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Total synthesis of a keramamide

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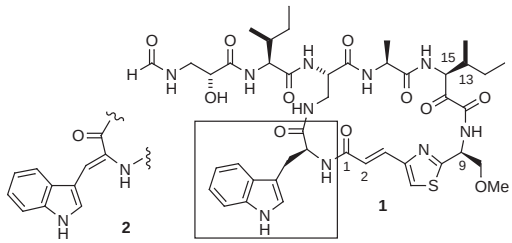
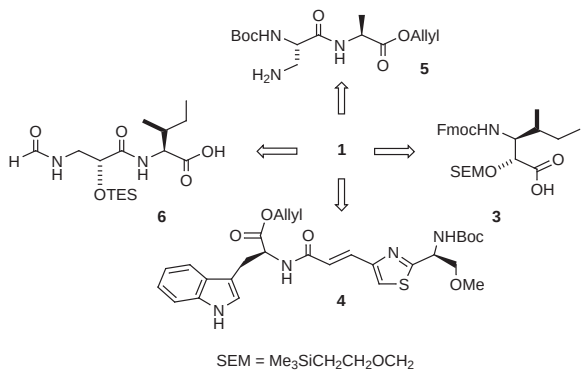
The first total synthesis of a molecule possessing the structure proposed for keramamide J is described, providing data indicating that the structure of this natural product should be re-examined.

Keramamide J (KJ; **1**)[1] is the simplest member of a class of *Theonella* natural products that collectively exhibit cytotoxic,[1–3] anti-fungal[4] 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**.



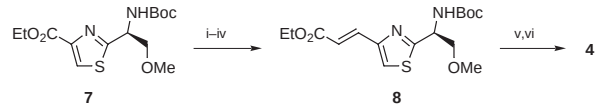
Our synthetic plan for constructing KJ is shown in Scheme 1.[6] A convergent route was devised, employing four fragments of comparable complexity (**3**–**6**). We decided to mask the electrophilic keto-amide moiety[7] as a protected α-hydroxy carbonyl group until the conclusion of the synthesis to avoid 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

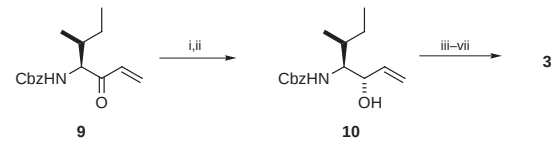


SEM = $Me_3SiCH_2CH_2OCH_2$

Scheme 1



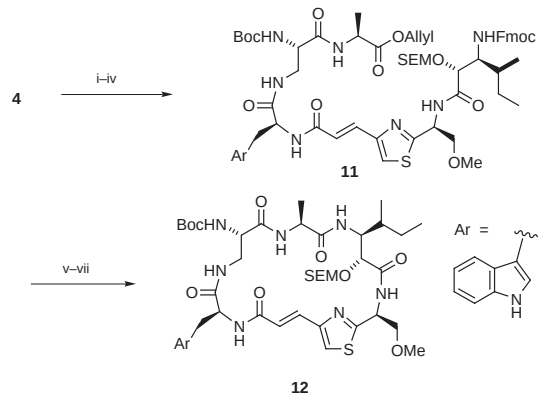
Scheme 2 *Reagents and conditions*: i, NaOH, MeOH; ii, BOP, Et_3N, HN(Me)OMe; iii, $LiAlH_4$; iv, $Ph_3P{=}CHCO_2Et$ (70% for 4 steps); v, NaOH, MeOH; vi, L-Trp-OAllyl, Ph_2POCl (68% for 2 steps).



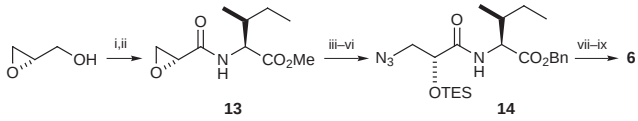
Scheme 3 *Reagents and conditions*: i, $NaBH_4$, $CeCl_3$; ii, separate (76% for 2 steps); iii, SEMCl, 2,6-lutidine; iv, O_3, DMS; v, $NaClO_2$, isobutene; vi, H_2, Pd/C; vii, FmocOSu, aq. $NaHCO_3$ (66% for 5 steps).

prepared from L-isoleucine *via* displacement of the corresponding Weinreb amide with vinyl magnesium bromide. Reduction of this enone with $NaBH_4$ under the Luche conditions[9] 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 $RuCl_3$ and $NaIO_4$,[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



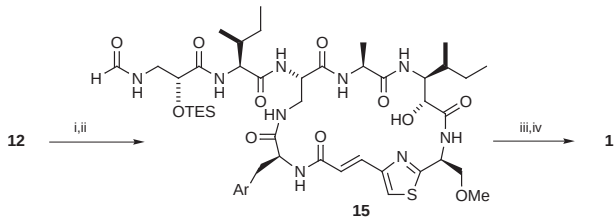
Scheme 4 *Reagents and conditions*: i, HCl, Et_2O; ii, **3**, DCC, HOBt, Pr^i_2EtN; iii, $Pd(PPh_3)_4$, dimedone; iv, **5**, DCC, HOBt, Pr^i_2EtN (56% for 4 steps); v, $Pd(PPh_3)_4$, dimedone; vi, Et_2NH; vii, $(PhO)_2PON_3$, $NaHCO_3$ (51% over 3 steps).



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*-$O_2NC_6H_4$-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 Et_2O–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 $\Delta\delta \leqslant 0.06$, ^{13}C $\Delta\delta \leqslant 1.3$ 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 and 0.1 M NaOH (room temperature, overnight), followed by 6 M HCl (110 °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 (~ 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.

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		
	H-9	H-13	C-9	C-13	$[\alpha]_D^{25}$ [a]
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 cm^2 g^{-1}.

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.

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