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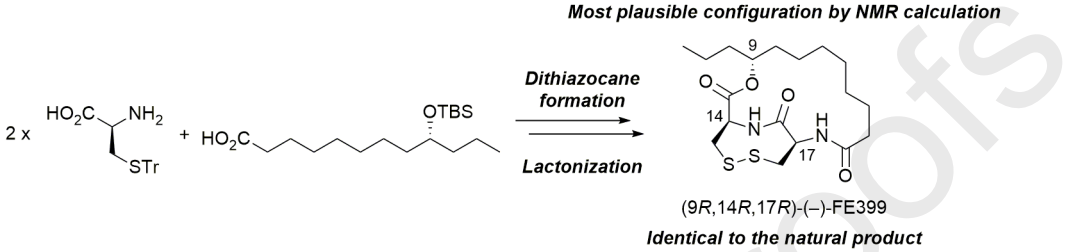

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Synthesis and Stereochemistry of (–)-FE399

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ABSTRACT

The stereochemistry of selective anticancer compound FE399 was determined to be *rel*-9*R*,14*R*,17*R* by theoretical and synthetic studies. Relative stereochemistry of FE399 was predicted by comparison of the ^{13}C NMR chemical shifts of the natural sample with that predicted by theoretical calculation for each possible stereoisomer. The first synthesis of (9*R*,14*R*,17*R*)-(–)-FE399 was achieved using an amide formation of a dithiazocane with a hydroxy dodecanoic acid derivatives and sixteen-membered macrolactonization as key steps. The overall yield was 18% in ten steps from L-cysteine and (*S*)-glycidyl tosylate.

Introduction

Antitumor compound FE399 (**1**) has been isolated from the fermentation broth of an endophytic filamentous fungus *Ascochyta* sp. AJ117309 by researchers from Ajinomoto Co., Inc.[1] Selective apoptosis-inducing activity against several cancer cell lines with a mutation in the *p53* gene has been reported. Mutations that cause inactivation of the p53 tumor suppressor protein network are often present in many malignant tumor cells; thus, a selective cytotoxic compound against those cell lines would be a potent lead compound for alternative anticancer chemotherapy.[2] Additionally, p53-mediated DNA damage response limits reprogramming to several stem cells.[3] FE399 is a structurally unique depsipeptide that has three chiral centers, as shown in Fig. 1. Two cysteines and a hydroxylated dodecanoic acid assemble into a bicyclic system containing a dithiazocane and a sixteen-membered lactone, and it has been reported to show at least two separable conformers in solution. However, only the planar structure of FE399 (**1**) was determined. To our knowledge, only a few structurally related compounds to FE399 has been reported, including the hydroxylated derivative, PM181110 (**2**)[4], and malformins A_1 (**3**) to C.[5] These compounds has also been reported to exhibit anticancer activities.[4,5] Significant biological activity and the structural features of FE399 allow us to determine its stereochemistry by theoretical NMR prediction and synthesis.

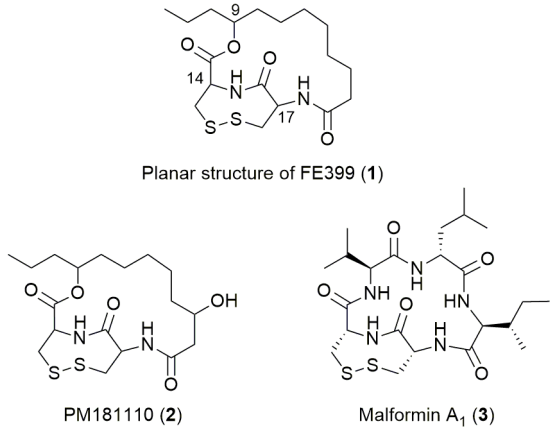

Figure 1. Structures of FE399 (**1**) and related compounds.

To estimate the relative stereochemistry of **1**, we performed theoretical NMR calculations of isomers. Because FE399 contains three chiral carbon atoms, four diastereomers, 9*R*,14*R*,17*R*, 9*R*,14*R*,17*S*, 9*R*,14*S*,17*R*, and 9*R*,14*S*,17*S*, are possible. The ^{13}C NMR chemical shifts of each diastereomer were predicted according to the method of the Hehre and Hashimoto group using Spartan'18 software (Wavefunction Inc., Irvine, CA, USA).[6,7] A conformational search using MMFF molecular mechanics, geometry optimization, NMR calculations with the ωB97X-D/6-31G* model, and the ωB97X-V/6-311+G(2df,2p)[6-311G*] energy calculation produced 20 to 31 low-energy conformers. On the other hand, the natural FE399 has been reported to show rotamers[1] and it was thought that is due to conformation of the dithiazocane moiety because conformational studies of related compounds suggested that dithiazocane conformers would be separately shown in solution NMR.[8] Thus,

giv ...IO_4

Reitz group, i.e. by amide geometry (*T*/*C*) and helicity about the S-S bond (+/–) on the dithiazocane moiety.[9,10] Then, the given ^{13}C chemical shifts of each conformer were individually corrected based on the Boltzmann distribution. A comparison of the natural and theoretical ^{13}C chemical shifts of FE399 is shown in Table 1. Within all calculated isomers, the both conformers of (9*R*,14*R*,17*R*)-isomer showed good agreement with the major isomer of the natural product (RMS($\delta_{nat.}$–$\delta_{calc.}$) < 2.0 ppm), whereas other isomers showed broad distinction (RMS > 3.0 ppm). This suggested that the relative stereochemistry of the natural FE399 is 9*R*,14*R*,17*R*. Because of the abundance of natural L-amino acids compared to the D-amino acids, we synthesized FE399 with the 9*R*,14*R*,17*R* configuration.

Our synthetic strategy for (9*R*,14*R*,17*R*)-isomer (**4**) was as follows. Because eight-membered rings are known to be strained, the construction of dithiazocane should be performed without further constraint due to the extra bridged ring. Accordingly, a sixteen-membered ring was planned for construction during the late stage of the synthesis, following the acylation of aminodithiazocane with a chiral carboxylic acid.

The synthesis of the dithiazocane and carboxylic acid are illustrated in Scheme 1. Commercially available H-Cys(Trt)-OH (**5**) was converted into aminoester **6** and *N*-Teoc derivative **7**. These materials were reacted under EDC/HOBt conditions to produce the protected dicysteine **8**.[11] Simultaneous detritylation and disulfide formation of **8** proceeded by treatment with iodine at a 3 mM substrate concentration to afford dithiazocane **9**. Performing the reaction using the dicholoromethane-methanol co-solvent system was essential to avoid the formation of oligomeric disulfides.[12,13] Subsequently, the protected 9-hydroxyundecanoic acid was synthesized. (*S*)-Glycidyl tosylate (**10**) was converted into hydroxytosylate **11** by addition of the cuprate generated from 8-bromo-oct-1-ene. Compound **11** was further treated with excess C_2 cuprate to produce alcohol **12**. Protection of the secondary hydroxy group with the TBS group produced the desired chiral carboxylic acid **14**.

The synthesis of FE399 via acylation and lactonization is illustrated in Scheme 2. After removal of the Teocgroup of **9** by treatment with excess trifluoroacetic acid (TFA), the corresponding TFA salt was smoothly acylated with acid **14** under EDC/HOBt conditions.[11,14] For the hydrolysis of the methyl ester of **15**, basic saponification conditions involving aqueous LiOH resulted in decomposition of the disulfide moiety.[15] However, the reaction using Me_3SnOH effectively proseeded to afford carboxylic acid **16**.[16] The TBS group of **16** was removed under acidic conditions. Finally, the corresponding hydroxy acid, **17**, was cyclized in the presence of 2-methyl-6-nitrobenzoic anhydride/DMAP[17] at 1 mM substrate concentration to produce (9*R*,14*R*,17*R*)-(–)-FE399 (**4**).[18]

As reported for the natural FE399, the synthetic material was also observed as a mixture of conformers in DMSO-d_6 solution.[1,18,19] As shown in Table 2, the ^{13}C NMR chemical shifts for the major conformer were identical to those of the natural product, and the stereochemistry of the natural FE399 was unambiguously determined to be *rel*-9*R*,14*R*,17*R*. Of note, no information regarding the absolute configuration of FE399, such as the specific rotations or circular dichroism, has been previously reported. That makes determination of the absolute stereochemistry of FE399 at this stage difficult.

In conclusion, the first synthesis of (–)-FE399 was achieved via amide formation of a dithiazocane with a hydroxy dodecanoic acid derivative and macrolactonization as key steps. The overall yield was 18% in ten steps starting from L-cysteine and (*S*)-glycidyl tosylate. The stereochemistry of natural FE399 was determined to be *rel*-9*R*,14*R*,17*R* via comparison of the spectral data of natural FE399 and with the synthetic material. Determination of the absolute configuration, conformational analyses of the rotamers, and synthesis of related natural products are under way.

Table 1. Comparison of selected ^{13}C chemical shifts (δ, ppm) of natural and calculated FE399 isomers

Position[a]	Natural[b,1]	Calculated							
Stereochemistry		9*R*,14*R*,17*R*		9*R*,14*R*,17*S*		9*R*,14*S*,17*R*		9*R*,14*S*,17*S*	
Conformation[c,9]		*T'*–	*C*+	*T*+	*C*–	*T*+	*C*–	*T*+	*C*–
Relative energy (kJ/mol)[d]		±0.0	+1.6	±0.0	+4.7	±0.0	+0.3	±0.0	+1.2
1	173.1	174.0	174.0	174.7	177.7	176.1	169.5	176.1	170.5
2	34.9	36.8	37.0	34.6	36.3	37.8	32.6	38.4	35.1
9	74.2	75.7	78.1	78.1	78.1	75.9	78.4	75.1	81.0
10	36.0	33.0	35.4	34.3	34.8	33.3	30.6	29.5	33.2
11	18.3	18.9	19.3	16.2	18.2	17.8	14.9	18.9	20.2
13	170.3	170.5	171.7	171.2	170.2	171.5	169.8	170.9	170.2
14	53.3	56.8	54.7	54.7	60.4	55.8	57.5	56.0	58.5
15	44.1	43.6	39.6	44.5	42.1	42.2	41.7	42.4	44.8
16	174.8	176.1	173.4	172.4	174.3	172.8	174.7	172.3	175.5
17	52.6	50.5	52.8	61.7	49.0	61.3	52.0	61.6	52.4
18	47.4	52.1	43.9	41.9	38.2	42.9	38.1	42.6	39.0
RMS ($\delta_{nat.}$-$\delta_{calc.}$)		1.95	1.99	3.08	3.41	2.88	3.87	3.35	3.43
Max. abs. err.		4.7	3.9	9.1	9.2	8.7	9.3	9.0	8.4

[a] Numbers of positions are according to ref 1; see Scheme 2

[b] 150 MHz in DMSO-d_6

[c] Two low energy dithiazocane conformers per each diastereomer are shown.

[d] Relative energies indicate energy distance from the global minimum of each diastereomer.

Table 2. Comparison of the ^{13}C chemical shifts (δ, ppm) of natural and synthetic (9*R*,14*R*,17*R*)-(–)-FE399 (4)

Position[a]	Natural[b]	Synthetic[b]
1	173.1	173.1
2	34.9	34.9
3	24.8	24.8
4	26.6	26.6
5	26.6	26.6
6	26.9	26.9
7	21.6	21.6
8	32.3	32.3
9	74.2	74.1
10	36.0	36.0
11	18.3	18.3
12	13.7	13.7
13	170.3	170.3
14	53.3	53.3
15	44.1	44.1
16	174.8	174.8
17	52.6	52.6
18	47.4	47.4

[a] Numbers of positions are according to ref 1; see Scheme 2

[b] 150 MHz in DMSO-d_6

Acknowledgments

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13. With another solvent (e.g.: MeOH, CH_2Cl_2, toluene), corresponding trimer and tetramer were observed in this reaction, which was confirmed by MS spectra.
14. Acylation of aminodithiazocane **9**: To a solution of dithiazocane **9** (200 mg, 0.526 mmol) in CH_2Cl_2 (20 mL) was added trifluoroacetic acid (1.00 mL). After being stirred for 90 min., the reaction mixture was diluted with toluene and concentrated in vacuo. Corresponding crude TFA salt (c.a. 200 mg) was dissolved in DMF (15 mL). Under argon atmosphere, carboxylic acid **14** (173 mg, 0.526 mmol), EDC•HCl (303 mg, 1.58 mmol) and HOBt (142 mg, 1.05 mmol) were added successively to the solution of the crude amide. The reaction mixture was stirred at ambient temperature for 17.5 h and poured into ice cold water. It was extracted with EtOAc, and the combined organic layer was washed with water and brine, dried over anhydrous Na_2SO_4. After concentration in vacuo, the residue was chromatographed over silica gel. Elusion with hexanes:EtOAc (4:1 to 1:1) produced **15** (225 mg, 78% in two steps) as colorless amorphous solids.
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18. Synthesis of (9*R*,14*R*,17*R*)-(–)-FE399 (**4**): A mixture of carboxylic acid **16** (53.0 mg, 99.1 μmol), HCl-washed Amberlite® IR120 (300 mg) and acetone-water (2.5 mL, 10:1) was stirred for 2 days at ambient temperature and the reaction mixture was filtered and concentrated in vacuo. Resulting crude **17** (45 mg) was used to the next reaction without further purification. A solution of crude **17** in THF (100 mL) was slowly added to a solution of 2-methyl-6-nitrobenzoic anhydride (47.5 mg, 138 μmol), 4-DMAP (2.6 mg, 21 μmol) and triethylamine (29.5 μL, 212 μmol) during 12 h at ambient temperature. The mixture was stirred for 4 h at the same temperature and poured into ice cold saturated $NaHCO_3$ solution. It was extracted with EtOAc, dried over anhydrous Na_2SO_4. After concentration in vacuo, the residue was chromatographed over silica gel. Elusion with $CHCl_3$:MeOH (93:7) produced **4** (29.7 mg, 69% in two steps) as colorless amorphous solids.
$[\alpha]_D^{26} = -49$ (*c* 0.1, $CHCl_3$); IR (KBr): ν = 3427, 2927, 2858, 1719, 1670, 1637, 1536, 1250 cm^{-1}; major conformer: ^1H NMR (600 MHz, DMSO-d_6, approximately 5:3 mixture of conformers, δ) signals for major conformer: 0.84 (t, *J* = 7.4 Hz, 12-H, 3H), 1.05-1.35 (m, 4-H, 5-H, 6-H, 11-H, 8H), 1.15 (m, 7-Ha, 1H), 1.30-1.50 (m, 8-H, 2H), 1.36 (m, 7-Hb, 1H), 1.41 (m, 10-Ha, 1H), 1.43 (m, 3-Ha, 1H), 1.48 (m, 10-Hb, 1H), 1.57 (m, 3-Hb, 1H), 2.09 (m, 2-Ha, 1H), 2.20 (m, 2-Hb, 1H), 3.19 (m, 18-Ha, 1H), 3.22 (m, 15-Ha, 1H), 3.42 (dd, *J* = 14.2, 10.5 Hz, 15-Hb, 1H), 3.43 (dd, *J* = 14.2, 2.4 Hz, 18-Hb, 1H), 4.14 (m, 17-H, 1H), 4.82 (m, 9-H, 1H), 4.83 (m, 14-H, 1H), 7.74 (d, *J* = 10.3 Hz, 14-NH, 1H), 8.09 (br, 17-NH, 1H); signals for minor conformer: 0.84 (t, *J* = 7.4 Hz, 12-H, 3H), 1.02 (m, 7-Ha, 1H), 1.05-1.35 (m, 4-H, 5-H, 6-H, 8-Ha, 11-H, 9H), 1.35-1.55 (m, 3-H, 7-Hb, 8-Hb, 10-H, 6H), 1.95 (dt, *J* = 12.6, 5.2 Hz, 2-Ha, 1H), 2.22 (m, 2-Hb, 1H), 3.06 (brd, *J* = 15.4 Hz, 15-Ha, 1H), 3.20 (m, 18-Ha, 1H), 3.50-3.65 (m, 15-Hb, 18-Hb, 2H), 3.93 (m, 17-H, 1H), 4.80-4.90 (m, 9-H, 14-H, 2H), 8.02 (d, *J* = 8.6 Hz, 14-NH, 1H), 8.81 (br, 17-NH, 1H);^{13}C NMR (150 MHz, DMSO-d_6, δ) signals for major conformer: 13.7, 18.3, 21.6, 24.8, 26.6, 26.6, 26.9, 32.3, 34.9, 36.0, 44.1, 47.4, 52.6, 53.3, 74.1, 170.3, 173.1, 174.8; signals for minor conformer: 13.8, 18.3, 22.6, 25.2, 26.2, 27.8, 27.8, 33.4, 35.3, 35.6, 42.7, 47.9, 52.1, 70.2, 172.0, 173.4, two of signals for dithiazocane consisting carbon were not found probably due to conformational flexibility or overlapping to other signals; ESI-TOFMS *m/z* calcd. For $C_{18}H_{30}N_2O_5S_2Na^+$ $[M+Na]^+$ 425.1539 found 425.1542.
The ratio of two conformers was determined by integration of 17-H (4.14 ppm for major conformer and 3.93 ppm for minor comformer).

corresponding to conformers (Kanto Chemical co.,inc. Mightysil RP-18 GP, 250 mm x 4.6 Φ, eluent: 55% CH_3CN/H_2O, flow rate 0.8 mL/min.,10.5 min. for major and 13.2 min. for minor conformers). Peak for the minor conformer sometimes splitted into two. A separation of conformers were also achieved using the avobe conditions. However separated rotamers were immediately reached equilibrium of conformers as a DMSO-d_6 solution. In contrast to this the isomerisation could be partially reduced during ^{1}H NMR time scale at 20 °C as a CD_3CN solution.

Supplementary Material

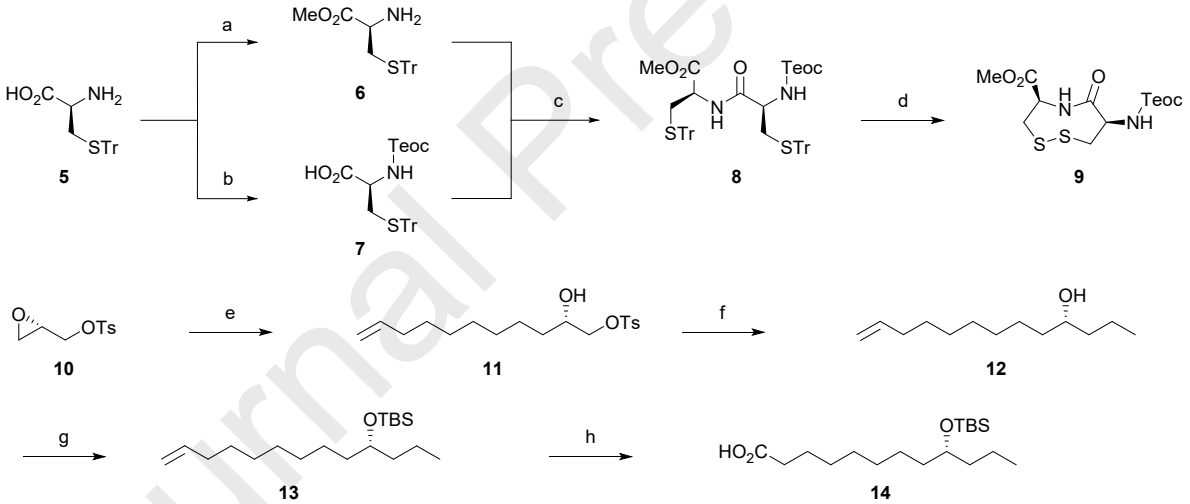
Supplementary data (NMR spectra and HPLC chromatogram of synthetic (9*R*,14*R*,17*R*)-(–)-FE399, cartesian coordinate, energy and ^{13}C chemical shift of calculated (9*R*,14*R*,17*R*)-FE399 conformers) associated with this article can be found, in the online version, at https://doi.org/XXX. These data include MOL

this article.

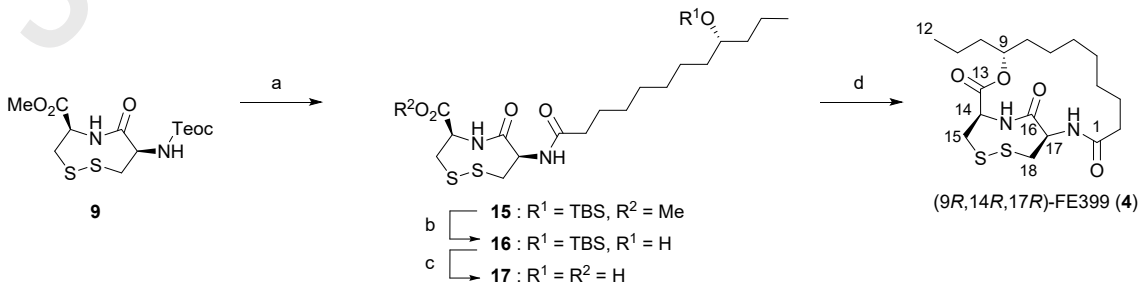
Determination of the relative stereochemistry of FE399 using theoretical and synthetic methods

Efficient construction of the unique bicyclic system containing dithiazocane and sixteen-membered lactone

The first synthesis of (–)-FE399



Scheme 1. Synthesis of **11** and **15**. Reagents and conditions: a) $SOCl_2$, MeOH, quant.; b) TeocOSu, Et_3N, H_2O, 1,4-dioxane, 79%; c) EDC•HCl, HOBt, Et_3N, DMF, 96%; d) I_2, CH_2Cl_2/MeOH (10/1), 3 mM, 77%; e) Mg, 8-bromooct-1-ene, Li_2CuCl_4, THF, –78 °C to –50 °C 75%; f) Mg, EtBr, CuCl, Et_2O, THF, –30 °C 74%; g) TBSOTf, 2,6-lutidine, CH_2Cl_2, 97%; h) $KMnO_4$, $NaIO_4$, phosphate buffer (pH 7.2), *t*-BuOH, 71%



Scheme 2. Synthesis of FE399 epimers. Reagents and conditions: a) TFA, CH_2Cl_2, then **14**, EDC•HCl, HOBt, Et_3N, DMF, 78% in 2 steps; b) Me_3SnOH, $(ClCH_2)_2$, 60 °C, 89%; c) Amberlite® IR120-H, acetone, H_2O; d) 2-methyl-6-nitrobenzoic anhydride, Et_3N, 4-DMAP, THF, 69% in 2 steps