

Total synthesis of a keramamide

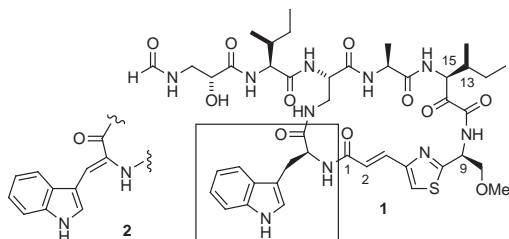
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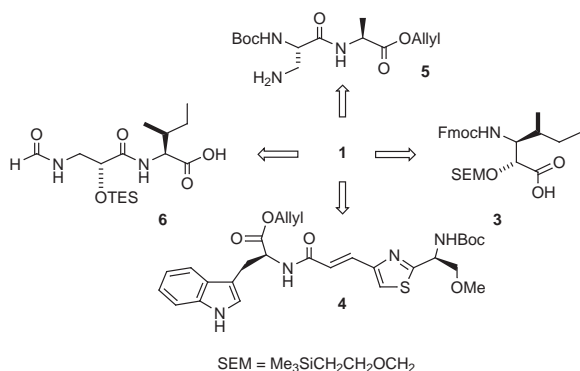
The first total synthesis of a molecule possessing the structure proposed for keramamide **J** is described, providing data indicating that the structure of this natural product should be re-examined.

Keramamide **J** (KJ; **1**)¹ is the simplest member of a class of *Theonella* natural products that collectively exhibit cytotoxic,^{1–3} anti-fungal⁴ and anti-oxidant activity.⁵ To study the biological activities of these molecules, we required a general synthetic route that would be amenable to the synthesis of all members of this series.⁴ Herein, we report our progress towards this goal, including the first total synthesis of a keramamide possessing structure **1**.

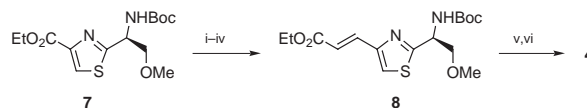


Our synthetic plan for constructing KJ is shown in Scheme 1.⁶ A convergent route was devised, employing four fragments of comparable complexity (**3–6**). We decided to mask the electrophilic keto-amide moiety⁷ as a protected α -hydroxy carbonyl group until the conclusion of the synthesis to avoid nucleophilic attack at this center. In addition, we elected to attempt ring closure between the amine of fragment **3** and the C-terminus of the alanine residue, which was anticipated to be less prone to epimerization than the tryptophan residue and easier to couple than the α -hydroxy acid. Late incorporation of side chain fragment **6** was selected to increase convergency and minimize potential side reactions during the critical macrocyclization reaction.

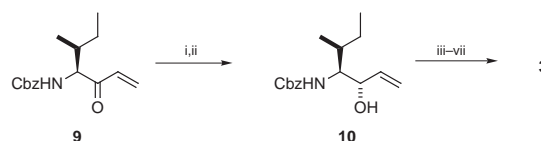
Thiazole **7**⁸ was extended *via* reduction of the ester to an aldehyde and Wittig olefination to introduce the *E*-double bond (Scheme 2). Saponification of the ethyl ester, followed by coupling to L-tryptophan allyl ester provided fragment **4**. To obtain the hexanoic acid fragment **3**, enone **9** (Scheme 3) was



Scheme 1



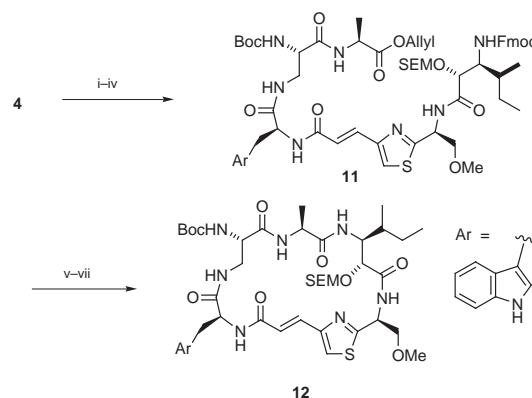
Scheme 2 Reagents and conditions: i, NaOH, MeOH; ii, BOP, Et₃N, HN(Me)OMe; iii, LiAlH₄; iv, Ph₃P=CHCO₂Et (70% for 4 steps); v, NaOH, MeOH; vi, L-Trp-OAllyl, Ph₂POCl (68% for 2 steps).



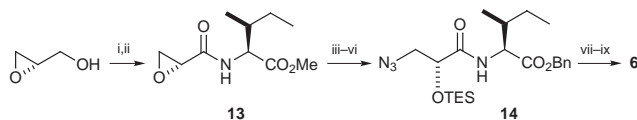
Scheme 3 Reagents and conditions: i, NaBH₄, CeCl₃; ii, separate (76% for 2 steps); iii, SEMCl, 2,6-lutidine; iv, O₃, DMS; v, NaClO₂, isobutene; vi, H₂, Pd/C; vii, FmocOSu, aq. NaHCO₃ (66% for 5 steps).

prepared from L-isoleucine *via* displacement of the corresponding Weinreb amide with vinyl magnesium bromide. Reduction of this enone with NaBH₄ under the Luche conditions⁹ provided a 4 : 1 mixture of alcohols which could be separated by column chromatography. Protection of the major product (**10**)¹⁰ as its (trimethylsilyl)ethoxymethyl (SEM) acetal, followed by a two step oxidation of the double bond, provided the carboxylic acid. Hydrogenolytic cleavage of the Cbz group and subsequent treatment with FmocOSu[†] gave compound **3**. Deprotection of fragment **4**, followed by condensation with acid **3**, gave the corresponding tripeptide in 77% yield (Scheme 4). Subsequent cleavage of the allyl ester and coupling to dipeptide **5** gave linear precursor to the KJ macrocycle **11**. Following deprotection of the C-terminus, and removal of the Fmoc group under standard conditions, macrocyclization proceeded smoothly to provide cyclic peptide **12** in 34–51% overall yields from intermediate **11**.

To prepare the KJ side chain, (*S*)-glycidol was oxidized to glycidic acid using RuCl₃ and NaIO₄,¹¹ then coupled with L-isoleucine methyl ester to give peptide **13** as a single diastereomer (Scheme 5). Attack of the oxirane by azide ion at



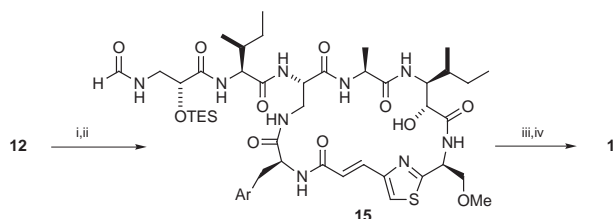
Scheme 4 Reagents and conditions: i, HCl, Et₂O; ii, **3**, DCC, HOBT, Pr₂EtN; iii, Pd(PPh₃)₄, dimedone; iv, **5**, DCC, HOBT, Pr₂EtN (56% for 4 steps); v, Pd(PPh₃)₄, dimedone; vi, Et₂NH; vii, (PhO)₂PON₃, NaHCO₃ (51% over 3 steps).



Scheme 5 Reagents and conditions: i, RuCl_3 , NaIO_4 ; ii, L-Ile-OMe, DCC, HOBt (36% for 2 steps); iii, NaN_3 , MgSO_4 ; iv, LiOH ; v, CsCO_3 , BnBr ; vi, TESCl , imidazole (48% for 4 steps); vii, PPh_3 , H_2O ; viii, $p\text{-O}_2\text{NC}_6\text{H}_4\text{-OCHO}$; ix, H_2 , Pd/C (79% for 3 steps).

the less substituted position, followed by transesterification and protection of the hydroxy function produced azido peptide **14**. Staudinger reduction of the azide to a primary amine,¹² followed by formylation with *p*-nitrophenyl formate and hydrogenolytic cleavage of the ester, provided fragment **6**.

Treatment of macrocycle **12** with HCl in $\text{Et}_2\text{O-MeOH}$ - CHCl_3 deprotected the amine and secondary alcohol, and fragment **6** was attached using HATU[†] to produce alcohol **15** (Scheme 6).¹³ Oxidation of the alcohol was performed under mild, non-acid conditions, using IBX[†] in DMSO.¹⁴ Finally, removal of the TES group was achieved by stirring the peptide over Amberlite IR-120 suspended in EtOAc . Purification of the final product by silica gel chromatography followed by reversed-phase HPLC provided material that exhibits ^1H , ^{13}C , ^1H - ^1H COSY and inverse detected ^1H - ^{13}C HMQC NMR spectra consistent with the proposed structure **1**, and a high resolution mass spectrum corresponding to the expected molecular formula.



Scheme 6 Reagents and conditions: i, HCl , MeOH ; ii, **6**, HATU, 2,4,6-collidine (48% for 2 steps); iii, IBX, DMSO; iv, Amberlite IR-120 (95% for 2 steps).

From the NMR data, it is apparent that our synthetic keramamide is not the same compound reported by Kobayashi and co-workers.^{1,15} Comparison of the NMR spectra leads us to conclude that this synthetic keramamide and KJ are configurational isomers. In support of the assignment of structure **1** to the synthetic product, a very close correlation is observed between the spectral data for this compound and the data published for KF (**2**; Table 1), which differs only in the replacement of the tryptophan in structure **1** by *Z*-didehydrotryptophan. In particular, the ^1H and ^{13}C chemical shifts at C-9 and C-13 for these two compounds are in excellent agreement (^1H $\Delta\delta \leq 0.06$, ^{13}C $\Delta\delta \leq 1.3$ ppm). In contrast, the published data for KJ more closely resemble the data for keramamide G (KG) which is epimeric to KF at carbon-13.^{1,16} We note that the degradation conditions used by Kobayashi to determine the absolute configuration at carbon-13 in KJ have been found previously to cause epimerization at this center in a closely related molecule and possibly could have been misleading.³ Degradation of the synthetic keramamide under milder conditions using 30% H_2O_2

Table 1 Selected ^1H and ^{13}C NMR resonances, and optical rotations published for keramamides F, G, J and observed for compound **1**

	δ_{H}		δ_{C}		$[\alpha]_{\text{D}}^{25}$ ^a
	H-9	H-13	C-9	C-13	
Keramamide G	4.81	5.49	53.7	56.7	+10.0
Keramamide J	4.75	5.49	53.7	56.1	+8.4
Compound 1	5.31	5.19	51.4	61.0	-10.0
Keramamide F	5.33	5.25	51.7	59.7	-25

^a Given in units of 10^{-1} degrees $\text{cm}^2 \text{g}^{-1}$.

and 0.1 M NaOH (room temperature, overnight), followed by 6 M HCl (110 $^\circ\text{C}$, 24 h), produced L-isoleucine containing less than 10% D-*allo*-isoleucine by chiral HPLC analysis, supporting assignment of the L-configuration to carbon-13 in the synthetic material.¹⁷ Upon standing in aqueous solution, the synthetic material partially converted ($\sim 10\%$) to a new product possessing ^1H NMR resonances that match those observed for KJ, consistent with the notion that these two molecules differ at a single, epimerizable center.

Based on the preceding analysis, we conclude that our synthesis proceeded as intended to correctly provide a molecule possessing structure **1**. This work strongly indicates that the structure of KJ should be revised. However, since no natural KJ is presently available,¹⁵ structural re-assignment will require either re-isolation or total synthesis of the correct structure.

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Notes and references

[†] Abbreviations: HATU = *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, IBX = 1-hydroxy-1,3-dihydro-1,2-benziodoxol-3-one 1-oxide, BOP = benzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophosphate, FmocOSu = *N*-(fluoren-9-ylmethoxycarbonyloxy)succinimide, HOBt = 1-hydroxybenzotriazole hydrate.

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- 15 Authentic samples of KJ and KF are not available. We thank Professor Kobayashi for providing a copy of the ^1H NMR spectrum for natural KJ.
- 16 Chemical degradation of KG converts the homo-Ile fragment to D-isoleucine indicating that KG possesses the (*R*) configuration at both C-13 and C-15.
- 17 A peak corresponding to L-alanine was also detected in this analysis, indicating that this residue did not epimerize during the cyclization reaction.