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Biakamides A–D, unique polyketides from a marine sponge, act as selective growth inhibitors of tumor cells adapted to nutrient starvation

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Biakamides A–D, unique polyketides from a marine sponge, act as selective growth inhibitors of tumor cells adapted to nutrient starvation

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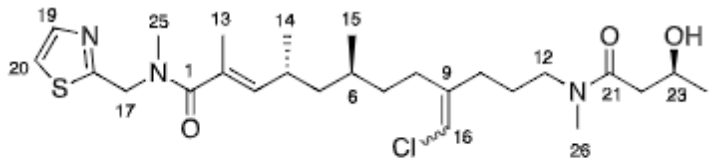
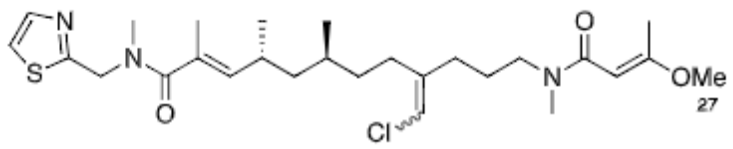


biakamide A (**1**): 9*E*-isomer
biakamide B (**2**): 9*Z*-isomer



biakamide C (**3**): 9*E*-isomer
biakamide D (**4**): 9*Z*-isomer

Abstract

Biakamides A–D, novel unusually unique polyketides, were isolated from an Indonesian marine sponge (*Petrosaspongia* sp.) with a constructed bioassay using PANC-1 human pancreatic cancer cells. Through detailed analyses of the one- and two-dimensional nuclear magnetic resonance (NMR) spectra of biakamides, planar chemical structures possessing a terminal thiazole, two *N*-methyl amides, a chloromethylene, and a substituted butyryl moiety were obtained. After elucidation of the configuration of the secondary alcohol moiety in biakamides A and B, the absolute stereostructures of the two secondary methyl groups in biakamides A–D were determined by the asymmetric total syntheses of all possible stereoisomers from the optically pure monoprotected 2,4-dimethyl-1,5-diol. Biakamides A–D showed selective antiproliferative activities against PANC-1 cells cultured under glucose-deficient conditions in a

concentration-dependent manner. The primary mode of action of biakamides was found to be inhibition of complex I in the mitochondrial electron transport chain.

Keywords: biakamides; polyketide; marine natural product; structure elucidation; total synthesis; glucose-deficient condition; mitochondrial electron transport chain

Introduction

The microenvironment surrounding tumors is an important target for anticancer drug discovery. Because of the disordered vascular network, solid tumors have large areas exhibiting hypoxia and/or nutrient starvation.[1] Cancer cells are known to acquire tolerance toward such severe conditions, allowing them to survive. Furthermore, these cancer cells also acquire resistance to cancer chemotherapy and irradiation and aggravate the pathology of cancer by promoting tumor growth and metastasis.[2] Therefore, compounds that exhibit selective growth inhibitory activity against tumor cells adapted to the tumor microenvironment may be novel, promising anticancer drugs and important chemical tools for identification of new molecule(s) responsible for the adaptation of tumor cells to hypoxia and/or nutrient starvation.

Marine natural products have been shown to be a rich and promising source of drug candidates, particularly in the field of anticancer drug discovery.[3,4] During our assessment of bioactive substances from marine organisms, we have focused on searching for compounds targeting the tumor microenvironment. From our studies, we have identified several active compounds, such as furospinosulin-1[5] and dictyoceratin[6],

showing hypoxia-selective growth inhibitory activity against cancer cells. Furthermore, we have also conducted synthetic studies and target analyses of the identified compounds to validate their potential as anticancer drugs.[7-11]

In contrast, some reports have described the search for selective growth inhibitors against cancer cells adapted to nutrient starvation conditions. According to the so-called "anti-austerity" strategy,[12,13] several active compounds, including kigamycin D and arctigenin, have been identified from microbial secondary metabolites or traditional herbal medicines.[14-17] However, the adaptation system of cancer cells to nutrient deprivation has not been fully elucidated. Recently, we also established a screening system to search for substances that selectively inhibit the growth of PANC-1 human pancreatic cancer cells cultured under glucose-deficient conditions, and we isolated a new 3-alkylpyridine alkaloid (*N*-methylniphatyne A) and 3,4,5-tribromo-2-(2',4'-dibromophenoxy)-phenol as active substances through bioassay-guided separation.[18,19] Further screening of the extract library of marine organisms led us to isolate unique polyketides named biakamides A–D (**1**–**4**) from an Indonesian marine sponge (*Petrosaspongia* sp.).

Here, we report the isolation, structure elucidation, total synthesis, and biological evaluation of biakamides A–D (Figure 1).

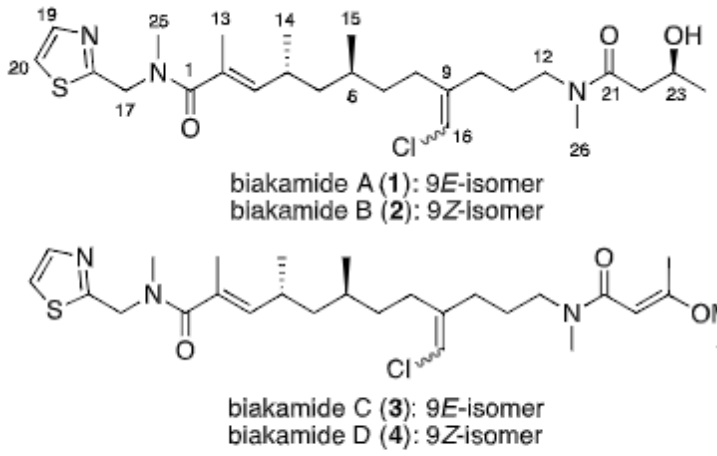


Figure 1. Chemical Structures of Biakamides A (**1**), B (**2**), C (**3**) and D (**4**).

Results and Discussion

Isolation and structure elucidation of biakamides A–D

The MeOH extract (240 g) of the marine sponge *Petrosaspongia* sp. (05A01), collected in Biak, Indonesia in 2005, showed selective growth inhibitory activity against PANC-1 cells cultured in glucose-deficient medium. The MeOH extract was partitioned into an H_2O and AcOEt mixture, and the AcOEt soluble portion was further partitioned into an *n*-hexane and 90% methanol mixture. Using the bioassay, the active 90% methanol soluble portion (34 g) was fractionated by successive silica gel column

chromatography and reversed-phase high-performance liquid chromatography (HPLC) to yield four active constituents, designated biakamides A (**1**, 2.8 mg), B (**2**, 1.4 mg), C (**3**, 6 mg), and D (**4**, 6 mg).

The matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) results for biakamide A (**1**) showed pseudomolecular ion peaks at *m/z* 534 and 536 $[M+Na]^+$ with a ratio of 3:1. These data suggested that there may be a chlorine atom within this molecule. The molecular formula of **1** was determined to be $C_{26}H_{42}O_3N_3SCl$ by high-resolution (HR) MALDI-TOF MS, indicating that this compound possessed seven double bond equivalents. Many signals in the ^1H and ^{13}C NMR spectra of **1** were anomalously broadened, irrespective of solvent, and some others were observed as doubled signals (Figure 2). Brief analyses of the two-dimensional (2D)-nuclear magnetic resonance (NMR) spectra indicated that two *N*-methylamide moieties were contained in the molecule, implying the presence of distinguishable *cis*/*trans* rotamers. Thus, structure elucidation of **1** was executed using the NMR signals of the respective conformers, as described below (Table 1).

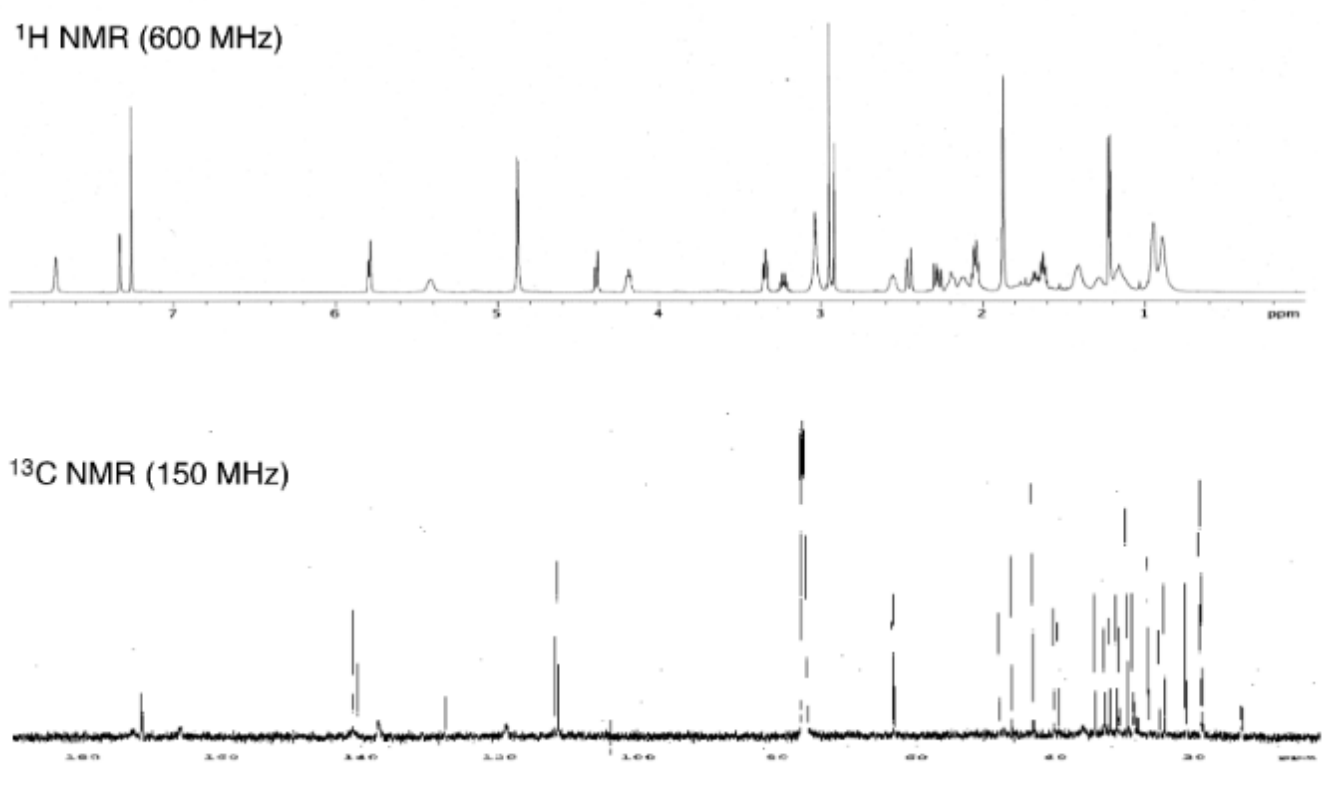


Figure 2. ^{1}H and ^{13}C NMR Spectra of Biakamide A (**1**).

Table 1. NMR chemical shifts of biakamide A (**1**) in $CDCl_3$.

Pos.	Major conformer		Pos.	Minor conformer [a]	
	δ_C	δ_H		δ_C	δ_H
1	173.7 (br)		1		
2	128.9		2		
3	138.4 (br)	5.42 (br s)	3		
4	29.8	2.56 (m)	4		
5	44.2	1.26 (br), 1.13 (br)	5		
6	30.6	1.41 (br)	6		
7	34.0	1.42 (br), 1.18 (br)	7		
8	27.6	2.11 (m), 2.20 (m)	8	27.5	
9	142.0		9	141.5	
10	32.1	2.05 (m)	10	31.8	
11	25.2	1.63 (m)	11	26.1	1.68 (m)
12	47.3	3.34 (t, 7.5 Hz)	12	49.0	3.24 (m)
13	14.2 (br)	1.88 (s)	13		
14	20.0	0.95 (br s)	14		
15	19.8	0.89 (br s)	15		
16	112.4	5.79 (s)	16	113.0	5.80 (s)
17	48.5 (br)	4.88 (s)	17	52.6 (br)	
18	167.0 (br)		18		

19	119.9 (br)	7.33 (br)	19		
20	142.3 (br)	7.72 (br)	20		
21	172.6		21	172.4	
22	41.2	2.44 (m), 2.30 (m)	22	40.6	2.46 (m), 2.28 (m)
23	64.1	4.19 (m)	23	64.3	
24	22.2	1.22 (d, 6.3 Hz)	24	22.3	1.217 (d, 6.3 Hz)
25	37.0 (br)	3.04 (br s)	25	33.3 (br)	
26	35.2	2.95 (s)	26	33.1	2.92 (s)
OH		4.59 (d, 4.3 Hz) [b]	OH		4.61 (d, 4.3 Hz) [b]

[a] Only distinguishable signals were indicated. [b] Observed in DMSO-d_6.

Analyses of the ^{13}C NMR and heteronuclear single quantum coherence (HSQC) spectra of biakamide A (**1**) revealed that this molecule consisted of six methyls (four aliphatic and two nitrogen-substituted), eight methylenes (six aliphatic and two heteroatom-substituted), seven methines (two aliphatic, one oxymethine, and four olefinic), and five quaternary carbons (two olefinic and three possible carbonyls). Detailed analyses of some 2D-NMR spectra (correlation spectroscopy [COSY], HSQC, and heteronuclear multiple bond correlation [HMBC]) of **1** demonstrated the presence of seven partial structures, **A** to **G** (Figure 3). The characteristic 2-substituted thiazole structure (partial structure **A**) has been found in some marine-derived metabolites, such as barbamide or dysideathiazole.[20,21] The chloromethylene moiety in the partial structure **E** was deduced from the HMBC correlations between H-16/C-8, C-9, and C-10 and from a comparison of the chemical shifts of the sp2 carbons with those of

malyngamides and jamaicamides.[22,23] Furthermore, the HMBC between H-13 and C-1, together with an exceptionally upfield-shifted vinylic proton[24] (H-3, δ_H 5.42), indicated that the two partial structures **B** and **C** were connected to form a *N*-methylated α,β-unsaturated amide. The HMBCs between H-22, H-26/C-21, and H-26/C-12 led us to deduce the connectivities of another terminal (structures **E**–**G**). Some pairs of the distinguishable NMR signals corresponding to this region were observed, probably because of the presence of the two conformers (ratio of approximately 3:2) for the *N*-methylamide group at C-21. The nuclear Overhauser effect spectroscopy (NOESY) correlation between H-22 and H-26 was observed in the spectrum of the major *trans*-conformer of this *N*-methyl amide moiety, whereas the H-22/H-12 NOESY correlation was observed in the minor *cis*-conformer.

In contrast, no correlation signals around the partial structure **D** were observed in the HMBC and H-H COSY spectra of biakamide A (**1**). Most ^{1}H and ^{13}C NMR signals corresponding to the left half of the molecule were exceptionally broadened, probably because of the presence of a large energy barrier of bond rotation around the *N*-methylamide group. Therefore, we anticipated that an elevated temperature would

accelerate bond rotation to yield averaged and sharpened NMR signals. As expected, the ^{1}H NMR spectrum of **1** measured at 50°C in DMSO-d_6 gave somewhat sharpened signals. Under this condition, the magnetization relay (from H-15 to H-4, H-5, H-7, and H-8) was observed in the total correlation spectroscopy (TOCSY) results. Furthermore, the HMBCs between H-15/C-5 and C-7 were observed under these conditions, providing evidence for the connectivities between the partial structures **C**, **D**, and **E**. The geometries of the two olefins (Δ^{2} and $\Delta^{9(16)}$) were determined as *E* from the NOESY correlations among H-13/H-4, H-16/H-10, and H-11. Finally, the absolute configuration of the secondary alcohol moiety at C-23 was found to be *S* by applying the modified Mosher method[25] (Figure 4, Supporting Information). In this way, most of the chemical structure of biakamide A (**1**), except for configurations of the two secondary methyls at C-4 and C-6, could be determined as depicted in Figure 4.

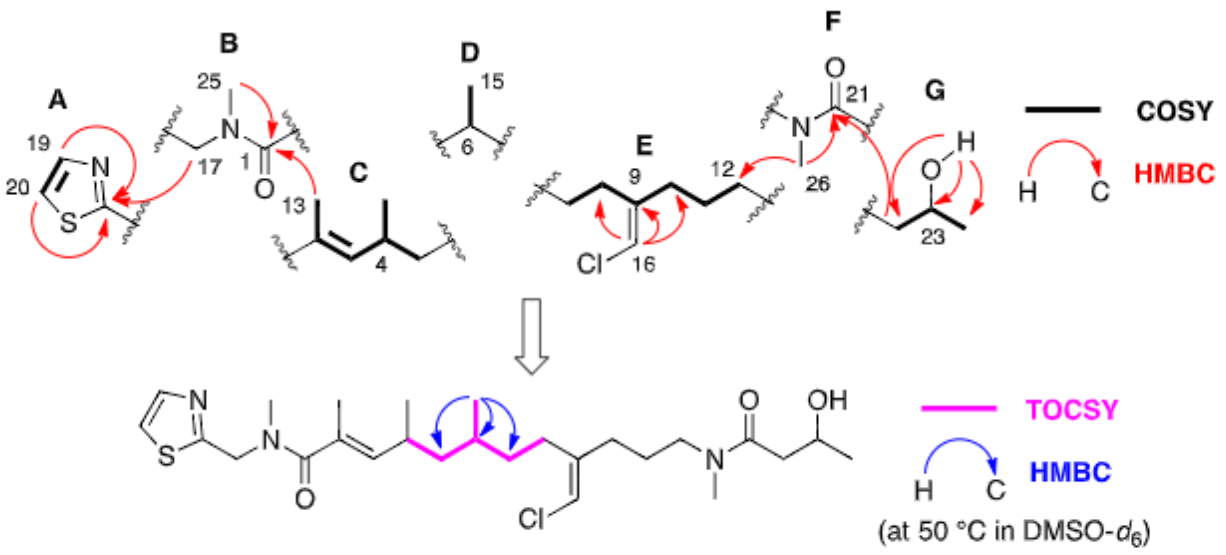


Figure 3. Partial Structures and 2D NMR correlations of Biakamide A (**1**).

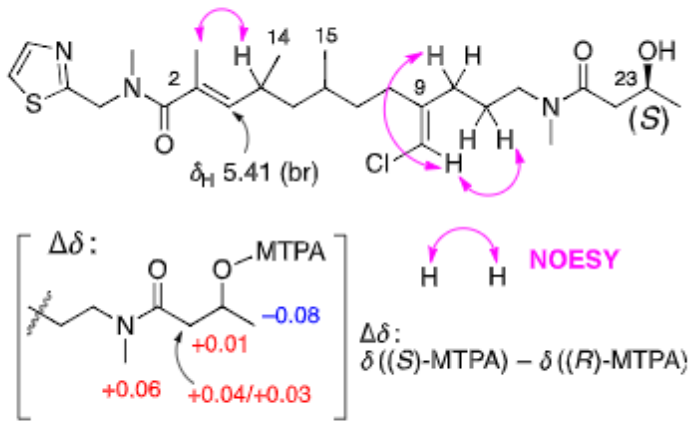


Figure 4. Selected NOEs and Modified Mosher Analysis of Biakamide A (**1**).

From the HR-MALDI-TOF MS analysis, the molecular formula of biakamide B (**2**) was found to be the same as that of biakamide A (**1**). The one-dimensional (1D) and 2D NMR analyses revealed that biakamide B (**2**) possessed almost the same planar structure as **1**, except for the geometry of the chloromethylene moiety, in which the NOESY correlations between H-16/H-7 and H-8 were observed to define the geometry

of the $\Delta^{9(16)}$ olefin as *Z* (Figure 5).

The molecular formula of biakamide C (**3**) was determined to be $C_{27}H_{42}O_3N_3SCl$, by HR-MALDI-TOF MS. Initial attempts to determine the structure of **3** revealed that many signals in the ^{1}H-NMR spectrum of **3** were further broadened compared with those of **1**. Additionally, compound **3** was unstable and gradually changed to another compound during prolonged NMR measurement in $CDCl_3$ solvent. Considering the acid lability of **3**, the $CDCl_3$ that passed through basic alumina just before preparation of the NMR sample was used to suppress the decomposition of **3**. Then, the chemical structure of **3** could be deduced by detailed analysis of the 2D NMR spectra, which was similar to that of **1**, except that a methoxy group (δ_H 3.60) and an olefinic proton (δ_H 5.15) were observed in the terminal acyl moiety. Through analysis of HMBCs (H-22/C-21 and C-23, H-24/C-22 and C-23, and H-27/C-23), as depicted in Figure 5, biakamide C (**3**) was found to possess a 3-methoxy-2-butenoyl moiety at its terminal. Some reported metabolites, such as ajudazole,[26] also had the same functionality. Furthermore, the geometry of the Δ^{22} olefin in **3** was determined as *E* from nuclear Overhauser effect (NOE) experiments. Additionally, biakamide D (**4**) was assigned as the geometrical

counterpart of biakamide C (**3**) at the $\Delta^{9(16)}$ chloromethylene moiety (Figure 5).

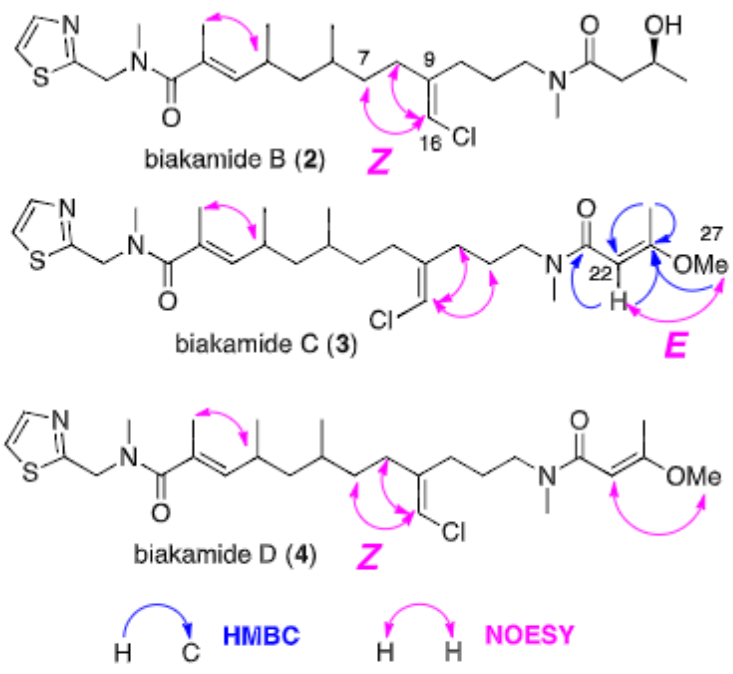


Figure 5. Planar Structures and Selected NOEs of Biakamides B (**2**), C (**3**) and D (**4**).

Total syntheses of biakamides A– D (1–4)

Finally, we aimed to determine the relative/absolute stereostructures of the two secondary methyl groups at C-4 and C-6 for biakamides A–D (**1–4**) . As described above, the ^{1}H NMR signals at the C-4–C-7 positions in biakamides were too broadened, and we could not apply empirical methods[27, 28] to analyze the differences in ^{1}H NMR chemical shifts. Accordingly, we decided to carry out the total synthesis of **1–4** in order to determine the absolute stereostructures of these moieties and to generate sufficient

amounts of the compounds for further biological analyses.

An outline of our synthetic plan is depicted in Figure 6. To obtain all possible stereo-isomers of biakamides A–D (**1**–**4**), we planned to use the monoprotected 2,4-dimethyl-1,5-pentanediol as a chiral synthon, which could be obtained in an optically pure form through the known enzymatic desymmetrization and kinetic resolution of the *syn*-isomer and *anti*-isomer, respectively.[29] The central polyketide skeleton **II** could be prepared through the Corey-Seebach coupling reaction[30] between the 1,3-dithiane **III** and the nitrogen-contained alkyl halide, and the following successive introduction of the unsaturated amide and chloromethylene moieties was expected to provide the common precursor **I** of biakamides. The final acylation using the corresponding carboxylic acid would afford biakamides A–D (**1**–**4**).

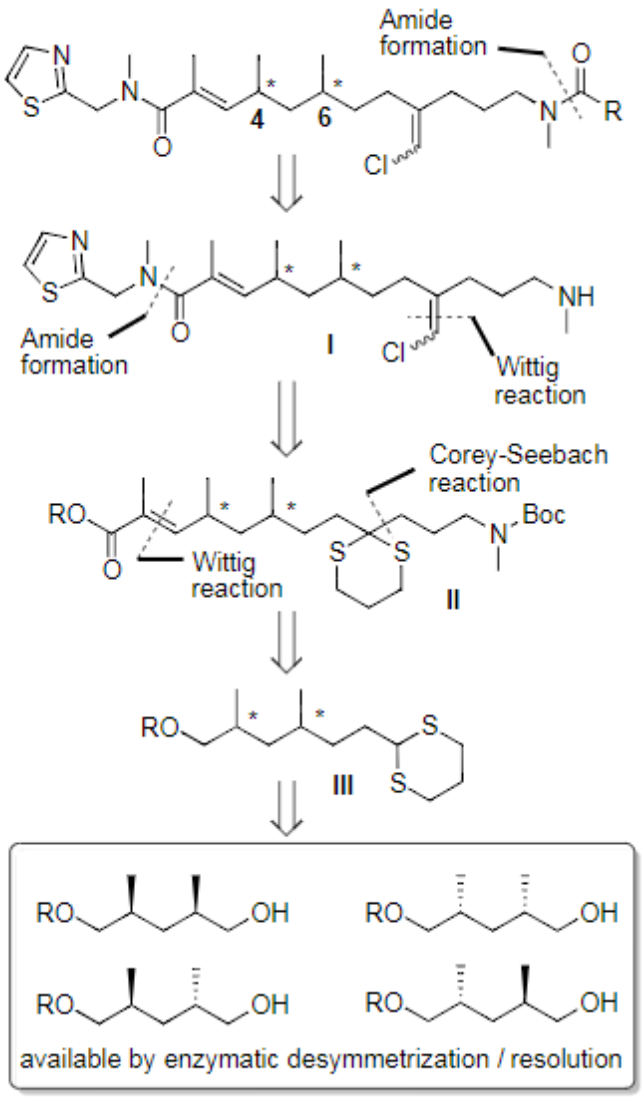
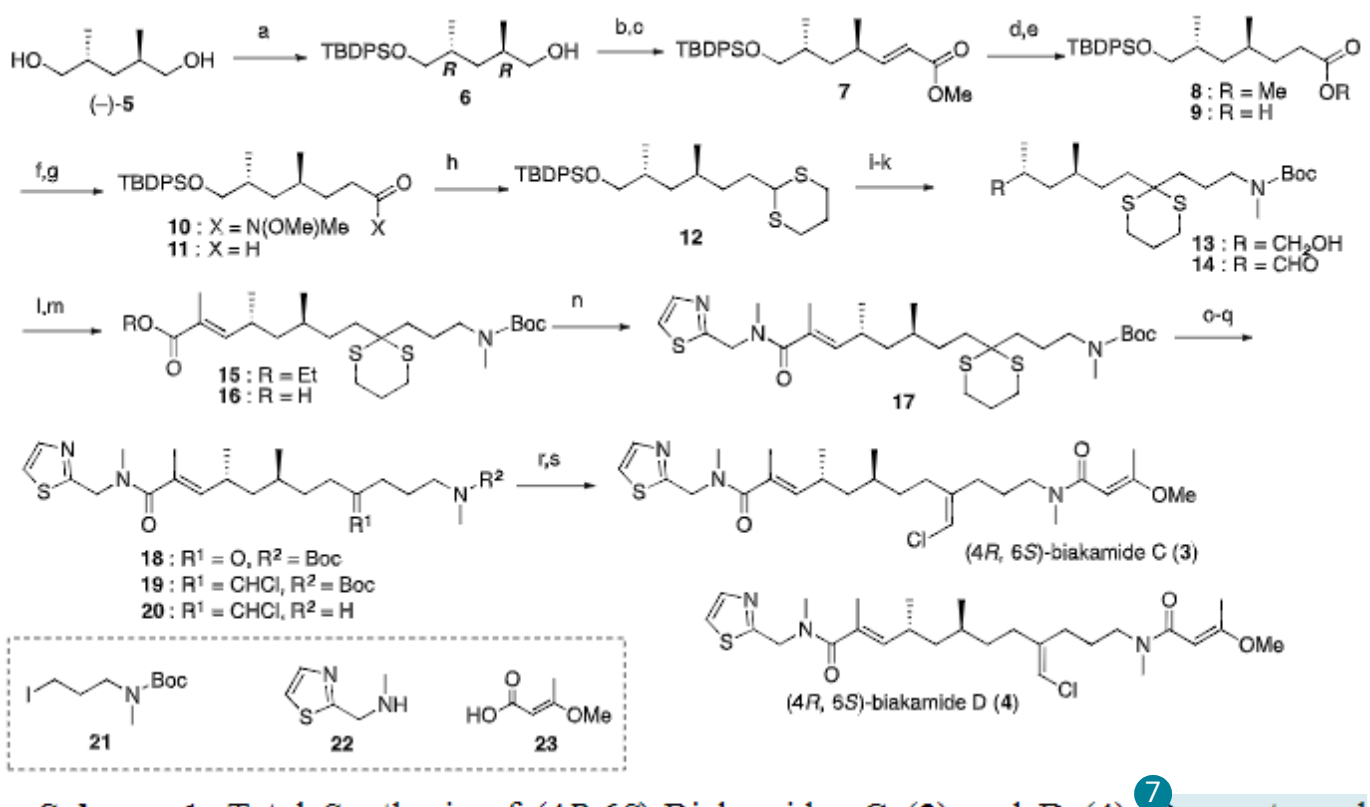


Figure 6. Retrosynthesis of Biakamides.

Initially, the syntheses of biakamides C (**3**) and D (**4**) were carried out as shown in Scheme 1 because the structure determination process for **3** and **4** was expected to be simpler. The (*R*, *R*)-2,4-dimethyl-1,5-pentanediol ((–)-**5**) was prepared by the known enzymatic optical resolution method[29] using lipase AK. According to a recent report,[31] monoprotection of the two hydroxyl groups of (–)-**5** as *tert*-butyldiphenylsilyl (TBDPS) ether was achieved in acceptable yield. Subsequent one-pot TEMPO oxidation/Wittig

reaction[32] proceeded smoothly to give an unsaturated ester (**7**). The double bond of **7** was reduced by treatment with magnesium in MeOH, and the subsequent hydrolysis of the resulting ester (**8**) provided a carboxylic acid (**9**). Then, **9** was converted into the corresponding Weinreb amide (**10**), which was reduced with DIBAL to give an aldehyde (**11**). Treatment of **11** with 1,3-propanedithiol and a catalytic amount of iodine[33] gave a 1,3-dithiane (**12**), the designated precursor **III** of the central fragment. As expected, the Corey-Seebach coupling reaction between a carbanion generated by the *n*-BuLi treatment of **12** and an alkyl iodide (**21**)[34], followed by TBAF treatment, afforded the desired compound (**13**) in good yield. TPAP oxidation of the liberated primary alcohol of **13** gave an aldehyde (**14**), and the subsequent Wittig homologation with a stabilized ylide yielded an α,β-unsaturated ester (**15**). The concomitant *Z*-isomer was separated by SiO_2 column chromatography. Subsequent hydrolysis of the ester moiety with NaOH or KOH in aqueous methanol or 1,4-dioxane was found to be very sluggish, whereas TMSOK treatment[35] resulted in decomposition. Gratifyingly, hydrolysis with LiOH proceeded efficiently to provide a carboxylic acid (**16**) in good yield. Epimerization at C-4, the γ-position of the unsaturated ester moiety, did not occur

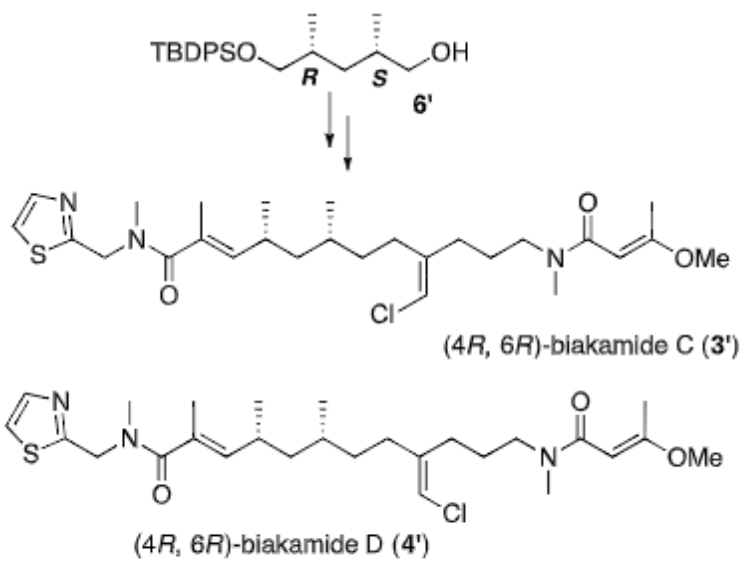
during hydrolysis. Subsequent condensation with a secondary amine (**22**)[36] yielded an *N*-methylamide (**17**). We found that almost all the signals in the ^{1}H NMR spectrum of the amide **17** broadened exceptionally, whereas the normal and sharp signals were observed in the ^{1}H NMR spectrum of the acid **16**. These data clearly indicated that the presence of the unsaturated *N*-methylamide moiety interrupted free bond rotation. Iodine treatment of **17** under basic conditions resulted in clean removal of 1,3-dithiane to give a ketone (**18**), and subsequent introduction of chloromethylene moiety was quantitatively proceeded to give **19** through the Wittig reaction using (chloromethyl)triphenylphosphonium chloride (*E*/*Z* = 3:2). Finally, removal of the Boc moiety by TFA treatment and subsequent condensation with a known *E*-3-methoxy-2-butenoic acid (**23**)[37] afforded (4*R*, 6*S*)-biakamides C (**3**) and D (**4**). These two compounds were separated by reversed-phase HPLC under the established conditions.



Scheme 1. Total Synthesis of (4*R*,6*S*)-Biakamides C (**3**) and D (**4**). Reagents and Conditions: (a) TBDPSCl, Et_3N, CH_3CN/Hexane, 50%; (b) TEMPO, $PhI(OAc)_2$, CH_2Cl_2; (c) $Ph_3P{=}CHCO_2Me$, toluene, 100 °C, 82% (2 steps); (d) Mg, MeOH, quant.; (e) LiOH, MeOH, 96%; (f) MeONHMe·HCl, EDCI·HCl, HOBt, Et_3N, CH_2Cl_2; (g) DIBAL, THF, 0 °C, 49% (2 steps); (h) 1,3-propanedithiol, I_2, $CHCl_3$, 90%; (i) **21**, *n*-BuLi, THF; (j) TBAF, THF, 61% (2 steps); (k) TPAP, NMO, MS4A, CH_2Cl_2, 79%; (l) $Ph_3P{=}C(Me)CO_2Et$, toluene, 100 °C, quant.; (m) LiOH, THF/MeOH/H_2O, 77%; (n) **22**, EDCI·HCl, HOBt, Et_3N, CH_2Cl_2, 92%; (o) I_2, $NaHCO_3$, aq. CH_3CN, 0 °C, 47%; (p) (chloromethyl)triphenylphosphonium chloride, LHMDS, THF, 99%; (q) TFA, CH_2Cl_2; (r) **23**, EDCI·HCl, HOBt, Et_3N, CH_2Cl_2, 78%; (s) HPLC separation.

According to the synthetic route described above, (4*R*, 6*R*)-biakamides C (**3'**) and D (**4'**) were synthesized starting from (2*S*, 4*R*)-2,4-dimethyl-1,5-pentanediol 5-TBDPS ether (**6'**) (Scheme 2).[38] Each reaction proceeded without problem, resulting in comparable yields. A comparison of the ^{1}H-NMR spectra of these compounds revealed

that the ^{1}H signals of the natural biakamide C (**3**) showed good accordance with those of the (4*R*, 6*S*)-isomer, whereas some discrepancies were observed with those of the (4*R*, 6*R*)-isomer, typically at H-6 and H-7 signals (Figure 7). Furthermore, the specific rotation ($[\alpha]_D = -17.2$) of the (4*R*, 6*S*)-isomer matched well with that ($[\alpha]_D = -18.0$) of the natural biakamide C (**3**). Similar results were obtained in the case of biakamide D (**4**) (data not shown). Therefore, the absolute stereostructures both of biakamides C (**3**) and D (**4**) were unambiguously determined to be (4*R*, 6*S*), as shown in Figure 1.



Scheme 2. Synthesis of (4*R*,6*R*)-Biakamides C (**3'**) and D (**4'**).

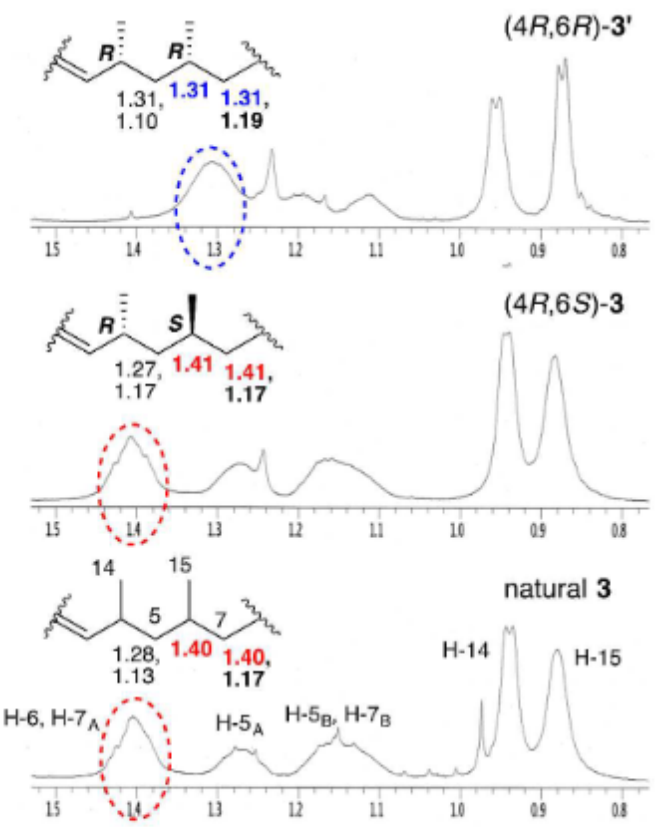
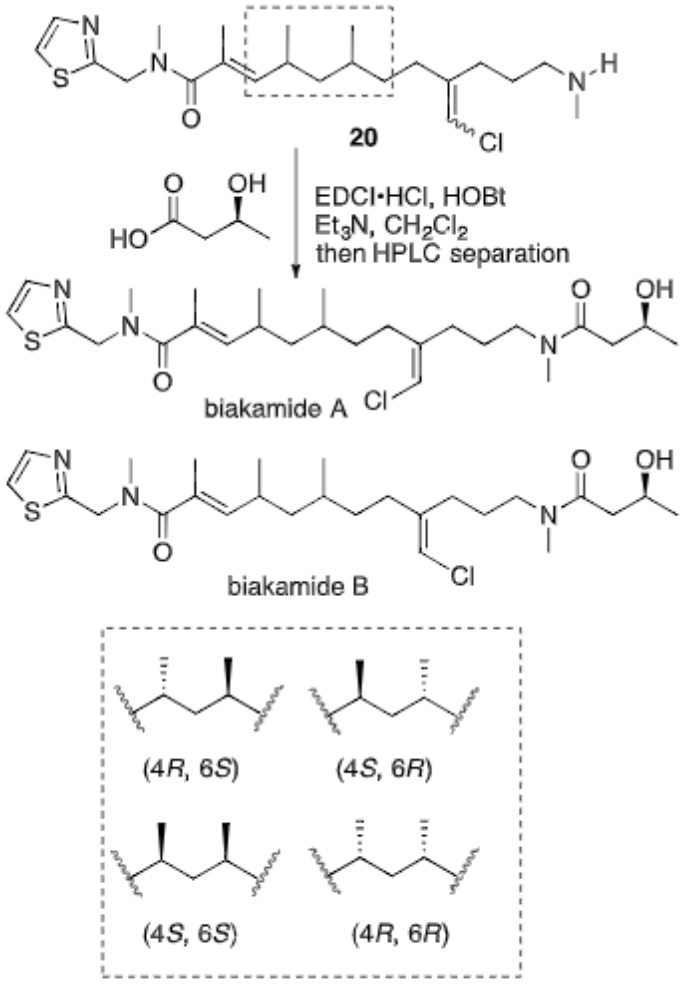


Figure 7. Comparison of ^{1}H NMR Spectra of Natural biakamide C (**3**) and Synthetic (4*R*,6*S*)-**3** or (4*R*,6*R*)-**3'**.

Next, the synthesis of biakamides A (**1**) and B (**2**) was also investigated. The 23*S* configuration for these two compounds was determined as described above. However, a comparison of the NMR spectra and specific rotations between the four synthetic stereoisomers [(4*R*, 6*S*)-, (4*S*, 6*R*)-, (4*S*, 6*S*)-, and (4*R*, 6*R*)-] and the natural biakamides A (**1**) and B (**2**) is needed to finalize this stereochemical analysis.

In the same manner as described above, the (4*S*, 6*R*)- and (4*S*, 6*S*)-isomers of the amine **20** were synthesized starting from the (2*S*, 4*S*)- or (2*R*, 4*S*)-congeners of the

2,4-dimethyl-1,5-pentanediol 5-TBDPS ether, respectively. Condensation between commercially available (*S*)-3-hydroxybutyric acid and the respective isomer of the amine **20** proceeded without problem, and subsequent HPLC separation afforded all possible stereoisomers of biakamides A (**1**) and B (**2**) (Scheme 3).



Scheme 3. Synthesis of All Possible Isomers of Biakamides A (**1**) and B (**2**).

Next, we compared the NMR spectra and specific rotations of the four diastereomers with those of the natural biakamide A (**1**) (Figure 8). The potential (4*S*,

6*S*) and (4*R*, 6*R*) configurations were easily excluded by considering the obvious differences in the chemical shifts of the ^{1}H-NMR signals at H-6 and H-7 between the isomers and the natural biakamide A (**1**) (Figure 8A). In contrast, the ^{1}H-NMR spectra of the (4*S*, 6*R*)- and (4*R*, 6*S*)-isomers were found to similar to each other throughout the molecule. Fortunately, only the multiplicity of the signals at H-12 of the (4*S*, 6*R*)-isomer was different from that of the (4*R*, 6*S*)-isomer and the natural biakamide A (**1**) (Figure 8B). In addition, the specific rotation of the natural biakamide A (**1**) ($[\alpha]_D$ = +6.3) was similar to that of the (4*R*, 6*S*)-isomer ($[\alpha]_D$ = +1.2), whereas a large value ($[\alpha]_D$ = +33.4) was observed in the case of the (4*S*, 6*R*)-isomer.

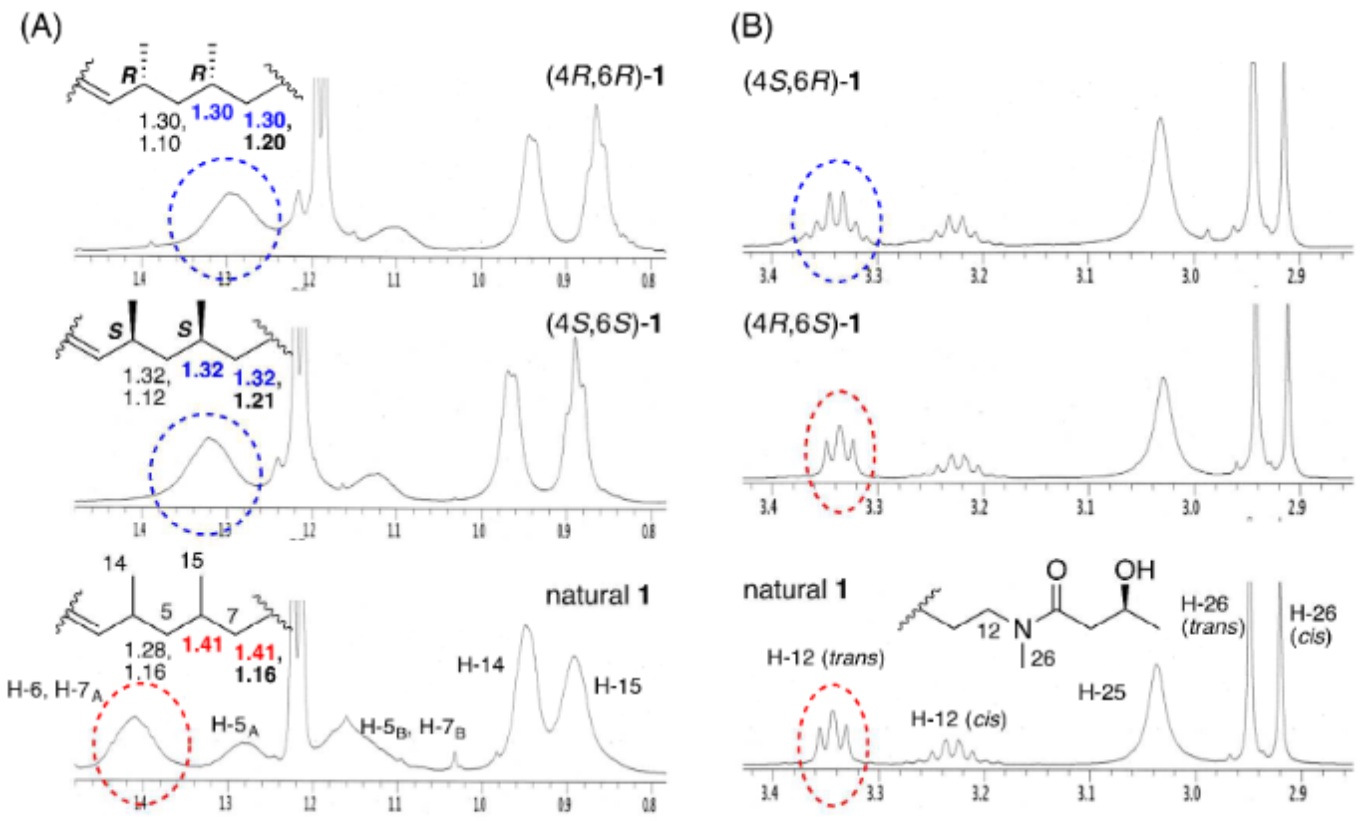


Figure 8. Comparison of ^{1}H NMR Spectra of Natural biakamide A (**1**) and Synthetic four isomers of **1**.

Furthermore, crucial evidence was obtained from a comparison of the CD spectra of these compounds. Thus, the CD spectrum of the (4*S*, 6*R*)-isomer showed a positive maximum at 210 nm derived from the α,β-unsaturated amide moiety, whereas both the (4*R*, 6*S*)-isomer and natural biakamide A (**1**) showed a negative maximum (Figure 9). Similar data were obtained in the case of biakamide B (**2**) (data not shown). Thus, the absolute stereochemistry both of biakamides A (**1**) and B (**2**) was found to be (4*R*, 6*S*), which was similar to those of biakamides C (**3**) and D (**4**).

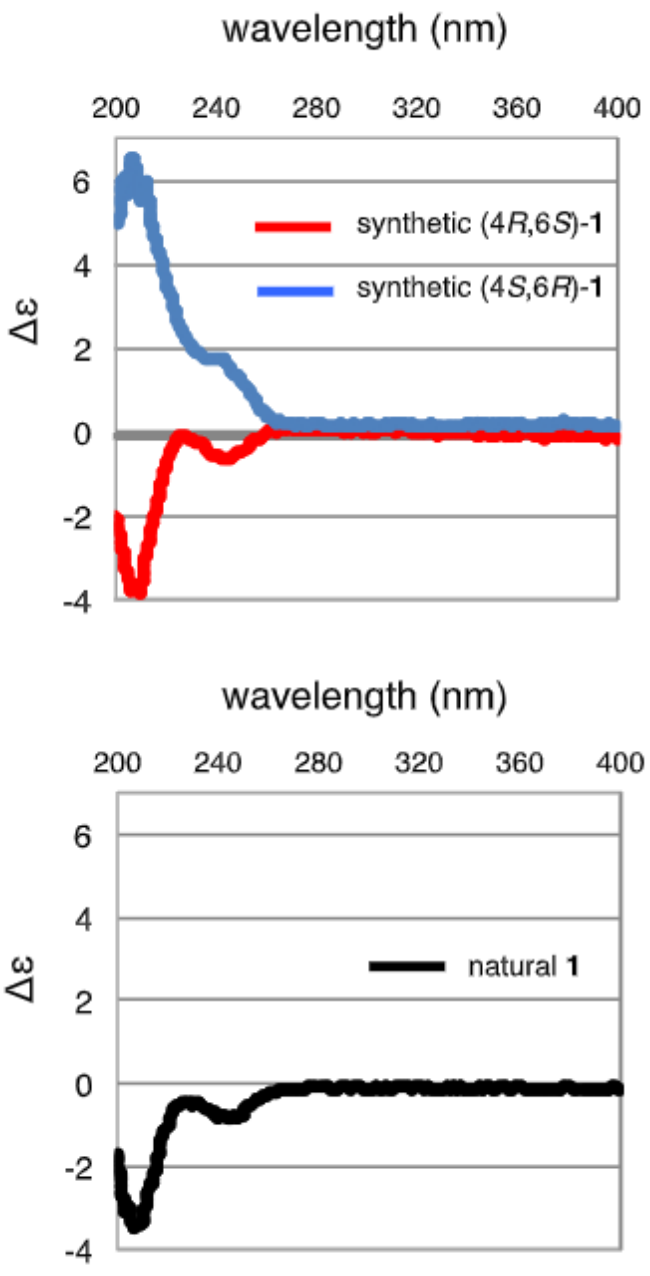


Figure 9. Comparison of CD Spectra of Natural biakamide A (**1**) and Synthetic (4*R*,6*S*)-**1** or (4*S*,6*R*)-**1**.

Bioactivity of biakamides A–D (1–4)

Biakamides A–D (**1**–**4**) exhibited selective antiproliferative activity against PANC-1 cells cultured under glucose-deficient conditions, in a concentration-dependent manner

(Table 2). Among the four compounds, biakamides C (**3**) and D (**4**) showed more potent activity, with a half-maximal inhibitory concentration (IC_{50}) of 0.5 μM. Additionally, when used at a concentration of less than 30 μM, these biakamides showed almost no toxicity against PANC-1 cells cultured in normal medium containing 25 mM glucose. These data indicated that these biakamides may be promising anticancer drugs and that the anticancer effects of these compounds were realized by the targeting of cancer cells adapted to a glucose-deficient microenvironment.

Table 2. Growth inhibition of biakamides A (**1**) – D (**4**) against PANC-1 cells.

	1		**2**		**3**		**4**	
	IC_{50}	S.I.	IC_{50}	S.I.	IC_{50}	S.I.	IC_{50}	S.I.
Glucose (–)	1.0	-	4.0	-	0.5	-	0.5	-
Glucose (+)	>100	>100	>100	>25	50	100	35	70

IC_{50} = μM; S.I = selective index: IC_{50} in Glucose (+) medium / IC_{50} in Glucose (–) medium.

Next, we performed a mechanistic analysis of biakamides. Recent studies have revealed that cancer cells adapt to nutrient starvation by activating the phosphoinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) signaling pathway and the unfolded protein response (UPR), such as induction of glucose-regulated protein 78

(GRP78). Moreover, inhibitors of mitochondrial function or GRP78 are known to exhibit selective growth-inhibitory activity against cancer cells cultured under glucose-deprived conditions.[39,40] Indeed, we recently revealed that 3,4,5-tribromo-2-(2',4'-dibromophenoxy)-phenols exhibit antiproliferative activity against PANC-1 cells cultured under glucose-starved conditions through weak inhibition of Akt phosphorylation and GRP78 expression. We clarified that the antiproliferative activity of this compound may be mediated by inhibition of complex II in the mitochondrial electron transport chain.[19] We then analyzed the effect of biakamides on these signaling pathways or mitochondrial function using western blotting and Mito Check Complex Activity Assays (Cayman Chemical).

Western blot analysis revealed that Akt phosphorylation and GRP78 expression were induced in PANC-1 cells cultured in glucose-deficient medium in comparison with those cultured in general glucose medium (Figure 10, lanes 1 and 2). Additionally, biakamide C (**3**) weakly inhibited Akt phosphorylation and GRP78 expression in PANC-1 cells cultured in glucose-deficient medium (lanes 5 and 6). A similar phenomenon was observed for antimycin A, a known anti-austerity agent that inhibits

complex III in the mitochondrial electron transport chain (lanes 3 and 4).

Furthermore, we found that biakamide C (**3**) selectively inhibited complex I in the mitochondrial electron transport chain, with an IC_{50} of 0.45 μM. This inhibitory concentration was similar to the IC_{50} of the antiproliferative activity of **3** against PANC-1 cells cultured under glucose-deficient conditions, as shown in Table 3. We also found that rotenone, a known inhibitor of complex I, also exhibited selective growth inhibitory activity against PANC-1 cells cultured under glucose-deficient conditions and showed inhibitory properties similar to those observed for Akt and GRP78 (Figure S1, Supporting Information). These results strongly implied that biakamide C (**3**) showed selective growth inhibitory activity against cancer cells adapted to glucose-deficient conditions through inhibition of complex I in the mitochondrial electron transport chain.

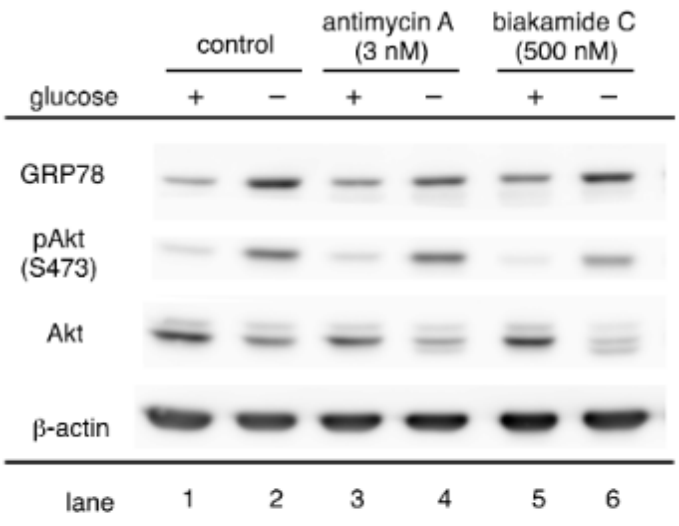


Figure 10. Effect of biakamide C (**3**) on the Akt signaling and induction of GRP78 by western blot analysis. The PANC-1 cells cultured in the Glucose Deficient Medium (Glucose (—)) or General Glucose Medium (Glucose (+)) were treated with biakamide C (**3**) or antimycin A. Cell lysate was resolved by using SDS-PAGE and detected with antibodies against the indicated proteins.

Table 3. Effect of biakamide C (**3**) on the mitochondrial electron transfer chain.

	IC_{50} value (μM)				
	complex I	complex II	complex II/III	complex IV	complex V
biakamide C (**3**)	0.45	>100	>100	>100	37
positive control[a]	0.13	18	0.003	7.7	0.36

[a] Compounds used as positive control are rotenone, thenoyltrifluoroacetone, antimycin A, KCN and oligomycin for complex I, II, III, IV and V, respectively.

Conclusion

Based on the constructed bioassay, we isolated four novel polyketides, designated biakamides A–D (**1**–**4**), from the Indonesian marine sponge (*Petrosaspongia* sp.), as selective growth inhibitors against the PANC-1 human pancreatic cancer cells cultured

under glucose-deprived conditions. The unique chemical structures of these compounds could be unambiguously elucidated by spectral analyses and the asymmetric total syntheses of all possible stereoisomers.

The primary mode of action of biakamides was found to be inhibition of complex I in the mitochondrial electron transport chain. Further biological evaluation, including *in vivo* studies, mechanistic analyses, and structure-activity relationship studies using the synthetic analogs, are now underway.

Experimental Section

General experimental

The following instruments were used to obtain physical data: a JASCO P-2200 digital polarimeter (L = 50 mm) for specific rotations; a JEOL ECS-300 (^{1}H-NMR: 300 MHz, ^{13}C-NMR: 75 MHz), ECA-500 (^{1}H-NMR: 500 MHz, ^{13}C-NMR: 125 MHz) and an Agilent NMR system (^{1}H-NMR: 600 MHz, ^{13}C-NMR: 150 MHz) spectrometer for ^{1}H and ^{13}C NMR data using tetramethylsilane as an internal standard; a JASCO FT/IR-5300 infrared spectrometer for IR spectra; a JEOL JMS-S3000 mass spectrometer for MALDI-TOF MS; a Waters Q-Tof Ultima API mass spectrometer for ESI-TOF MS. Silica gel (Kanto, 40-100 μm) and pre-coated thin layer chromatography (TLC) plates (Merck, 60F_{254}) were used for column chromatography and TLC. Spots on

TLC plates were detected by spraying acidic *p*-anisaldehyde solution (*p*-anisaldehyde: 25 mL, *c*-H_2SO_4: 25 mL, AcOH: 5 mL, EtOH: 425 mL) or phosphomolybdic acid solution (phosphomolybdic acid: 25 g, EtOH: 500 mL) with subsequent heating. Unless otherwise noted, all the reaction was performed under a N_2 atmosphere. After workup, the organic layer was dried over Na_2SO_4.

Extraction and isolation of active compounds

The dried marine sponge of *Petrosaspongia* sp. (2.4 kg), which was collected in 2005 at Biak, Indonesia, was extracted with MeOH. On the guidance of bioassay, the MeOH extract (240 g, IC_{50} (Glucose Deficient Medium) = 50 μg/mL, IC_{50} (General Glucose Medium) = >100 μg/mL) was partitioned into a water-EtOAc mixture (1:1). The active EtOAc soluble portion was further partitioned into a hexane-90%MeOH mixture (1:1). The active 90%MeOH soluble portion [34 g, IC_{50} (Glucose Deficient Medium) = 10 μg/mL, IC_{50} (General Glucose Medium) = >30 μg/mL] was fractionated by SiO_2 gel column chromatography [$CHCl_3$-MeOH-H_2O (lower phase)] to give six fractions (Fr. 1- Fr. 6). The active Fr. 2 [6.3 g, IC_{50} (Glucose Deficient Medium) = 5 μg/mL, IC_{50} (General Glucose Medium) = 30 μg/mL] was fractionated by SiO_2 gel column chromatography [$CHCl_3$-MeOH-H_2O (lower phase)] to give six fractions (Fr. 2-1 - Fr. 2-6). The active Fr. 2-5 was then further purified by ODS HPLC (Cosmosil MS-II, CH_3CN-H_2O = 55:45) to obtain six fractions (Fr. 2-5-1 - Fr. 2-5-6). As a result of bioassay, the Fr. 2-5-2 and the Fr. 2-5-4 showed the cytotoxic activity against PANC-1 cell in the Glucose Deficient Medium selectively. Then, the Fr. 2-5-2 (7.4 mg) was then

further purified by ODS HPLC (Cosmosil MS-II, MeOH-H_2O = 6:4) to afford biakamide A (**1**, 2.8 mg) and biakamide B (**2**, 1.4 mg). And the Fr. 2-5-4 (21.1 mg) was then further purified by ODS HPLC (Cosmosil MS-II, MeOH-H_2O = 7:3) to afford biakamide C (**3**, 6.1 mg) and biakamide D (**4**, 6.3 mg).

Biakamide A (**1**): Colorless amorphous powder.

$[\alpha]_D^{20}$ +6.3 (c = 0.22, $CHCl_3$).

MALDI-TOF-MS *m/z*: 534 $[M+Na]^+$.

HR-MALDI-TOF-MS: Calcd for $C_{26}H_{42}N_3O_3SClNa$ 534.25276. Found 534.25333.

UV λ_{max} (MeOH) nm (ε): 242 (13200).

IR ν_{max} (KBr) cm^{-1}: 3611, 2926, 1628, 1456, 1397.

^1H NMR (600 MHz, δ_H), ^{13}C NMR (150 MHz, δ_C): Table 1

Biakamide B (**2**): Colorless amorphous powder.

$[\alpha]_D^{20}$ +11.9 (c = 0.09, $CHCl_3$).

MALDI-TOF-MS *m/z*: 534 $[M+Na]^+$.

HR-MALDI-TOF-MS: Calcd for $C_{26}H_{42}N_3O_3SClNa$ 534.25276. Found 534.25310.

UV λ_{max} (MeOH) nm (ε): 240 (11200).

IR ν_{max} (KBr) cm^{-1}: 3561, 2926, 1624, 1458, 1397.

^1H NMR (600 MHz, δ_H), ^{13}C NMR (150 MHz, δ_C): Table S1

Biakamide C (**3**): Colorless amorphous powder.

$[\alpha]_D^{20}$ –18.0 (c = 0.40, $CHCl_3$).

MALDI-TOF-MS *m/z*: 546 $[M+Na]^+$.

HR-MALDI-TOF-MS: Calcd for $C_{27}H_{42}N_3O_3SClNa$ 546.25276. Found 546.25260.

UV λ_{max} (MeOH) nm (ε): 255 (10043).

IR ν_{max} (KBr) cm^{-1}: 2953, 2926, 1720, 1640, 1501, 1454, 1393.

^{1}H NMR (600 MHz, δ_H), ^{13}C NMR (150 MHz, δ_C): Table S2

Biakamide D (**4**): Colorless amorphous powder.

$[\alpha]_D^{20}$ –18.4 (*c* = 0.41, $CHCl_3$).

MALDI-TOF-MS *m/z*: 546 $[M+Na]^+$.

HR-MALDI-TOF-MS: Calcd for $C_{27}H_{42}N_3O_3SClNa$ 546.25276. Found 546.25309.

UV λ_{max} (MeOH) nm (ε): 258 (11875).

IR ν_{max} (KBr) cm^{-1}: 2955, 2928, 1647, 1456, 1381, 1240, 1146, 1074.

^{1}H NMR (600 MHz, δ_H), ^{13}C NMR (150 MHz, δ_C): Table S3

Preparation of (*S*)- or (*R*)-MTPA ester of **1** and **2**:

(*S*)- or (*R*)-MTPA (20 equiv.), DMAP (5 equiv.), DCC (20 equiv.) were added to a solution of **1** or **2** in THF, and the whole mixture was stirred for 4 days. Sat. $NaHCO_3$ aq. was added to the mixture and the whole mixture was extracted with AcOEt. Removal of the solvent from AcOEt extract under reduced pressure gave a crude product, which was purified by SiO_2 column [hexane/AcOEt = 1:1 → $CHCl_3$/MeOH/H_2O = 30:3:1 (lower phase)], and by reversed-phase HPLC (Cosmosil 5C_{18}-MS-II, 10 mm i.d. × 250 mm, CH_3CN/H_2O = 55:45 → 70:30 → 80:20, flow rate = 4.0 mL/min, detection UV = 240 nm) to give the corresponding MTPA esters.

(*S*)-MTPA ester of **1**:

^{1}H NMR (600 MHz, $CDCl_3$: only key resonances of the major conformer are listed):

δ 5.64 (1H, m, H-23), 2.93 (3H, s, H-26), 2.78, 2.44 (total 2H, m, H-22), 1.37 (3H, d, *J* = 6.2 Hz, H-24).

(*R*)-MTPA ester of **1**:

^{1}H NMR (600 MHz, $CDCl_3$: only key resonances of the major conformer are listed):

δ 5.63 (1H, m, H-23), 2.87 (3H, s, H-26), 2.74, 2.41 (total 2H, m, H-22), 1.45 (3H, d, *J* = 6.3 Hz, H-24).

HR-ESI-TOF-MS: Calcd for $C_{36}H_{49}N_3O_5F_3NaSCl$ 750.2931. Found 750.2968.

(*S*)-MTPA ester of **2**:

^{1}H NMR (600 MHz, $CDCl_3$: only key resonances of the major conformer are listed):

δ 5.64 (1H, m, H-23), 2.95 (3H, s, H-26), 2.78, 2.44 (total 2H, m, H-22), 1.37 (3H, d, *J* = 6.2 Hz, H-24).

HR-ESI-TOF-MS: Calcd for $C_{36}H_{49}N_3O_5F_3NaSCl$ 750.2931. Found 750.2968.

(*R*)-MTPA ester of **2**:

^{1}H NMR (600 MHz, $CDCl_3$: only key resonances of the major conformer are listed):

δ 5.64 (1H, m, H-23), 2.89 (3H, s, H-26), 2.75, 2.42 (total 2H, m, H-22), 1.45 (3H, d, *J* = 6.3 Hz, H-24).

HR-ESI-TOF-MS: Calcd for $C_{36}H_{49}N_3O_5F_3NaSCl$ 750.2931. Found 750.2978.

Total synthesis of biakamides A - D

(2*R*,4*R*)-2,4-Dimethylpentane-1,5-diol (5)

The compound was obtained through the reported method.[29] The optical purity was confirmed by NMR measurement of the corresponding bis-(*R*)-MTPA ester.

(2*R*,4*R*)-5-((*tert*-Butyldiphenylsilyl)oxy)-2,4-dimethylpentan-1-ol (6)

Et_3N (575 mL, 4.1 mmol, 1.2 equiv.) and TBDPSCl (894 mL, 3.4 mmol, 1.0 equiv.)

were added to a solution of **5** (454.4 mg, 3.4 mmol) in CH_3CN / Hexane (3:1, 34 mL), and the whole mixture was stirred for 19 h at rt. Sat. $NaHCO_3$ aq. was added to the mixture, and the whole mixture was extracted with AcOEt. Removal of the solvent from AcOEt extract under reduced pressure gave a crude product, which was purified by SiO_2 column (Hexane/AcOEt = 5:1 to 1:1) to give **6** (636 mg, 50%) as a colorless oil. The spectroscopic and physical data were identical to those reported.[41]

Methyl (4*R*,6*R*,*E*)-7-((*tert*-butyldiphenylsilyl)oxy)-4,6-dimethylhept-2-enoate (7)

Iodobenzene diacetate (1.22 g, 2.8 mmol, 2.3 equiv.) and TEMPO (25.6 mg, 0.17 mmol, 0.1 equiv.) were added to a solution of 7 (607 mg, 1.6 mmol) in CH_2Cl_2, and the whole mixture was stirred for 3 h at rt. Then toluene (30 mL) and methyl 2-(triphenyl-λ^5-phosphanylidene)acetate (658 mg, 2.0 mmol, 1.2 equiv.) was added to the mixture, and the whole mixture was stirred for 28 h at 100 °C. $Na_2S_2O_3$ / $NaHCO_3$ aq. was added to the mixture, and the whole mixture was extracted with AcOEt. The AcOEt extract was successively washed with sat. NH_4Cl aq., $NaHCO_3$ aq., and brine. Removal of the solvent from the AcOEt extract under reduced pressure gave a crude product, which was purified by SiO_2 column (Hexane/AcOEt = 15:1) to give 7 (571 mg, 82%) as a colorless oil.

$[\alpha]_D^{26}$ –21.9 (*c* 1.17, $CHCl_3$). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.66 (4H, t, *J* = 6.5 Hz), 7.45-7.38 (6H, m), 6.86 (1H, dd, *J* = 15.8, 7.9 Hz), 5.71 (1H, d, *J* = 15.8 Hz), 3.73 (3H, s), 3.50 (1H, dd, *J* = 9.7, 5.2 Hz), 3.44 (1H, dd, *J* = 9.7, 6.1 Hz), 2.32 (1H, quint, *J* = 7.0 Hz), 1.69 (1H, q-like, *J* = 6.3 Hz), 1.46 (1H, dt, *J* = 13.7, 6.9 Hz), 1.18 (1H, dt, *J* = 13.7,

7.1 Hz), 1.06 (9H, s), 0.99 (3H, d, J = 6.5 Hz), 0.92 (3H, d, J = 6.8 Hz). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 167.4, 155.3, 135.62 (2C), 135.60 (2C), 133.9, 133.8, 129.6 (2C), 127.6 (4C), 119.0, 68.4, 51.4, 39.5, 34.1, 33.2, 26.9 (3C), 19.5, 19.3, 17.3. IR (KBr): 2957, 1725, 1657, 1429, 1111 cm^{-1}. MS (ESI-TOF) *m/z*: 447 $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 447.2331 calcd for $C_{26}H_{36}O_3SiNa$; Found: 447.2321.

Methyl (4*S*,6*R*)-7-((*tert*-butyldiphenylsilyl)oxy)-4,6-dimethylheptanoate (8)

Magnesium (160 mg, 6.6 mmol, 5.0 equiv.) was added to a solution of **7** (557 mg, 1.3 mmol) in MeOH (10 mL), and whole mixture was stirred for 1 h at rt. Sat. NH_4Cl aq. was added to the mixture, and the whole mixture was extracted with AcOEt, and the AcOEt extract was washed with Sat. NH_4Cl aq. and brine. Removal of the solvent from the AcOEt extract under reduced pressure gave a crude product, which was purified by SiO_2 column (Hexane/AcOEt = 10:1) to give **8** (559 mg, quant.) as a colorless oil.

$[\alpha]_D^{27}$ +9.5 (*c* 1.39, $CHCl_3$). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.67 (4H, d, J = 6.7 Hz), 7.44-7.37 (6H, m), 3.66 (3H, s), 3.47 (1H, dd, J = 9.7, 5.9 Hz), 3.43 (1H, dd, J = 9.7, 6.2 Hz), 2.35-2.23 (2H, m), 1.76-1.71 (1H, m), 1.62-1.57 (1H, m), 1.50-1.42 (2H, m), 1.24-1.20 (1H, m), 1.06 (9H, s), 1.06-1.02 (1H, m), 0.88 (3H, d, J = 6.7 Hz), 0.84 (3H, d, J = 6.1 Hz). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 174.5, 135.6 (4C), 134.04, 134.03, 129.5 (2C), 127.6 (4C), 69.4, 51.5, 40.3, 33.1, 32.7, 31.9, 29.7, 26.9 (3C), 19.3, 19.1, 16.6. IR (KBr): 2956, 1740, 1428, 1111 cm^{-1}. MS (ESI-TOF) *m/z*: 449 $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 449.2488 calcd for $C_{26}H_{38}O_3SiNa$; Found: 449.2486.

(4*S*,6*R*)-7-((*tert*-Butyldiphenylsilyl)oxy)-4,6-dimethylheptanoic acid (9)

LiOH (153 mg, 6.6 mmol, 5.0 equiv.) was added to a solution of **8** (543 mg, 1.3 mmol) in MeOH (10 mL), and the whole mixture was stirred for 7 h at rt. 5% HCl was added to the mixture, and the whole mixture was extracted with AcOEt. Removal of the solvent from the AcOEt extract under reduced pressure gave a crude product, which was purified by SiO_2 column (Hexane/AcOEt = 2:1) to give **9** (504 mg, 96%) as a colorless oil.

$[\alpha]_D^{26}$ +9.9 (*c* 0.37, $CHCl_3$). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.66 (4H, d, *J* = 6.8 Hz), 7.43-7.36 (6H, m), 3.47 (1H, dd, *J* = 9.7, 5.9 Hz), 3.43 (1H, dd, *J* = 9.7, 6.3 Hz), 2.38-2.27 (2H, m), 1.76-1.70 (1H, m), 1.63-1.58 (1H, m), 1.50-1.42 (2H, m), 1.25-1.20 (1H, m), 1.06 (9H, s), 1.06-1.02 (1H, m), 0.88 (3H, d, *J* = 6.8 Hz), 0.84 (3H, d, *J* = 6.2 Hz). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 179.5, 135.6 (4C), 134.0 (2C), 129.5 (2C), 127.6 (4C), 69.4, 40.3, 33.1, 32.4, 31.6, 29.6, 26.9 (3C), 19.3, 19.0, 16.6. IR (KBr): 3300, 2858, 1709, 1428, 1111 cm^{-1}. MS (ESI-TOF) *m/z*: 435 $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 435.2331 calcd for $C_{25}H_{36}O_3SiNa$; Found: 435.2340.

(4*S*,6*R*)-7-((*tert*-Butyldiphenylsilyl)oxy)-4,6-dimethylheptanal (11)

HOBt (229 mg, 1.7 mmol, 1.4 equiv.), MeONHMe•HCl (165 mg, 1.7 mmol, 1.4 equiv.), Et_3N (0.85 mL, 6.1 mmol, 5.0 equiv.) and EDCI· HCl (325 mg, 1.7 mmol, 1.4 equiv.) were added to a solution of **9** (499 mg, 1.2 mmol) in CH_2Cl_2 (20 mL), and the whole mixture was stirred for 3 h at rt. Sat. NH_4Cl aq. was added to the mixture, and the whole mixture was extracted with AcOEt, and the AcOEt extract was washed with Sat.

NH_4Cl aq., $NaHCO_3$ aq., and brine. Removal of the solvent from the AcOEt extract under reduced pressure gave crude Weinreb amide **10**, which was used in the next reaction without further purification. The crude **10** was dissolved in anhydrous THF (12 mL). DIBAL-H solution (1.0 M in THF, 1.4 mL, 1.4 mmol, 1.2 equiv.) was added to the solution at 0 °C, and the whole mixture was stirred for 1 h at 0 °C. Sat. NH_4Cl aq. was added to the mixture, and the whole mixture was extracted with AcOEt, and the AcOEt extract was washed with sat. NH_4Cl aq. and brine. Removal of the solvent from the AcOEt extract gave a crude product, which was purified by SiO_2 column (Hexane/AcOEt = 12:1) to give **11** (226 mg, 0.58 mmol, 49%) as a colorless oil.

$[\alpha]_D^{26}$ +10.1 (*c* 1.79, $CHCl_3$). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 9.76 (1H, s), 7.68 (4H, d, *J* = 7.0 Hz), 7.44-7.38 (6H, m), 3.49 (1H, dd, *J* = 9.7, 5.9 Hz), 3.45 (1H, dd, *J* = 9.7, 6.5 Hz), 2.46-2.35 (2H, m), 1.78-1.72 (1H, m), 1.64-1.57 (1H, m), 1.52-1.42 (2H, m), 1.28-1.23 (1H, m), 1.07 (9H, s), 1.06-1.03 (1H, m), 0.90 (3H, d, *J* = 6.8 Hz), 0.85 (3H, d, *J* = 6.5 Hz). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 202.9, 135.5 (4C), 133.9 (2C), 129.4 (2C), 127.5 (4C), 69.3, 41.6, 40.3, 33.0, 29.5 (2C), 26.8 (3C), 19.2, 19.0, 16.5. IR (KBr): 2958, 1727, 1428, 1111 cm^{-1}. MS (ESI-TOF) *m/z*: 419 $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 419.2382 calcd for $C_{25}H_{36}O_2SiNa$; Found: 419.2368.

(((2*R*,4*S*)-6-(1,3-Dithian-2-yl)-2,4-dimethylhexyl)oxy)(*tert*-butyl)diphenylsilane (12)

1,3-Propanedithiol (76 μL, 0.74 mmol, 1.3 equiv.) and iodine (14.5 mg, 0.06 mmol, 0.10 equiv.) were added to a solution of **11** (226 mg, 0.57 mmol) in $CHCl_3$ (10 mL), and

the whole mixture was stirred for 1.3 h at rt. $Na_2S_2O_3$ / $NaHCO_3$ aq. was added to the mixture, and the whole mixture was extracted with $CHCl_3$. Removal of the solvent from the $CHCl_3$ extract under reduced pressure gave a crude product, which was purified by SiO_2 column (Hexane/AcOEt = 12:1) to give **12** (249 mg, 90%) as a colorless oil. $[\alpha]_D^{26}$ +6.8 (*c* 1.02, $CHCl_3$). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.69 (4H, d, *J* = 6.8 Hz), 7.45-7.38 (6H, m), 4.02 (1H, t, *J* = 6.9 Hz), 3.50 (1H, dd, *J* = 9.7, 5.8 Hz), 3.45 (1H, dd, *J* = 9.7, 6.5 Hz), 2.91-2.81 (4H, m), 2.13-2.10 (1H, m), 1.90-1.83 (1H, m), 1.80-1.69 (3H, m), 1.52-1.47 (2H, m), 1.37-1.32 (1H, m), 1.28-1.22 (1H, m), 1.09 (9H, s), 1.07-1.03 (1H, m), 0.91 (3H, d, *J* = 6.8 Hz), 0.85 (3H, d, *J* = 6.4 Hz). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 135.6 (4C), 133.9 (2C), 129.4 (2C), 127.5 (4C), 69.4, 48.0, 40.4, 34.6, 33.1, 33.0, 30.5, 30.4, 29.8, 26.8 (3C), 26.0, 19.30, 19.26, 16.7. IR (KBr): 2929, 1426, 1108 cm^{-1}. MS (ESI-TOF) *m/z*: 509 $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 509.2344 calcd for $C_{28}H_{42}OSiS_2Na$; Found: 509.2339.

***tert*-butyl**

(3-(2-((3*S*,5*R*)-6-Hydroxy-3,5-dimethylhexyl)-1,3-dithian-2-yl)propyl)(methyl)carbamate (13)

n-BuLi (1.59 M in *n*-hexane, 770 μL, 0.77 mmol, 1.5 equiv.) was added to a solution of **12** (249 mg, 0.51 mmol) in anhydrous THF (10 mL), and the whole mixture was stirred for 15 min. A solution of **21** (557 mg, 1.4 mmol, 2.7 equiv) in anhydrous THF (5 mL) was added dropwise to the mixture via cannula over 5 min at rt, and the whole mixture was stirred for 0.5 h at rt. Sat. NH_4Cl aq. was added to the mixture, and the

whole mixture was extracted with AcOEt, and the AcOEt extract was washed with NH_4Cl aq. and brine. After removal of the solvent from AcOEt extract under reduced pressure, the residue was dissolved to THF (10 mL). TBAF solution (1 M in THF, 3.0 mL) was added to the solution at rt, and the whole mixture was stirred for 8 h at rt. Sat. NH_4Cl aq. was added to the mixture, and the whole mixture was extracted with AcOEt, and the AcOEt extract was washed with NH_4Cl aq. and brine. Removal of the solvent from the AcOEt extract under reduced pressure gave a crude product, which was purified by SiO_2 column (Hexane/AcOEt = 3:1 to 1:1) to give **13** (131 mg, 61%) as a colorless oil.

$[\alpha]_D^{26}$ +7.6 (*c* 0.41, $CHCl_3$). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 3.47 (1H, dd, *J* = 10.3, 5.9 Hz), 3.41-3.34 (1H, m), 3.28-3.17 (2H, m), 2.89 (3H, s), 2.79 (4H, d-like, *J* = 5.6 Hz), 1.95-1.90 (2H, m), 1.89-1.75 (4H, m), 1.74-1.65 (2H, m), 1.65-1.60 (2H, m), 1.53-1.45 (1H, m), 1.45 (9H, s), 1.45-1.38 (1H, m), 1.30-1.22 (1H, m), 1.20-1.14 (1H, m), 1.12-1.07 (1H, m), 0.88 (3H, d, *J* = 6.5 Hz), 0.87 (3H, d, *J* = 5.8 Hz). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 155.8, 79.3, 68.8, 53.0, 48.5, 40.6, 35.7, 34.7, 34.2, 33.2, 31.6, 30.2, 28.5 (3C), 25.99, 25.98, 25.5, 22.7, 19.5, 16.5. IR (KBr): 3467, 2928, 1693, 1365, 1151 cm^{-1}. MS (ESI-TOF) *m/z*: 442 $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 442.2426 calcd for $C_{21}H_{41}NO_3S_2Na$; Found: 442.2416.

***tert*-Butyl**

(3-(2-((3*S*,5*R*)-3,5-dimethyl-6-oxohexyl)-1,3-dithian-2-yl)propyl)(methyl)carbamate

(14)

MS4A (130 mg) and NMO (51.1 mg, 0.44 mmol, 1.5 equiv.) were added to a solution of **13** (118 mg, 0.29 mmol) in CH_2Cl_2 (5 mL), and the whole mixture was stirred for 15 min at rt. Then, TPAP (5.1 mg, 0.015 mmol, 0.05 equiv.) was added to the mixture, and the solution was stirred for 1 h at rt. $Na_2S_2O_3$ / $NaHCO_3$ aq. and sat. $CuSO_4$ aq. were added to the mixture, and the whole mixture was extracted with CH_2Cl_2. Removal of the solvent from the CH_2Cl_2 extract under reduced pressure gave a crude product, which was purified by SiO_2 column (Hexane/AcOEt = 3:1) to give **14** (93 mg, 79%) as a colorless oil.

$[\alpha]_D^{27}$ –4.1 (*c* 0.39, $CHCl_3$). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 9.60 (1H, d, *J* = 1.8 Hz), 3.23 (2H, br), 2.85 (3H, br s), 2.79 (4H, br), 2.43 (1H, q-like, *J* = 6.5 Hz), 1.95-1.90 (2H, m), 1.89-1.78 (4H, m), 1.67-1.61 (2H, m), 1.57-1.53 (1H, m), 1.52-1.45 (2H, m), 1.45 (9H, s), 1.31-1.26 (2H, m), 1.07 (3H, d, *J* = 6.7 Hz), 0.91 (3H, d, *J* = 6.2 Hz). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 205.2, 155.8, 79.3, 52.9, 48.7, 48.0, 44.2, 37.4, 35.8, 34.9, 34.1, 31.2, 30.5, 28.5 (3C), 26.0 (2C), 25.4, 22.8, 19.3, 13.4. IR (KBr): 2931, 1723, 1693, 1394, 1148 cm^{-1}. MS (ESI-TOF) *m/z*: 440 $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 440.2269 calcd for $C_{21}H_{39}NO_3S_2Na$; Found: 440.2252.

Ethyl

(4*R*,6*S*,*E*)-8-(2-(3-((*tert*-Butoxycarbonyl)(methyl)amino)propyl)-1,3-dithian-2-yl)-2,4,6-trimethyloct-2-enoate (15)

(Carbetoxyethylidene)triphenylphosphorane (302 mg, 0.84 mmol, 4.0 equiv.) was added to the solution of **14** (87 mg, 0.21 mmol) in toluene (8 mL), and whole mixture

stirred for 44 h at 100 °C. Sat. NH_4Cl aq. was added to the mixture, and the whole mixture was extracted with AcOEt, and the AcOEt extract was washed with NH_4Cl aq. and brine. Removal of the solvent from the AcOEt extract under reduced pressure gave a crude product, which was purified by SiO_2 column (Hexane/AcOEt = 10:1 to 1:1) to give **15** (110 mg, quant.) as a colorless oil.

$[\alpha]_D^{27}$ –20.3 (*c* 0.89, $CHCl_3$). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 6.52 (1H, d, *J* = 9.1 Hz), 4.17 (2H, q, *J* = 7.2 Hz), 3.23 (2H, br), 2.88-2.75 (7H, br), 2.63-2.58 (1H, m), 1.95-1.90 (2H, br), 1.87-1.70 (2H, m), 1.84 (3H, d, *J* = 1.1 Hz), 1.67-1.61 (2H, m), 1.57-1.53 (1H, m), 1.52-1.45 (2H, m), 1.45 (9H, s), 1.41-1.15 (5H, m), 1.29 (3H, t, *J* = 7.2 Hz), 0.96 (3H, d, *J* = 6.4 Hz), 0.88 (3H, d, *J* = 6.4 Hz). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 168.5, 155.8, 148.1, 126.0, 79.3, 60.4, 53.0, 48.8, 47.9, 44.1, 35.6, 34.9, 34.7, 34.1, 30.7 (2C), 30.5, 28.5 (3C), 25.97, 25.95, 25.4, 22.8, 22.5, 20.1, 19.9, 14.3, 12.5. IR (KBr): 2927, 1697, 1365, 1149 cm^{-1}. MS (ESI-TOF) *m/z*: 524 $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 524.2844 calcd for $C_{26}H_{47}NO_4S_2Na$; Found: 524.2848.

(4*R*,6*S*,*E*)-8-(2-(3-((*tert*-Butoxycarbonyl)(methyl)amino)propyl)-1,3-dithian-2-yl)-2,4,6-trimethyloct-2-enoic acid (16)

LiOH (48 mg, 2.0 mmol, 10 equiv.) was added to a solution of **15** (100 mg, 0.2 mmol) in THF / MeOH / H_2O (1:1:1, 12 mL), and the whole mixture was stirred for 24 h at rt. 5% HCl was added to the mixture, and the whole mixture was extracted with AcOEt. Removal of the solvent from the AcOEt extract under reduced pressure gave a crude product, which was purified by SiO_2 column (Hexane/AcOEt = 1:1) to give **16**

(72.6 mg, 77%) as a colorless oil.

^{1}H-NMR (600 MHz, $CDCl_3$) δ: 6.67 (1H, d, J = 8.9 Hz), 3.23 (2H, br), 2.88-2.75 (7H, br), 2.68-2.60 (1H, m), 1.95-1.90 (2H, br), 1.87-1.70 (3H, m), 1.86 (3H, s), 1.67-1.61 (2H, m), 1.52-1.45 (1H, m), 1.46 (9H, s), 1.41-1.15 (5H, m), 0.99 (3H, d, J = 6.7 Hz), 0.89 (3H, d, J = 6.1 Hz). IR (KBr): 3200 (br), 2962, 1696, 1261, 1030 cm^{-1}. MS (ESI-TOF) *m/z*: 496 $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 496.2531 calcd for $C_{24}H_{43}NO_4S_2Na$; Found: 496.2552.

tert-Butyl methyl(3-(2-((3*S*,5*R*,*E*)-3,5,7-trimethyl-8-(methyl(thiazol-2-ylmethyl)amino)-8-oxooct-6-en-1-yl)-1,3-dithian-2-yl)propyl)carbamate (17)

HOBt (35.8 mg, 0.27 mmol, 1.4 equiv.), *N*-methyl-1-(thiazol-2-yl)methanamine (36.4 mg, 0.28 mmol, 1.5 equiv.), Et_3N (148 μL, 1.06 mmol, 4.0 equiv.) and EDCI· HCl (50.8 mg, 0.27 mmol, 1.4 equiv.) were added to a solution of **16** (72.6 mg, 0.19 mmol) in CH_2Cl_2 (15 mL), and the whole mixture was stirred for 48 h at rt. Sat. NH_4Cl aq. was added to the mixture, and the whole mixture was extracted with AcOEt, and the AcOEt extract was washed with Sat. NH_4Cl aq., $NaHCO_3$ aq., and brine. Removal of the solvent from the AcOEt extract under reduced pressure gave a crude product, which was purified by SiO_2 column (Hexane/AcOEt = 1:2) to give **17** (86.1 mg, 92%) as a colorless oil.

$[\alpha]_D^{27}$ –24.7 (*c* 0.43, $CHCl_3$). Mixture of rotamers. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.66 (1H, br s), 7.28 (1H, d, J = 2.6 Hz), 5.33 (1H, br), 4.82 (2H, s), 3.16 (2H, br), 2.98 (3H,

br s), 2.80-2.76 (7H, br), 2.55-2.48 (1H, m), 1.95-1.50 (8H, m), 1.83 (3H, d, J = 1.1 Hz), 1.48-1.00 (5H, m), 1.39 (9H, s), 0.88 (3H, br), 0.81 (3H, br). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 173.4, 166.6, 155.6, 141.9, 138.2, 128.8, 119.9, 119.5, 79.1, 60.2, 52.9, 52.8, 48.6, 48.2, 47.7, 44.2, 36.8, 35.5, 34.7, 34.5, 33.9, 30.6, 30.3, 30.2, 29.6, 28.3 (3C), 25.8 (2C?), 25.2, 22.6, 22.3, 20.0, 19.9, 14.1. IR (KBr): 2926, 1692, 1632, 1393, 1147 cm^{-1}. MS (ESI-TOF) *m/z*: 606 $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 606.2834 calcd for $C_{29}H_{49}N_3O_3S_3Na$; Found: 606.2857.

tert-Butyl methyl((7*S*,9*R*,*E*)-7,9,11-trimethyl-12-(methyl(thiazol-2-ylmethyl)amino)-4,12-dioxododec-10-en-1-yl)carbamate (18)

Iodine (142 mg, 0.56 mmol, 4 equiv.) was added to a solution of **17** (81.4 mg, 0.1400 mmol) in a mixture of CH_3CN (6 mL) and sat. $NaHCO_3$ aq. (3 mL) at 0 °C, and the whole mixture was stirred for 1.3 h at 0 °C. $Na_2S_2O_3$ / $NaHCO_3$ aq. was added to the mixture, and the whole mixture was extracted with AcOEt, and the AcOEt extract was washed with $Na_2S_2O_3$ / $NaHCO_3$ aq. and brine. Removal of the solvent from the AcOEt extract under reduced pressure gave a crude product, which was purified by SiO_2 column (Hexane/AcOEt = 1:3 to 0:1) to give **18** (32.6 mg, 47%) as a colorless oil.

$[\alpha]_D^{27}$ –16.2 (*c* 1.11, $CHCl_3$). Mixture of rotamers. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.57 (1H, br s), 7.21 (1H, d, J = 3.0 Hz), 5.26 (1H, br), 4.73 (2H, s), 3.06 (2H, t-like), 2.89 (3H, br s), 2.67 (3H, br s), 2.43-2.38 (1H, m), 2.30-2.15 (2H, m), 2.25 (2H, t, J = 7.0 Hz), 1.73 (3H, br s), 1.62 (2H, quint, J = 7.0 Hz), 1.52-1.44 (1H, br), 1.30 (9H, s),

1.20-0.90 (3H, m), 0.79 (3H, br), 0.69 (3H, br). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 210.0, 209.7, 173.2, 166.4, 155.5, 155.3, 141.8, 137.9, 128.6, 119.7, 78.9, 78.8, 52.3, 48.0, 47.7, 47.2, 43.9, 39.9, 39.1, 39.0, 36.6, 33.6, 29.9, 29.7, 29.3, 28.0, 21.5, 21.1, 19.7, 19.3, 13.9. IR (KBr): 2926, 1692, 1632, 1393, 1169 cm^{-1}. MS (ESI-TOF) *m/z*: 516 $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 516.2872 calcd for $C_{26}H_{43}N_3O_4SNa$; Found: 516.2887.

tert-Butyl ((4*EZ*,7*S*,9*R*,10*E*)-4-(chloromethylene)-7,9,11-trimethyl-12-(methyl(thiazol-2-ylmethyl)amino)-12-oxododec-10-en-1-yl)(methyl)carbamate (19)

LHMDS solution (1.0 M in THF, 320 μL, 0.32 mmol, 4.8 equiv.) was added to a solution of (chloromethyl)triphenylphosphonium chloride (114 mg, 0.33 mmol, 5.0 equiv.) in anhydrous THF (3 mL), and the whole mixture was stirred for 10 min. A solution of **18** (32.6 mg, 0.066 mmol) in anhydrous THF (1 mL) was added dropwise to the mixture via cannula over 5 min at rt, and the whole mixture was stirred for 1 h at rt. Sat. NH_4Cl aq. was added to the mixture, and the whole mixture was extracted with AcOEt, and the AcOEt extract was washed with NH_4Cl aq. and brine. Removal of the solvent from AcOEt extract under reduced pressure gave a crude product, which was purified by SiO_2 column (Hexane/AcOEt = 1:2) to give **19** (34.5 mg, 99%) as a colorless oil.

E/*Z* mixture (*E*/*Z* = 3:2). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.72 (1H, br s), 7.32 (1H, d, *J* = 3.0 Hz), 5.77 (1H, br s), 5.41 (1H, br), 4.87 (2H, s), 3.18 (2H, br), 3.03 (3H, br s),

2.83 (3H, br), 2.60-2.50 (1H, m), 2.20-2.05 (2H, m), 2.04-1.92 (2H, m), 1.87 (0.6x3H, s), 1.86 (0.4x3H, s), 1.73 (1H, br), 1.64-1.56 (2H, m), 1.443 (0.4x9H, s), 1.440 (0.6x9H, s), 1.44-1.10 (4H, m), 0.93 (3H, br), 0.88 (0.6x3H, br), 0.85 (0.4x3H, br). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 173.7, 166.9, 155.8, 155.7, 142.2, 138.4, 128.86, 128.82, 120.1, 112.2, 79.3, 79.2, 48.8, 48.5, 48.2, 48.1, 44.18, 44.16, 37.0, 34.9, 34.2, 33.9, 32.3, 32.0, 30.6, 30.2, 29.84, 29.80, 28.5, 27.6, 27.5, 25.8, 25.6, 25.5, 25.0, 20.0, 19.9, 19.8, 19.7, 14.3. IR (KBr): 2926, 1693, 1632, 1393, 1168 cm^{-1}. MS (ESI-TOF) *m/z*: 548/550 (3:1) $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 548.2690 calcd for $C_{27}H_{44}N_3O_3S^{35}ClNa$; Found: 548.2692.

Biakamide A (1) and B (2)

TFA (33 μL, 0.45mmol, 10 equiv.) was added to a solution of **19** (23.5 mg, 0.045 mmol) in CH_2Cl_2 (1 mL), and the whole mixture was stirred for 7 h. Sat. $NaHCO_3$ aq. was added to the mixture, and the whole mixture was extracted with AcOEt. Removal of the solvent from AcOEt extract under reduced pressure gave an amine **20**, which was used in the next reaction without further purification.

38% aliquot of the above amine was dissolved to CH_2Cl_2 (3 mL). HOBt (3.9 mg, 0.047 mmol, 1.4 equiv.), (S)-3-hydroxybutyric acid (6.0 mg, 0.05 mmol, 1.5 equiv.), Et_3N (24 μL, 0.17 mmol, 6.0 equiv.) and EDCI· HCl (5.6 mg, 0.029 mmol, 1.4 equiv.) were added to the solution, and the whole mixture was stirred for 15 h at rt. Sat. NH_4Cl aq. was added to the mixture, and the whole mixture was extracted with AcOEt, and the AcOEt extract was washed with Sat. NH_4Cl aq., $NaHCO_3$ aq., and brine. Removal of

the solvent from AcOEt extract under reduced pressure gave a crude product, which was purified by SiO_2 column (AcOEt/MeOH = 5:1) to give a mixture of **1** and **2** (7.6 mg, 74%, two steps) as a white powder. HPLC separation (Cosmosil $5C_{18}$-MS-II, 10 mm i.d. × 250 mm, MeOH/H_2O = 60:40, flow rate = 3.5 mL/min) yielded pure **1** and **2**.

(4*R*,6*S*)-biakamide A (**1**): $[\alpha]_D^{28}$ +1.2 (*c* 0.57, $CHCl_3$). Mixture of rotamers. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.72 (1H, br s), 7.32 (1H, br s), 5.79 (0.4x1H, s), 5.78 (0.6x1H, s), 5.41 (1H, br), 4.87 (2H, s), 4.40 (0.4x1H, s), 4.38 (0.6x1H, s), 4.20-4.16 (1H, m), 3.36-3.30 (0.6x2H, t-like), 3.27-3.18 (0.4x2H, m), 3.03 (3H, br s), 2.94 (0.6x3H, s), 2.91 (0.4x3H, s), 2.55 (1H, br), 2.45 (1H, dd, *J* = 16.5, 2.1 Hz), 2.27 (1H, dd, *J* = 16.5, 9.6 Hz), 2.23-2.15 (1H, m), 2.15-2.08 (1H, m), 2.07-2.05 (2H, m), 1.87 (3H, s), 1.80 (1H, br), 1.70-1.59 (2H, m), 1.45-1.35 (2H, br), 1.33-1.08 (3H, m), 1.21 (3H, d, *J* = 6.6 Hz), 0.94 (3H, br), 0.88 (3H, br). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 173.6, 172.5, 172.4, 166.9, 142.2, 142.0, 141.4, 138.0, 128.87, 128.82, 120.0, 112.9, 112.4, 64.2, 64.1, 52.8, 49.0, 48.3, 47.2, 44.1, 41.1, 40.5, 37.0, 35.2, 33.9, 33.8, 33.0, 32.1, 31.7, 30.5, 29.78, 29.75, 29.6, 27.54, 27.50, 26.0, 25.1, 22.2, 22.1, 20.04, 20.01, 19.3, 14.1. IR (KBr): 3434, 2925, 1631, 1394 cm^{-1}. MS (ESI-TOF) *m/z*: 534/536 (3:1) $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 534.2533 calcd for $C_{26}H_{42}N_3O_3S^{35}ClNa$; Found: 534.2513.

(4*R*,6*S*)-biakamide B (**2**): $[\alpha]_D^{26}$ +4.1 (*c* 0.42, $CHCl_3$). Mixture of rotamers. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.72 (1H, br s), 7.33 (1H, d, *J* = 2.4 Hz), 5.84 (0.4x1H, s), 5.78 (0.6x1H, s), 5.41 (1H, br), 4.87 (2H, s), 4.45 (1H, br), 4.22-4.15 (1H, m), 3.38 (0.6x2H, t-like), 3.30-3.22 (0.4x2H, m), 3.03 (3H, br s), 2.96 (0.6x3H, s), 2.93 (0.4x3H, s), 2.52 (1H, br), 2.46 (1H, ddd, *J* = 16.2, 9.0, 1.8 Hz), 2.29 (1H, ddd, *J* = 16.2, 9.6, 6.6 Hz),

2.23-2.14 (2H, m), 2.13-2.07 (1H, br), 2.02-1.94 (1H, br), 1.86 (3H, s), 1.71-1.60 (2H, m), 1.44-1.35 (1H, br), 1.25-1.08 (3H, m), 1.22 (3H, d, J = 6.6 Hz), 0.94 (3H, br), 0.85 (3H, br). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 173.5, 172.5, 172.4, 166.8, 142.0 (br), 142.0, 141.4, 138.4, 128.84, 128.77, 120.0, 113.1, 112.5, 64.3, 64.1, 52.6, 49.3, 48.4, 47.2, 44.1, 41.1, 40.4, 36.9, 35.1, 34.9, 33.1, 32.5, 32.2, 30.2, 30.1, 29.74, 29.71, 27.4, 27.3, 25.6, 24.6, 22.2, 22.1, 19.92, 19.89, 19.6, 14.2. IR (KBr): 3435, 2962, 1628, 1398, 1261 cm^{-1}. MS (ESI-TOF) *m/z*: 534/536 (3:1) $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 534.2533 calcd for $C_{26}H_{42}N_3O_3S^{35}ClNa$; Found: 534.2525.

Biakamide C (3) and D (4)

62% aliquot of the above amine **20** was dissolved to CH_2Cl_2 (3 mL). HOBt (6.4 mg, 0.047 mmol, 1.4 equiv.), (*E*)-3-methoxybut-2-enoic acid (5.6 mg, 0.050 mmol, 1.5 equiv.), Et_3N (39 μL, 0.28 mmol, 6.0 equiv.) and EDCI· HCl (9.0 mg, 0.047 mmol, 1.4 equiv.) were added to the solution, and whole mixture stirred for 25 h at rt. Sat. NH_4Cl aq. was added to the mixture, and the whole mixture was extracted with AcOEt, and the AcOEt extract was washed with Sat. NH_4Cl aq., $NaHCO_3$ aq., and brine. Removal of the solvent from AcOEt extract under reduced pressure gave a crude product, which was purified by SiO_2 column (AcOEt/MeOH = 50:1) to give a mixture of **3** and **4** (13.8 mg, 78%) as a white powder. HPLC separation (Cosmosil 5C_{18}-MS-II, 10 mm i.d. × 250 mm, MeOH/H_2O = 70:30, flow rate = 4.0 mL/min) yielded pure **3** and **4**.

(4*R*,6*S*)-biakamide C (**3**): $[\alpha]_D^{27}$ –17.3 (*c* 0.48, $CHCl_3$). Mixture of rotamers. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.72 (1H, br s), 7.33 (1H, d, J = 3.0 Hz), 5.78 (1H, s), 5.41 (1H,

br), 5.15 (1H, s), 4.87 (2H, s), 3.61 (0.6x3H, s), 3.59 (0.4x3H, s), 3.40-3.34 (0.6x2H, br), 3.32-3.25 (0.4x2H, br), 3.03 (3H, br s), 3.00 (0.6x3H, s), 2.94 (0.4x3H, s), 2.59-2.51 (1H, br), 2.19 (3H, s), 2.20-1.08 (2H, m), 2.05 (2H, t, J = 7.5 Hz), 1.87 (3H, s), 1.76-1.62 (4H, m), 1.45-1.36 (2H, m), 1.30-1.25 (1H, m), 1.20-1.10 (2H, m), 0.94 (3H, d, J = 3.6 Hz), 0.88 (3H, br). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 173.7, 168.6, 168.2, 168.0, 166.9, 142.3, 142.2, 141.7, 138.4, 128.8, 120.0, 112.7, 112.2, 91.2, 90.9, 54.8, 52.6, 49.7, 48.3, 47.2, 44.1, 37.1, 36.0, 33.9, 33.4, 32.2, 31.6, 30.5, 29.8, 29.7, 27.6, 26.0, 25.5, 20.0, 19.8, 18.7, 14.2. IR (KBr): 2924, 1639, 1453, 1379, 1239 cm^{-1}. MS (ESI-TOF) *m/z*: 546/548 (3:1) $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 546.2533 calcd for $C_{27}H_{42}N_3O_3S^{35}ClNa$; Found: 546.2512.

(4*R*,6*S*)-biakamide D (**4**): $[\alpha]_D^{29}$ –11.4 (*c* 0.35, $CHCl_3$). Mixture of rotamers. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.72 (1H, br s), 7.32 (1H, d, J = 3.0 Hz), 5.81 (0.5x1H, br s), 5.77 (0.5x1H, br s), 5.40 (1H, br), 5.17 (1H, s), 4.87 (2H, s), 3.60 (3H, s), 3.42 (0.5x2H, t-like), 3.33 (0.5x2H, t-like), 3.02 (3H+0.5x3H, br s), 2.95 (0.5x 3H, br s), 2.52 (1H, br), 2.19 (3H, s), 2.20-2.16 (2H, m), 2.14-2.06 (1H, m), 2.02-1.96 (1H, m), 1.86 (3H, s), 1.77-1.60 (4H, m), 1.42-1.05 (4H, m), 0.93 (3H, br), 0.85 (3H, br). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 173.6, 168.4, 168.1, 168.0, 166.8, 142.4, 142.1, 141.8, 138.4, 128.8, 120.0, 112.8, 112.2, 91.3, 91.0, 54.84, 54.78, 52.8, 50.2, 48.4, 47.3, 44.1, 36.9, 35.9, 34.9, 33.5, 32.32, 32.25, 30.1, 29.8, 27.6, 27.5, 26.0, 24.9, 19.9, 19.6, 18.7, 14.2. IR (KBr): 2962, 1645, 1261, 1102 cm^{-1}. MS (ESI-TOF) *m/z*: 546/548 (3:1) $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 546.2533 calcd for $C_{27}H_{42}N_3O_3S^{35}ClNa$; Found: 546.2513.

Synthesis of (4*R*,6*R*)-isomer of biakamides

Using the same procedures as those for the synthesis of (4*R*,6*S*)-isomers, (4*R*,6*R*)-isomers of biakamides were obtained starting from (2*S*,4*R*)-5-((*tert*-Butyldiphenylsilyl)oxy)-2,4-dimethylpentan-1-ol (**6'**).[42]

Methyl (4*S*,6*R*,*E*)-7-((*tert*-butyldiphenylsilyl)oxy)-4,6-dimethylhept-2-enoate (7')

Yield: 86%. $[\alpha]_D^{27}$ +18.7 (*c* 3.19, $CHCl_3$). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.68 (4H, d, *J* = 7.7 Hz), 7.45-7.38 (6H, m), 6.84 (1H, dd, *J* = 15.8, 8.4 Hz), 5.81 (1H, d, *J* = 15.8 Hz), 3.75 (3H, s), 3.50-3.43 (2H, m), 2.42 (1H, quint, *J* = 6.5 Hz), 1.70-1.65 (1H, m), 1.59-1.53 (1H, m), 1.16-1.11 (1H, m), 1.08 (9H, s), 1.04 (3H, d, *J* = 6.4 Hz), 0.92 (3H, d, *J* = 6.4 Hz). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 167.3, 154.8, 135.644 (2C), 135.637 (2C), 133.90, 133.86, 129.6 (2C), 127.6 (4C), 119.4, 69.0, 51.4, 39.8, 34.2, 33.4, 26.9 (3C), 20.5, 19.3, 16.7. IR (KBr): 2958, 1725, 1626, 1273, 1112 cm^{-1}. MS (ESI-TOF) *m/z*: 447 $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 447.2331 calcd for $C_{26}H_{36}O_3SiNa$; Found: 447.2323.

Methyl (4*R*,6*R*)-7-((*tert*-butyldiphenylsilyl)oxy)-4,6-dimethylheptanoate (8')

Yield: 80%. $[\alpha]_D^{27}$ +6.4 (*c* 1.59, $CHCl_3$). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.67 (4H, d, *J* = 6.8 Hz), 7.44-7.37 (6H, m), 3.66 (3H, s), 3.50 (1H, dd, *J* = 9.7, 5.4 Hz), 3.42 (1H, dd, *J* = 9.7, 6.5 Hz), 2.33 (1H, ddd, *J* = 15.5, 10.0, 5.6 Hz), 2.25 (1H, ddd, *J* = 15.5, 9.8, 6.2 Hz), 1.78-1.72 (1H, m), 1.71-1.64 (1H, m), 1.51-1.44 (1H, m), 1.40-1.32 (2H, m), 1.06 (9H, s), 0.96-0.91 (1H, m), 0.93 (3H, d, *J* = 6.7 Hz), 0.84 (3H, d, *J* = 6.7 Hz). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 174.6, 135.64 (2C), 135.63 (2C), 134.0 (2C), 129.5 (2C), 127.6

(4C), 68.8, 51.5, 40.7, 33.0, 31.64, 31.57, 29.7, 26.9 (3C), 19.9, 19.3, 17.6. IR (KBr): 2955, 1740, 1428, 1171, 1112 cm^{-1}. MS (ESI-TOF) *m/z*: 449 $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 449.2488 calcd for $C_{26}H_{38}O_3SiNa$; Found: 449.2478.

(4*R*,6*R*)-7-((*tert*-Butyldiphenylsilyl)oxy)-4,6-dimethylheptanoic acid (9')

Yield: 80%. $[\alpha]_D^{26}$ +6.8 (*c* 1.06, $CHCl_3$). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.67 (4H, d, *J* = 6.7 Hz), 7.44-7.36 (6H, m), 3.49 (1H, dd, *J* = 9.7, 5.3 Hz), 3.42 (1H, dd, *J* = 9.7, 6.4 Hz), 2.37 (1H, ddd, *J* = 15.9, 10.0, 5.6 Hz), 2.29 (1H, ddd, *J* = 15.9, 9.7, 6.5 Hz), 1.77-1.65 (2H, m), 1.53-1.47 (1H, m), 1.41-1.33 (2H, m), 1.06 (9H, s), 0.96-0.92 (1H, m), 0.93 (3H, d, *J* = 6.8 Hz), 0.85 (3H, d, *J* = 6.5 Hz). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 180.3, 135.64 (2C), 135.63 (2C), 134.0 (2C), 129.5 (2C), 127.6 (4C), 68.8, 40.7, 33.0, 31.6, 31.3, 29.6, 26.9 (3C), 19.8, 19.3, 17.6. IR (KBr): 3100 (br), 2957, 1709, 1427, 1112 cm^{-1}. MS (ESI-TOF) *m/z*: 435 $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 435.2331 calcd for $C_{25}H_{36}O_3SiNa$; Found: 435.2317.

(4*R*,6*R*)-7-((*tert*-Butyldiphenylsilyl)oxy)-4,6-dimethylheptanal (11')

Yield: 66% (2 steps). $[\alpha]_D^{26}$ +6.3 (*c* 2.03, $CHCl_3$). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 9.76 (1H, s), 7.68 (4H, d, *J* = 6.7 Hz), 7.44-7.38 (6H, m), 3.50 (1H, dd, *J* = 9.9, 5.4 Hz), 3.44 (1H, dd, *J* = 9.9, 6.3 Hz), 2.45-2.33 (2H, m), 1.76-1.71 (1H, m), 1.68-1.63 (1H, m), 1.53-1.47 (1H, m), 1.42-1.33 (2H, m), 1.07 (9H, s), 0.97-0.92 (1H, m), 0.94 (3H, d, *J* = 6.7 Hz), 0.85 (3H, d, *J* = 6.8 Hz). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 202.9, 135.58 (2C), 135.56 (2C), 133.9 (2C), 129.5 (2C), 127.5 (4C), 68.7, 41.4, 40.6, 33.0, 29.6, 28.5, 26.8

(3C), 19.9, 19.3, 17.6. IR (KBr): 2957, 1726, 1471, 1427, 1111 cm^{-1}. MS (ESI-TOF) *m/z*: 419 $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 419.2382 calcd for $C_{25}H_{36}O_2SiNa$; Found: 419.2383.

(((2*R*,4*R*)-6-(1,3-Dithian-2-yl)-2,4-dimethylhexyl)oxy)(*tert*-butyl)diphenylsilane (12')

Yield: 80%. $[\alpha]_D^{27}$ +7.1 (*c* 1.12, $CHCl_3$). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.67 (4H, d, *J* = 7.6 Hz), 7.45-7.37 (6H, m), 3.99 (1H, t, *J* = 6.9 Hz), 3.50 (1H, dd, *J* = 9.9, 5.2 Hz), 3.40 (1H, dd, *J* = 9.9, 6.6 Hz), 2.90-2.80 (4H, m), 2.13-2.10 (1H, m), 1.90-1.82 (1H, m), 1.80-1.65 (3H, m), 1.55-1.43 (2H, m), 1.38-1.33 (1H, m), 1.28-1.22 (1H, m), 1.06 (9H, s), 0.93-0.88 (1H, m), 0.93 (3H, d, *J* = 6.8 Hz), 0.83 (3H, d, *J* = 6.7 Hz). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 135.6 (4C), 134.0 (2C), 129.5 (2C), 127.6 (4C), 68.8, 48.0, 40.9, 33.6, 33.0, 32.9, 30.55, 30.47, 29.9, 26.9 (3C), 26.1, 20.1, 19.3, 17.8. IR (KBr): 2930, 1470, 1427, 1111 cm^{-1}. MS (ESI-TOF) *m/z*: 509 $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 509.2344 calcd for $C_{28}H_{42}OSiS_2Na$; Found: 509.2332.

***tert*-Butyl (3-(2-((3*R*,5*R*)-6-Hydroxy-3,5-dimethylhexyl)-1,3-dithian-2-yl)propyl)(methyl)carbamate (13')**

Yield: 69% (2 steps). $[\alpha]_D^{27}$ +6.4 (*c* 1.87, $CHCl_3$). Mixture of rotamers. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 3.46 (1H, br), 3.35 (1H, dd, *J* = 10.6, 6.8 Hz), 3.20 (2H, br), 2.83 (3H,

br), 2.77 (4H, br), 1.95-1.59 (9H, m), 1.50-1.44 (1H, m), 1.43 (9H, s), 1.35-1.32 (1H, m), 1.22-1.16 (1H, m), 0.97-0.92 (1H, m), 0.90 (3H, d, J = 6.4 Hz), 0.89 (3H, d, J = 6.2 Hz). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 155.8, 79.3, 68.2, 53.0, 48.7, 48.1, 40.8, 40.5, 35.4, 35.2, 34.8, 34.5, 34.1, 33.3, 33.1, 30.3, 30.2, 29.9, 28.4 (3C), 25.9, 25.4, 22.8, 22.4, 20.4, 17.2. IR (KBr): 3456, 2928, 1694, 1395, 1151 cm^{-1}. MS (ESI-TOF) *m/z*: 442 $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 442.2426 calcd for $C_{21}H_{41}NO_3S_2Na$; Found: 442.2421.

tert-Butyl (3-(2-((3*R*,5*R*)-3,5-dimethyl-6-oxohexyl)-1,3-dithian-2-yl)propyl)(methyl)carbamate (14')

Yield: 84%. $[\alpha]_D^{27}$ –0.1 (*c* 2.06, $CHCl_3$). Mixture of rotamers. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 9.56 (1H, d, J = 2.0 Hz), 3.20 (2H, br), 2.83 (3H, br s), 2.77 (4H, br), 2.41 (1H, q-like, J = 6.4 Hz), 1.95-1.70 (6H, m), 1.64-1.59 (2H, m), 1.47-1.42 (1H, m), 1.43 (9H, s), 1.25-1.20 (1H, m), 1.13 (1H, quint, J = 7.0 Hz), 1.07 (3H, d, J = 7.1 Hz), 0.90 (3H, d, J = 6.4 Hz). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 205.1, 155.7, 79.2, 52.8, 48.6, 47.8, 43.9, 37.9, 35.4, 34.9, 34.6, 34.0, 30.6, 30.5, 28.4 (3C), 25.8, 25.3, 22.7, 22.3, 19.7, 14.0. IR (KBr): 2932, 1723, 1693, 1393, 1149 cm^{-1}. MS (ESI-TOF) *m/z*: 440 $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 440.2269 calcd for $C_{21}H_{39}NO_3S_2Na$; Found: 440.2277.

Ethyl (4*R*,6*R*,*E*)-8-(2-(3-((_tert_-Butoxycarbonyl)(methyl)amino)propyl)-1,3-dithian-2-yl)-2,4,6-trimethyloct-2-enoate (15')

Yield: 87%. $[\alpha]_D^{27}$ –8.0 (*c* 2.45, $CHCl_3$). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 6.45 (1H, d, *J* = 10.3 Hz), 4.18-4.13 (2H, m), 3.20 (2H, br), 2.82 (3H, br s), 2.80-2.70 (4H, br), 2.62-2.55 (1H, m), 1.91 (2H, br s), 1.85-1.75 (3H, m), 1.81 (3H, s), 1.65-1.58 (2H, m), 1.43 (9H, s), 1.40-1.32 (2H, m), 1.31-1.12 (5H, m), 1.27 (3H, t, *J* = 7.0 Hz), 0.95 (3H, d, *J* = 6.4 Hz), 0.83 (3H, d, *J* = 6.4 Hz). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 168.3, 155.7, 147.7, 126.3, 79.2, 60.3, 52.9, 48.7, 47.9, 44.2, 35.6, 34.8, 34.0, 31.5, 30.9, 30.7, 28.4 (3C), 25.9, 25.4, 22.6, 20.6, 19.4, 14.2, 12.4. IR (KBr): 2930, 1698, 1392, 1263, 1150 cm^{-1}. MS (ESI-TOF) *m/z*: 524 $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 524.2844 calcd for $C_{26}H_{47}NO_4S_2Na$; Found: 524.2842.

(4*R*,6*R*,*E*)-8-(2-(3-((*tert*-Butoxycarbonyl)(methyl)amino)propyl)-1,3-dithian-2-yl)-2,4,6-trimethyloct-2-enoic acid (16')

Yield: quant. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 6.57 (1H, d, *J* = 8.9 Hz), 3.17 (2H, br), 2.80-2.72 (7H, br), 2.62-2.54 (1H, m), 1.90-1.83 (2H, br), 1.80-1.70 (4H, m), 1.79 (3H, s), 1.60-1.55 (2H, m), 1.39 (9H, s), 1.38-1.10 (5H, m), 0.93 (3H, d, *J* = 6.8 Hz), 0.80 (3H, d, *J* = 7.6 Hz). IR (KBr): 3100 (br), 2930, 1690, 1395, 1276, 1152 cm^{-1}. MS (ESI-TOF) *m/z*: 496 $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 496.2531 calcd for $C_{24}H_{43}NO_4S_2Na$; Found: 496.2531.

***tert*-Butyl**

methyl(3-(2-((3*R*,5*R*,*E*)-3,5,7-trimethyl-8-(methyl(thiazol-2-ylmethyl)amino)-8-oxo oct-6-en-1-yl)-1,3-dithian-2-yl)propyl)carbamate (17')

Yield: 84%. $[\alpha]_D^{27}$ –6.2 (*c* 1.57, $CHCl_3$). Mixture of rotamers. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.71 (1H, br s), 7.32 (1H, d, *J* = 2.4 Hz), 5.35 (1H, br), 4.86 (2H, s), 3.20 (2H, br), 3.02 (3H, br s), 2.85-2.70 (7H, br), 2.56 (1H, br), 1.95-1.65 (6H, m), 1.87 (3H, s), 1.63-1.58 (2H, m), 1.43 (9H, s), 1.38-1.10 (5H, m), 0.94 (3H, br), 0.86 (3H, br). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 173.7, 166.8, 155.8, 142.3, 138.2, 129.3, 120.1, 79.3, 60.2, 53.0, 52.9, 48.7, 47.9, 44.4, 37.0, 35.6, 34.9, 34.6, 34.0, 31.5, 30.9, 29.9, 28.5 (3C), 25.9 (2C?), 25.4, 22.8, 22.5, 20.9, 19.5, 14.3. IR (KBr): 2927, 1692, 1631, 1393, 1149 cm^{-1}. MS (ESI-TOF) *m/z*: 606 $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 606.2834 calcd for $C_{29}H_{49}N_3O_3S_3Na$; Found: 606.2855.

***tert*-Butyl**

methyl((7*R*,9*R*,*E*)-7,9,11-trimethyl-12-(methyl(thiazol-2-ylmethyl)amino)-4,12-dioxododec-10-en-1-yl)carbamate (18')

Yield: 98%. $[\alpha]_D^{26}$ –12.2 (*c* 1.72, $CHCl_3$). Mixture of rotamers. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.49 (1H, br s), 7.15 (1H, br s), 5.14 (1H, br), 4.65 (2H, s), 2.98 (2H, t-like), 2.81 (3H, br s), 2.59 (3H, br s), 2.35 (1H, br), 2.20-2.10 (2H, m), 2.16 (2H, t, *J* = 7.0 Hz), 1.65 (3H, s), 1.53 (2H, quint, *J* = 7.0 Hz), 1.35-1.25 (1H, br), 1.22 (9H, s), 1.15-0.95 (3H, m), 0.90 (1H, br), 0.74 (3H, br), 0.62 (3H, br). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 209.7, 209.4, 172.9, 166.2, 155.2, 155.1, 141.6, 137.3, 128.8, 119.6, 78.6, 78.5, 52.2, 47.8, 47.5, 46.9, 43.9, 39.8, 38.8, 37.7, 36.5, 33.4, 32.7, 30.6, 29.9, 29.3, 27.9, 21.3, 20.9, 20.3, 18.7, 13.8. IR (KBr): 2927, 1693, 1632, 1393, 1169 cm^{-1}. MS (ESI-TOF) *m/z*: 516 $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 516.2872 calcd for

$C_{26}H_{43}N_3O_4SNa$; Found: 516.2856.

***tert*-Butyl**

((4*EZ*,7*R*,9*R*,10*E*)-4-(chloromethylene)-7,9,11-trimethyl-12-(methyl(thiazol-2-ylmethyl)amino)-12-oxododec-10-en-1-yl)(methyl)carbamate (19')

Yield: 89%. *E*/*Z* mixture (*E*/*Z* = 3:2). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.60 (1H, br s), 7.23 (1H, br s), 5.64 (1H, br s), 5.26 (1H, br), 4.76 (2H, s), 3.06 (2H, br), 2.92 (3H, br s), 2.71 (3H, br), 2.50-2.40 (1H, m), 2.05-1.95 (2H, m), 1.89 (2H, t, *J* = 7.4 Hz), 1.77 (3H, s), 1.50-1.42 (2H, m), 1.33 (9H, s), 1.25-0.95 (5H, m), 0.85 (3H, br), 0.78 (0.6x3H, br), 0.74 (0.4x3H, br). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 173.3, 166.5, 155.38, 155.37, 141.8, 137.7, 129.1, 129.0, 119.8, 111.8, 78.9, 78.8, 52.4, 48.5, 48.1, 47.9, 47.7, 44.1, 44.0, 36.7, 35.2, 34.3, 33.9, 31.9, 31.5, 30.5, 30.2, 29.64, 29.58, 28.2, 27.3, 27.1, 25.5, 25.3, 25.2, 24.7, 20.65, 20.61, 19.1, 19.0, 14.0. IR (KBr): 2926, 1694, 1633, 1393, 1168 cm^{-1}. MS (ESI-TOF) *m/z*: 548/550 (3:1) $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 548.2690 calcd for $C_{27}H_{44}N_3O_3S^{35}ClNa$; Found: 548.2701.

(4*R*,6*R*)-Biakamide A (1') and B (2')

Combined yield: 85%. HPLC separation provided (4*R*,6*R*)-biakamide A (**1'**) and B (**2'**).

(4*R*,6*R*)-biakamide A (**1'**): $[\alpha]_D^{26}$ +5.7 (*c* 2.50, $CHCl_3$). Mixture of rotamers. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.69 (1H, br s), 7.31 (1H, br s), 5.75 (0.4x1H, s), 5.73 (0.6x1H, s), 5.34 (1H, br), 4.85 (2H, s), 4.39 (1H, br), 4.20-4.13 (1H, m), 3.35-3.27 (0.6x2H, m),

3.25-3.15 (0.4x2H, m), 3.00 (3H, br s), 2.92 (0.6x3H, s), 2.89 (0.4x3H, s), 2.55 (1H, br), 2.43 (1H, dd, J = 16.5, 2.2 Hz), 2.26 (1H, ddd, J = 16.5, 9.6, 2.2 Hz), 2.11 (2H, br), 2.04-1.97 (2H, m), 1.85 (3H, s), 1.65-1.53 (2H, m), 1.40-1.05 (5H, m), 1.19 (3H, d, J = 6.2 Hz), 0.94 (3H, br), 0.86 (3H, br). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 173.5, 172.5, 172.3, 166.8, 142.1, 141.9, 141.3, 138.1, 129.3, 129.2, 120.0, 112.8, 112.3, 64.2, 64.0, 52.6, 48.9, 48.3, 47.1, 44.2, 41.1, 40.5, 37.0, 35.1, 34.61, 34.57, 33.0, 31.9, 31.6, 30.8, 29.91, 29.88, 27.5, 27.4, 26.0, 25.1, 22.2, 22.1, 20.9, 20.8, 19.3, 14.2. IR (KBr): 3434, 2925, 1627, 1454, 1396 cm^{-1}. MS (ESI-TOF) m/z: 534/536 (3:1) $[M+Na]^+$. HRMS (ESI-TOF) m/z: 534.2533 calcd for $C_{26}H_{42}N_3O_3S^{35}ClNa$; Found: 534.2541.

(4R,6R)-biakamide B (**2'**): $[\alpha]_D^{26}$ +11.1 (c 0.81, $CHCl_3$). Mixture of rotamers. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.71 (1H, br s), 7.32 (1H, br s), 5.81 (0.4x1H, s), 5.76 (0.6x1H, s), 5.33 (1H, br), 4.86 (2H, s), 4.45 (1H, br), 4.20-4.14 (1H, m), 3.37 (0.6x2H, t, J = 7.4 Hz), 3.29-3.20 (0.4x2H, m), 3.02 (3H, br s), 2.96 (0.6x3H, s), 2.92 (0.4x3H, s), 2.55 (1H, br), 2.46 (1H, dd, J = 16.1, 7.6 Hz), 2.28 (1H, dt?, J = 16.1, 2.2 Hz), 2.20-2.10 (2H, m), 2.09-1.90 (2H, br), 1.86 (3H, s), 1.69-1.58 (2H, m), 1.35-1.05 (5H, m), 1.21 (3H, d, J = 6.2 Hz), 0.95 (3H, br), 0.84 (3H, br). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 173.5, 172.5, 172.4, 166.7, 142.1, 142.0, 141.3, 137.9, 129.4, 129.3, 120.1, 113.0, 112.4, 64.2, 64.1, 52.7, 49.2, 48.3, 47.1, 44.44, 44.38, 41.1, 40.4, 37.0, 35.6, 35.5, 35.0, 33.0, 32.2, 32.1, 30.5, 29.9, 29.8, 27.4, 27.3, 26.0, 24.6, 22.2, 22.1, 20.94, 20.89, 19.22, 19.21, 14.2. IR (KBr): 3435, 2925, 1628, 1395, 1258 cm^{-1}. MS (ESI-TOF) m/z: 534/536 (3:1) $[M+Na]^+$. HRMS (ESI-TOF) m/z: 534.2533 calcd for $C_{26}H_{42}N_3O_3S^{35}ClNa$; Found: 534.2529.

(4*R*,6*R*)-Biakamide C (3') and D (4')

Combined yield: 76%. HPLC separation provided (4*R*,6*R*)-biakamide C (**3'**) and D (**4'**).

(4*R*,6*R*)-biakamide C (**3'**): $[\alpha]_D^{27}$ –11.9 (*c* 1.32, $CHCl_3$). Mixture of rotamers. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.71 (1H, d, *J* = 1.9 Hz), 7.32 (1H, d, *J* = 1.9 Hz), 5.75 (1H, s), 5.36 (1H, br), 5.14 (0.6x1H, s), 5.13 (0.4x1H, s), 4.86 (2H, s), 3.60 (0.6x3H, s), 3.58 (0.4x3H, s), 3.40-3.30 (0.6x2H, br), 3.32-3.15 (0.4x2H, br), 3.02 (3H, s), 2.99 (0.6x3H, br s), 2.92 (0.4x3H, s), 2.56 (1H, br), 2.18 (3H, s), 2.12 (2H, br), 2.02 (2H, t, *J* = 7.5 Hz), 1.87 (3H, s), 1.67-1.60 (2H, m), 1.40-1.05 (6H, m), 0.95 (3H, d, *J* = 5.5 Hz), 0.86 (3H, d, *J* = 5.2 Hz). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 173.6, 168.6, 168.5, 168.1, 168.0, 166.8, 142.3, 142.2, 141.6, 138.1, 129.3, 129.2, 120.0, 112.6, 112.1, 91.2, 90.9, 54.8, 52.6, 49.6, 48.4, 47.2, 44.2, 36.9, 36.0, 34.6, 33.4, 32.1, 31.6, 30.8, 29.7, 29.6, 27.6, 27.5, 26.0, 25.5, 20.9, 19.4, 18.7, 14.1. IR (KBr): 2924, 1638, 1450, 1380, 1239 cm^{-1}. MS (ESI-TOF) *m/z*: 546/548 (3:1) $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 546.2533 calcd for $C_{27}H_{42}N_3O_3S^{35}ClNa$; Found: 546.2511.

(4*R*,6*R*)-biakamide D (**4'**): $[\alpha]_D^{26}$ –6.8 (*c* 0.69, $CHCl_3$). Mixture of rotamers. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.71 (1H, br s), 7.32 (1H, br s), 5.79 (0.5x1H, br s), 5.74 (0.5x1H, br s), 5.33 (1H, b), 5.16 (1H, s), 4.86 (2H, s), 3.60 (0.5x3H, s), 3.59 (0.5x3H, s), 3.41 (0.5x2H, t-like), 3.31 (0.5x2H, t-like), 3.01 (3H+0.5x3H, br s), 2.95 (0.5x3H, br s), 2.55 (1H, br), 2.19 (3H, s), 2.20-2.10 (2H, m), 2.08-1.90 (2H, m), 1.86 (3H, s), 1.69-1.60 (2H, m), 1.38-1.05 (6H, m), 0.95 (3H, d, *J* = 5.3 Hz), 0.83 (3H, br). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 173.6, 168.4, 168.2, 168.1, 167.8, 166.8, 142.4, 142.2, 141.8, 137.9,

129.5, 129.4, 120.1, 112.8, 112.2, 91.3, 91.0, 54.9, 54.8, 53.0, 50.3, 48.4, 47.3, 44.5, 37.0, 35.9, 35.7, 33.5, 32.33, 32.27, 30.5, 29.9, 29.7, 27.6, 27.5, 26.0, 24.9, 21.0, 19.3, 18.7, 14.3. IR (KBr): 2925, 1644, 1380, 1072 cm^{-1}. MS (ESI-TOF) *m/z*: 546/548 (3:1) $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 546.2533 calcd for $C_{27}H_{42}N_3O_3S^{35}ClNa$; Found: 546.2543.

(4*S*,6*R*)-Biakamide A

Using the same procedures as those for the synthesis of the (4*R*,6*S*)-isomer, (4*S*,6*R*)-biakamide A was obtained starting from (2*S*,4*S*)-5-((*tert*-Butyldiphenylsilyl)oxy)-2,4-dimethylpentan-1-ol. [41]

(4*S*,6*R*)-biakamide A: $[\alpha]_D^{28}$ +34.0 (*c* 0.70, $CHCl_3$). Mixture of rotamers. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.71 (1H, br s), 7.32 (1H, d, *J* = 2.4 Hz), 5.79 (0.4x1H, s), 5.77 (0.6x1H, s), 5.40 (1H, br), 4.86 (2H, s), 4.39 (0.4x1H, s), 4.38 (0.6x1H, s), 4.20-4.15 (1H, m), 3.35-3.27 (0.6x2H, m), 3.25-3.17 (0.4x2H, m), 3.02 (3H, br s), 2.94 (0.6x3H, s), 2.91 (0.4x3H, s), 2.55 (1H, br), 2.44 (1H, d, *J* = 16.8 Hz), 2.27 (1H, dd, *J* = 16.8, 9.6 Hz), 2.22-2.08 (2H, m), 2.06-2.00 (2H, m), 1.86 (3H, s), 1.70-1.58 (3H, m), 1.40 (2H, br), 1.34-1.10 (3H, m), 1.21 (3H, d, *J* = 6.0 Hz), 0.94 (3H, br), 0.88 (3H, br). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 173.6, 172.5, 172.4, 166.9, 142.2, 142.0, 141.4, 138.4, 128.9, 128.8, 120.1, 112.9, 112.4, 64.2, 64.1, 52.6, 49.0, 48.4, 47.2, 44.1, 41.1, 40.5, 37.0, 35.2, 33.86, 33.83, 33.0, 32.1, 31.7, 30.5, 29.78, 29.76, 27.54, 27.49, 26.0, 25.1, 22.2, 22.1, 20.03, 20.00, 19.8, 14.2. IR (KBr): 3409, 2961, 1626, 1454, 1397 cm^{-1}. MS (ESI-TOF) *m/z*: 534/536 (3:1) $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 534.2533 calcd for

$C_{26}H_{42}N_3O_3S^{35}ClNa$; Found: 534.2533.

(4*S*,6*S*)-Biakamide A

Using the same procedures as those for the synthesis of the (4*R*,6*S*)-isomer, (4*S*,6*S*)-biakamide A was obtained starting from (2*R*,4*S*)-5-((*tert*-Butyldiphenylsilyl)oxy)-2,4-dimethylpentan-1-ol.[43]

(4*S*,6*S*)-biakamide A: $[\alpha]_D^{28}$ +32.5 (*c* 0.80, $CHCl_3$). Mixture of rotamers. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.72 (1H, br s), 7.32 (1H, br s), 5.77 (0.4x1H, s), 5.76 (0.6x1H, s), 5.37 (1H, br), 4.87 (2H, s), 4.40 (1H, br), 4.20-4.15 (1H, m), 3.35-3.30 (0.6x2H, m), 3.25-3.15 (0.4x2H, m), 3.03 (3H, br s), 2.94 (0.6x3H, s), 2.91 (0.4x3H, s), 2.57 (1H, br), 2.45 (1H, dd, *J* = 16.8, 2.4 Hz), 2.28 (1H, ddd, *J* = 16.8, 9.6, 2.4 Hz), 2.13 (2H, br), 2.06-1.97 (2H, m), 1.88 (3H, s), 1.70-1.58 (2H, m), 1.40-1.08 (5H, m), 1.19 (3H, d, *J* = 6.0 Hz), 0.97 (3H, d, *J* = 4.8 Hz), 0.89 (3H, br). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 173.6, 172.6, 172.4, 166.9, 142.2, 141.9, 141.4, 138.1, 129.4, 129.3, 120.1, 112.9, 112.4, 64.2, 64.1, 52.6, 49.0, 48.3, 47.2, 44.3, 41.1, 40.5, 37.0, 35.2, 34.7, 34.6, 33.1, 32.0, 31.7, 30.8, 29.99, 29.96, 27.6, 27.5, 26.0, 25.2, 22.3, 22.2, 21.0, 20.9, 19.4, 14.3. IR (KBr): 3435, 2925, 1627, 1454, 1396 cm^{-1}. MS (ESI-TOF) *m/z*: 534/536 (3:1) $[M+Na]^+$. HRMS (ESI-TOF) *m/z*: 534.2533 calcd for $C_{26}H_{42}N_3O_3S^{35}ClNa$; Found: 534.2548.

Biological evaluation of biakamides A-D

Materials

Dulbecco's Modified Eagle's medium (DMEM), WST-8 colorimetric reagent, and

KCN were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Fetal bovine serum (FBS) and Dialyzed FBS were purchased from Equitech-Bio Inc. (Kerrville, TX, USA) and Thermo Fisher Scientific Inc. (Waltham, MA, USA), respectively. Anti-Akt, Anti-phosphorylated Akt, anti-GRP78, and anti-β-tubulin antibodies were obtained from Cell Signaling Technology, Inc. (Danvers, MA, USA). Horseradish peroxidase (HRP)-linked anti-rabbit IgG antibody (GE Healthcare Life Sciences, Buckinghamshire, UK) was used as secondary antibody. Mito Check Complex Activity Assay Kit (Cayman Chemical, Ann Arbor, MI, USA) was used to evaluate the effect of biakamide C (**3**) on the mitochondrial complex I–V. Rotenone, thenoyltrifluoroacetone (TTFA), antimycin A, and oligomycin mixture were obtained from Tokyo Chemical Industry Co., LTD. (Tokyo, Japan), Wako Pure Chemical Industries, Ltd. (Osaka, Japan), LKT Laboratories, Inc. (St. Paul, MN, USA), and Cayman Chemical (Ann Arbor, MI, USA), respectively. Other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Kishida Chemical Co., Ltd. (Osaka, Japan).

Cell cultures

Human pancreatic cancer cell line PANC-1 was cultured in DMEM supplemented with heat-inactivated 10% fetal bovine serum (FBS) and kanamycin (50 μg/mL) in a humidified atmosphere of 5% CO_2 at 37 °C. In the case of the conditions of nutrient starvation, PANC-1 cells was cultured in the Glucose Deficient Medium [Basal Medium (25 mM HEPES buffer (pH 7.4) supplemented with 6.4 g/L NaCl, 700 mg/L $NaHCO_3$, 400 mg/L KCl, 265 mg/L $CaCl_2$•$2H_2O$, 200 mg/L $MgSO_4$•$7H_2O$, 125 mg/L NaH_2PO_4,

0.1 mg/L $Fe(NO_3)•9H_2O$, 15 mg/L Phenol red, 10 mL/L MEM vitamin solution (×100) (GIBCO, Carlsbad, CA), 200 mmol/L L-glutamine solution (GIBCO, Carlsbad, CA), 25 mg/L kanamycin) containing 10% dialyzed FBS]. The General Glucose Medium [Basal Medium supplemented with 10% FBS and 2.0 g/L glucose (final 25 mM)] was also used for bioassay as the general culture conditions to compare the activity of the sample under the conditions of nutrient starvation.

Assay for growth inhibitory activity under glucose-deprived condition

The PANC-1 cells in the culture medium were plated into each well of 96-well plates (1×10^4 cells/well/100 μL) for 12 h in a humidified atmosphere of 5% CO_2 at 37 °C. After removal of the medium, the cells in each well were rinsed with PBS twice. Then, the plates were incubated in either General Glucose Medium or Glucose Deficient Medium inducing adaptation toward austerity. After 12 h incubation, testing compounds were added as an EtOH solution (1 μL), and then the plates were incubated for an additional 12 h. The anti-proliferative activities of the testing compounds under the respective conditions were evaluated by colorimetric assay using WST-8 reagent, and the growth inhibition rate was calculated as percentage of parallel negative controls.

Western blotting analysis

PANC-1 cells (3.0×10^5 cells/2 mL in 6 well plastic plate) were pre-incubated in the DMEM supplement with 10 % FBS for 24 h. The medium was then replaced with either General Glucose or Glucose Deficient Medium. After 12 h incubation, biakamide C (**3**,

0.5 μM) or antimycin A (3.0 nM) as a positive control was added, and the cells were incubated for additional 12 h in a humidified atmosphere of 5 % CO_2 at 37 °C. Then, the cells were rinsed with ice-cold PBS and lysed in the lysis buffer (50 mM Tris–HCl (pH 8.0) containing 137 mM NaCl, 5 mM EDTA-2Na, 0.1% NP-40, 1% glycerol, 1% protease inhibitor cocktail, and 1% phosphatase inhibitor cocktail). The cell lysate was subjected to SDS–PAGE and transferred onto PVDF membranes (GE Healthcare Life Sciences Buckinghamshire, UK). The membranes were then incubated with appropriate primary antibodies and HRP-conjugated secondary antibodies, and the immunopositive bands were visualized using an ECL kit (GE Healthcare Life Sciences). The luminescent signals were analyzed using an ImageQuant LAS4010 Scanner (GE Healthcare Life Sciences).

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Supporting Information Available

^{1}H and ^{13}C NMR signal assignments of biakamides B (**2**), C (**3**), and D (**4**), 1D and 2D NMR spectra of the biakamides A–D (**1**–**4**) and synthetic compounds **1**–**20**, and the result of western blot analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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