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Revised absolute stereochemistry of natural kulokekahilide-2

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Abstract

Kulokekahilide-2 is a potent cytotoxic depsipeptide isolated from the Hawaiian marine mollusk *Philinopsis speciosa*. The structure of kulokekahilide-2 was reported to be composed of five amino acids (L-Ala, L-Ile, MeGly, L-MePhe, D-Ala) and two hydroxy acids (D-Hica, 5*S*,6*S*,7*S*-Dtda); however, following its total synthesis, the ^{1}H NMR spectrum of the synthetic compound was found to be different from that of the natural one, suggesting that the stereochemistry of the reported structure was incorrect. To determine the stereochemistry of the natural compound, arrays of analogues have been prepared using different sets of chiral amino acids, and the absolute stereochemistry of kulokekahilide-2 has been unambiguously confirmed to involve the combination 21-L-Ala, 24-D-MePhe, and 43-D-Ala.

Keywords: Kulokekahilide-2; Macrolactamization; Cytotoxicity; cis–trans Conformations

Kulokekahilide-2 (**1**) is a potent cytotoxic depsipeptide isolated from the Hawaiian marine mollusk *Philinopsis speciosa*.[1] The structure of kulokekahilide-2 was reported as **1b**, which contains five amino acids (L-Ala, L-Ile, MeGly, L-MePhe, D-Ala), and two hydroxy acids (D-Hica, 5*S*,6*S*,7*S*-Dtda); however, the total synthesis of **1b** was attempted by macrolactonization of the corresponding seco acid, which afforded the 43-epi isomer (**1a**), the complete inverse of the target product (**1b**) at this position. On the other hand, macrolactamization of the corresponding amino acid did indeed afford **1b**, but the NMR spectral data of this product were not identical to those of natural **1**, suggesting that the stereochemistry of the proposed structure of **1b** was incorrect.[2]

In view of the lack of natural **1**, we attempted its synthesis by the use of various chiral amino acids with a view to confirming its stereochemistry.

Aurilide (**2**), from the Japanese sea hare *Dolabella auricularia*, is a potent cytotoxic 26-membered cyclodepsipeptide similar to natural **1**. The stereochemistries at 21-L-Val and 24-D-MeLeu in **2** are the reverse of those at 21-D-Ala and 24-L-MePhe in the incorrect structure of **1b**.[3] These differences seemed strange because stereochemistry is usually retained when different amino acid residues are substituted in peptides biosynthesized by non-ribosomal peptide synthetases (NRPS). Also, racemization occurred easily at the 43-position, and this was clearly related to the stability of the macrolactone ring and/or to the cytotoxicity. Thus, the 21-, 24-, and 43-positions of the relevant amino acids were considered to be related to the cytotoxic activities, positions at which mistakes could easily be made in assigning the absolute stereochemistry.

To address this issue, arrays of analogues with different combinations of the key amino acids were synthesized: 21-L-Ala, 24-L-MePhe, and 43-D-Ala (**1c**), 21-L-Ala, 24-D-MePhe, and 43-L-Ala (**1d**), and 21-L-Ala, 24-D-MePhe, and 43-D-Ala (**1e**).

Subsequent investigations using these analogues revealed that the structure of the natural product (**1**) was in fact **1e**. The NMR and mass spectral data of **1e** were in complete accordance with those of the natural product

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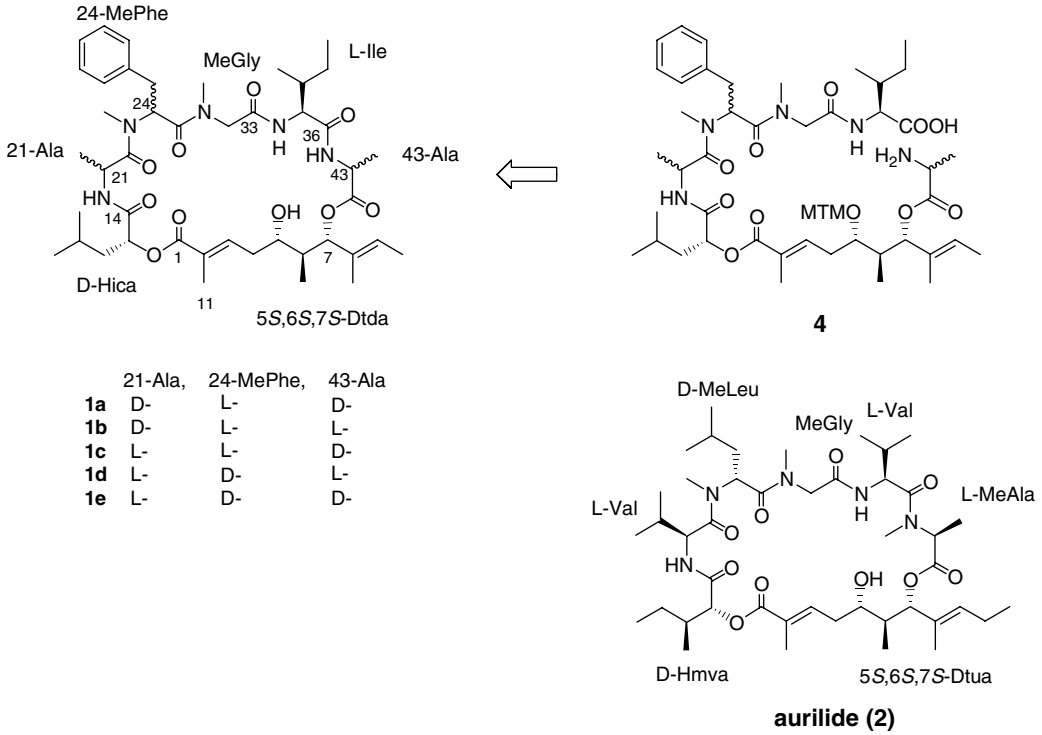

	21-Ala,	24-MePhe,	43-Ala
1a	D-	L-	D-
1b	D-	L-	L-
1c	L-	L-	D-
1d	L-	D-	L-
1e	L-	D-	D-

Fig. 1. Structure of kulokekahilide-2 analogues.

and the cytotoxicity of **1e** was just as strong as that of **1** (see Fig. 1).[1]

The strategy used to prepare the analogues of **1** involved a macrolactamization as in the synthesis of **1b** and aurilide.[2,3] Macrolactamization between the *C*-terminus of L-Ile and the N-terminus of the Ala moiety in the amino acid **4** was carried out using EDCI-HCl and 1-hydroxy-7-azabenzotriazole (HOAt). The MTM protecting group was subsequently removed to afford the target products in yields of 59–70% (Table 1).[4] Marfey analysis indicated that the absolute configurations of all component amino acids had been maintained,[5] and reductive degradation of **1c**–**e** revealed that the absolute configurations of the hydroxy acids were also unchanged. The ^{1}H NMR spectrum of **1e** was in complete accordance with that of the reported natural **1**, as was its cytotoxicity, as indicated by the potent IC_{50} values against various cell lines. Interestingly, **1c**, and **1d**: with the aurilide-type configuration, were also highly potent in terms of their cytotoxic activities, as shown in Table 1.

Table 1
Yields and cytotoxicity of kulokekahilide-2 and its analogues

	Lactamization yield (%)	Deprotection of MTM yield (%)	$[\alpha]_D$	*c* (MeOH)	Cell lines (IC_{50}: μg/ml)	
					P388	HeLa
1a	60	65	−28	0.27	>10	>10
1b	74	91	−78	0.29	10	>10
1c	70	Quant	−62	0.57	0.40	0.039
1d	65	Quant	−19	0.81	0.016	0.0053
1e	77	76	−4	0.10	0.016	0.0032
Natural	—	—	−14	0.04	0.004	—

This may be rationalized by considering that the absolute configuration at the 21-position of the 26-membered ring of depsipeptides **1** and **2** greatly influences their cytotoxicities, whereas the configurations at the 24- and 43-positions do not have such a marked influence. By comparison with **2**, the stereochemistry is seen to be a more important factor for activity than small changes in the number of carbon atoms of the different constituent amino acids and hydroxy acids.

The NMR spectra of these compounds revealed the presence of two conformers as a result of cis–trans isomerization about the amide bonds between MeGly and L-MePhe.[1] Moreover, the four characteristic methyl group signals from the two conformers of **1c** became eight signals a few hours after isolating this compound. This was attributed to the generation of further conformational isomers about the amide bonds between L-Ala and L-MePhe.[6] In fact, the *N*-methyl signals of MeGly and L-MePhe in **1c** appeared at δ 3.36, 3.25, 2.71, and 2.61, but after a few hours the corresponding resonances gave rise to double signals at δ 3.38, 3.36, 3.33, 3.25, 2.82, 2.71, 2.69, and 2.61. Thus, analysis of the ^{1}H NMR spectrum of **1c** was difficult because of rapid conformational change and complexity.

We are currently carrying out detailed conformational analyses as well as studies of the structure–cytotoxicity relationships of kulokekahilide-2 analogues.

References and notes

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4. *MTM ether of* **1e**: $[\alpha]_D$ −8.9 (*c* 0.35, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): Characteristic signals of two conformations were shown, but some methyl and amide protons could not be determined because of overlapping (major rotamer): $\delta = 1.46$ (d, $J = 7.2$ Hz, H-44), 2.12 (s, CH_3S–), 3.01 (s, H-35), 3.04 (s, H-32), 3.00–3.05 (overlapping, H-25), 3.12 (dd, $J = 14.1$, 9.0 Hz, H-25), 3.50 (d, $J = 16.8$ Hz, H-34), 3.85 (d, $J = 16.8$ Hz, H-34), 3.82 (dt, $J = 9.4$, 3.0 Hz, H-5), 4.25 (dd, $J = 8.2$, 8.2 Hz H-37), 4.47–4.50 (overlapping, H-43), 4.60 (s, –SCH_2O–), 4.77 (dq, $J = 7.1$, 6.8 Hz, H-21), 5.02 (d, $J = 10.4$ H-7), 5.22 (dd, $J = 6.7$, 6.1 Hz, H-15), 5.45–5.49 (overlapping, H-9, H-24), 6.78 (br t, $J = 7.8$ Hz, H-3); ^{13}C NMR (126 MHz, $CDCl_3$) $\delta_C = 13.7$ (S–CH_3), 30.8 (C-32), 35.0 (C-25), 35.8 (C-35), 44.4 (C-21), 48.0 (C-43), 51.7 (C-34), 54.7 (C-24), 57.8 (C-37), 72.2 (C-15), 73.4 (–SCH_2O–), 74.4 (C-5), 81.9 (C-7), 126.6 (C-9), 140.4 (C-3); (minor rotamer): $\delta = 1.44$ (d, $J = 7.1$ Hz, H-44), 2.06 (s, CH_3S–), 2.87 (s, H-35), 3.09 (s, H-32), 3.10–3.17 (overlapping, H-25), 3.21 (dd, $J = 14.1$, 7.0 Hz, H-25), 3.56 (d, $J = 16.5$ Hz, H-34), 3.91 (dt, $J = 5.6$, 2.4 Hz, H-5), 4.47–4.50 (overlapping, H-37, H-43), 4.60 (s, –SCH_2O–), 4.61 (d, $J = 16.5$ Hz, H-34), 4.77 (dq, $J = 7.1$, 6.8 Hz, H-21), 4.92 (dd, $J = 9.8$, 3.5 Hz, H-15), 4.97 (d, $J = 10.7$ Hz, H-7), 5.54 (dq, $J = 6.4$, 1.2 Hz, H-9), 5.65 (dd, $J = 7.6$, 7.6 Hz, H-24), 6.78 (br t, $J = 7.8$ Hz, H-3); ^{13}C NMR (126 MHz, $CDCl_3$) $\delta_C = 13.6$ (CH_3S–), 30.3 (C-32), 35.0 (C-25), 35.7 (C-35), 45.0 (C-21), 48.6 (C-43), 51.2 (C-34), 54.3 (C-24), 57.8 (C-37), 72.9 (C-15), 72.7 (–SCH_2O–), 75.2 (C-5), 82.2 (C-7), 126.3 (C-9), 140.8 (C-3); HR-FABMS: $[M+H]^+$ *m/z* 886.5002 (*m/z* 886.5000 calcd for $C_{46}H_{72}N_5O_{10}S$).
Compound **1c**: $[\alpha]_D$ −62.3 (*c* 0.57, MeOH); ^1H NMR (500 Hz, CD_2Cl_2) (major rotamer): $\delta = 0.78$ (d, $J = 7.1$ Hz, H-12), 1.26 (d, $J = 7.0$ Hz, H-22), 1.32 (d, $J = 6.8$ Hz, H-44), 1.58 (m, H-10), 1.72 (m, H-16), 2.71 (s, H-35), 2.76 (d, $J = 15.6$ Hz, H-34), 3.04 (dd, $J = 13.0$, 5.6 Hz, H-25), 3.19 (dd, $J = 13.0$, 9.8 Hz, H-25), 3.36 (s, H-32), 3.86 (m, H-5), 4.01 (dd, $J = 8.2$, 8.2 Hz, H-37), 4.14 (d, $J = 15.6$ Hz, H-34), 4.44 (dq, $J = 7.5$, 6.8 Hz, H-43), 4.85 (dq, $J = 7.1$, 7.0 Hz, H-21), 4.95 (d, $J = 11.3$ Hz, H-7), 5.22 (m, H-15), 5.50 (m, H-9), 5.56 (dd, $J = 9.8$, 5.6 Hz, H-24), 6.91 (dd, $J = 10.7$, 5.2 Hz, H-3), 6.97 (d, $J = 7.5$ Hz, C43–NH), 7.41 (d, $J = 7.1$ Hz, C21–NH); HR-FABMS: $[M+Na]^+$ *m/z* 848.4804 (*m/z* 848.4786 calcd for $C_{44}H_{67}N_5O_{10}Na$).
Compound **1d**: $[\alpha]_D$ −19.2 (*c* 0.81, MeOH); ^1H NMR (500 MHz, CD_2Cl_2) (major rotamer): $\delta = 0.78$ (d, $J = 7.1$ Hz, H-12), 0.79 (d, $J = 7.0$ Hz, H-22), 0.90 (d, $J = 6.7$ Hz, H-41), 0.92 (d, $J = 6.4$ Hz, H-18), 0.93 (d, $J = 6.3$ Hz, H-19), 0.95 (t, $J = 7.4$ Hz, H-40), 1.29 (m, H-39), 1.34 (d, $J = 7.0$ Hz, H-44), 1.59 (m, H-39), 1.60 (br d, $J = 7.1$ Hz, H-10), 1.61 (br s, H-13), 1.66 (m, H-16, -17), 1.79 (m, H-16), 1.87 (br s, H-11), 1.92 (m, H-38), 2.16 (m, H-6), 2.26 (m, H-4), 2.96 (s, H-35), 2.99 (s, H-32), 3.02 (dd, $J = 14.4$, 6.0 Hz, H-25), 3.10 (dd, $J = 14.4$, 9.9 Hz, H-25), 3.54 (d, $J = 17.0$ Hz, H-34), 3.75 (dt, $J = 6.4$, 4.4 Hz, H-5), 3.92 (d, $J = 17.0$ Hz, H-34), 4.01 (dd, $J = 8.0$, 8.0 Hz, H-37), 4.18 (dq, $J = 7.0$, 6.7 Hz, H-43), 4.68 (dq, $J = 7.4$, 7.0 Hz, H-21), 4.98 (d, $J = 11.2$ Hz, H-7), 5.03 (dd, $J = 9.2$, 4.7 Hz, H-15), 5.47 (dd, $J = 9.9$, 6.0 Hz, H-24), 5.55 (dq, $J = 6.4$, 1.2 Hz, H-9), 6.66 (br s, C43–NH), 6.86 (d, $J = 7.7$ Hz, C21–NH), 6.96 (d, $J = 6.6$ Hz, C37–NH), 6.99 (br t, $J = 7.3$ Hz, H-3), 7.15–7.30 (m, overlapping, H-27, -28, -29, -30, -31); ^{13}C NMR (126 MHz, CD_2Cl_2) $\delta_C = 10.8$ (q), 11.6 (q), 12.7 (q), 12.8 (q), 13.2 (q), 15.5 (q), 16.6 (q), 17.7 (q), 21.9 (q), 23.3 (q), 25.1 (d), 25.2 (t), 30.6 (q), 31.9 (t), 35.6 (q), 36.2 (t), 38.1 (d), 40.6 (d), 41.0 (t), 45.3 (d), 49.6 (d), 52.2 (t), 54.4 (d), 57.6 (d), 71.3 (d), 73.4 (d), 83.0 (d), 126.5 (d), 127.1 (d), 128.6 (d, 2C), 128.8 (d), 129.7 (d, 2C), 132.5 (s), 137.0 (s), 142.6 (d), 168.1 (s), 168.9 (s), 169.8 (s), 170.5 (s), 171.3 (s), 171.7 (s), 173.6 (s); HR-FABMS: $[M+Na]^+$ *m/z* 848.4804 (*m/z* 848.4786 calcd for $C_{44}H_{67}N_5O_{10}Na$).
Compound **1e**: MTM ether of **1e** (192 mg, 0.217 mmol) was treated with 2,6-lutidine (4.34 mmol) and $AgNO_3$ (8.68 mmol) in THF–H_2O solution (4: 1 v/v; 5.3 mL) at 65 °C for 1.5 h to afford **1e** (137 mg, 0.165 mmol, 76%) as colorless oil. $[\alpha]_D$ −4 (*c* 0.10, MeOH); UV (MeOH) λ_{max} 204 nm (ε 3.3×10^4); IR (KBr disk) 3296, 2962, 1687 (sh), 1658, 1634 (sh), 1530, 1456, 1382, 1269, 1096, 752 cm^{-1}; ^1H and ^{13}C NMR spectra were identical to those of natural **1**;[1] HR-FABMS: $[M+Na]^+$ *m/z* 848.4763 (*m/z* 848.4786 calcd for $C_{44}H_{67}N_5O_{10}Na$). After acid hydrolysis of **1e**, Marfey analysis indicated D-Ala, L-Ala, and D-MePhe which were used as starting amino acids.[5]
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