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Reinvestigation of the stereochemistry of kulokekahilide-2

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Abstract—An attempt to carry out a total synthesis of kulokekahilide-2 (**1**) by macrolactonization of a seco acid prepared from a suitably protected hexapeptide and a dioxy acid moiety unexpectedly resulted in the formation of the 43-epimer (**1a**) of the cytotoxic depsipeptide, for which structure **1b** has previously been proposed. A second attempt involving macrolactamization of the corresponding amino acid gave the target product, **1b**, but the spectral data of the product did not match those of natural **1**. Furthermore, neither **1a** nor **1b** showed any cytotoxicity, from which it is concluded that the structure of natural **1** is incorrect and should be re-examined.

We have previously reported the isolation, structural elucidation, and biological activity of kulokekahilide-2 (**1**), which showed potent cytotoxicity against several cell lines (IC_{50} values ranging from 4.2 to 59.1 nM against P388, SK-OV-3, MDA-MB-435, and A-10).[1,2] Compound **1** contains five amino acids, namely L- and D-alanine (L-Ala, D-Ala), L-isoleucine (L-Ile), *N*-methylglycine (MeGly), and L-*N*-methylphenylalanine (L-MePhe), as well as two hydroxy acids, D-2-hydroxyisocaproic acid (D-Hica) and (5*S*,6*S*,7*S*,2*E*,8*E*)-2,6,8-trimethyl-5,7-dihydroxy-2,8-decadienoic acid (Dtda).[1] These residues are similar to those of aurilide (**2**), a compound isolated from the Japanese mollusk *Dolabella auricularia*,[3,4] but when the structures of **1** and **2** are compared, the stereochemistries at L-Val and D-MeLeu in **2** are seen to be reversed to D-Ala and L-MePhe in the proposed structure of **1** (Scheme 1). However, we were unable to further analyze the stereochemistry because of a lack of natural **1**. In order to re-examine the stereochemistry of **1**, a synthetic approach to **1** (=**1b**) was attempted. We found that macrolactonization as the final step was difficult and resulted in the formation of the 43-epimer (**1a**) as the sole product. In contrast, lactamization rather than lactonization as the final macrocyclization step proceeded without epimerization and afforded **1b**, which has identical stereochemistry to that proposed for natural **1**. To our surprise, however, the NMR spectral data of **1b** did not match those of natural **1**, and the potent cytotoxicity seen for **1** was not observed for either **1a** or **1b**.

In this Letter, we report the synthesis of **1a** and **1b** and the basis for our conclusion that the proposed structure of **1** is incorrect.

The strategy used for the synthesis of **1** (=**1b**) involved the macrolactonization of a seco acid (**3**) obtained by the coupling of a hexapeptide (**4**) and a dioxy acid moiety (**5**) as shown in Scheme 1. D-Hica, a component of **4** which is not commercially available, was obtained from commercially available L-Hica by means of a Mitsunobu reaction.[5] Hexapeptide **4** was synthesized starting from L-Ala-OTce-HCl by means of the usual condensation steps of peptide synthesis, the final step being the condensation with D-Hica using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride salt (EDCI-HCl) and 1-hydroxy benzotriazole (HOBt) (Scheme 2).[6,7]

The dioxy acid moiety (**5**), which consists of a chain similar to that found in **2** but shorter by one carbon,

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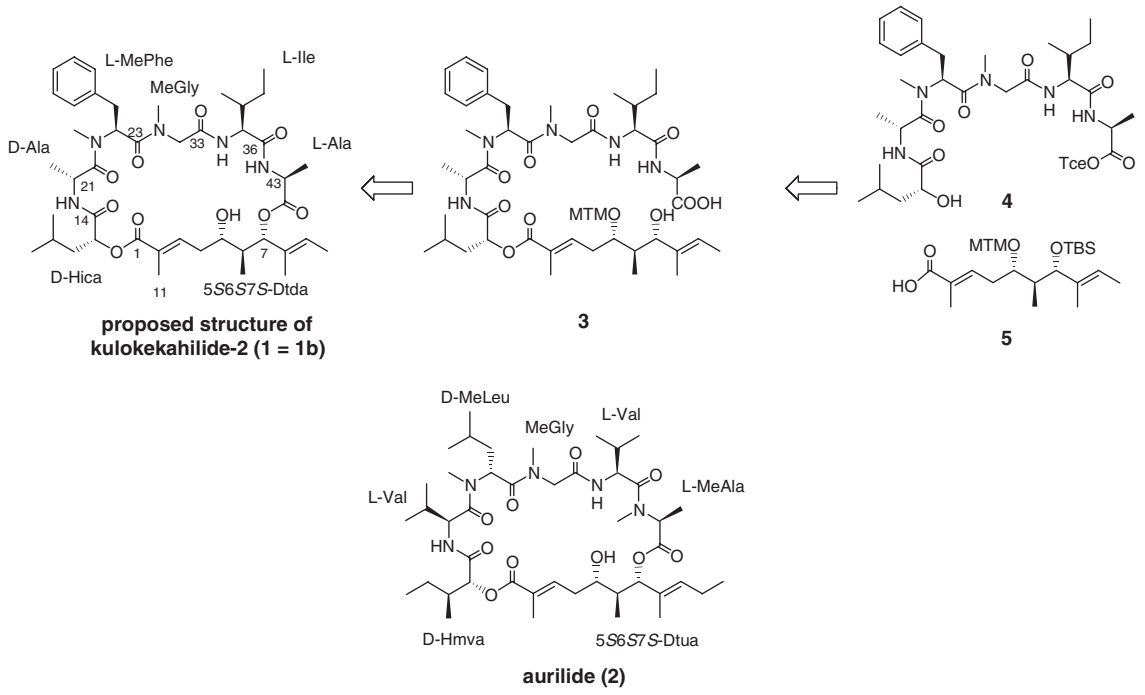
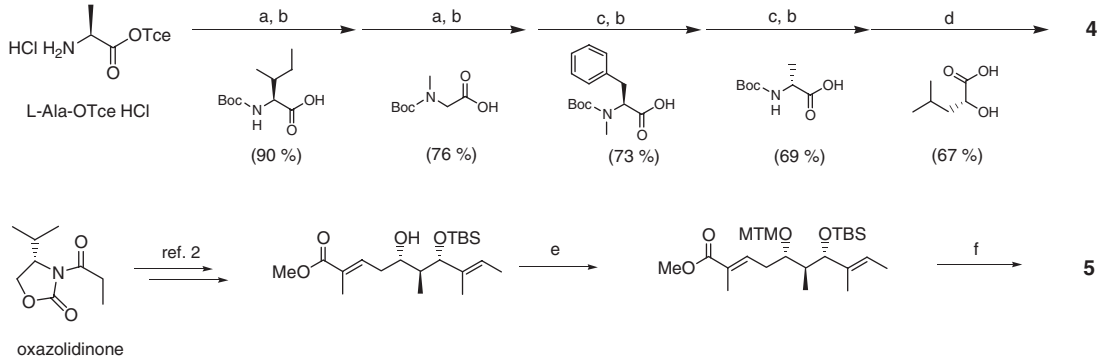


Scheme 1.



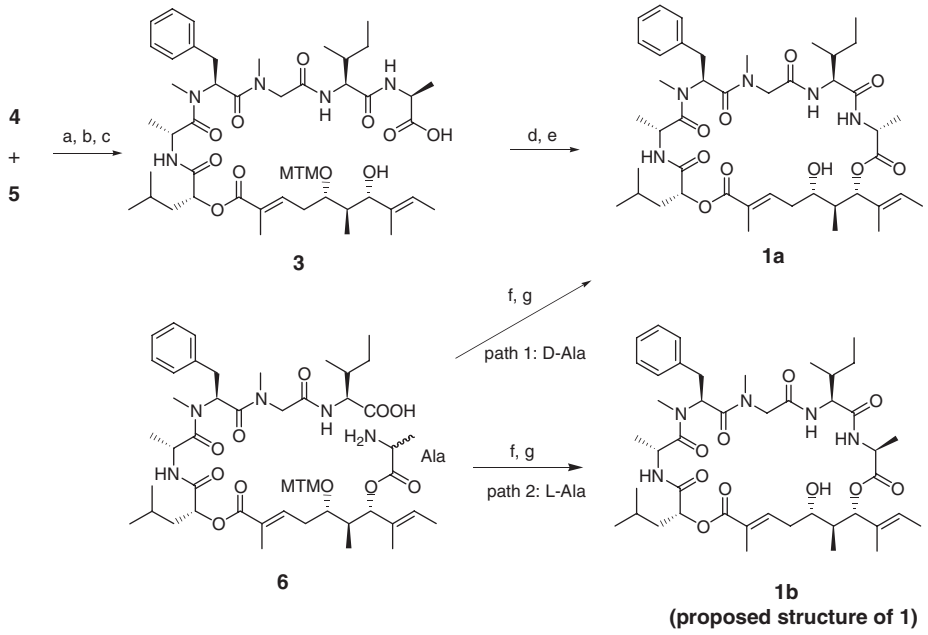
Scheme 2. Reagents and conditions: (a) EDCI-HCl,[6] HOBt, Et_3N, CH_2Cl_2:DMF (1:1), rt; (b) 4 M HCl/dioxane, rt; (c) PyBOP,[7] *i*-Pr_2NEt, CH_2Cl_2:DMF (1:1), rt; (d) EDCI-HCl, HOBt, Et_3N, DMF, rt; (e) Ac_2O, AcOH, DMSO, rt (84%); (f) LiOH–H_2O, MeOH, H_2O, rt (88%).

was synthesized by enantioselective aldol condensation from the corresponding oxazolidinone derivative followed by protection with a suitable protecting group (Scheme 2).[1,3]

Coupling of **4** and **5** in the presence of EDCI-HCl and *N*,*N*-dimethylaminopyridine (DMAP) afforded the corresponding protected depsipeptide, and subsequent deprotection of the *tert*-butyldimethylsilyl (TBS) and trichloroethyl (Tce) groups produced seco acid **3**. The key step of macrolactonization of **3** was attempted using a condensation reagent, EDCI-HCl, and DMAP. However, it was not successful, and the starting material was quantitatively recovered. Thus, EDCI-HCl showed low reactivity toward macrolactonization, and therefore, presumably, also toward aurilide synthesis.[3b] Another attempt at macrolactonization was carried out using 2-methyl-6-nitrobenzoic anhydride (MNBA) as a condensation reagent and DMAP,[8] whereupon the reaction proceeded smoothly to give the target product in 60% yield. Deprotection of the methylthiomethyl (MTM) group in the cyclized product afforded **1a**.[9]

Although the FABMS data for **1a** were identical to those of natural **1**, the ^1H NMR spectra of the two compounds were different; for example, the N-methyl signals of MeGly and L-MePhe in **1a** appeared at $\delta = 3.11$, 2.92, 2.91, and 2.86, while the corresponding resonances for **1** were observed at $\delta = 2.97$, 2.95, 2.91, and 2.71 (two conformers). The signals observed for **1a** suggest the presence of a common conformational isomer as for natural **1**.

The stereochemistry of **1a** was examined by reductive degradation, chiral HPLC analysis, and Marfey analysis.[10] Compound **1a** was treated with $LiAlH_4$ to give a triol corresponding to that obtained from **1**.[1] After acid hydrolysis of **1a**, the stereochemistry of Hica was



Scheme 3. Reagents and conditions: (a) EDCI-HCl, DMAP, CH_2Cl_2, (45%); (b) HF–Py, pyridine, THF, (97%); (c) Zn powder, $AcONH_4$ (93%); (d) MNBA, DMAP, CH_2Cl_2, (60%); (e) $AgNO_3$, 2,6-lutidine, THF, H_2O (65%); (f) EDCI-HCl, HOAt, CH_2Cl_2, DMF (path 1: 57 %, path 2: 70 %); (g) $AgNO_3$, 2,6-lutidine, THF, H_2O (path 1: 75% path 2: 81%).

confirmed as D by chiral HPLC analysis. However, Marfey analysis of the component amino acids indicated the presence of L-Ile, L-MePhe, and D-Ala, but not L-Ala. When the seco acid (**3**) was subjected to a similar examination, there was no change in the configurations of L-Ile, L-MePhe, and both D- and L-Ala compared to the starting amino acids. This result suggested that the configuration of L-Ala was completely reversed during the macrolactonization step. We concluded that **1a** was the 43-epimer of the proposed structure (**1b**).

In order to confirm this, **1a** was synthesized by macrolactamization based on the synthesis of aurilide.[3b,c] Macrolactamization between the C-terminus of L-Ile and the N-terminus of D-Ala in the amino acid (**6**) was carried out using EDCI-HCl and 1-hydroxy-7-azabenzotriazole (HOAt), giving the target product in 57% yield (Scheme 3, path 1). The product was found to be identical to **1a** formed by macrolactonization, revealing that the reaction proceeded without epimerization of the L-Ile corresponding to L-Val in **2**.[3c]

Therefore, we attempted the synthesis of the proposed natural product **1** by a macrolactamization method using L-Ala instead of D-Ala (Scheme 3, path 2). However, the spectral data of the product (**1b**)[11] were different from those of **1**; for example, the N-methyl groups of MeGly and L-MePhe resonated at $\delta = 2.97$ and 2.88. Marfey analysis indicated that the absolute configurations of all of the amino acids were maintained. In addition, **1b** showed no potent cytotoxicity against P388 or Hela cells.

The phenomenon of complete inversion during the macrolactonization step and the existence of conformational isomers about the amide bond between MeGly and MePhe is very intriguing with regard to cytotoxic activity.[1]

This work has revealed that the proposed stereochemistry of natural **1** is incorrect. In order to confirm the structure of **1**, the synthesis of several other stereoisomers is currently in progress.

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9. *Compound* **1a**: $[\alpha]_D$ −28.0° ($c = 0.27$, CH_3OH); ^1H NMR (500 MHz, CD_2Cl_2) (major rotamer): $\delta = 0.69$ (d, $J = 6.8$ Hz, H-22), 0.85 (t, $J = 6.7$ Hz, H-40), 0.86 (d, $J = 6.8$ Hz, H-12), 0.91 (d, $J = 4.5$ Hz, H-41), 0.92 (d, $J = 5.2$ Hz, H-18, -19), 1.12 (m, H-39a), 1.33 (d, $J = 7.3$ Hz, H-44), 1.46 (m, H-39b), 1.62 (d, $J = 6.8$ Hz, H-10), 1.63 (s, H-13), 1.60–1.73 (m, H-16a, -16b, -17, overlapping), 1.80 (m, H-38), 1.88 (s, H-11), 2.22–2.34 (m, H-4a, -4b, -6, overlapping), 2.86 (s, H-35), 2.91 (s, H-32), 3.02 (dd, $J = 14.2$, 7.7 Hz, H-25a), 3.15 (dd, $J = 14.2$, 6.7 Hz, H-25b), 3.52 (d, $J = 17.2$ Hz, H-34a), 3.75 (ddd, $J = 10.6$, 3.2, 3.2 Hz, H-5), 4.39 (bd, $J = 17.2$ Hz, H-34b), 4.40 (m, H-43), 4.41 (dd, $J = 7.4$, 7.2 Hz, H-37), 4.69 (m, H-21), 5.05 (d, $J = 11.0$ Hz, H-7), 5.18 (m, H-15), 5.60 (m, H-9), 5.63 (m, H-24), 6.22 (d, $J = 7.2$ Hz, C37-NH), 6.47 (d, $J = 8.6$ Hz, C43-NH), 6.87 (d, $J = 8.2$ Hz, C21-NH), 6.98 (dt, $J = 7.4$, 1.3 Hz, H-3), 7.20–7.24 (m, H-27, -28, -29, -30, -31, overlapping); ^{13}C NMR (CD_2Cl_2): $\delta_c = 10.3$ (q), 11.0 (q), 11.5 (q), 12.8 (q), 13.3 (q), 15.8 (q), 17.8 (q), 18.1 (q), 22.0 (q), 23.3 (q), 25.0 (d), 25.1 (t), 30.4 (q), 31.1 (t), 35.1 (q), 35.3 (t), 38.3 (d), 40.3 (d), 41.4 (t), 45.0 (d), 49.5 (d), 52.5 (t), 53.5 (d), 58.0 (d), 70.3 (d), 73.2 (d), 83.4 (d), 126.8 (d), 126.9 (s), 127.5 (d), 128.6 (d, 2C), 129.9 (d, 2C), 132.5 (s), 137.0 (s), 141.5 (d), 167.2 (s), 168.3 (s), 170.0 (s), 170.1 (s), 170.2 (s), 170.6 (s), 173.1 (s); HR-FABMS: $[M+Na]^+$ m/z 848.4807 (m/z 848.4786 calcd. for $C_{44}H_{67}N_5O_{10}Na$).
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11. *Compound* **1b**: $[\alpha]_D$ −78.0° ($c = 0.29$, CH_3OH); ^1H NMR (500 MHz, CD_2Cl_2) (major rotamer): $\delta = 0.80$ (d, $J = 6.9$ Hz, H-12), 0.81 (d, $J = 6.9$ Hz, H-22), 0.87 (t, $J = 7.3$ Hz, H-40), 0.92 (d, $J = 6.0$ Hz, H-18, -19, overlapping), 0.95 (d, $J = 6.4$ Hz, H-41), 1.32 (d, $J = 7.3$ Hz, H-44), 1.48 (m, H-39a, -39b), 1.62 (d, $J = 6.0$ Hz, H-10), 1.62 (s, H-13), 1.60–1.72 (m, H-16a, -16b, -17, overlapping), 1.80 (m, H-38), 1.90 (s, H-11), 2.19 (m, H-6), 2.20 (m, H-4a), 2.43 (ddd, $J = 14.9$, 10.1, 7.5 Hz, H-4b), 2.88 (s, H-35), 2.97 (dd, $J = 14.7$, 6.0 Hz, H-25a), 2.97 (s, H-32), 3.06 (dd, $J = 14.7$, 10.0 Hz, H-25b), 3.56 (d, $J = 17.4$ Hz, H-34a), 4.13 (m, H-5), 4.17 (dq, $J = 7.3$, 7.3 Hz, H-43), 4.24 (d, $J = 17.4$ Hz, H-34b), 4.50 (dd, $J = 8.9$, 6.2 Hz, H-37), 4.65 (dq, $J = 7.3$, 6.8 Hz, H-21), 5.03 (m, H-15), 5.09 (d, $J = 11.0$ Hz, H-7), 5.52 (dd, $J = 10.0$, 6.0 Hz, H-24), 5.57 (m, H-9), 6.47 (d, $J = 4.6$ Hz, C43-NH), 6.67 (d, $J = 7.3$ Hz, C21-NH), 6.91 (d, $J = 9.2$ Hz, C37-NH), 7.17 (m, H-3), 7.20–7.24 (m, H-27, -28, -29, -30, -31, overlapping); ^{13}C NMR (CD_2Cl_2): $\delta_c = 10.7$ (q), 11.5 (q), 11.7 (q), 12.9 (q), 13.2 (q), 15.3 (q), 16.3 (q), 17.0 (q), 21.9 (q), 23.3 (q), 25.0 (d), 25.1 (t), 30.4 (q), 33.0 (t), 35.1 (q), 35.2 (t), 38.6 (d), 40.8 (d), 41.4 (t), 45.0 (d), 50.2 (d), 52.0 (t), 53.7 (d), 57.9 (d), 71.3 (d), 73.7 (d), 82.6 (d), 126.2 (s), 126.8 (d), 127.2 (d), 128.5 (d, 2C), 129.7 (d, 2C), 132.7 (s), 137.3 (s), 144.6 (d), 168.2 (s), 168.9 (s), 170.1 (s), 170.9 (s), 171.7 (s), 172.3 (s), 172.9 (s); HR-FABMS: $[M+Na]^+$ m/z 848.4763 (m/z 848.4786 calcd. for $C_{44}H_{67}N_5O_{10}Na$).