Tetrahedron 68 (2012) 659–669

Contents lists available at SciVerse ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

The total synthesis and structure–activity relationships of a highly cytotoxic depsipeptide kulokekahilide-2 and its analogs

Yuuki Takada [a], Masahiro Umehara [a], Ryosuke Katsumata [b], Yoichi Nakao [b], Junji Kimura [a,*]

[a] Department of Chemistry and Biological Science, College of Science and Engineering, Aoyama Gakuin University, 5-10-1 Fuchinobe, Chuo-ku, Sagamihara, Kanagawa 252-5258, Japan
[b] Department of Chemistry and Biochemistry, School of Advanced Science and Engineering, Waseda University, 3-4-1 Okubo, Shinjuku-ku, Tokyo 169-8555, Japan

ARTICLE INFO

Article history:
Received 3 October 2011
Received in revised form 26 October 2011
Accepted 27 October 2011
Available online 3 November 2011

Keywords:
Kulokekahilide-2
Cyclic depsipeptide
Cytotoxicity
Total synthesis
Structure–activity relationships

ABSTRACT

We successfully completed the total synthesis of kulokekahilide-2, a cytotoxic depsipeptide from the Cephalaspidean mollusk *Phillinopsis speciosa*. We have revised the absolute stereochemistry of kulokekahilide-2 to 21-L-Ala, 24-D-MePhe, 37-L-Ile, 43-D-Ala, 15-D-Hica, and 5*S*,6*S*,7*S*-Dtda. We also investigated the cause of the mis-assignment of the configuration in the originally proposed kulokekahilide-2 and concluded that methanolysis using MeONa caused partial racemization, which led to the mis-assignment. The structure–activity relationships of kulokekahilide-2 and its analogs indicate the importance of an L-amino acid at position 21.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Marine organisms are well known as a rich source of bioactive compounds that can be potential drug leads.[1] Among their various bioactivities, cytotoxicity is a desirable characteristic, and numerous marine cytotoxic compounds have been reported.[2] Cytotoxic cyclic depsipeptides, as exemplified by aurilide isolated from the Japanese sea hare *Dolabella auricularia*[3] and palau'amide from the marine cyanobacterium *Lyngbya majuscula*,[4] are potential anticancer leads. A related 26-membered cyclic depsipeptide, kulokekahilide-2 (**1**), which was isolated from a Cephalaspidean mollusk *Philinopsis speciosa*, also 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 cells).[5a] The structure of kulokekahilide-2 was originally proposed as **1b** composed of five amino acids (L- and D-alanine (43-L-Ala, 21-D-Ala), L-isoleucine (37-L-Ile), *N*-methylglycine (MeGly), and L-*N*-methylphenylalanine (24-L-MePhe)) as well as two hydroxy acids (D-2-hydroxyisocaproic acid (15-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)).[5a] Investigation into its stereochemistry using synthetic **1b** revealed that the proposed stereochemistry (**1b**) was incorrect.[5b] Therefore, we successfully completed the total synthesis of an array of analogs with plausible stereochemistries (**1c**: 21-L-Ala, 24-D-MePhe, and 43-L-Ala, and **1d**: 21-L-Ala, 24-D-MePhe, and 43-D-Ala, **1e**: 21-L-Ala, 24-L-MePhe, and 43-D-Ala). By comparison of the spectral data with those of the natural compound, we conclude that the absolute stereostructure of kulokekahilide-2 is **1d** (Fig. 1).[5c]

In this paper, we describe the details of the total synthesis of kulokekahilide-2 and its analogs, which allowed us to revise the configuration of the proposed structure from **1b** to **1d**. The cause of the mis-assignment of the initial stereochemistry was also investigated. Finally, structure–activity relationships (SARs) are discussed using the kulokekahilide-2 analogs prepared in this study.

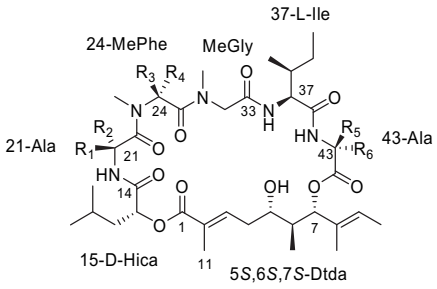
2. Result and discussion

2.1. Total synthesis of kulokekahilide-2

2.1.1. Synthesis by macrolactonization. Our first synthetic strategy followed the suggested biosynthetic route, including macrolactonization at the final step.[6] We attempted to prepare kulokekahilide-2 (**1b**) by macrolactonization of the corresponding seco acid (**2**) as the key reaction (Scheme 1, route A). Compound **2** was prepared by the coupling of hexapeptide (**3**) and dihydroxy acid (**4**). Hexapeptide **3** was synthesized from five protected amino

* Corresponding author. Tel.: +81 42 759 6229; fax: +81 42 759 6493; e-mail address: kimura@chem.aoyama.ac.jp (J. Kimura).

0040-4020/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2011.10.094



Compounds	21-Ala R_1	21-Ala R_2	24-MePhe R_3	24-MePhe R_4	43-Ala R_5	43-Ala R_6
1a	H	Me	Bn	H	H	Me
1b	H	Me	Bn	H	Me	H
1c	Me	H	H	Bn	Me	H
kulokekahilide-2 (**1d**)	Me	H	H	Bn	H	Me
1e	Me	H	Bn	H	H	Me
1f	H	Me	H	Bn	H	Me

Fig. 1. Structures of revised kulokekahilide-2 (**1d**) and its analogs.

acids and D-Hica. Dihydroxy acid (**4**) was prepared by enantioselective aldol condensation as described previously.[5a,b]

Hexapeptide **3** was synthesized via the usual condensation steps of peptide synthesis using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride salt (EDCI-HCl)[7] or benzotriazol-1-yl-oxy-trispyrrolydino phosphonium hexafluorophosphate (PyBOP)[8] in the presence of 1-hydroxy benzotriazole (HOBt) or *N*,*N*-dimethylaminopyridine (DMAP) (Scheme 2).

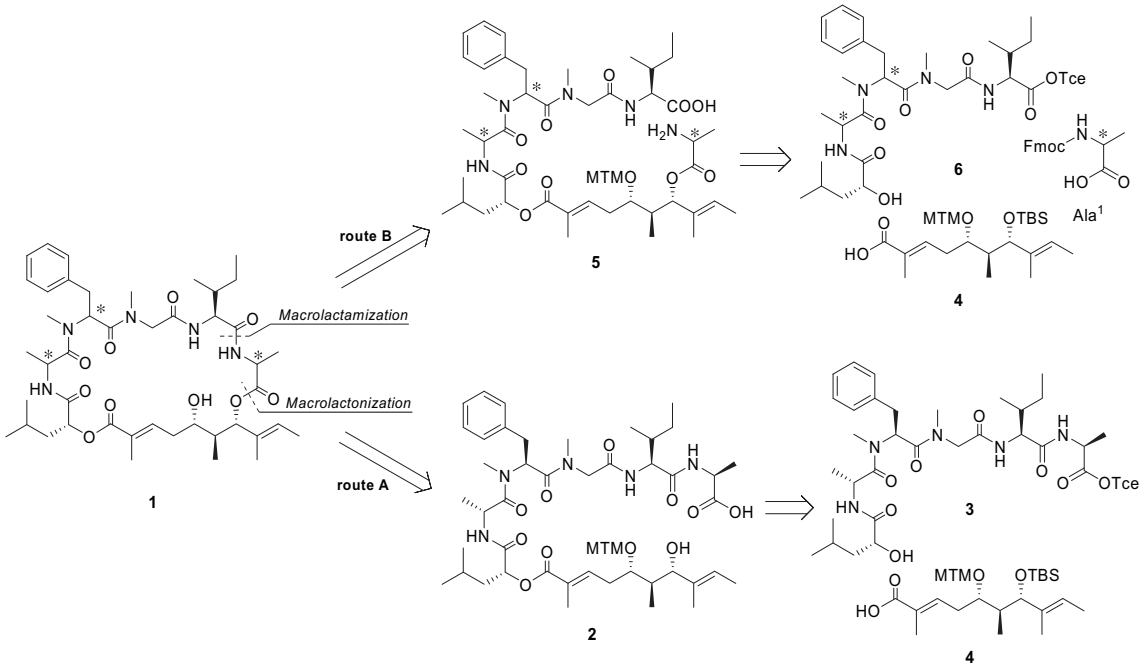
Coupling of suitably protected hexapeptide **3** and dihydroxy acid **4** in the presence of EDCI-HCl gave the corresponding depsipeptide **11** in 54% yield. When this coupling reaction was carried out using Shiina's reagent,[9] 2-methyl-6-nitrobenzoic anhydride (MNBA), and DMAP, the yield of **11** was increased up to 67%. Thus, the combination of MNBA and DMAP was more effective in this coupling than that of EDCI-HCl and DMAP. Treatment with HF-Py and subsequently with zinc powder provided seco acid **2**.

The macrolactonization of **2** was first attempted utilizing EDCI-HCl and DMAP, but resulted in quantitative recovery of the starting material. The coupling reagent showed only low reactivity in the macrolactonization reaction, as was the case for the synthesis of aurilide. When macrolactonization was implemented with a low concentration of dichloromethane (2.0 mM) by Shiina's reagent, the reaction proceeded smoothly to give the cyclized product (**12a**) in 63% yield. Yamaguchi lactonization[10] produced **12a** with a maximum of only 48% yield. Deprotection of the methylthiomethyl (MTM) group in **12a** by $AgNO_3$ afforded **1a** (75%).[11]

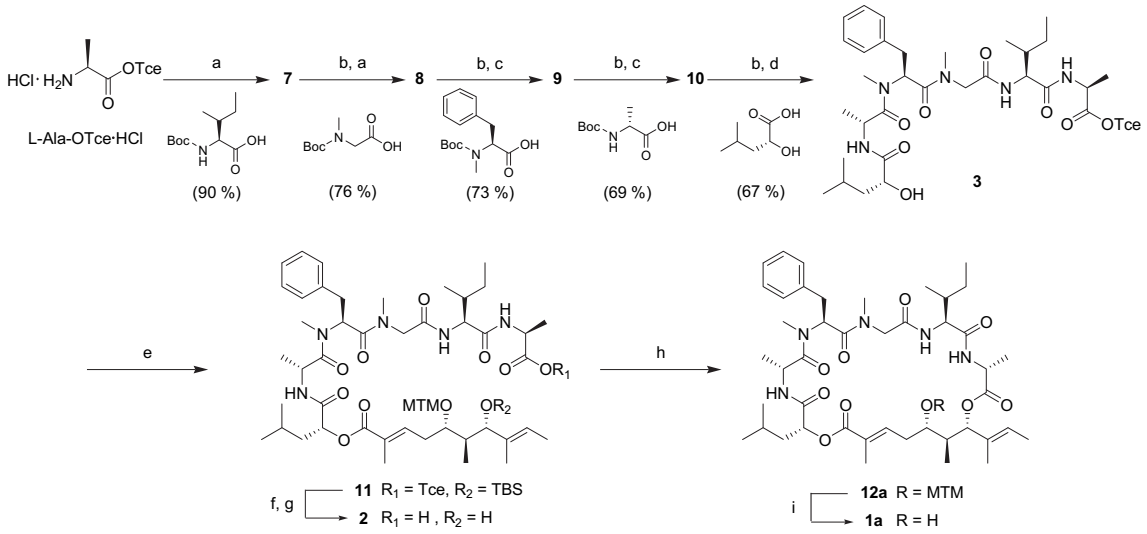
To our great disappointment, the ^{1}H NMR spectrum of **1a** was not identical to that of natural kulokekahilide-2 (Fig. 2). Thus, the absolute stereochemistry of each amino acid in **1a** was re-assessed by Marfey's analysis,[12] which led to more confusion due to the results that the component amino acids from **1a** were L-Ile, L-MePhe, and D-Ala. The loss of the expected L-Ala indicated that 43-L-Ala had completely inverted its stereochemistry; therefore, **1a** was concluded to be the 43-epimer of the originally proposed **1b**.[5b]

Racemization occasionally occurs at the optically active C-terminal amino acids when DMAP is used for condensation.[13] The complete inversion of the stereochemistry at C-terminal 43-Ala from L- to D-form was presumed to be caused during the final macrolactonization step, which did not proceed with C-terminal 43-L-Ala because of the steric hindrance but proceeded for the 43-*epi* seco acid derived from the epimerization equilibrium between L-Ala and D-Ala of **2**.

2.1.2. Synthesis by macrolactamization. To confirm the structure of **1a** with 43-D-Ala, we synthesized **1a** through the independent route B by macrolactamization of the linear precursor **5** (see



Scheme 1. Retrosynthetic analysis of kulokekahilide-2.



Scheme 2. Synthesis of **1a** via macrolactonization (route A). Reagents and conditions: (a) EDCI-HCl, HOBt, Et_3N, CH_2Cl_2/DMF (1:1), rt; (b) 4 M HCl/dioxane; (c) PyBOP, *i*-Pr_2NEt, CH_2Cl_2/DMF (1:1), rt; (d) EDCI-HCl, HOBt, DMF, rt; (e) **4**, EDCI-HCl, DMAP, CH_2Cl_2, rt, (54%), or **4**, MNBA, DMAP, CH_2Cl_2, rt, (67%); (f) HF-pyridine/prydine/THF (1:1:4), rt, (97%); (g) Zn powder, 1 M $AcONH_4$, rt, (97%); (h) MNBA, DMAP, CH_2Cl_2, rt, (63%) or 2,4,6-trichlorobenzoyl chloride, *i*-Pr_2NEt, benzene, rt then DMAP, benzene, rt, (48%); (i) $AgNO_3$, 2,6-lutidine, THF/H_2O (4:1), 65 °C, (75%).

Scheme 1, route B and Scheme 3).[3b,c] Synthesis of **1a** by lactamization was performed as below.

Pentapeptide **6a** was prepared from L-Ile-OTce-HCl as the starting material following standard peptide synthetic procedures.[14] Coupling of pentapeptide **6a** with protected dihydroxy acid **4** was effected by EDCI-HCl and DMAP to furnish ester **16a** (83%), which was deprotected to yield alcohol **17a** (87%). Esterification of **17a** with *N*-Fmoc-D-Ala instead of *N*-Fmoc-L-Ala gave the protected **18a** (88%)

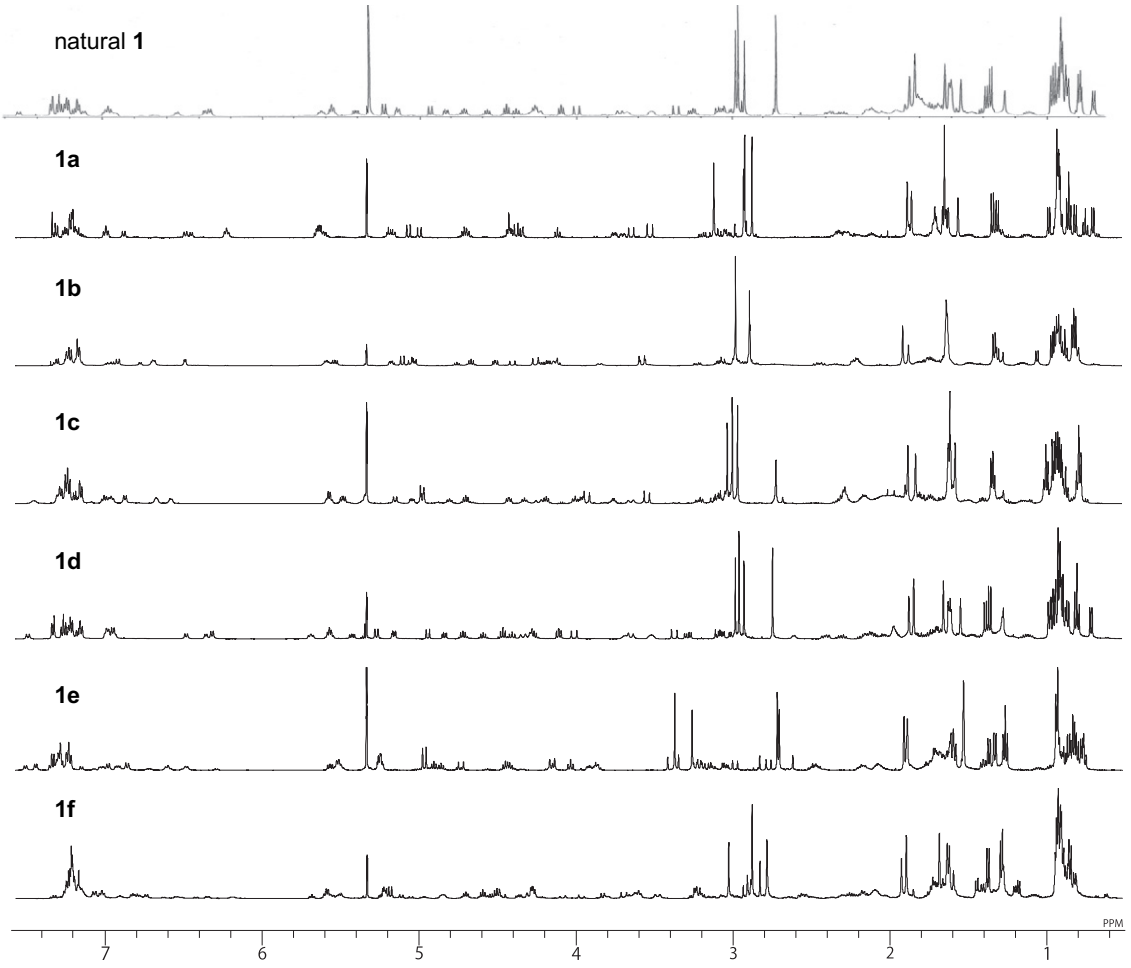
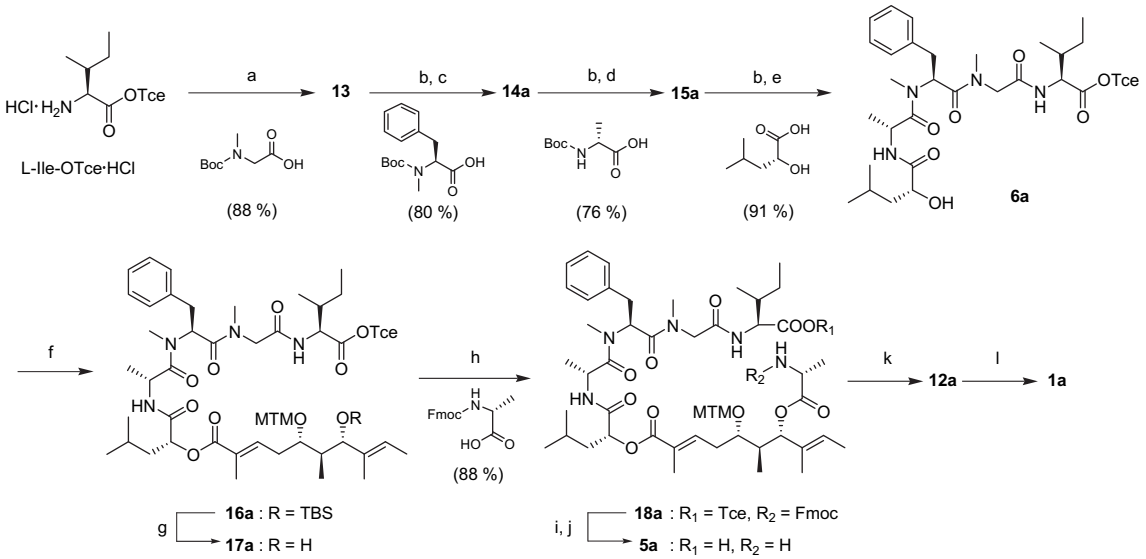


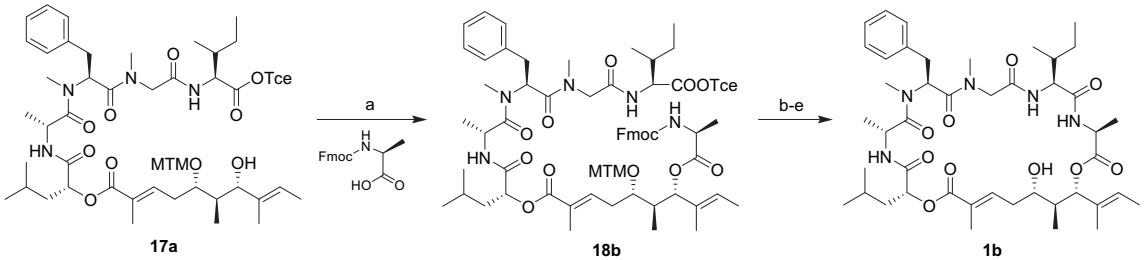
Fig. 2. ^1H NMR spectra of kulokekahilide-2 (**1**) and its analogs (**1a**–**f**) in CD_2Cl_2.



Scheme 3. Synthesis of **1a** via macrolactamization (route B). Reagents and conditions: (a) EDCI-HCl, HOBt, Et_3N, CH_2Cl_2/DMF (1:1), rt; (b) 4 M HCl/dioxane; (c) TBTU, *i*-Pr_2NEt, CH_2Cl_2/DMF (1:1), rt; (d) HBTU, *i*-Pr_2NEt, CH_2Cl_2/DMF (1:1), rt; (e) EDCI-HCl, HOBt, Et_3N, DMF, rt; (f) **4**, EDCI-HCl, DMAP, CH_2Cl_2, rt, (83%); (g) HF-pyridine/prydine/THF (1:1:4), rt, (87%); (h) EDCI-HCl, DMAP, CH_2Cl_2, rt, (88%); (i) Zn powder, 1 M $AcONH_4$, rt, (75%); (j) Et_2NH, MeCN, rt, (84%); (k) EDCI-HCl, HOAt, CH_2Cl_2/DMF (10:1), rt, (57%); (l) $AgNO_3$, 2,6-lutidine, THF/H_2O (4:1), 65 °C, (75%).

without racemization. Deprotection of **18a** generated **5a** and macrolactamization between 37-L-Ile and 43-D-Ala was carried out using EDCI-HCl and 1-hydroxy-7-azabenzotriazole (HOAt)[15] to yield cyclized **12a** (57%). Deprotection of **12a** at the 5-hydroxy group afforded **1a**. The NMR spectrum of the obtained **1a** was identical to that of the previously prepared **1a**. Epimerization of 37-L-Ile did not occur, and the corresponding L-Val in aurilide was inverted to D-Val under the similar lactamization condition and yielded the byproduct 6-*epi*-aurilide.[3c]

These results indicated that the macrolactamization using 37-L-Ile as the C-terminus in **5a** was more practical without racemization than the macrolactonization using 43-Ala as the C-terminus. Therefore, we attempted to synthesize the proposed **1b** (21-D-Ala, 24-L-MePhe, and 43-L-Ala, abbreviated as D, L, L) by macrolactamization of **18b** in which 43-L-Ala was moved to the N-terminus of the seco-peptide derived from **17a** (Scheme 4).



Scheme 4. Synthesis of **1b**. Reagents and conditions: (a) EDCI-HCl, DMAP, CH_2Cl_2, rt, (95%); (b) Zn powder, 1 M $AcONH_4$, rt; (c) Et_2NH, MeCN, rt; (d) EDCI-HCl, HOAt, CH_2Cl_2/DMF (10:1), rt, (70% in three steps); (e) $AgNO_3$, 2,6-lutidine, THF/H_2O (4:1), 65 °C, (91%).

Deprotection and macrolactamization of **18b** yielded **1b**. The NMR data of the obtained product yielded further confusion. The ^1H NMR spectrum of **1b** was not identical to that of natural kulokekahilide-2 (**1**). All of the stereocenters, including 43-Ala in **1b**, were confirmed, as shown in Fig. 2. Therefore, we concluded that the proposed structure **1b** in our original report was incorrect.[5b]

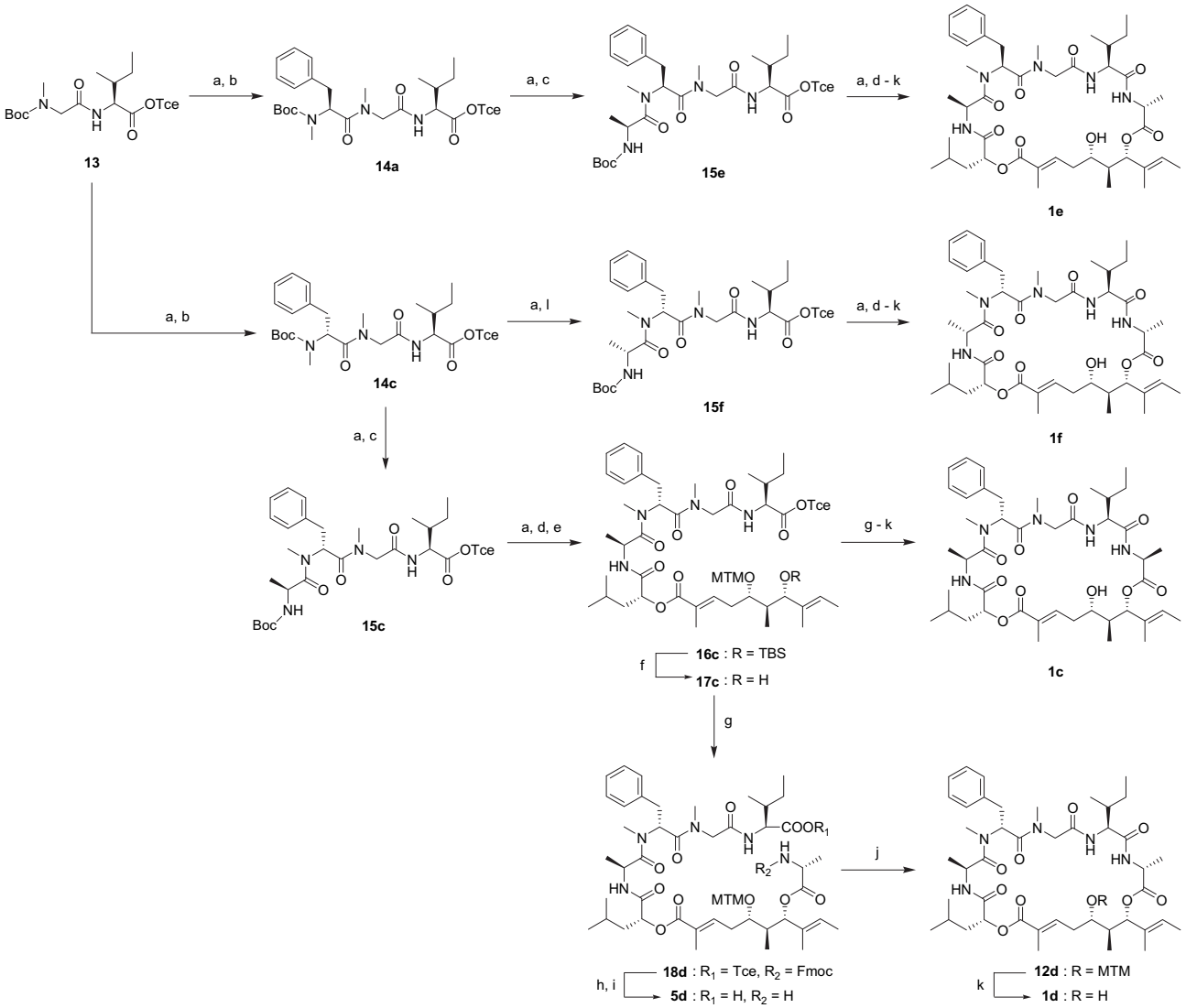
2.1.3. Determination of the true structure of kulokekahilide-2 by synthesis of analogs. We reexamined the stereochemistry of **1** in comparison to that of aurilide and palau'amide. The strangest differences were reversed configurations at positions 21 and 24 in **1b** (21-D and 24-L) to that of related compounds (21-L and 24-D). Therefore, we synthesized an array of analogs to evaluate different combinations of the configurations: 21-L, 24-D; 21-L, 24-L; and 21-D, 24-D. Namely, 21-L-Ala, 24-D-MePhe, and 43-L-Ala (abbreviated as L, D, L: **1c**); 21-L-Ala, 24-D-MePhe, and 43-D-Ala (L, D, D: **1d**); 21-L-Ala, 24-L-MePhe, and 43-D-Ala (L, L, D: **1e**); and 21-D-Ala, 24-D-MePhe, and 43-D-Ala (D, D, D: **1f**) were prepared through the same macrolactamization reaction applied for **1a** and **1b** (Scheme 5).

The ^1H and ^{13}C NMR data in CD_2Cl_2 for the major isomer of **1a**–**f** are summarized in Table 1. All of the ^1H and ^{13}C spectral data were assigned based on DQF-COSY, HMQC, and HMBC experiments. Comparison of the ^1H NMR spectra of **1a**–**f** indicated that **1d** (L, D, D) corresponds to the natural **1** (Fig. 2). From these results, we concluded that the absolute stereostructure of **1** is 21-L-Ala, 24-D-MePhe, and 43-D-Ala.

Comparison of spectral data for these analogs revealed that **1a**–**f** adopt two conformers derived from the *cis*–*trans* amide isomerism between MeGly and MePhe in CD_2Cl_2. The ROESY analysis indicated that only the *trans* conformer was seen for **1d** in DMSO-d_6[5d] and that CH_3-35 and C5-OH are closely located. The presence of D-phenylalanine and an *N*-methylated amino acid might stabilize the specific ring conformation[16] in DMSO, potentially showing a similar conformation to that of **1d** under physiological conditions.[17]

2.2. Consideration of the mis-assignment of the absolute stereochemistry

With the correct configuration confirmed, we next investigated the reason for the mis-assignment of the absolute



Scheme 5. Synthesis of analogs **1c**–**f**. Reagents and conditions: (a) 4 M HCl/dioxane; (b) Boc-L- or D-*N*MePhe, HBTU, *i*-Pr_2NEt, CH_2Cl_2/DMF (1:1), rt; (c) Boc-L-Ala, PyBOP, *i*-Pr_2NEt and/or Et_3N, CH_2Cl_2/DMF (1:1), rt; (d) D-Hica, EDCI-HCl, HOBt, Et_3N, DMF, rt; (e) **4**, EDCI-HCl, DMAP, CH_2Cl_2, rt; (f) HF-pyridine/pyridine/THF (1:1:4), 40 °C; (g) Fmoc-L- or D-Ala, EDCI-HCl, DMAP, CH_2Cl_2, rt; (h) Zn powder, 1 M $AcONH_4$, rt; (i) Et_2NH, MeCN, rt; (j) EDCI-HCl, HOAt, CH_2Cl_2/DMF (10:1), rt; (k) $AgNO_3$, 2,6-lutidine, THF/H_2O (4:1), 65 °C; (l) Boc-D-Ala, HBTU, Et_3N, *i*-Pr_2NEt, CH_2Cl_2/DMF (1:1), rt.

stereochemistry of kulokekahilide-2. In a previous report, the stereochemistries of all of the amino acids as well as dihydroxy acid **4** were confirmed to not be isomerized during the processes of reductive degradation and acid hydrolysis.[5b,c] Therefore, we suspected that the alkaline methanolysis determined the amino acid configuration in the original structure.[5a] Compound **1d** was subjected to alkaline methanolysis by MeONa, and the reaction mixture was purified by HPLC (column: COSMOSIL 5C_{18}-MS-II, 10 mm ID×250 mm, UV at 220 nm; mobile phase: 0.05% TFA in CH_3CN/H_2O (60/40) at a flow rate of 2.5 mL/min), yielding three hexapeptides as the main peaks. These peptides were acid hydrolyzed and examined by Marfey analysis. The first peptide contained the same configurations of amino acids as **1d** (35%), while the second and the third contained 24-L-MePhe (corresponding to **1e**, 12%) and 43-L-Ala (corresponding to **1c**, 20%), respectively.

These observations suggested that the hexapeptide used for the hydrazinolysis might have been racemized in the preceding methanolysis; consequently, 43-Ala was misassigned as being in the L-form in the subsequent Marfey analysis.

2.3. Structure–activity relationships (SARs)

Structure–activity relationships were investigated based on the cytotoxicity of the synthetic analogs **1a**–**f**. The cytotoxicities of these analogs against two cell lines (P388 and HeLa) are summarized in Table 2.

As expected, **1d** with the natural conformation L, D, D exhibited the same potent cytotoxicity (IC_{50} values of 16 and 3.2 ng/mL against P388 and HeLa cells, respectively) as natural kulokekahilide-2. Analog **1c** (L, D, L) with the exact same configuration as aurilide (L, D, L) with high cytotoxicity (IC_{50} values of 11 ng/mL against HeLa cells[3c]) as described in Fig. 3 showed a similar cytotoxicity (IC_{50} values of 16 and 5.3 ng/mL, respectively).

By contrast, the cytotoxicity of **1e** (L, *l*, D) was reduced to 1/20th–1/10th of the level (IC_{50} values of 400 and 39 ng/mL, respectively) of **1d**. Aurilide B (L, *l*, L) and C (L, *l*, L) also exhibited a lower LC_{50} value of 10–130 nM against NCI-H460 and neuro-2a mouse neuroblastoma cell lines.[18] The configuration at C-24-D seemed critical to the cytotoxicity (Fig. 3, black line square).

Table 1
^{1}H and ^{13}C NMR data for the major rotamer of compound **1a**–**f** in CD_2Cl_2

Unit	Position	**1a**		**1b**		**1c**		**1d**		**1e**		**1f**	
		δ_H, mult., (*J* in Hz)[a]	δ_C[b]	δ_H, mult., (*J* in Hz) [a]	δ_C[b]	δ_H, mult., (*J* in Hz) [a]	δ_C[b]	δ_H, mult., (*J* in Hz) [a]	δ_C[b]	δ_H, mult., (*J* in Hz) [a]	δ_C [b,c]	δ_H, mult., (*J* in Hz) [a]	δ_C[b]
Dtda	1	—	167.2	—	168.2	—	168.1	—	166.9	—		—	166.5
	2	—	126.9	—	126.2	—	126.5	—	128.2	—		—	129.4
	3	6.98 dt 7.4, 1.3	141.5	7.17 m	144.6	6.99 bt 7.3	142.6	6.97 dt 8.4, 1.1	141.9	6.91 m	140.4	7.01 bddd 8.4, 6.4, 1.3	140.3
	4a	2.30–2.34 m	31.1	2.20 m	33.0	2.26 m	31.9	2.37 dd 14.3, 8.4	32.5	1.99–2.22 m		2.25 m	34.0
	4b	2.30–2.34 m		2.43 ddd 14.9, 10.1, 7.5				2.14 m		2.37–2.51 m		2.55 ddd 14.7, 8.4, 2.1	
	5	3.75 ddd 10.6, 3.2, 3.2	70.3	4.13 m	71.3	3.75 dt 6.4, 4.4	71.3	3.51 bdd 8.7, 5.6	72.1	3.85–3.93 m		3.59 dt 7.7, 2.1	74.4
	6	2.30–2.34 m	40.3	2.19 m	40.8	2.16 m	40.6	2.11 m	41.4	1.99–2.22 m		2.16 m	40.5
	7	5.05 d 11.0	83.4	5.09 d 11.0	82.6	4.98 d 11.2	83	5.22 d 9.8	83.5	4.89–4.97 m		5.17 d 9.3	85.2
	8	—	132.5	—	132.7	—	132.5	—	133.2	—		—	133.3
	9	5.60 m	126.8	5.57 m	126.8	5.55 qd 6.4, 1.2	127.1	5.55 qd 6.6, 1.1	125.9	5.45–5.50 m		5.57 bq 6.8	125.9
	10	1.62 d 6.8	13.3	1.62 d 6.0	13.2	1.60 bd 7.1	13.2	1.61 dq 6.6, 0.9	13.1	1.55–1.80 m		1.62 bd 6.8	13.2
	11	1.88 s	12.8	1.90 s	12.9	1.87 s	12.7	1.83 bs	12.7	1.52 s		1.88 bs	13.0
	12	0.86 d 6.8	11.5	0.80 d 6.9	11.7	0.78 d 7.1	11.6	0.79 d 6.9	11.6	0.70–0.96 m		0.84 d 7.1	13.3
	13	1.63 s	11.0	1.62 s	10.7	1.61 br s	12.8	1.64 bs	11.2	1.88 m		1.67 bs	11.6
Hica	14	—	170.2	—	170.9	—	169.8	—	170.4	—		—	170.0
	15	5.18 m	73.2	5.03 m	73.7	5.03 dd 9.2, 4.7	73.4	5.14 dd 10.3, 5.8	72.6	5.18–5.24 m	73.0	5.21 dd 8.2, 3.7	73.1
	16a	1.60–1.73 m	41.4	1.60–1.72 m	41.4	1.66 m	41.0	1.77 m	40.9	1.55–1.80 m		1.64–1.75 m	41.4
	16b	1.60–1.73 m		1.60–1.72 m		1.79 m		1.71 m		1.55–1.80 m		1.64–1.75 m	
	17	1.60–1.73 m	25.0	1.60–1.72 m	25.0	1.66 m	25.1	1.69 m	24.9	1.55–1.80 m		—[c]	24.9
	18	0.91 d 5.2	22.0	0.92 d 6.0	21.9	0.92 d 6.4	21.9	0.91 d 6.3	21.9	0.70–0.96 m		0.88–0.94 m	22.1
	19	0.91 d 5.2	23.3	0.92 d 6.0	23.3	0.93 d 6.3	23.3	0.91 d 6.3	23.3	0.70–0.96 m		0.88–0.94 m	23.3
Ala-1	20	—	173.1	—	172.9	—	173.6	—	173.0	—		—	172.7
	21	4.69 m	45.0	4.65 dq 7.3, 6.8	45.0	4.68 dq 7.7, 7.0	45.3	4.71 dq 7.8, 6.7	45.5	4.89–4.97 m		4.58 dq 7.0, 6.8	46.0
	22	0.69 d 6.8	17.8	0.81 d 6.9	17.0	0.79 d 7.0	17.7	0.87 d 6.7	17.5	1.24 d 6.8		1.28 d 6.8	18.1
	NH	6.87 d 8.2	—	6.67 d 7.3	—	6.86 d 7.7	—	7.10 d 7.8	—	7.46 d 7.2	—	—[c]	—
N-MePhe	23	—	170.1	—	170.1	—	171.7	—	172.5	—		—	171.6
	24	5.63 m	53.5	5.52 dd 10.1, 6.0	53.7	5.47 dd 9.9, 6.0	54.4	5.62 dd 9.1, 7.0	56.3	5.54 dd 10.0, 6.0		—[c]	—[c]
	25a	3.02 dd 14.2, 7.7	35.3	2.97 dd 14.7, 7.8	35.2	3.02 dd 14.4, 6.0	36.2	3.25 dd 14.1, 7.0	35.3	3.01–3.22 m		3.22 m	34.9
	25b	3.15 dd 14.2, 6.7		3.06 dd 14.7, 10.1		3.10 dd 14.4, 9.9		3.07 dd 14.1, 9.1		3.01–3.22 m		3.22 m	
	26	—	137.0	—	137.3	—	137.0	—	136.9	—		—	—[c]
	27	7.20–7.24 m	129.9	7.20–7.24 m	129.7	7.15–7.30 m	129.7	7.32 bd 8.0	129.9	7.12–7.34 m		7.16–7.24 m	129.8
	28		128.6		128.5		128.6	7.25 dd 8.0, 7.1	128.6				128.8
	29		127.5		127.2		128.8	7.21 t 7.1	127.2				127.2
	30		128.6		128.5		128.6	7.25 dd 8.0, 7.1	128.6				128.8
	31		129.9		129.7		129.7	7.32 bd 8.0	129.9				129.8
	32	2.91 s	30.4	2.97 s	30.4	2.99 s	30.6	2.95 s	31.2	3.24 s		3.02 s	30.7
N-MeGly	33	—	170.0	—	168.9	—	168.9	—	169.7	—		—	169.7
	34a	3.52 d 17.2	52.5	3.56 d 17.4	52.0	3.54 d 17.0	52.2	4.28 bd	52.9	2.98 d 16.1		3.47 d 14.1	53.8
	34b	4.39 d 17.2		4.24 d 17.4		3.94 d 17.0		3.72 bd		4.71 d 16.1		4.29 d 14.1	
	35	2.86 s	35.1	2.88 s	35.1	2.96 s	35.6	2.71 s	36.3	2.71 s	33.5	2.87 s	37.2
Ile	36	—	168.3	—	171.7	—	170.5	—	170.8	—		—	170.7
	37	4.41 dd 7.4, 7.2	58.0	4.50 dd 9.2, 6.2	57.9	4.01 dd 8.0, 8.0	57.6	4.09 dd 8.2, 7.1	58.8	4.15 t 6.8		4.27 dd 9.0, 5.2	58.2
	38	1.80 m	38.3	1.80 m	38.6	1.92 m	38.1	1.96 m	35.8	1.55–1.80 m		2.08 m	35.5
	39a	1.12 m	25.1	1.48 m	25.1	1.29 m	25.2	1.48 m	25.1	0.96–1.05 m		1.07 m	24.8
	39b	1.46 m		1.48 m		1.59 m		1.12 m		1.21–1.33 m		1.36–1.41 m	
	40	0.85 t 6.7	10.3	0.87 t 7.3	11.5	0.95 t 7.4	10.8	0.88 t 7.5	11.3	0.70–0.96 m		0.88–0.94 m	11.8
	41	0.91 d 4.5	15.8	0.95 d 6.4	15.3	0.90 d 6.7	15.5	0.92 d 5.7	16.0	0.70–0.96 m		0.88–0.94 m	16.2
	NH	6.22 d 7.2	—	6.91 d 9.2	—	6.96 d 7.5	—	6.95 d 8.2	—	6.63 bd 8.2	—	7.06 d 9.0	—
Ala-2	42	—	170.6	—	172.3	—	171.3	—	171.6	—		—	171.7
	43	4.40 m	49.5	4.16 dd 7.3, 4.6	50.2	4.18 dq 7.0, 6.7	49.6	4.44 dq 7.6, 7.1	48.7	4.44 dq 7.5, 7.4	48.7	4.49 dq 8.4, 7.2	48.9
	44	1.33 d 7.3	18.1	1.32 d 7.3	16.3	1.34 d 7.0	16.6	1.35 d 7.1	18.5	1.32 d 7.4		1.36 d 7.2	18.2
	NH	6.47 d 8.6	—	6.47 d 4.6	—	6.66 bd	—	6.32 d 7.6	—	7.06 bd	—	—[c]	—

[a] Recorded at 500 MHz.
[b] Recorded at 126 MHz.
[c] Signals were not assigned due to overlap.

Table 2
Cyototoxicity of kulokekahilide-2 and its analogs against two cell lines

Compound	Chirality at each residue H-Ala-MePhe-MeGly-Ile-Ala-OH	Cell lines (IC_{50}: ng/mL)	
		P388	HeLa
1a	DL—LD	>10,000	>10,000
1b	DL—LL	10,000	>10,000
1c	LD—LL	16	5.3
1d	LD—LD	16	3.2
1e	LL—LD	400	39
1f	DD—LD	4500	2000

Compound **1f** (*d*, D, D) showed only weak cytotoxicity against P388 and HeLa cells (IC_{50} values of 4500 and 2000 ng/mL, respectively), while **1a** (*d*, L, D) and **1b** (*d*, L, L) completely lost activity (IC_{50} value of ≥10,000 ng/mL against both P388 and HeLa cells). Therefore, the configuration at C-21-L is also critical to the cytotoxicity (dotted line square).

In recent years, deoxyaurilide and aurilide analogs with an L-Lys residue instead of 2-L-MeAla, 6- and 21-L-Val (corresponding to positions 43, 37 and 21 in **1d**, respectively) have been synthesized. The cytotoxicities of these analogs were 10–100 times less than that of aurilide. Moreover, 6-*epi*-aurilide with 6-D-Val instead of L-Val (corresponding to position 37 in **1d**) showed no cytotoxicity,[3e] indicating that the absolute configuration at position 37 contributes to the cytotoxicity. Therefore, the reversed stereochemistry of potent cytotoxic lagnamides at the position corresponding to that of C-7 in **1d** is interesting.[19]

We have reported that **1d** (L, D, D) undergoes intramolecular ester exchange to yield 24-membered products, such as paula'amide (L, D, L) in nonpolar solvents. The cytotoxicity of the 24-membered isomer of **1d** (IC_{50} values of 7.2 and 40 ng/mL against P388 and Hela cells, respectively)[5d] showed equivalent activity to that (IC_{50} values of 16 and 3.2 ng/mL, respectively) of **1d**. Also, the 24-membered paula'amide exhibited potent cytotoxicity against KB cells (IC_{50} value of 13 nM).[4a] On the other hand, **1b** and **1c** containing 43-L-Ala did not undergo intramolecular ester exchange and thus did not yield any 24-membered isomers. The effect of the ring size on the cytotoxicity and the relationship between cytotoxicity and intramolecular esterification will be described in more detail in a future publication (gray line square).

3. Conclusion

We completed the total synthesis of kulokekahilide-2 (**1d**: L, D, D) and its analogs **1a**–**c**, **1e**, and **1f** (**1a**: D, L, D, **1b**: D, L, L, **1c**: L, D, L, **1e**: L, L, D, and **1f**: D, D, D) by macrolactamization.

We investigated the reason for the mis-assignment of the configuration in the original report using synthetic **1d**. We concluded that the methanolysis of **1d** by MeONa caused partial racemization, which led to the mis-assignment.

Evaluation of the cytotoxicities of these compounds (**1a**–**f**) showed that the 21-L and 24-D configurations of **1d** are critical to the cytotoxicity, while the C-43 stereochemistry has less influence. The whole ring conformation affected by the C-21 and C-24 configurations was likely more important for the cytotoxicity than the side chain substitutions. Investigation of the target molecules for **1d** and its analogs is currently in progress.

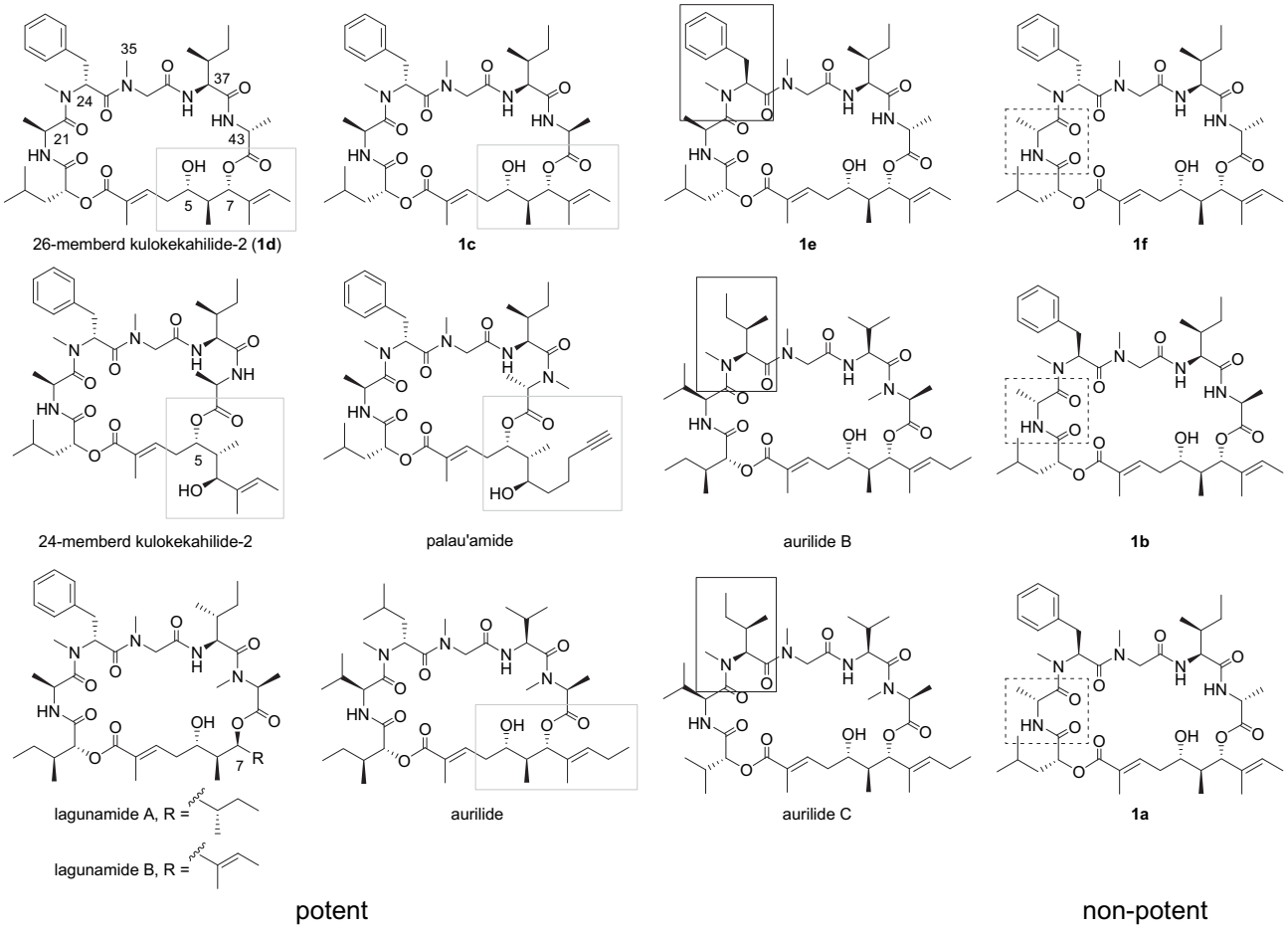


Fig. 3. Structurally related cyclic depsipeptides.

4. Experimental section

4.1. General procedures

All moisture-sensitive reactions were carried out under an argon atmosphere. The solvents used were distilled from the following drying reagents: tetrahydrofuran (THF: sodium benzophenone), dichloromethane (CH_2Cl_2: calcium chloride and 4-Å molecular sieves), dimethylformamide and dimethylsulfoxide (DMF and DMSO: calcium hydride). The other anhydrous solvents were purchased from Aldrich or Kanto Kagaku. *N*,*N*-Dimethylaminopyridine (DMAP) was recrystallized from EtOAc prior to use. Open and flash column chromatography were performed using Fuji Silysia silica gel BW-300 and BW-350, respectively. Analytical thin-layer chromatography (TLC) was performed using TLC plates precoated with Merck Silica gel 60 F_{254} (0.25 mm layer thickness). The ^1H (500 MHz) and ^{13}C (126 MHz) NMR spectra were measured on a JEOL JNM-ECP500 spectrometer. Chemical shifts are reported in δ parts per million calibrated using residual undeuterated solvent as an internal reference. The following abbreviations are used to explain the multiplicities: s=singlet, d=doublet, t=triplet, q=quartet, qu=quintet, m=multiplet, br=broadened. FABMS (Fast Atom Bombardment Mass Spectrometry) spectra were obtained on a JEOL MStation JMS-700A spectrometer. Nitrobenzyl alcohol (NBA) was used as a matrix for the FABMS measurements. Polyethylene glycol was used as a marker for HR-FABMS. Optical rotations were measured on a Perkin–Elmer-340 digital spectropolarimeter. IR spectra are reported in wavenumbers (cm^{-1}) and were measured on a Shimadzu FTIR-8200A infrared spectrometer. HPLC was carried out with a Shimadzu LC-10 liquid chromatograph equipped with a Shimadzu SPD-10A UV–vis detector and Integrator C-R6A Chromatopac and with a JASCO 880-PV liquid chromatograph equipped with a JASCO 875-UV UV–vis detector and Sic chromatocorder 12.

4.2. Synthesis of revised kulokekahilide-2 (1d)

*4.2.1. Dipeptide Boc-N-MeGly-L-Ile-OTce (**13**).* 2,2,2-Trichloroethanol (1.06 mL, 11.0 mmol), DMAP (122 mg, 1.00 mmol), and EDCI-HCl (2.30 g, 12.0 mmol) were added to a stirred solution of Boc-L-Ile-OH (2.32 mg, 10.0 mmol) in CH_2Cl_2 (20 mL), and the mixture was stirred at room temperature for 1 h. The reaction mixture was evaporated to dryness and dissolved in EtOAc (40 mL). The EtOAc solution was washed with 1 M HCl (40 mL) and 5% aqueous $NaHCO_3$ (40 mL), dried over $MgSO_4$, and evaporated to dryness. The residue was treated with 4 M HCl in dioxane (20 mL) at room temperature for 2 h to yield L-Ile-OTce HCl salt (2.88 mg, 9.64 mmol, 96%).

Boc-*N*-MeGly-OH (2.08 g, 11.0 mmol), Et_3N (1.39 μL, 10.0 mmol), HOBt (1.49 g, 11.0 mmol), and EDCI-HCl (4.22 g, 22.0 mmol) were added to a stirred solution of L-Ile-OTce HCl salt (2.88 g, 9.64 mmol) in CH_2Cl_2/DMF (1:1, 20 mL), and the mixture was stirred at room temperature for 6 h. The reaction mixture was concentrated, diluted with 1 M HCl (40 mL) and extracted with CH_2Cl_2 (40 mL×2). The combined extracts were washed with 5% aqueous $NaHCO_3$ (40 mL), dried over $MgSO_4$, and evaporated to dryness. The residue was subjected to column chromatography on silica gel [*n*-hexane/EtOAc, (7:3)] to give dipeptide **13** as a colorless oil (3.71 g, 8.57 mmol, 86%). $[\alpha]_D^{20}$ +7.6 (*c* 1.0, $CHCl_3$); IR (KBr disk) 3315, 2968, 1760, 1699, 1669, 1636, 1534, 1455, 1392, 1146, 767, 719, 574 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.93 (t, 3H, *J*=7.3 Hz, Ileδ-H), 0.98 (d, 3H, *J*=6.9 Hz, Ileγ-H), 1.20 (m, 1H, Ileγ-Ha), 1.48 (m, 1H, Ileγ-Hb), 1.47 (s, 9H, $OC(CH_3)_3$), 2.03 (m, 1H, Ileβ-H), 2.96 (s, 3H, Gly–*N*Me), 3.81 (d, 1H, *J*=16.0 Hz, Glyα-Ha), 3.98 (d, 1H, *J*=16.0 Hz, Glyα-Hb), 4.64 (d, 1H, *J*=11.9 Hz, CH_2CCl_3), 4.75 (m, 1H, Ileα-H), 4.90 (d, 1H, *J*=11.9 Hz, CH_2CCl_3), 6.41–6.83 (br d, 1H, Ile-NH); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 11.4 (q), 15.5 (q), 24.8 (t), 28.2 (q, 3C), 35.7 (q), 37.5 (d), 53.1 (t), 56.2 (d), 74.4 (t), 80.8 (s), 94.3 (s), 169.9 (s, 3C); HR-FABMS: $[M+H]^+$ *m*/*z* 433.1086 (*m*/*z* 433.1064 calcd for $C_{16}H_{28}N_2O_5^{35}Cl_3$).

*4.2.2. Tripeptide Boc-N-Me-D-Phe-N-MeGly-L-Ile-OTce (**14c**).* Dipeptide **13** (3.71 g, 8.57 mmol) was stirred with 4 M HCl in dioxane (20 mL) at 0 °C for 1 h, and then the solvent was removed to give *N*-MeGly-L-Ile-OTce HCl salt (3.10 g, 8.38 mmol, 98%). Boc-*N*-Me-D-Phe-OH (486 mg, 1.74 mmol), *i*-Pr_2NEt (910 μL, 5.22 mmol), and HBTU (792 mg, 2.09 mmol) were added to a stirred solution of *N*-MeGly-L-Ile-OTce HCl salt (644 mg, 1.74 mmol) in DMF/CH_2Cl_2 (1:1, 6 mL), and the mixture was stirred at room temperature for 12 h. The reaction mixture was diluted with 1 M HCl (40 mL) and extracted with CH_2Cl_2 (40 mL×2). The combined extracts were washed with 5% aqueous $NaHCO_3$ (40 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was subjected to flash column chromatography on silica gel [*n*-hexane/EtOAc, (8:2) to (1:1)] to give tripeptide **14c** as a colorless amorphous powder (882 mg, 1.48 mmol, 85%). $[\alpha]_D^{20}$ +78.9 (*c* 0.50, $CHCl_3$); IR (KBr disk) 3328, 2968, 1757, 1696, 1658, 1636, 1455, 1388, 1141, 754, 701, 575 cm^{-1}. Characteristic signals of four conformations were observed, but some carbons could not be determined due to overlap (major rotamer). ^1H NMR (500 MHz, $CDCl_3$) δ 0.91 (t, 3H, *J*=7.1 Hz, Ileδ-H), 0.92 (d, 3H, *J*=6.9 Hz, Ileγ-H), 1.18 (m, 1H, Ileγ-Ha), 1.32 (s, 9H, $OC(CH_3)_3$), 1.45 (m, 1H, Ileγ-Hb), 1.98 (m, 1H, Ileβ-H), 2.80 (s, 3H, Gly–*N*Me), 2.94 (dd, 1H, *J*=14.0, 8.2 Hz, Pheβ-Ha), 3.03 (s, 3H, Phe–*N*Me), 3.14 (dd, 1H, *J*=14.0, 7.1 Hz, Pheβ-Hb), 3.84 (d, 1H, *J*=14.9 Hz, Glyα-Ha), 4.12 (d, 1H, *J*=14.9 Hz, Glyα-Hb), 4.59 (d, 1H, *J*=11.9 Hz, CH_2CCl_3), 4.66 (m, 1H, Ileα-H), 4.88 (d, 1H, *J*=11.9 Hz, CH_2CCl_3), 5.35 (dd, 1H, *J*=8.2, 7.1 Hz, Pheα-H), 6.67 (d, 1H, *J*=8.5 Hz, Ile–NH), 7.14–7.27 (m, 5H, PheAr–H); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 11.5 (q), 15.6 (q), 24.8 (t), 28.1 (q, 3C), 29.7 (q), 35.2 (t), 36.3 (q), 37.2 (d), 52.6 (t), 55.5 (d), 56.4 (d), 74.4 (t), 80.1 (s), 94.4 (s), 126.4 (d), 128.4 (d, 2C), 129.3 (d, 2C), 137.2 (s), 155.3 (s), 168.7 (s), 169.9 (s), 171.7 (s); HR-FABMS: $[M+Na]^+$ *m*/*z* 618.1675 (*m*/*z* 618.1699 calcd for $C_{26}H_{38}N_3O_6^{35}Cl_2^{37}ClNa$). The ratio of isomers is 10:5.7:2:1.

*4.2.3. Tetrapeptide Boc-L-Ala-N-Me-D-Phe-N-MeGly-L-Ile-OTce (**15c**).* Tripeptide **14c** (2.24 g, 3.76 mmol) was treated with 4 M HCl in dioxane (8.0 mL) at 0 °C for 1 h, and then the solvent was removed to give *N*-Me-D-Phe-*N*-MeGly-L-Ile-OTce HCl salt (2.02 g, 3.76 mmol, 100%). Boc-L-Ala-OH (1.05 g, 5.53 mmol), *i*-Pr_2NEt (1.31 mL, 7.52 mmol), Et_3N (0.52 mL, 3.76 mmol), and HBTU (2.10 g, 5.64 mmol) were added to a stirred solution of *N*-Me-D-Phe-*N*-MeGly-L-Ile-OTce HCl salt (2.02 g, 3.76 mmol) in DMF/CH_2Cl_2 (1:1, 16 mL), and the mixture was stirred at room temperature for 14 h. The reaction mixture was diluted with 1 M HCl (40 mL) and extracted with CH_2Cl_2 (20 mL×3). The combined extracts were washed with 5% aqueous $NaHCO_3$ (20 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was subjected to flash column chromatography on silica gel [*n*-hexane/EtOAc, (8:2) to (1:1)] to give tetrapeptide **15c** as a colorless powder (2.07 g, 3.11 mmol, 83%). $[\alpha]_D^{20}$ +48.6 (*c* 0.50, $CHCl_3$); IR (KBr disk) 3320, 2968, 1757, 1694, 1653, 1636, 1497, 1455, 1367, 1173, 755, 719, 574 cm^{-1}; (major rotamer); ^1H NMR (500 MHz, $CDCl_3$) δ 0.87 (d, 3H, *J*=6.9 Hz, Alaβ-H), 0.93 (t, 3H, *J*=7.6 Hz, Ileδ-H), 0.96 (d, 3H, *J*=6.9 Hz, Ileγ-H), 1.22 (m, 1H, Ileγ-H), 1.39 (s, 9H, $OC(CH_3)_3$), 1.45 (m. 1H, Ileγ-H), 2.00 (m, 1H, Ileβ-H), 3.00 (s, 3H, Gly–*N*Me), 3.03 (s, 3H, Phe–*N*Me), 3.05 (dd, 1H, *J*=14.7, 9.2 Hz, Pheβ-H), 3.17 (dd, 1H, *J*=14.7, 6.9 Hz, Pheβ-H), 3.97 (d, 1H, *J*=15.6 Hz, Glyα-H), 4.06 (d, 1H, *J*=15.6 Hz, Glyα-H), 4.44 (dq, 1H, *J*=8.2, 6.9 Hz, Alaα-H), 4.62 (d, 1H, *J*=11.9 Hz, CH_2CCl_3), 4.70 (dd, 1H, *J*=8.7, 4.6 Hz, Ileα-H), 4.90 (d, 1H, *J*=11.9 Hz, CH_2CCl_3), 5.26 (d, 1H, *J*=8.2 Hz, Ala–NH), 5.77 (dd, 1H, *J*=9.2, 6.9 Hz, Pheα-H), 6.68 (d, 1H, *J*=8.7 Hz, Ile–NH), 7.14–7.27 (m, 5H, PheAr–H); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 11.6 (q), 15.5 (q), 17.5 (q), 24.9 (t), 28.2 (q, 3C), 30.3 (q), 35.1 (t), 36.5 (q), 37.4 (d), 46.5 (d), 52.5 (t), 53.8 (d), 56.3 (d), 74.4 (t), 79.4 (s), 94.3 (s), 126.7

(d), 128.3 (d, 2C), 129.2 (d, 2C), 136.2 (s), 155.1 (s), 168.4 (s), 170.1 (s), 171.1 (s), 173.9 (s); HR-FABMS: $[M+Na]^+$ *m/z* 689.2094 (*m/z* 689.2071 calcd for $C_{29}H_{43}N_4O_7^{35}Cl_2^{37}Cl$ Na). The ratio of isomers is 3.1:1.

4.2.4. Pentapeptide D-Hica-L-Ala-*N*-Me-D-Phe-*N*-MeGly-L-Ile-OTce (**6c**). Tetrapeptide **15c** (2.14 g, 3.21 mmol) was treated with 4 M HCl in dioxane (10 mL) at room temperature for 2 h, and then the solvent was removed to give L-Ala-*N*-Me-D-Phe-*N*-MeGly-L-Ile-OTce HCl salt (1.93 g, 3.20 mmol, 100%). D-Hica (465 mg, 3.52 mmol), Et_3N (446 μL, 3.20 mmol), HOBt (865 mg, 6.40 mmol), and EDCI-HCl (920 mg, 4.80 mmol) were added to a stirred solution of L-Ala-*N*-Me-D-Phe-*N*-Me-Gly-L-Ile-OTce HCl salt (1.93 g, 3.20 mmol) in DMF (6.4 mL), and the mixture was stirred at room temperature for 17 h. The reaction mixture was diluted with 1 M HCl (40 mL) and extracted with EtOAc (20 mL×2). The combined extracts were washed with 5% aqueous $NaHCO_3$ (40 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was subjected to flash column chromatography on silica gel [*n*-hexane/EtOAc, (7:3) to EtOAc] to give pentapeptide **6c** as a colorless powder (1.64 g, 2.41 mmol, 75%). $[\alpha]_D^{20}$ +37.5 (*c* 0.50, $CHCl_3$); IR (KBr disk) 3320, 2960, 1757, 1696, 1653, 1636, 1455, 1140, 754, 720, 574 cm^{-1}; (major rotamer); ^1H NMR (500 MHz, $CDCl_3$) δ 0.93 (d, 3H, *J*=6.6 Hz), 0.93 (d, 3H, *J*=6.8 Hz), 0.94 (t, 3H, *J*=7.2 Hz), 0.95 (d, 3H, *J*=6.9 Hz), 0.96 (d, 3H, *J*=6.9 Hz), 1.48 (m, 1H), 1.48 (m, 1H), 1.57 (overlapping m, 2H), 1.83 (m, 1H), 2.02 (m, 1H), 2.99 (s, 3H), 3.05 (s, 3H), 3.05 (dd, 1H, *J*=13.9, 9.0 Hz), 3.18 (dd, 1H, *J*=13.9, 6.9 Hz), 3.86 (d, 1H, *J*=17.2 Hz), 3.99 (d, 1H, *J*=17.2 Hz), 4.07 (dd, 1H, *J*=10.0, 3.4 Hz), 4.62 (d, 1H, *J*=11.9 Hz), 4.69 (dd, 1H, *J*=8.7, 4.8 Hz), 4.78 (dd, 1H, *J*=7.8, 6.8 Hz), 4.91 (d, 1H, *J*=11.9 Hz), 5.73 (dd, 1H, *J*=9.0, 6.9 Hz), 6.67 (d, 1H, *J*=8.7 Hz), 7.13 (d, 1H, *J*=7.8 Hz), 7.15–7.28 (m, 5H), A signal from one proton (OH) was not observed. ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 11.4 (q), 15.5 (q), 17.5 (q), 21.3 (q), 23.4 (q), 24.3 (d), 24.9 (t), 30.5 (q), 35.2 (t), 36.5 (q), 37.3 (d), 43.5 (t), 45.1 (d), 52.3 (t), 53.9 (d), 56.4 (d), 70.4 (d), 74.5 (t), 94.3 (s), 126.8 (d), 128.3 (d, 2C), 129.1 (d, 2C), 136.2 (s), 168.4 (s), 170.2 (s), 170.9 (s), 172.7 (s), 174.0 (s); HR-FABMS: $[M+Na]^+$ *m/z* 703.2221 (*m/z* 703.2228 calcd for $C_{30}H_{45}N_4O_7^{35}Cl_2^{37}Cl$ Na). The ratio of isomers is 2.9:1.

4.2.5. Depsihexapeptide (**16c**). Dihydroxy acid **4** (167 mg, 0.400 mmol), DMAP (48.9 mg, 0.400 mmol), and EDCI-HCl (199 mg, 1.04 mmol) were added to a stirred solution of pentapeptide **6c** (407 mg, 0.598 mmol) in CH_2Cl_2 (1.1 mL), and the mixture was stirred at room temperature for 22 h. The reaction mixture was diluted with 1 M HCl (40 mL) and extracted with CH_2Cl_2 (20 mL×2). The combined extracts were washed with 5% aqueous $NaHCO_3$ (40 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was subjected to flash column chromatography on silica gel [*n*-hexane/EtOAc, (8:2) to (1:1)] to give depsihexapeptide **16c** as a colorless powder (383 mg, 0.355 mmol, 90%). $[\alpha]_D^{20}$ +22 (*c* 0.25, $CHCl_3$); IR (KBr disk) 3314, 2964, 1757, 1696, 1640, 1144, 750, 576 cm^{-1}; (major rotamer); ^1H NMR (500 MHz, $CDCl_3$) δ −0.06 (s, 3H), 0.01 (s, 3H), 0.71 (d, 3H, *J*=7.1 Hz), 0.82 (d, 3H, *J*=6.9 Hz), 0.88 (overlapping s and m, 15H), 0.93 (t, 3H, *J*=7.3 Hz), 0.96 (d, 3H, *J*=6.6 Hz), 1.20 (m, 1H), 1.33 (dd, 1H, *J*=14.8, 7.0 Hz), 1.47 (m, 1H), 1.59 (br s, 3H), 1.61 (br d, 3H, *J*=6.9 Hz), 1.69 (overlapping m, 2H), 1.89 (s, 3H), 2.04 (m, 2H), 2.12 (s, 3H), 2.35 (m, 2H), 3.01 (s, 3H), 3.04 (overlapping s and dd, 4H), 3.18 (dd, 1H, *J*=14.5, 6.5 Hz), 3.68 (d, 1H, *J*=9.2 Hz), 3.95 (d, 1H, *J*=15.1 Hz), 4.10 (overlapping d and dt, 2H), 4.54 (d, 1H, *J*=11.7 Hz), 4.62 (d, 1H, *J*=11.9 Hz), 4.63 (d, 1H, *J*=11.5 Hz), 4.69 (m, 2H), 4.90 (d, 1H, *J*=11.9 Hz), 5.15 (m, 1H), 5.38 (q, 1H, *J*=6.8 Hz), 5.83 (dd, 1H, *J*=15.6, 6.3 Hz), 6.64 (d, 1H, *J*=7.6 Hz), 6.64 (d, 1H, *J*=8.5 Hz), 7.00 (t, 1H, *J*=6.3 Hz), 7.15–7.27 (m, 5H); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C −5.4 (q), −4.6 (q), 10.2 (q), 10.5 (q), 11.5 (q), 12.7 (q), 12.8 (q), 14.0 (q), 15.5 (q), 17.1 (s), 18.0 (q), 21.5 (q), 23.0 (q), 24.4 (d), 24.9 (t), 25.7 (q, 3C), 28.7 (t), 30.3 (q), 35.1 (t), 36.5 (q), 37.3 (d), 38.3 (d), 40.7 (t), 45.1 (d), 52.6 (t), 53.7 (d), 56.3 (d), 72.4 (d), 73.2 (t), 74.4 (t), 75.6 (d), 80.8 (d), 94.3 (s), 122.2 (d), 126.7 (d), 127.7 (s), 128.3 (d, 2C), 129.1 (d, 2C), 136.2 (s), 136.3 (s), 141.9 (d), 166.6 (s), 168.3 (s), 170.0 (s), 170.1 (s), 170.9 (s), 172.3 (s); HR-FABMS: $[M+Na]^+$ *m/z* 1101.4530 (*m/z* 1101.4548 calcd for $C_{51}H_{83}N_4O_{10}^{35}Cl_2^{37}Cl$ SSiNa).

4.2.6. Alcohol (**17c**). Depsihexapeptide **16c** (487 mg, 0.451 mmol) was dissolved in HF-pyridine/pyridine/THF (1:1:4, 27 mL). The solution was stirred at 40 °C for 12 h. After cooling, the reaction mixture was diluted with EtOAc (100 mL) and poured into saturated $NaHCO_3$ (160 mL). The mixture was separated and extracted with EtOAc (20 mL). The combined extracts were washed with 1 M HCl (20 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was subjected to flash column chromatography on silica gel [*n*-hexane/EtOAc, (7:3) to (3:7)] to give alcohol **17c** as a colorless powder (383 mg, 0.397 mmol, 88%). $[\alpha]_D^{20}$ +33.2 (*c* 0.50, $CHCl_3$); IR (KBr disk) 3321, 2961, 1696, 1642, 1523, 1457, 756, 668 cm^{-1}; (major rotamer); ^1H NMR (500 MHz, $CDCl_3$) δ 0.72 (d, 3H, *J*=7.0 Hz), 0.85 (d, 3H, *J*=6.8 Hz), 0.90 (d, 3H, *J*=5.7 Hz), 0.91 (d, 3H, *J*=5.6 Hz), 0.93 (t, 3H, *J*=7.4 Hz), 0.96 (d, 3H, *J*=6.9 Hz), 1.20 (m, 1H), 1.46 (m, 1H), 1.61 (overlapping s and d, 6H), 1.69 (m, 2H), 1.90 (s, 3H), 2.03 (overlapping m, 3H), 2.17 (s, 3H), 2.40 (m, 1H), 2.50 (m, 1H), 3.02 (s, 3H), 3.04 (overlapping s and dd, 4H), 3.18 (dd, 1H, *J*=14.4, 6.5 Hz), 3.77 (d, 1H, *J*=9.7 Hz), 3.97 (d, 1H, *J*=15.1 Hz), 4.06 (d, 1H, *J*=15.1 Hz), 4.15 (dt, 1H, *J*=7.3, 4.6 Hz), 4.61 (d, 1H, *J*=12.0 Hz), 4.65 (s, 2H), 4.73 (m, 2H), 4.90 (d, 1H, *J*=11.9 Hz), 5.23 (dd, 1H, *J*=9.2, 3.8 Hz), 5.43 (q, 1H, *J*=5.6 Hz), 5.80 (dd, 1H, *J*=9.0, 6.5 Hz), 6.66 (d, 1H, *J*=8.8 Hz), 6.77 (d, 1H, *J*=7.6 Hz), 7.03 (t, 1H, *J*=7.3 Hz), 7.14–7.27 (m, 5H). A signal from one proton (OH) was not observed. ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 10.2 (q), 11.3 (q), 11.4 (q), 12.6 (q), 12.9 (q), 14.1 (q), 15.5 (q), 17.3 (q), 21.6 (q), 23.0 (q), 24.4 (d), 24.8 (t), 29.4 (t), 30.4 (q), 35.0 (t), 36.5 (q), 37.3 (d), 38.1 (d), 40.6 (t), 45.0 (d), 52.5 (t), 53.7 (d), 56.3 (d), 72.3 (d), 73.3 (t), 74.4 (t), 77.3 (d), 80.7 (d), 94.3 (s), 122.7 (d), 126.7 (d), 127.8 (s), 128.3 (d, 2C), 129.1 (d, 2C), 136.1 (s), 136.6 (s), 141.1 (d), 166.4 (s), 168.3 (s), 169.8 (s), 170.0 (s), 170.7 (s), 172.4 (s); HR-FABMS: $[M+Na]^+$ *m/z* 987.3654 (*m/z* 987.3680 calcd for $C_{45}H_{69}N_4O_{10}^{35}Cl_2^{37}ClSNa$).

4.2.7. Depsiheptapeptide (**18d**). Fmoc-D-Ala-OH (286 mg, 0.918 mmol), DMAP (56.2 mg, 0.460 mmol), and EDCI-HCl (176 mg, 0.918 mmol) were added to a stirred solution of alcohol **17c** (444 mg, 0.460 mmol) in CH_2Cl_2 (1.8 mL) and maintained at 0 °C for 2 h and then at room temperature for 4 h. The reaction mixture was diluted with 1 M HCl (40 mL) and extracted with EtOAc (20 mL×2). The combined extracts were washed with 5% aqueous $NaHCO_3$ (20 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was subjected to flash column chromatography on silica gel [*n*-hexane/EtOAc, (7:3) to (3:7)] to give depsiheptapeptide **18d** as a colorless amorphous powder (533 mg, 0.423 mmol, 92%). $[\alpha]_D^{20}$ +14.5 (*c* 0.35, $CHCl_3$); IR (KBr disk) 3325, 2928, 1759, 1689, 1647, 1526, 1462, 1054, 836, 755, 573 cm^{-1}. Characteristic signals of two rotamers are shown, but some protons could not be determined due to overlap (major rotamer). ^1H NMR (500 MHz, $CDCl_3$) δ 0.73–0.96 (overlapping m, 18H), 1.20 (m, 1H), 1.49 (overlapping m, 4H), 1.59 (overlapping m, 6H), 1.73 (overlapping m, 3H), 1.89 (s, 3H), 2.01 (m, 1H), 2.11 (s, 3H), 2.33 (overlapping m, 3H), 3.00 (s, 3H), 3.03 (s, 3H), 3.13 (m, 1H), 3.17 (dd, 1H, *J*=14.0, 6.7 Hz), 3.88 (m, 1H), 3.95 (d, 1H, *J*=15.1 Hz), 4.07 (d, 1H, *J*=15.1 Hz), 4.21 (dd, 1H, *J*=7.1, 6.9 Hz), 4.38 (overlapping m, 3H), 4.57 (d, 1H, *J*=11.8 Hz), 4.60 (d, 1H, *J*=11.7 Hz), 4.64 (overlapping d and m, 2H, *J*=11.7 Hz), 4.69 (dd, 1H, *J*=8.7, 8.7 Hz), 4.89 (d, 1H, *J*=11.9 Hz), 5.02 (d, 1H, *J*=10.1 Hz), 5.16 (dd, 1H, *J*=9.4, 3.7 Hz), 5.58 (br dd, 1H, *J*=6.9, 6.2 Hz), 5.82 (dd, 1H, *J*=8.8, 6.7 Hz), 6.63 (d, 1H, *J*=8.7 Hz), 6.89 (br t, 1H, *J*=7.6 Hz),

7.16–7.32 (m, 7H), 7.39 (dd, 2H, *J*=7.5, 7.5 Hz), 7.60 (dd, 2H, *J*=6.8, 4.2 Hz), 7.76 (d, 2H, *J*=7.6 Hz); HR-FABMS: $[M+Na]^+$ *m*/*z* 1278.4733 (*m*/*z* 1278.4750 calcd for $C_{63}H_{84}N_5O_{13}^{35}Cl_3$ SNa).

*4.2.8. O-MTM-cyclic depsipeptide (**12d**).* Activated Zn powder (1.89 g, 28.8 mmol) was added to a stirred solution of depsiheptapeptide **18d** (519 mg, 0.414 mmol) in THF (18 mL) and 1 M $AcONH_4$ (3.6 mL), and the mixture was stirred at room temperature for 22.5 h. The reaction mixture was filtered through a Celite pad, and the residue was washed with EtOAc (20 mL). The combined organic layer was evaporated. The residue was dissolved in EtOAc (20 mL), washed with 1 M HCl (20 mL), 5% $NaHCO_3$ (20 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was subjected to column chromatography on silica gel [*n*-hexane, *n*-hexane/EtOAc (6:4), (3:7), EtOAc, and $CHCl_3$/MeOH (9:1)] to give carboxylic acid as a colorless oil (436 mg, 0.387 mmol, 94%). $[\alpha]_D^{20}$ +3.3 (*c* 0.40, $CHCl_3$); IR (KBr disk) 3318, 2929, 1720, 1641, 1524, 1452, 1053, 755 cm^{-1}; (major rotamer); ^1H NMR (500 MHz, $CDCl_3$) δ 0.81 (d, 3H, *J*=6.9 Hz),0.90 (overlapping m, 12H), 0.97 (d, 3H, *J*=6.6 Hz), 1.20 (m, 1H), 1.50 (d, 3H, *J*=7.1 Hz), 1.55 (m, 1H), 1.60 (s, 3H), 1.63 (d, 3H, *J*=6.6 Hz), 1.65–1.77 (overlapping m, 3H), 1.87 (s, 3H), 1.92 (m, 1H), 2.11 (s, 3H), 2.27–2.38 (overlapping m, 3H), 2.90 (s, 3H), 2.94–2.99 (m, 1H), 3.02–3.07 (m, 1H), 3.05 (s, 3H), 3.65 (d, 1H, *J*=16.0 Hz), 3.90 (overlapping m, 1H), 3.95 (d, 1H, *J*=16.0 Hz), 4.21 (overlapping m, 2H), 4.32–4.42 (overlapping m, 3H), 4.53–4.65 (overlapping m, 3H), 4.77 (qu, 1H, *J*=6.9 Hz), 5.01 (d, 1H, *J*=10.3 Hz), 5.22 (dd, 1H, *J*=9.3, 3.6 Hz), 5.58 (m, 1H), 5.72 (br dd, 1H, *J*=7.6, 7.1 Hz), 6.74 (d, 1H, *J*=8.0 Hz), 6.87–6.91 (m, 1H), 7.06 (d, 1H, *J*=8.2 Hz), 7.15–7.25 (m, 5H), 7.30 (t, 2H, *J*=7.3 Hz), 7.39 (t, 2H, *J*=7.1 Hz), 7.59 (br d, 2H, *J*=7.1 Hz), 7.75 (d, 2H, *J*=7.3 Hz). Diethylamine (2.1 mL) was added to a stirred solution of carboxylic acid (426 mg, 0.378 mmol) in MeCN (21 mL). The mixture was stirred at room temperature for 4 h and evaporated to dryness. The reside was subjected to flash column chromatography on silica gel [*n*-hexane, *n*-hexane/EtOAc (1:1), EtOAc, and $CHCl_3$/MeOH (95:5), (9:1), (8:2)] to give amino acid **5d** as a colorless powder (329 mg, 0.364 mmol, 96%). Next, HOAt (166 mg, 1.22 mmol) and EDCI-HCl (234 mg, 1.22 mmol) were added to a solution of amino acid **5d** (111 mg, 0.122 mmol) in CH_2Cl_2/DMF (10:1, 121 mL), and the mixture was stirred at room temperature for 23 h. The mixture was then diluted with EtOAc (100 mL) and washed with 1 M HCl (30 mL×2), H_2O (30 mL), 5% aqueous $NaHCO_3$ (30 mL) and brine (30 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was subjected to flash column chromatography on silica gel [*n*-hexane, *n*-hexane/EtOAc (1:1), (3:7), EtOAc, and $CHCl_3$/MeOH (95:5)] to give cyclic depsipeptide **12d** as a colorless amorphous powder (83 mg, 0.0938 mmol, 77%). $[\alpha]_D^{20}$ −8.9 (*c* 0.35, $CHCl_3$); IR (KBr disk) 3296, 2959, 1659, 1634, 1269, 1054, 754 cm^{-1}, Characteristic signals of two rotamers are shown, but some protons could not be determined due to overlap (major rotamer). ^1H NMR (500 MHz, $CDCl_3$) δ 0.79–0.81 (overlapping m, 6H), 0.87–0.97 (overlapping m, 12H), 1.22 (m, 1H), 1.46 (d, 3H, *J*=7.2 Hz), 1.61 (overlapping s, d and m, 7H), 1.68–1.79 (overlapping m, 3H), 1.85 (s, 3H), 2.00 (m, 1H), 2.12 (s, 3H), 2.26 (m, 1H), 2.38 (m, 2H), 3.01 (overlapping s and m, 4H), 3.04 (s, 3H), 3.12 (dd, 1H, *J*=14.1, 9.0 Hz), 3.50 (d, 1H, *J*=16.8 Hz), 3.82 (dt, 1H, *J*=9.4, 3.0 Hz), 3.85 (d, 1H, *J*=16.8 Hz), 4.25 (dd, 1H, *J*=8.2, 8.2 Hz), 4.46–4.56 (overlapping m, 1H), 4.60 (s, 3H), 4.77 (dq, 1H, *J*=7.1, 6.8 Hz), 5.02 (d, 1H, *J*=10.4 Hz), 5.22 (dd, 1H, *J*=6.7, 6.1 Hz), 5.48 (overlapping m, 2H), 6.78 (overlapping m, 2H), 6.99 (d, 1H, *J*=7.0 Hz), 7.14–7.24 (m, 5H), 7.72 (d, 1H, *J*=9.4 Hz); δ_C 9.8 (q), 10.7 (q), 11.4 (q), 12.4 (q), 12.9 (q), 13.7 (q), 15.6 (q), 16.6 (q), 17.9 (q), 21.6 (q), 23.0 (q), 24.4 (d), 24.6 (t), 28.7 (t), 30.8 (q), 34.8 (d), 35.0 (t), 35.8 (q), 36.9 (d), 39.9 (t), 44.4 (d), 48.0 (d), 51.7 (t), 54.7 (d), 57.8 (d), 72.2 (d), 73.4 (t), 74.5 (d), 81.9 (d), 126.6 (d, 2C), 128.2 (s), 128.5 (2C, d), 129.1 (2C, d), 131.6 (s), 136.1 (s), 140.4 (d), 166.6 (s), 168.1 (s), 169.5 (s), 170.6 (s, 3C), 172.1 (s); HR-FABMS: $[M+H]^+$ *m*/*z* 886.5002 (*m*/*z* 886.5000 calcd for $C_{46}H_{72}N_5O_{10}S$).

*4.2.9. Revised kulokekahilide-2 (**1d**).* 2,6-Lutidine (500 μL, 4.32 mmol) and $AgNO_3$ (1.47 g, 8.68 mmol) were added to a solution of **12d** (192 mg, 0.217 mmol) in THF/H_2O (4:1, 6.3 mL), and the mixture was stirred at 65 °C for 1.5 h. The mixture was diluted with EtOAc (50 mL) and filtered through a Celite pad, and the residue was washed with EtOAc (50 mL). The filtrate and washings were combined, then washed with 1 M HCl (30 mL), H_2O (30 mL), 5% $NaHCO_3$ (30 mL), and brine (30 mL), dried over Na_2SO_4, and evaporated to dryness. The residual oil was subjected to flash column chromatography on silica gel [*n*-hexane/EtOAc, (7:3) to (3:7), EtOAc, $CHCl_3$/MeOH (95:5)] to give the revised kulokekahilide-2 (**1d**) as a colorless amorphous powder (137 mg, 0.165 mmol, 76%). $[\alpha]_D^{20}$ −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}. The ^1H and ^{13}C NMR spectra were identical to those of natural **1**; HR-FABMS: $[M+Na]^+$ *m*/*z* 848.4763 (*m*/*z* 848.4786 calcd for $C_{44}H_{67}N_5O_{10}Na$).

4.3. Procedures for IC_{50} measurements against P388 and HeLa S_3 cells

4.3.1. Leukemia cell culture. P388 cells were maintained in RPMI1640 medium containing 10% fetal bovine serum, 100 μg/mL kanamycine, and 10 μM 2-hydroxyethyldisulfide. The cells were washed and resuspended in the above-described medium to 1×10^4 cells/mL, and 100 μL of this cell suspension was placed in each well of a 96-well flat-bottom plate. Next, 100 μL of different concentrations of each test solution (**1a**–**f** in medium) were added, and 100 μL of medium was added into control wells. The cells were incubated for 96 h at 37 °C in 5% CO_2/air.

HeLa S_3 cells were maintained in MEM medium (GibcoBRL) containing 10% fetal bovine serum, 2 μg/mL gentamicine and 10 μg/mL antibiotics adjusted to pH 7.0–7.4 by 1 M HCl. The cells were washed and resuspended in the above medium to 1000 cells/mL, and 200 μL of this cell suspension was placed in each well of a 96-well flat-bottom plate. The cells were incubated for 24 h at 37 °C in 5% CO_2/air. After incubation, 2 μL of different concentrations of each test solution were added, and the cells were incubated for 96 h at 37 °C in 5% CO_2/air.

4.3.2. MTT assay. After terminating the cell culture, 50 μL of 3-(4,5-dimethyl-2-tiazoyl)-2,5-diphenyl-*H*tetrazolium bromide (MTT) in saline (1 μg/mL) was added to every well, and the plate was reincubated at 37 °C in 5% CO_2/air for an additional 3 h. The plate was then centrifuged at 800× *g* for 5 min to precipitate the cells and formazan. The supernatant was removed from every well, and 100 μL of DMSO was added to dissolve the formazan crystals. The plate was mixed on a microshaker for 10 min and then read on a microplate reader at 510 nm. A dose–response curve was plotted for each drug, and the concentration that yielded 50% inhibition of cell growth (IC_{50}) was calculated.

Acknowledgements

The authors thank Ms. Eriko Mori and Ms. Ei Horii at the Aoyama Gakuin University for support in the synthesis. This work was supported by a Grant-in-Aid for Scientific Research (KAKENHI) (No. 1931013B) and Strategic Research Foundation Grant-aided Project for Private Universities (No. S0901031) from the Japan Society for the Promotion of Science and the Ministry of Education, Culture, Sports, Science and Technology (MEXT).

Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.tet.2011.10.094. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. (a) Villa, F. A.; Gerwick, L. *Immunopharmacol Immunotoxicol.* **2010**, *32*, 228–237; (b) Benkendorff, K. *Biol. Rev.* **2010**, *85*, 757–775; (c) Penesyan, A.; Kjelleberg, S.; Egan, S. *Mar. Drugs* **2010**, *8*, 438–459; (d) Albericio, F.; Alvarez, M.; Cuevas, C.; Francesch, A.; Pla, D.; Tulla-Puche, J. In *Molecular Imaging for Integrated Medical Therapy and Drug Development*; Tamaki, N., Kuge, Y., Eds.; Springer: Tokyo, 2010; Part VI, pp 237–249.
2. Jiang, T.; Zhang, P.; Chen, S.; Li, G. In *Alternative and Complementary Therapies for Cancer*; Alaoui-Jamali, M., Ed.; Springer: New York, NY, 2010; Part IX, Chapter 24, pp 613–643.
3. (a) Suenaga, K.; Mutou, T.; Shibata, T.; Itoh, T.; Kigoshi, H.; Yamada, K. *Tetrahedron Lett.* **1996**, *37*, 6771–6774; (b) Mutou, T.; Suenaga, K.; Fujita, T.; Itoh, T.; Takada, N.; Hayamizu, K.; Kigoshi, H.; Yamada, K. *Synlett* **1997**, 199–201; (c) Suenaga, K.; Mutou, T.; Shibata, T.; Itoh, T.; Fujita, T.; Takada, N.; Hayamizu, K.; Takagi, M.; Irifune, T.; Kigoshi, H.; Yamada, K. *Tetrahedron* **2004**, *60*, 8509–8527; (d) Takahashi, T.; Nagamiya, H.; Doi, T.; Griffith, P. G.; Bray, A. M. *J. Comb. Chem.* **2003**, *5*, 414–428; (e) Suenaga, K.; Kajiwara, S.; Kuribayashi, S.; Handa, T.; Kigoshi, H. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3902–3905.
4. (a) Williams, P. G.; Yoshida, W. Y.; Quon, M. K.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **2003**, *66*, 1545–1549; (b) Zou, B.; Long, K.; Ma, D. *Org. Lett.* **2005**, *7*, 4237–4240; (c) Sugiyama, H.; Watanabe, A.; Teruya, T.; Suenaga, K. *Tetrahedron Lett.* **2009**, *50*, 7343–7345.
5. (a) Nakao, Y.; Yoshida, W. Y.; Takada, Y.; Kimura, J.; Yang, L.; Susan, L. M.; Scheuer, P. J. *J. Nat. Prod.* **2004**, *67*, 1332–1340; (b) Takada, Y.; Mori, E.; Umehara, M.; Nakao, Y.; Kimura, J. *Tetrahedron Lett.* **2007**, *48*, 7653–7656; (c) Takada, Y.; Umehara, M.; Nakao, Y.; Kimura, J. *Tetrahedron Lett.* **2008**, *49*, 1163–1165; (d) Umehara, M.; Takada, Y.; Nakao, Y.; Kimura, J. *Tetrahedron Lett.* **2009**, *50*, 840–843.
6. (a) Walsh, C. T. *Science* **2004**, *303*, 1805–1810; (b) Chen, H.; Thomas, M. G.; O'Connor, S. E.; Hubbard, B. K.; Burkart, M. D.; Walsh, C. T. *Biochemistry* **2001**, *40*, 11651–11659.
7. Sheehan, J. C.; Cruickshank, P. A.; Boshart, G. L. *J. Org. Chem.* **1961**, *26*, 2525–2528.
8. Coste, J.; Le-Nyuyen, D.; Castro, B. *Tetrahedron Lett.* **1990**, *31*, 205–208.
9. (a) Shiina, I.; Kubota, M.; Ibuka, R. *Tetrahedron Lett.* **2002**, *43*, 7535–7539; (b) Shiina, I.; Kubota, M.; Oshiumi, H.; Hashizume, M. *J. Org. Chem.* **2004**, *69*, 1822–1830.
10. Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989–1993.
11. Corey, E. J.; Bock, M. G. *Tetrahedron Lett.* **1975**, *16*, 3269–3270.
12. Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591–596.
13. (a) Gamet, J. P.; Jacqjier, R.; Verwcci, J. *Tetrahedron* **1984**, *40*, 1995–2001; (b) Wang, B.; Huang, P. H.; Chen, C. S.; Forsyth, C. J. *J. Org. Chem.* **2011**, *76*, 1140–1150.
14. (a) Bodansky, M.; Bodansky, A. *The Practice of Peptide Synthesis*, 2nd ed.; Springer: Berlin, 1994; 15; (b) Prasad, K. V. S. R. G.; Bharathi, K.; Haseena Banu, B. *Int. J. Pharm. Sci. Rev. Res.* **2011**, *8*, 108–119.
15. (a) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397–4398; (b) Carpino, L. A.; El-Faham, A. *J. Org. Chem.* **1995**, *60*, 3561–3564.
16. (a) Pan, P.-S.; Vasko, R. C.; Lapera, S. A.; Johnson, V. A.; Sellers, R. P.; Lin, C.-C.; Pan, C.-M.; Davis, M. R.; Ardi, V. C.; Mcalpine, S. R. *Bioorg. Med. Chem.* **2009**, *17*, 5806–5825; (b) Otrubova, K.; Lushington, G.; Velde, D. V.; McGuire, K. L.; McAlpine, S. R. *J. Med. Chem.* **2008**, *51*, 530–544.
17. Wenger, R. M.; France, J.; Bovermann, G.; Walliser, L.; Widmer, A.; Widmer, H. *FEBS Lett.* **1994**, *340*, 255–259.
18. Han, B.; Gross, H.; Goeger, D. E.; Mooberry, S. L.; Gerwick, W. H. *J. Nat. Prod.* **2006**, *69*, 572–575.
19. Tripathi, A.; Puddick, J.; Prinsep, M. R.; Rottomann, M.; Tan, L. T. *J. Nat. Prod.* **2010**, *73*, 1810–1814.