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Intramolecular ester exchange of potent cytotoxic kulokekahilide-2

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ABSTRACT

Kulokekahilide-2 is a 26-membered cyclic depsipeptide that exhibits potent cytotoxicity against HeLa and P388 cells with IC_{50} values of 3.2 and 16 ng/mL, respectively. We have achieved a total synthesis of natural kulokekahilide-2, the NMR spectra of which showed complex signals because of trans and cis conformers at the amide bond. Besides, the spectra revealed that a mixture of 26- and 24-membered cyclic depsipeptides had been produced due to intramolecular ester exchange. The isolated 24-membered compound was transformed into the 26-membered compound over a period of several days. The two isomers have been shown to be in equilibrium and to display almost the same cytotoxicity.

Cyclic depsipeptides from marine sources are of interest in the pharmaceutical and biochemical fields because of potent biological activity.[1]

We have reported the revised stereochemistry of natural kulokekahilide-2 (**1**), which displays potent bioactivity and consists of five amino acids (L-Ala, D-MePhe, MeGly, L-Ile, and D-Ala) and two hydroxyl acids (D-Hica and 5S,6S,7S-Dtda).[2] The synthetic **1** showed the expected potent cytotoxicity against various cell lines. In the course of the determination of the absolute stereochemistry of **1**, we also synthesized various analogues with different combinations of the key amino acids (Fig. 1), and it was noticed that these analogues existed as two pairs of isomers in some cases.[2] One pair comprised the trans and cis conformers about the amide bond of MeGly and MePhe, and we surmised that the other isomer was likely to be that about the amide bond of MePhe and L-Ala. In fact, the *N*-methyl signals of MeGly and MePhe in **1** (CD_2Cl_2) appeared at δ 2.97, 2.95, 2.91, and 2.71, but after a few days the corresponding resonances gave rise to double signals at δ 3.05, 2.98, 2.97, 2.95, 2.93, 2.91, 2.74, and 2.71.[3]

HPLC separation of **1** was carefully examined, and a small peak was seen at a retention time close to that of the main peak of **1**.[4] The fraction corresponding to this small peak was collected and proved to be the other isomer (**2**) observed by NMR. Also, the characteristic *N*-methyl signals of **2** gradually appeared resulting in twice the number as in the case of **1** (Fig. 2).

The molecular formula of **2** was established as $C_{44}H_{67}N_5O_{10}$ on the basis of HR FABMS (*m*/*z* 848.4805 $[M+Na]^+$; Δ +1.9 mmu). Marfey analysis of the component amino acids indicated the presence of L-Ile, D-MePhe, and D- and L-Ala, and there was no change in the configurations of the amino acids compared to **1**. In its ^1H NMR spectrum (CD_2Cl_2), **2** exhibited two sets of *N*-methyl signals (δ 2.98 and 2.93; 3.05 and 2.74) in a ratio of about 1.9:1, indicative of trans and cis conformers similar to those of **1**. Also, **2** showed characteristic doublet and double triplet peaks at δ 3.82 and 5.06, corresponding to those at δ 5.22 and 3.51, respectively, in the case of **1**.[3] COSY analysis of connected proton signals led from the olefinic proton H-3 (δ 6.69) via the allylic methylene protons H-4 (2.44, 2.58) and oxymethine proton H-5 (5.06) to the methine proton H-6 (1.95) showed correlations with the oxymethine proton H-7 (3.82) and the methyl proton at H-12 (0.71). HMBC analysis of these protons revealed correlations between H-7 (δ 3.82)/C-5, /C-6, /C-8, /C-9, and /C-12 and between H-5 (5.06)/C-3, /C-4, /C-6, /C-12, and /C-42. This revealed that the signals at δ 3.82 and 5.06 could be attributed to H-7 and H-5, respectively (Fig. 3, Table 1). From these results, it was clear that ester exchange occurred between the 5- and 7-hydroxyl groups of the lactone.

In order to prove this phenomenon, a solution of **1** in methanol was left to stand for 5 days. Unexpectedly, however, the methyl ester was not obtained. The reason for this was considered to be the adoption of a different conformation in this solvent.

Thus, we carried out NMR measurements in various solvents, specifically CD_2Cl_2, $CDCl_3$, CD_3OD, and DMSO-d_6. As expected, essentially only the trans conformer was seen in the polar solvents CD_3OD and DMSO-d_6 from ROESY spectra (Fig. 4), whereas a mixture of trans and cis conformers in a ratio of about 1:1 was seen in the less polar solvents CD_2Cl_2 and $CDCl_3$. The ester exchange reaction is seemingly favored by the conformation in CD_2Cl_2, whereas in the polar solvents CD_3OD and DMSO-d_6 it does not proceed. An NOE between H-35/C5-OH was observed in DMSO-d_6 (Fig. 4).

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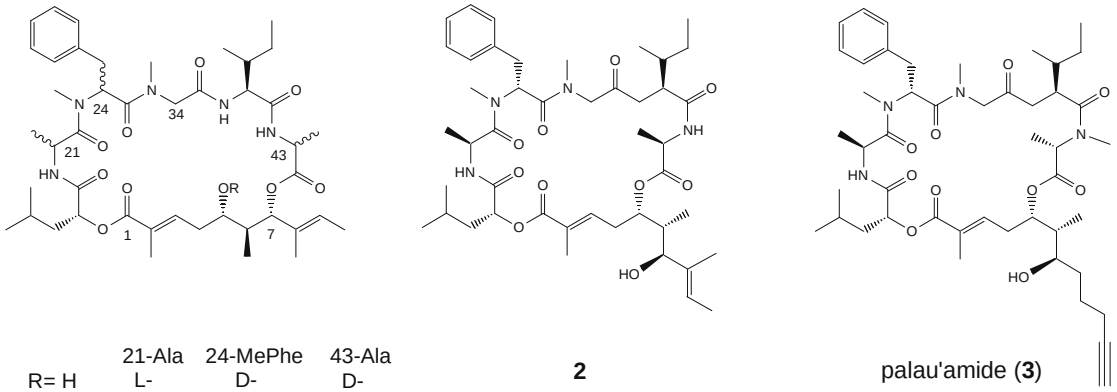


		21-Ala	24-MePhe	43-Ala
1	R= H	L-	D-	D-
1a	= H	L-	L-	D-
1b	= H	D-	L-	L-
1'	= MTM	L-	D-	D-

Figure 1. Structures of kulokekahilide-2 analogues and Palau'amide.

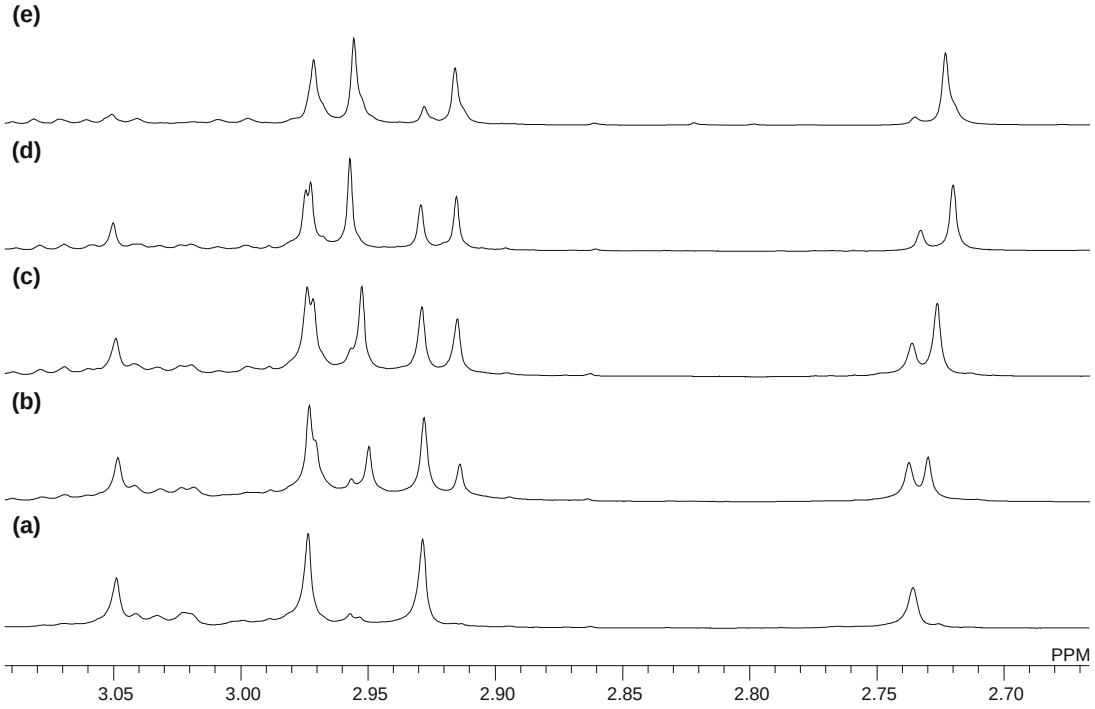


Figure 2. Partial ^{1}H NMR (500 MHz) spectra of **1** and **2** in CD_2Cl_2 : (a) compound **2**; (b) after 37 h; (c) after 85 h; (d) after 121 h; (e) compound **1**.

This further suggested that the occurrence of ester exchange was likely to be affected by different conformations in the various solvents. Unfortunately, ROESY spectra of **2** in CD_2Cl_2 and DMSO-d_6 could not be obtained because of spectral complexity. We also postulated that the configuration at 43-Ala and the associated conformation about the hydroxyl group and ester bond were of importance with regard to the ester exchange. In fact, analogue **1a** (43-D-Ala) behaved in the same manner as **1**, but **1b** incorporating 43-L-Ala did not undergo ester exchange.

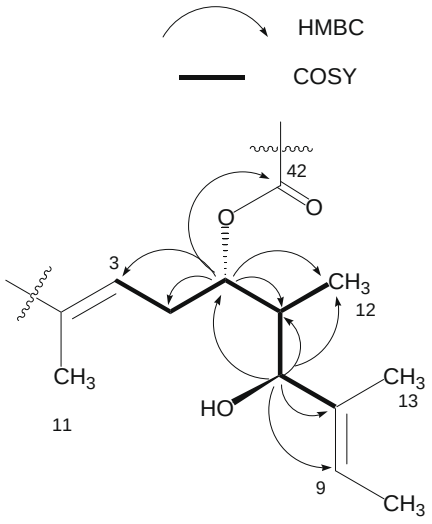
Palau'amide (**3**) is a potent cytotoxic depsipeptide from cyanobacterium Lyngbya,[5] which has been assigned a similar structure to that of **2**, having the same 24-membered depsipeptide ring. Compound **3** has been obtained by a total synthesis,[6] but the present work suggests that the reported structure may have been misassigned. Through our total synthesis of **1** and its analogues, it revealed that in order to have strong activity, the configurations of 21-L-Ala and 24-D-MePhe are related but 43-Ala is not so related in an amino acid part. The configurations of a dihydroxy acid part in **1** and synthetic **3** are 5*S*, 6*S*, and 7*S*, and 5*S*, 6*R*, and 7*R*, respectively, and in natural **3** are still ambiguous. However, **1**, natural and synthetic **3** have strong activity, therefore the configurations of a dihydroxy part might not be influenced in activity. Also, NMR data of **3** showed well-dispersed signals by MeOH-d_3 rather than $CDCl_3$, the same as those of **1**.[5] From these results, one conformational isomer of **3** might consist of a 26-membered cyclic depsipeptide, though there are still the problems of the different configurations at the 43-position and at the dihydroxy acid. These cyclic depsipeptides must be examined from every possible aspect of the total ring conformations.

This report might constitute the first instance of intramolecular ester exchange in a natural cyclic depsipeptide. It is anticipated that further investigation of this process by computer-aided analysis will clarify the conformation–bioactivity relationship.

Table 1
NMR data of compound **2** in CD_2Cl_2

Position	Main peak			Sub peak		
	^{13}C	^{1}H (δ, mult., Hz)	HMBC	^{13}C	^{1}H (δ, mult., Hz)	HMBC
1	168.6			166.9		
2	128.6					
3	139.9	6.69 br t 7.4	1,4,5,11	139.4	6.66 bt 7.4	1,4
4a	28.6	2.44 ddd 7.1, 7.5, 15.3	2,3,5	29.6	2.44 m*	2,3
4b		2.58 m*	2,3		2.53 m*	2,3,5
5	75.2	5.06 br dt 3.2, 6.1	3,4,6,12,42	74.7	5.37 m*	
6	39.5	1.95 m*	4,5,7,8,12	40.3	1.98 m*	
7	80.1	3.82 d 8.4	5,6,8,9,12	80.6	3.78 d 9.6	5,6,8,9,13
8	136.5			137.0		
9	123.3	5.44 m*	7,10	123.4	5.44 m*	
10	13.1	1.61 br d 8.6	9		1.55–1.62*	
11	12.7	1.89 br s	1,2,3	12.6	1.81 br s	1,2,3
12	11.5	0.71 d 7.1	5,6,7	11.9	0.78 d 7.0	5,6,7
13	11.1	1.57 br d 1.0	7,8,9	10.5	1.59 br d 0.7	7,8
14	171.1			172.7		
15	73.8	4.76 dd 4.1, 9.7	16,17	73.0	4.91 dd 4.5, 9.2	1,14,16,17
16a	41.0	1.80 m*	17,18	40.8	1.75 m*	
16b		1.80 m*			1.75 m*	
17	25.0	1.55 m	16,19	24.9	1.60 m*	19
18	21.8	0.91 d 6.5	16,17,19	22.0**	0.88–0.98**	
19	23.3	0.94 d 6.1	16,17,18	23.3**	0.88–0.98**	
20	173.6			172.6		
21	44.9	4.61 dq 8.0, 7.0	20,22	45.5	4.82 m*	22
22	16.6	0.82 d 7.0	20,21	17.2	0.98 d 6.9	20,21
NH		6.38 d 8.0	14,22		6.65 d 7.4	
23	170.0			170.7		
24	54.5	5.38 dd 6.8, 8.9	20,23,25,26,32	56.3	5.46 m*	23,25,26,32
25a	35.5	3.02 m*	24,26,27	35.3	2.98 m*	26,27
25b		3.05 m*			3.18 dd 6.6, 14.0	24,26,27,32
26	137.4			137.7		
27	129.8	7.13–7.25*	29	129.6	7.13–7.25*	25
28	128.4		26	128.6		
29	126.8		28,30	126.9		
30	128.4		26	128.6		
31	129.8		29	129.6		
32	30.4	2.98 s	20,24	31.2	3.05 s	20,24
33	169.3			169.6		
34a	51.6	3.24 d 18.1	23,35	51.6	3.43 d 16.4	23,35
34b		4.00 d 18.1	33,35		4.79 d 16.4	35
35	36.5	2.93 s	23,34	36.1	2.74 s	23,34
36	171.0			171.0		
37	57.8	4.35 dd 8.7, 9.0	38,39,41	58.0	4.16 dd 9.2, 9.3	36,38,39,41
38	36.8	2.00 m*	37	34.7	2.00 m*	
39a	24.9	1.30 m	40,41	25.2	1.13 m*	41
39b		1.68 m			1.55 m*	
40	11.2	0.95 t 7.8		11.0**	0.88–0.98**	
41	15.6	0.96 d 6.3	37	15.9	0.89 d 6.3	37
NH		7.64 d 9.0	33		6.50 d 9.2	33
42	171.6					
43	50.0	4.28 m*	42,44	48.8	4.27 m*	42
44	17.6	1.41 d 7.2	43	17.2	1.33 d 7.3	43
NH		6.55 br d 5.8	36,43		6.89 d 7.1	44

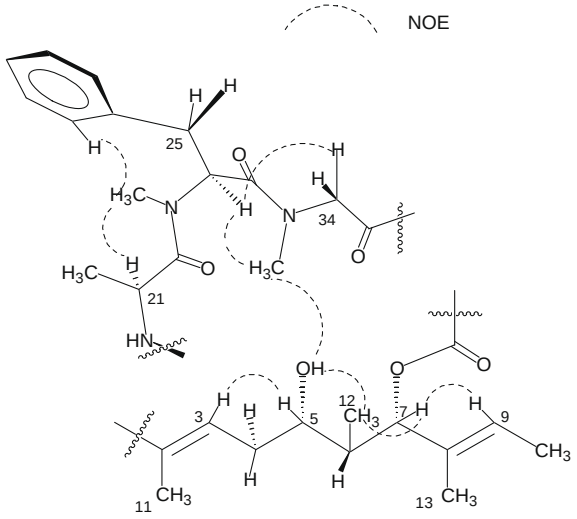
* The splitting is not clear because of overlapping.
** The signal could not be distinguished because of a small peak.



5-H: 5.06 (bdt, J = 3.2, 6.1 Hz)

7-H: 3.82 (d, J = 8.4 Hz) in CD_2Cl_2

Figure 3. Key COSY and HMBC correlations for **2**.



5-H: 3.51 (bdt, J = 5.6, 8.7 Hz)

7-H: 5.22 (d, J = 9.8 Hz) in CD_2Cl_2

Figure 4. Key ROESY correlations for **1** (in DMSO-d_6).

References and notes

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4. The retention times of the 26- and 24-membered compounds were 72.3 and 77.8 min, respectively (column: Cosmosil 5C18-MS, 250 × 10 mm, solvent: 50% aqueous MeCN, flow rate 2.5 mL/min, detection at 220 nm). In a large-scale synthesis of **1**, the 26-membered (**1**) and 24-membered (**2**) compounds could be separated by flash column chromatography to afford these products in quantitative yield in a 4:1 ratio following the final MTM-deprotection step (**1′**).Compound **2**: $[\alpha]_D$ −4.0 (*c* 0.76, MeOH); IC_{50} values against P388 and HeLa cells were 7.2 and 40 ng/mL, respectively. The ^1H and ^{13}C NMR spectra are summarized in Table 1. HR FABMS: $[M+Na]^+$ *m*/*z* 848.4805 (*m*/*z* 848.4786 calcd for $C_{44}H_{67}N_5O_{10}Na$).
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