L-Ile methyl ester (DCC, HOBt), gave the acylated amino ester **52** that underwent regiospecific epoxide opening by azide in the presence of a mild Lewis acid (NaN_3, $MgSO_4$) to provide azido intermediate that was transformed by ester interchange (OMe to OBn) and protection of the secondary OH group (TESCl, imidazole) to give benzyl ester **53**. Staudinger reduction of **53** (Ph_3P, H_2O) followed by saponification of the COOBn group and formylation of the liberated primary amine (*p*-$NO_2C_6H_4OCHO$) gave **47**.

Assembly of the peptide bonds and macrocyclisation (Scheme 9) began with removal of the Boc group from **45** (HCl, Et_2O) and peptide coupling with **44** (DCC, HOBt, Hünig's base), followed by removal of the allyl group ($Pd(Ph_3P)_4$, dimedone) and coupling with dipeptide **46** to deliver the cyclisation precursor **54** (56% over four steps).

After removal of both *N* and *C* terminal protecting groups of **54** in separate steps, a dilute solution of the product was cyclised with diphenylphosphoryl azide [$(PhO)_2P(O)N_3$, $NaHCO_3$] to afford the cyclic peptide **55** in 51% yield over three steps.

Completion of the synthesis required attachment of the *N*-formyl dipeptidyl chain **47** to the core macrocycle (Scheme 10). Treatment of **55** with mineral acid (HCl, MeOH) simultaneously removed both the Boc and SEM protecting groups, which allowed coupling of the amine product with fragment **47** to give **56**, the completed carbon framework of the target molecule.

Oxidation of the C-13 hydroxyl group in **56** (IBX, DMSO) followed by removal of the TES group in the presence of strong cation-exchange resin (Amberlite IR-120) gave a product **43** that, although of the same molecular mass as isolated keramamide J (**11**), was different by NMR.

From a thorough comparison of the NMR and chiroptical properties of the synthetic product, 13-*epi*-Keramamide J (**43**), and the highly similar 13*S* keramamide F (**12**), the authors made a strong case that their end product was the targeted 13*S* epimer, the structure assigned by Kobayashi, [8] but the natural product appears to be 13*R* (13-*epi*-keramamide J). It's possible that free 13*S*-Ile arose during oxidative degradation of natural 13*R*-keramamide J by epimerisation of the labile α-centre. The authors also noted, as did Fusetani with orbiculamide A (**5**),[6] that partial C-13 epimerisation of synthetic **43** occurs under the alkaline hydrogen peroxide conditions, or even spontaneously upon standing in solution.[23]

F. Other Synthetic Studies

As mentioned earlier, little has been published on the synthesis of AHPs, but key pilot studies show the way forward. Synthesis of peptide components for assembly of keramamide F (**12**)[9] were reported by Sowinsky and Toogood,[25] based largely on their successful synthesis of **48** (see Scheme 7) and a new method for preparation of Δ^1-Trp.[26].

Scleritodermin A (**20a**, Figure 3),[12] which resembles **16**-**d**,[13],[17] has been the subject of one report by Serra and coworkers who described the synthesis of the component aa units and assembly of the constituent dipeptide **57** (Scheme 11).[27] Efficient preparation of thiazole-homologated Tyr, **58**, starting with L-*p*-benzyloxy-Phe, was achieved using Wipf's oxazoline-thiazoline interconversion (H_2S, Et_3N, MeOH),[28] followed by oxidation-elimination (DAST, DBU, $BrCCl_3$). Conversion of the COOMe group of **58** to conjugated thiazole **59** was effected via the corresponding aldehydes through iterative Wittig-type olefination reactions. Finally, L-Ile was converted to the α-cyano phosphorane **60** (Scheme 12) which, after ozonolysis, was condensed with **59** using the Wasserman protocol[29] to give 'theoisoleucine' dipeptide **57** in 49% yield.

This approach cleverly avoids the masked α-hydroxycarboxamide derivatives of the Shioiri and Toogood approaches and attendant problems of oxidation-epimerisation and gives the dipeptide in fewer steps.

Conclusions

The marine sponge-derived azole-homologated peptides (AHPs) – based on a macrocyclic core of five aa residues, four of them non-proteinogenic – are remarkable in terms of

structural complexity and potential for discovery of inhibitors of serine proteases [e.g. elastase inhibitors, cyclotheonellazoles (**16b**–**c**)[17]).

Recent advances in metagenomic analysis have identified some of the genes responsible for keramamide biosynthesis in sponges: biosynthesis of these and other unrelated peptides are attributed to a single symbiotic γ-proteobacterium, *'Entotheonella'*, which lives interstitially within the sponge tissue.[19]

Published approaches to syntheses of AHPs have been few, and only one total synthesis – that of 13-*epi*-keramamide J (**43**) – have appeared. Nevertheless, the AHPs have inspired significant advances and creative endeavours towards the total syntheses of keramamide B (**2**), keramamide J (**11**) and the 'hybrid' peptide, scleritodermin A (**20a**). No doubt, design and execution of efficient targeted total synthetic schemes towards procurement of useful amounts of sample will evolve hand-in-hand with exploration of their biological properties.

Finally, a comment on stereo-assignment of AHPs is timely. To date, the aa residues in the macrocyclic cores of AHPs have been found to be L-configured with the exception of the α-keto-β-amino acid which appears to exhibit stereochemical plasticity. While mostly L-configured, this residue occasionally occurs in the D-configuration. Given the propensity for epimerisation (vide supra), the relatively acidic β-CH may responsible for the 13*R* configuration in 'keto-L-Ile' assigned to keramamide G (**9**) and possibly keramamide J (**11**), [8] but this alone does not account for an outstanding anomaly. Inversion of C-13 from L- to D- would give a 'keto-*allo*-D-Ile' residue that, upon oxidative-cleavage and acid hydrolysis, would yield the diastereomeric *allo*-D-Ile.[§§§§] The same residue in calyxamides A and B (**18a**,**b**)[15] is a more surprising finding: the two peptides have antipodal 'keto-Ile' residues corresponding to L-Ile and D-Ile, which implies both α- and β-stereocentres are inverted in **18b**. Finally, the corresponding residue in scleritodermin A (**20a**)[12] is replaced by 'keto-L-*allo*-Ile' that is inverted only at the β-stereocentre with respect to proteinogenic L-Ile; a rare, but not unprecedented finding. Findings of new AHPs should go hand-in-hand with critical stereochemical evaluation of α-keto-β-amino acid residue.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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[§§§§]Fusetani alludes to 'partially racemized Leu' obtained from strongly alkaline oxidative degradation of orbiculamide A (**5**); strictly, this is C-2 epimerization and the end product would be D-*allo*-Leu.[6] Other papers are also less clear on this point.

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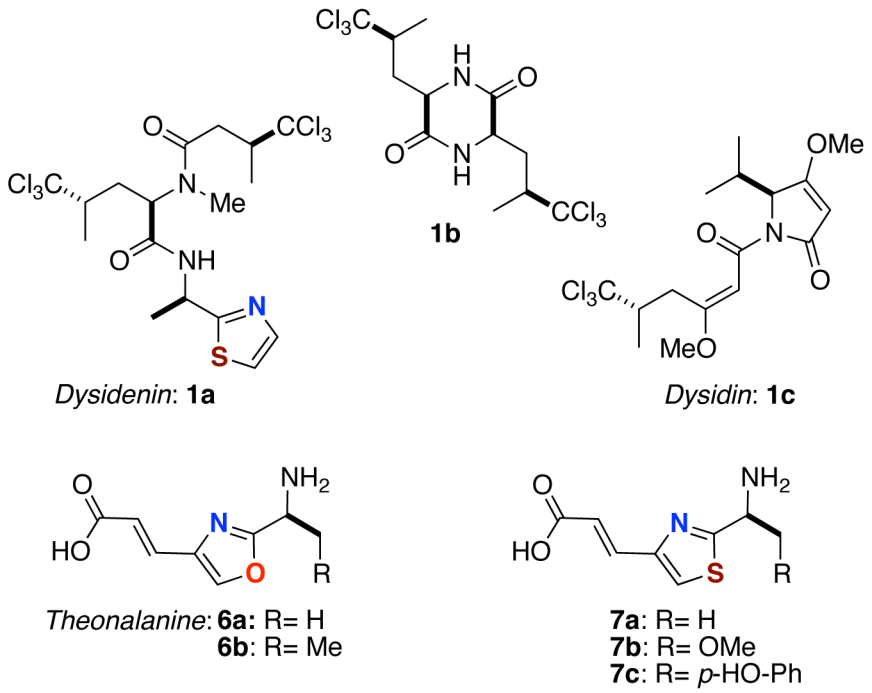


Figure 1.
Non-proteinogenic aa secondary metabolites

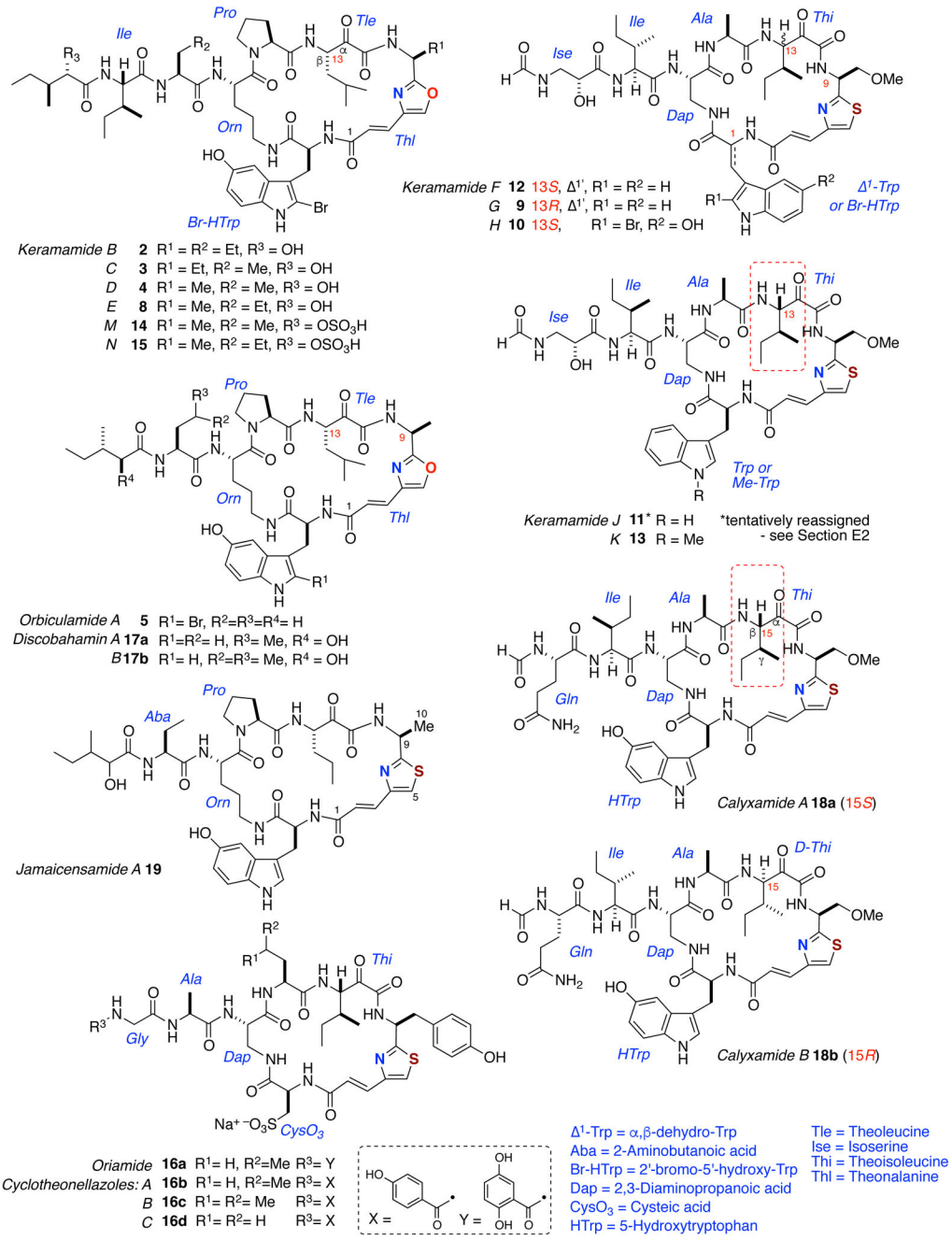


Figure 2.
Structures of azole-homologated peptides (AHPs).

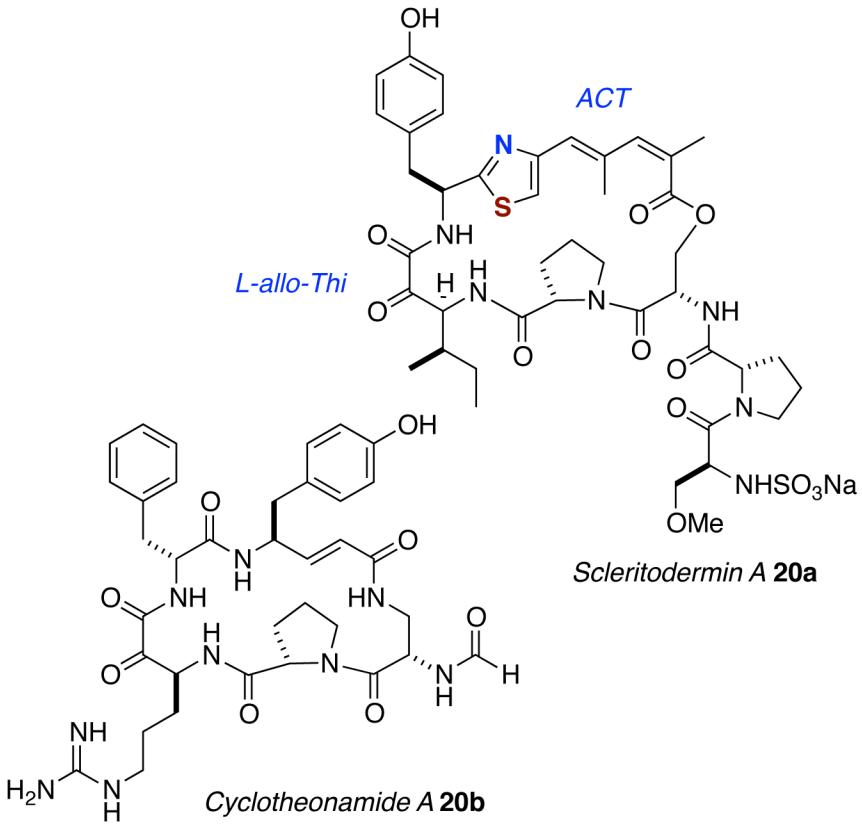
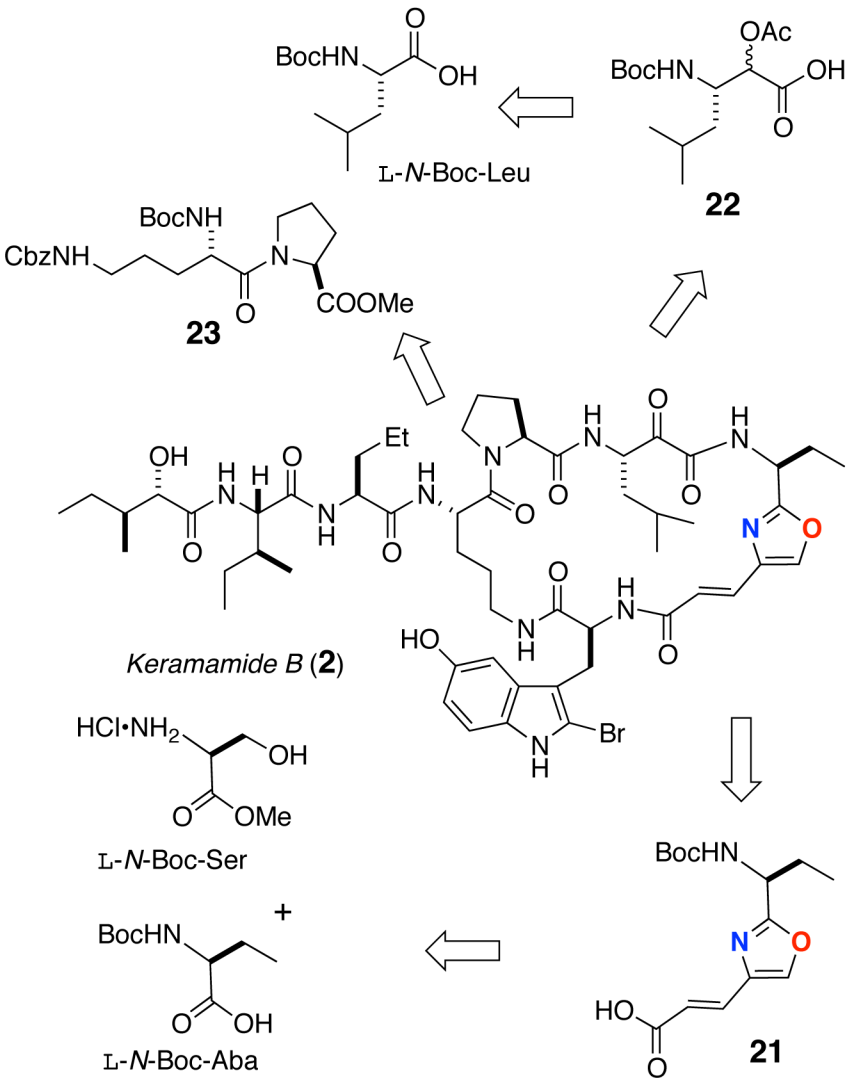
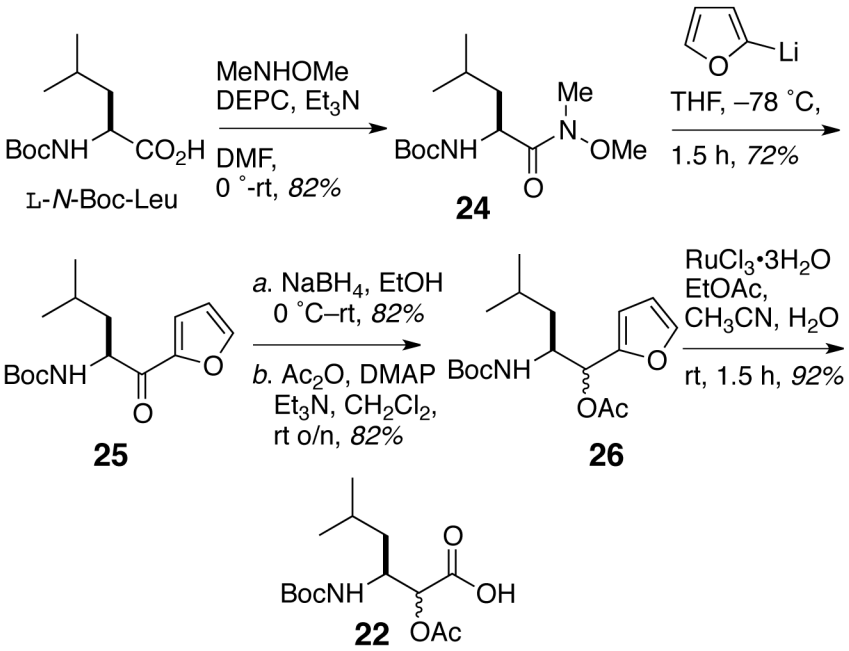


Figure 3.
Related α-keto-β-amino acid-containing peptides.

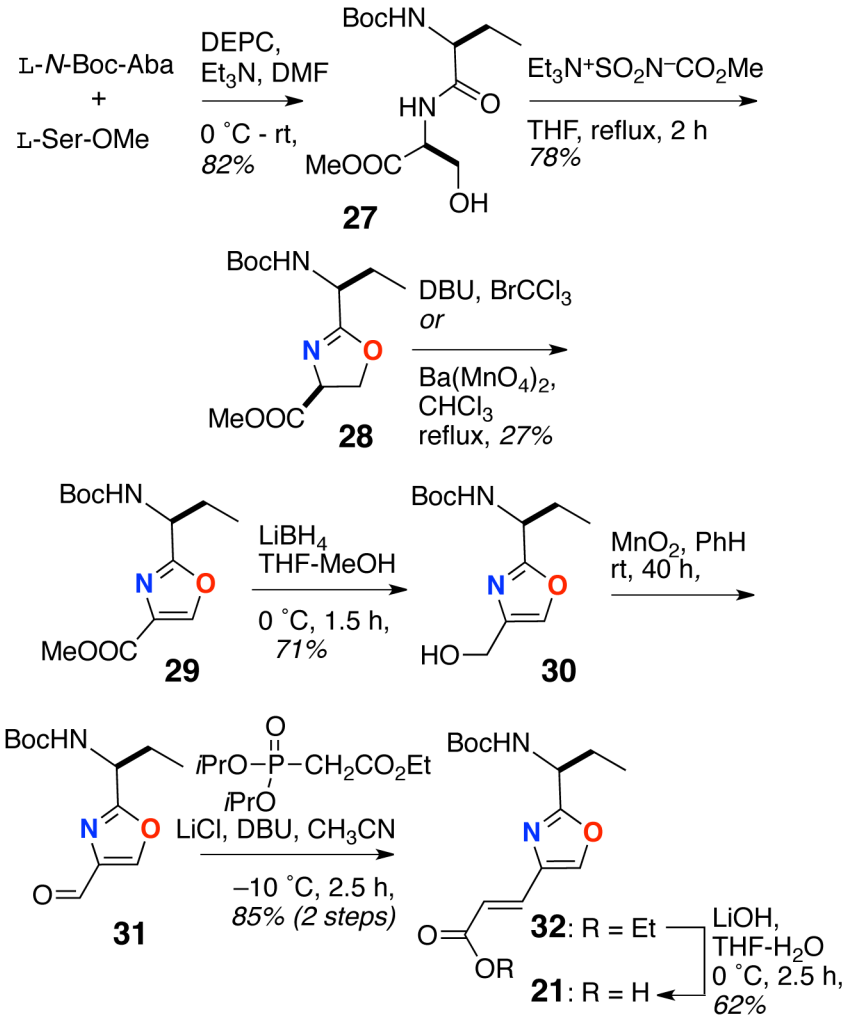


Scheme 1.
Shioiri's approach to keramamide B (**2**).



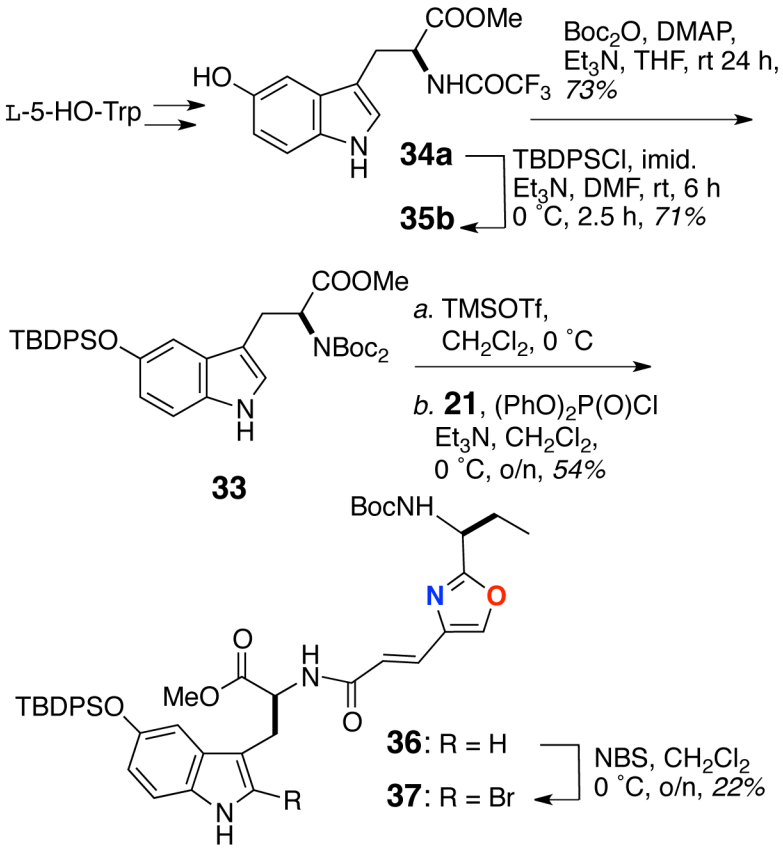
Scheme 2.

Keramamide B (**2**): Synthesis of the masked α-keto-β-amino acid **22**.



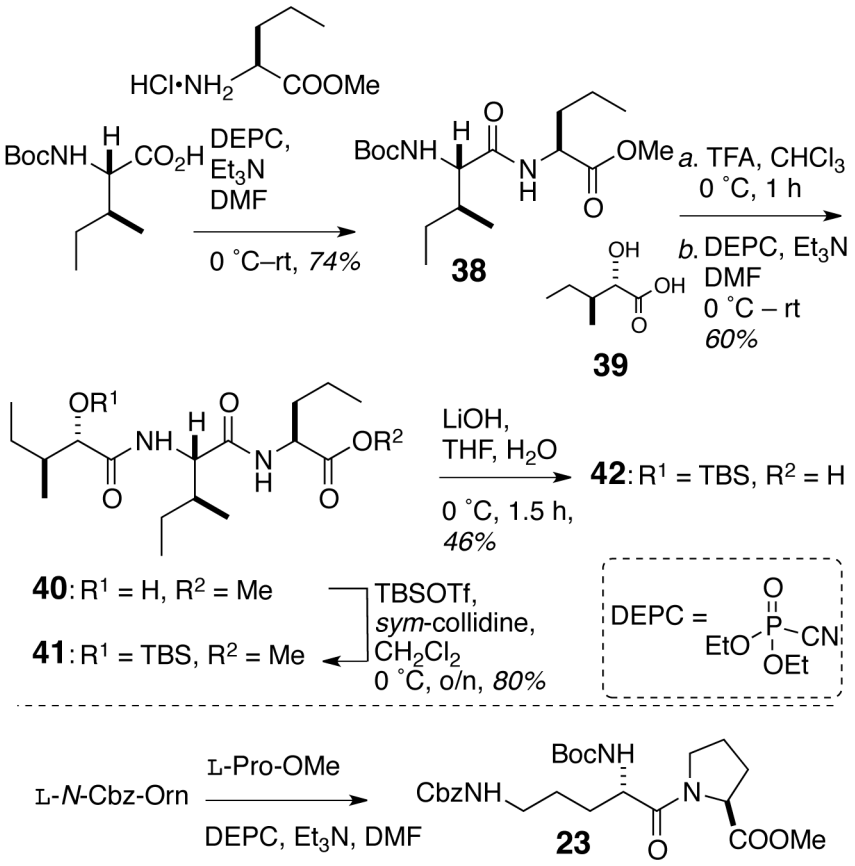
Scheme 3.

Keramamide B (**2**): Synthesis of protected oxazole-homologated aa residue **21**.



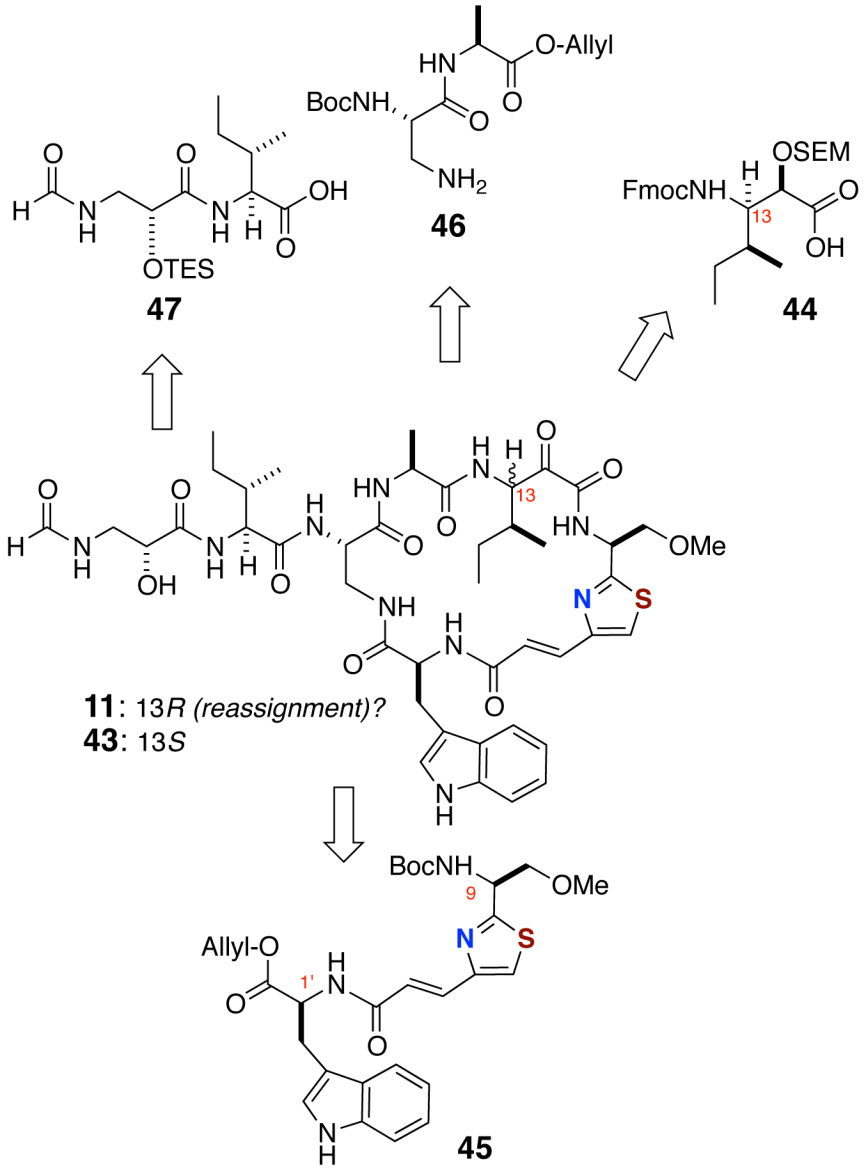
Scheme 4.

Keramamide B (**2**): Peptide coupling and Trp bromination to Trp-AHP **37**.



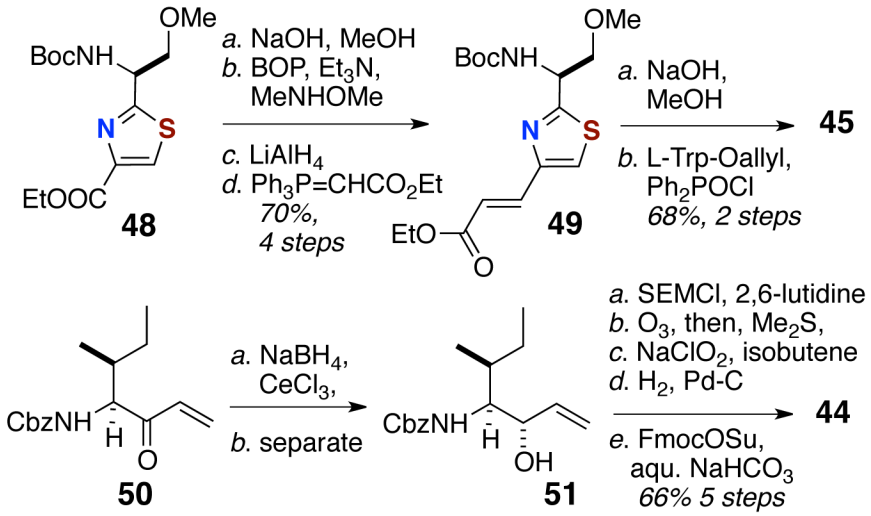
Scheme 5.

Keramamide B: Preparation of remaining dipeptide fragments, **23** and **42**.



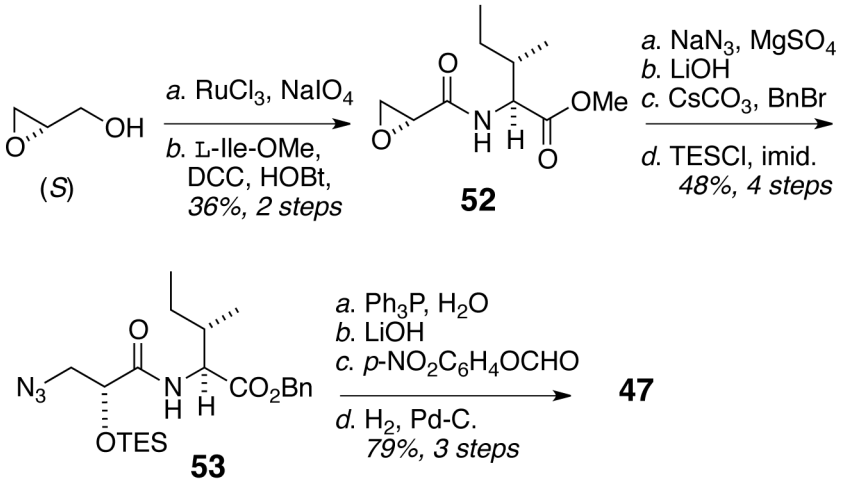
Scheme 6.

Toogood's synthesis of 13-*epi*-Keramamide J (**43**): Retrosynthesis.



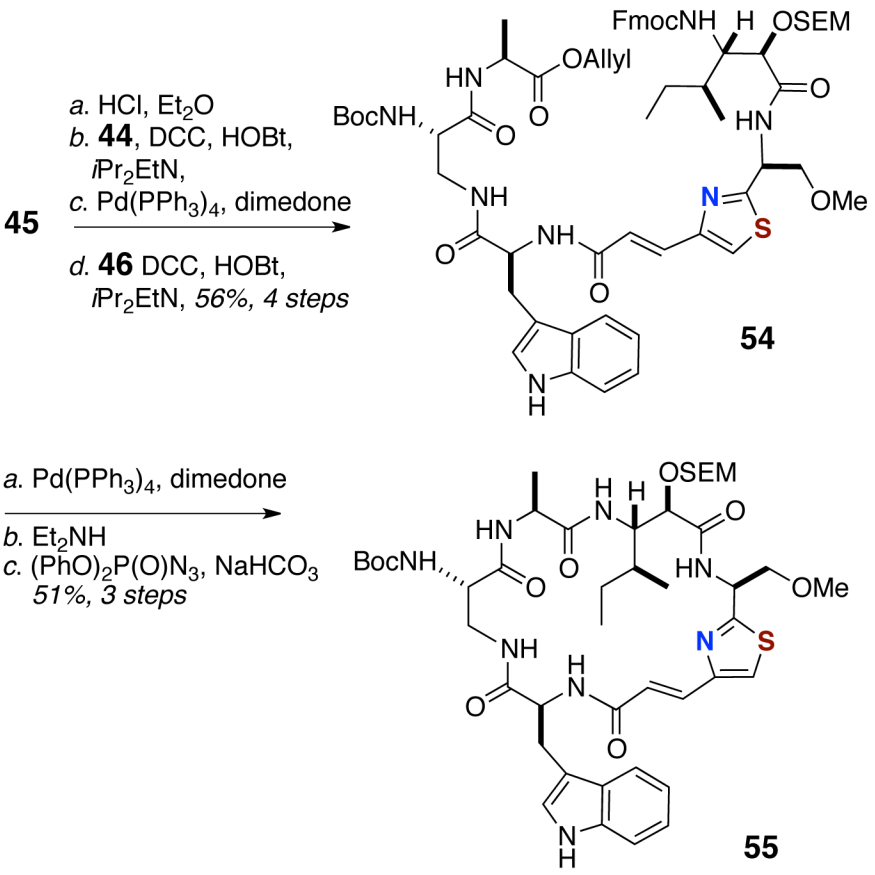
Scheme 7.

13-*epi*-Keramamide J: Preparation of protected α-keto-β-amino acid **44** and thiazole-homologated aa **45**.



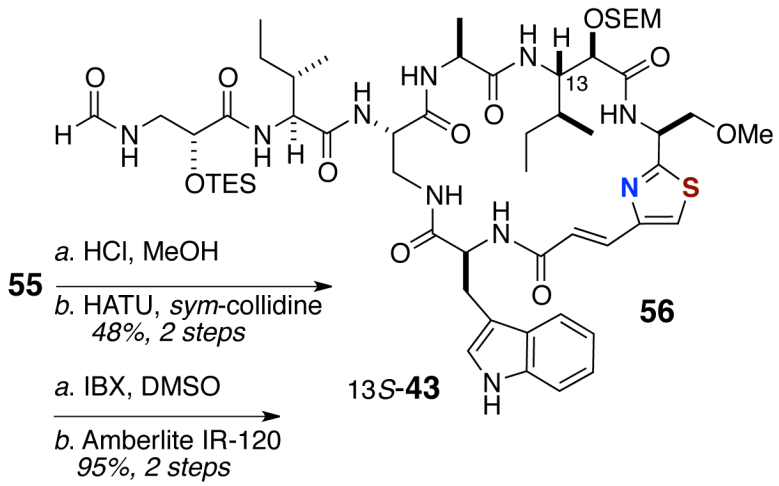
Scheme 8.

13-*epi*-Keramamide J : Preparation of *N*-formyl side chain peptide fragment **47**.



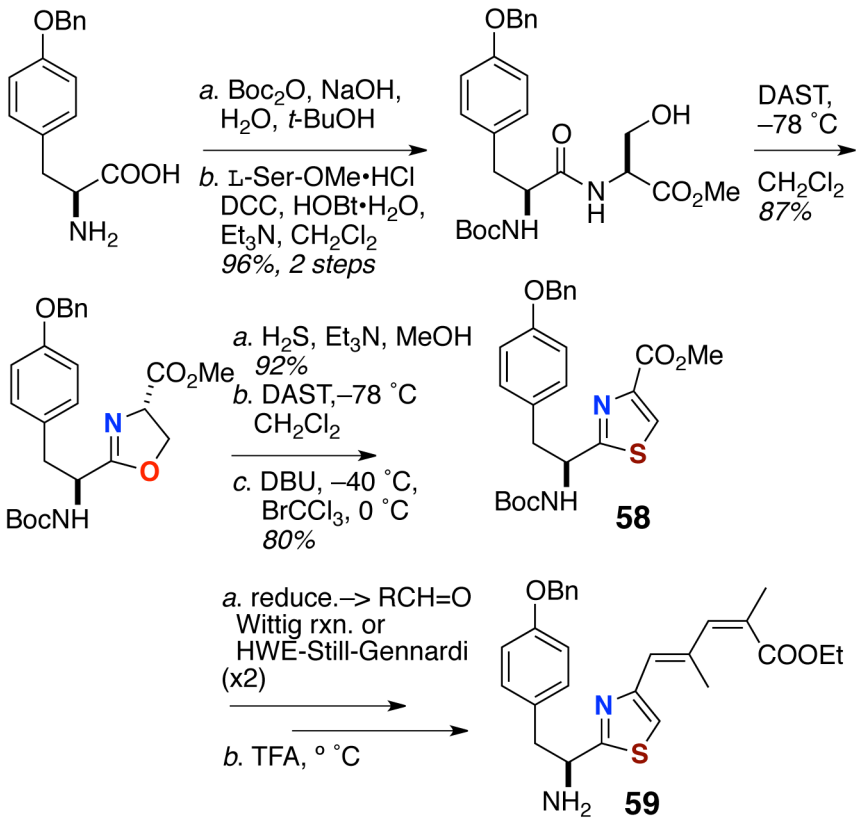
Scheme 9.

13-*epi*-Keramamide J: Fragment assembly by amide bond coupling-macrocyclisation to cyclic peptide **55**.



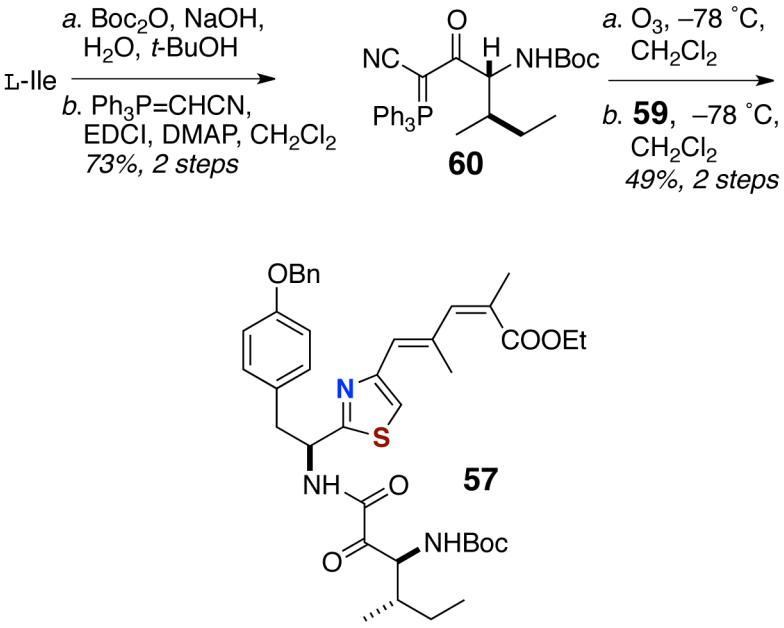
Scheme 10.

13-*epi*-Keramamide J: Final assembly (deprotection, oxidation) of 13-*epi*-Keramamide B (**43**).



Scheme 11.

Preparation of thiazole-homologated ‘theoisoleucine’ dipeptide **59** of scleritodermin A (**20a**).



Scheme 12.
Completion of ‘theo-*allo*-isoleucine’-AHP dipeptide **57**.