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Synthesis of Thelepamide via Catalyst-Controlled 1,4-Addition of Cysteine Derivatives and Structure Revision of Thelepamide

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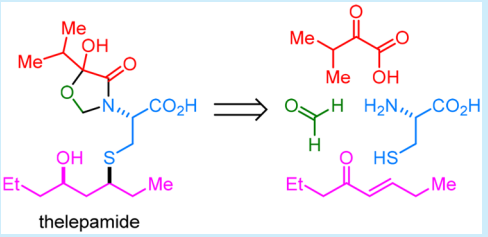
Supporting Information

ABSTRACT: The first enantioselective total synthesis and structural reassignment of (−)-thelepamide, a cytotoxic tetraketide−amino acid from the marine worm *Thelepus crispus*, is reported. A convergent approach provides access to all thelepamide diastereomers in six steps from four simple building blocks. Key features of the synthesis include the application of Melchiorre's organocatalytic thia-Michael reaction and a sonication-assisted assembly of an unprecedented *N,O*-acetal−hemiacetal moiety. The corrected structure was confirmed by NMR−DFT analysis.



Marine sponges and tunicates are an important source of structurally diverse bioactive secondary metabolites.[1] In 2013, the Rodríguez group[2] reported the isolation of (−)-thelepamide from the tidal zone-derived marine worm *Thelepus crispus*. Thelepamide (**1**) possesses an unprecedented *N,O*-acetal−hemiacetal motif that caught our interest as novel Val-Cys peptide mimetic.[3] While thelepamide's carbon backbone is identical to that of spongiacysteine (**2**), isolated by Kigoshi and co-workers[4] from a marine sponge, both molecules differ in the oxidation pattern at C2, C2″ and the *N*-methyl group (Figure 1).

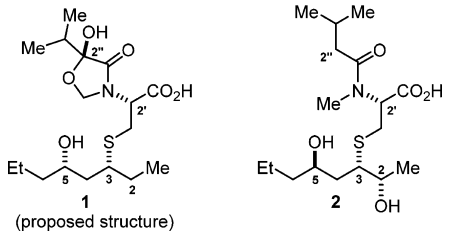


Figure 1. Structures of (−)-thelepamide (**1**: proposed) and spongiacysteine (**2**).

The relative configurations of thelepamide's four stereogenic centers were established on the basis of ROESY, J-based configurational analysis, and by comparison of calculated and experimental NMR shifts using Goodman's DP4 method.[5] Motivated by the unique 1,3-oxazolidin-2-one, the selective yet moderate biological activity against the leukemia cell line CCRF-CEM (IC_{50} = 13.2 μM), we decided to pursue its chemical synthesis.

In order to validate the structural assignment and probe structure−activity by synthetic analogues, a flexible synthetic access to thelepamide was sought. We identified the carbon−sulfur bond at C3 in thelepamide as the key disconnection between the tetraketide part of the molecule and a suitable cysteine derivative. In synthetic direction, two approaches to form this bond in a stereoselective fashion were considered. First, an S_N2 substitution of a tosylate leaving group under inversion of configuration was investigated. As a second option, we envisaged an asymmetric catalyst-controlled thia-Michael addition[6] of cysteine derivatives to (*E*)-oct-5-en-4-one and a subsequent diastereoselective reduction of the keto group.

The first synthetic route commenced with a Ru-catalyzed reduction[7] of β-ketoester **3** followed by Weinreb amide formation[8] to afford β-hydroxyamide **4** in 70% yield over two steps and 99% ee (Scheme 1). This material was subjected to ethylmagnesium bromide in Et_2O at 0 °C to give hydroxy ketone **5** in 64% yield.[9] The C3 stereogenic center was generated using an Evans−Tishchenko reduction.[10] The expected *anti*-configuration in **6** was confirmed using Rychnovsky's acetonide method.[11] After the free hydroxyl group had been converted to the corresponding tosylate **7**,[12] we carried out S_N2 reactions with D- and L-*N*-Boc-Cys-OMe. Due to partial epimerization at C2′ during the S_N2-reaction and problems occurring in the subsequent steps toward thelepamide, we decided to abandon this route in favor of the diastereoselective thia-Michael approach. Nevertheless, thioethers **9** and **10** turned out to be useful as reference standards for the following transformations.

In our second-generation approach, the synthetic sequence commenced with a stereoselective 1,4-addition of L-**8** to known enone **11**[13] (Table 1). Enantioselective thia-Michael additions have been studied by Wynberg since the late 1970s.[14] For our substrate, 20 mol % of unmodified cinchona alkaloids **12a−d** gave only moderate selectivities using dichloromethane as the solvent (entries 1−4). With Takemoto's thiourea catalyst **12e**[15]

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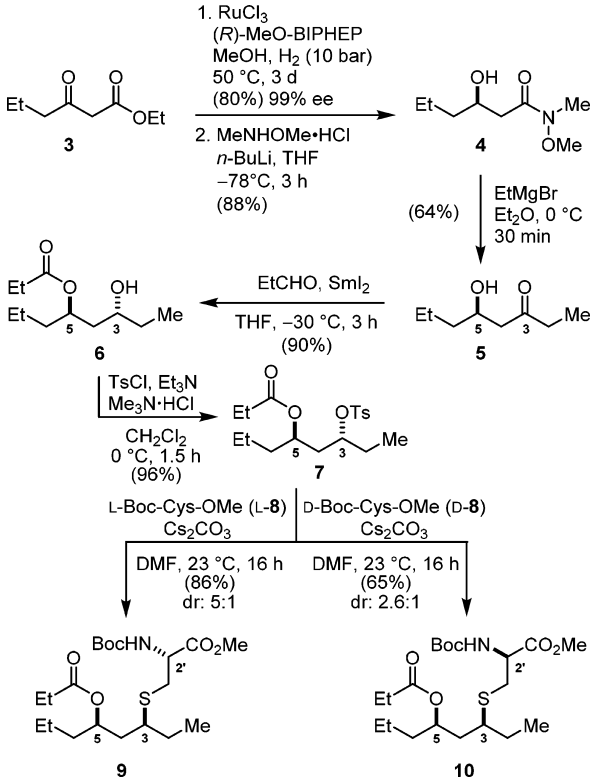
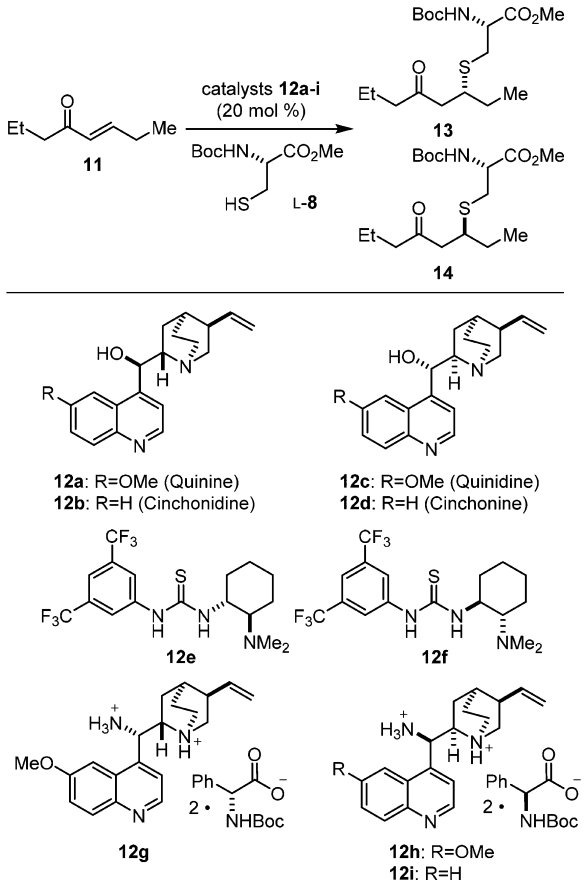
Scheme 1. First-Generation Route to Thelepamide



under the same conditions, the selectivity increased to 6.6:1 in favor of **13** (entry 5).[16] Surprisingly, using the enantiomeric Takemoto catalyst **12f**, no selectivity toward **14** could be induced (entry 6). In 2008, Melchiorre[17] reported that 1:2 adducts of cinchona alkaloid amines[18] and *N*-Boc-phenylglycine (Phg) derivatives[19] provide superior selectivities in thia-Michael reactions. Using their conditions at 0 °C provided **13** with 12.9:1 selectivity (entry 7). Lowering the temperature to −28 °C increased the selectivity to 24.5:1 but also raised the reaction time to 168 h (entry 8). Changing the amino acid to its enantiomer lowered the selectivity to 16.7:1, demonstrating its minor yet relevant influence (entry 9). Additionally, and in order to gain access to **14**, we screened the catalysts based on the pseudoenantiomeric cinchona alkaloids. Catalyst **12h** provided **14** in 9.3:1 ratio at 0 °C which increased to 12.9:1 at −28 °C (entries 10 and 11). The cinchonine-based catalyst **12i** gave **14** in 12:1 selectivity at 0 °C and 17.4:1 at −28 °C (entries 12 and 13). Again, the mismatching alkaloid−Phg combination lowered the selectivity to a 5:1 ratio (entry 14). On a preparative scale, **13** and **14** were obtained in 98% and 99% yield, respectively. It should be noted that this approach might also be useful in the stereoselective preparation of cysteine−S conjugates, which play an important role as precursor of plant aromas as well as in the formation of natural scents of cats and humans.[20]

Having developed a catalyst-controlled access to both diastereomers **13** and **14**, we then turned our attention to the diastereoselective reduction of the keto group and the completion of the synthesis of the proposed stereoisomer of thelepamide. As shown in Scheme 2, diastereoselective reduction of *β*-substituted ketone **13** with zinc borohydride[21] yielded a separable 3.8:1 mixture (89%). The relative and absolute configuration of the minor diastereomer **15a** was confirmed by X-ray-crystallography. The major diastereomer **15b** was acylated with propionic acid using the Steglich protocol[22] to give *ent*-**10**,

Table 1. Optimization of the Thia-Michael Addition[a]



entry	cat.	solvent	temp (°C)	time[b] (h)	dr (**13**:**14**)[c]
1	**12a**	CH_2Cl_2	23	18	1.9:1.0
2	**12b**	CH_2Cl_2	23	18	2.3:1.0
3	**12c**	CH_2Cl_2	23	18	1.0:1.2
4	**12d**	CH_2Cl_2	23	18	1.0:1.7
5	**12e**	CH_2Cl_2	23	30	6.6:1.0
6	**12f**	CH_2Cl_2	23	30	1.0:1.1
7	**12g**	PhMe	0	35	12.9:1.0
8	**12g**	**PhMe**	**−28**	**168**	**24.5:1.0**
9	**12g**[d]	PhMe	−28	168	16.7:1.0
10	**12h**	PhMe	0	35	1.0:9.3
11	**12h**	PhMe	−28	168	1.0:12.9
12	**12i**	PhMe	0	35	1.0:12.0
13	**12i**	**PhMe**	**−28**	**168**	**1.0:17.4**
14	**12i**[e]	PhMe	−28	168	1.0:5.0

[a]Reaction conditions (entries 1−6): 1.10 equiv of **11**, 1.00 equiv of L-**8**, cat. (20 mol %), CH_2Cl_2 (0.10 M), Reaction conditions (entries 7−14): 2.00 equiv of **11**, 1.00 equiv L-**8**, cat. (20 mol %), PhMe (0.24 M). [b]Reaction was run until starting material L-**8** was fully consumed. [c]The diastereomeric ratio was determined via GC analysis. [d]Reaction was carried out with L-*N*-Boc-phenylglycine. [e]Reaction was carried out with D-*N*-Boc-phenylglycine.

the enantiomer of **10** obtained in the initial route (Scheme 1). The Boc group of **15b** was removed with TFA in CH_2Cl_2 (**15b** → **16b**), followed by acylation with 2-ketoisovaleric acid to give *α*-ketoamide **17b**. Subsequently, methyl ester cleavage with lithium hydroxide afforded carboxylic acid **18b** in 75% yield. The reaction was accompanied by epimerization at C2′. Formation of the 5-hydroxyoxazolidin-4-one was achieved via *N*,*O*-acetal formation with formaldehyde by sonicating the reaction under

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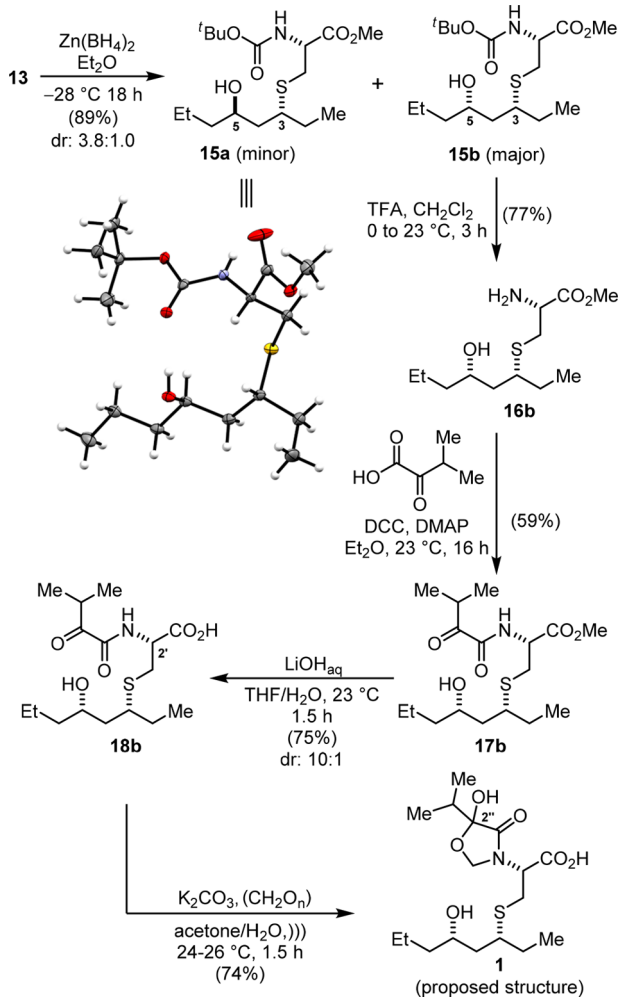
Scheme 2. Second-Generation Route to Thelepamide



basic conditions.[23] At this step it was necessary to maintain the reaction between 24 and 26 °C in order to suppress the formation of side products. Thus, the proposed structure of thelepamide (**1**) was obtained in 19% yield over six steps as 1:1 diastereomeric mixture at C2″.

In an analogous fashion to the route described above, 3,5-*epi*-thelepamide (3,5-*epi*-**1**) obtained from **14** and 5-*epi*-thelepamide (5-*epi*-**1**) obtained from **15a** were synthesized in six steps with 23% and 9% overall yield, respectively (Figure 2). Surprisingly, a comparison of the original thelepamide spectra with those of all obtained diastereomers showed a clear mismatch in both the ^{1}H and ^{13}C NMR spectra. The differences manifested in the magnitude of the chemical shifts as well as the diastereomeric

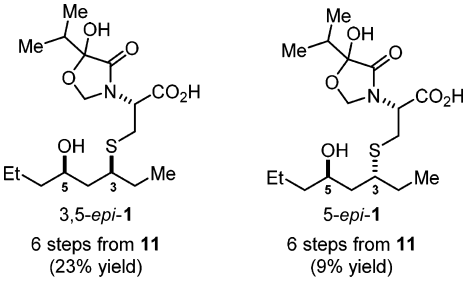


Figure 2. Structures of thelepamide diastereomers 3,5-*epi*-**1** and 5-*epi*-**1**.

ratios. From a previous project,[24] we were alerted that NMR spectra can be strongly affected by deprotonation of a carboxylic acid moiety. Gratifyingly, the ^{1}H NMR spectrum of 3,5-*epi*-**1** sodium carboxylate was superimposable with the original thelepamide spectrum.

In the ^{13}C NMR spectrum, the 44–71 ppm region was most indicative for comparing the thelepamide diastereomers (Figure 3). The sodium carboxylate of 3,5-*epi*-**1** and the reported

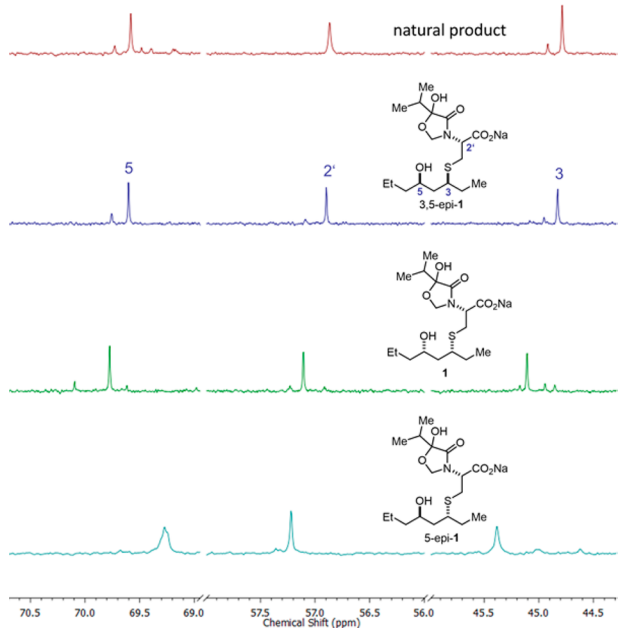
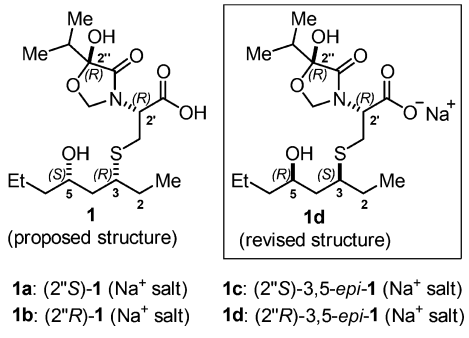


Figure 3. Comparison of the 44–71 ppm ^{13}C NMR spectral region of three synthetic thelepamide diastereomers with that of isolated material.

spectrum were nearly identical with an overall median ^{13}C NMR shift deviation of <0.1 ppm. These results were supported by HPLC comparison with an authentic thelepamide sample.[25]

While the previous DFT-NMR analysis[2] was carried out assuming that **1** was present as free carboxylic acid, we repeated the computational analysis with the corresponding carboxylates. The sodium carboxylates of four possible thelepamide diastereomers (**1a–d**, see Figure 4) were initially submitted to a conformational search with the Macromodel program using the protocol of Hoye et al.[26] Thus, 85 conformers for **1a**, 67 for **1b**, 90 for **1c**, and 51 for **1d** were found within a 3.0 kcal/mol window. All conformers were classified by energy and frequencies using the B3LYP[27]/6-31+G(d,p) functional. Finally,



1a: (2"*S*)-**1** (Na^+ salt)
1b: (2"*R*)-**1** (Na^+ salt)
1c: (2"*S*)-3,5-*epi*-**1** (Na^+ salt)
1d: (2"*R*)-3,5-*epi*-**1** (Na^+ salt)

Figure 4. Proposed thelepamide (**1**) and four diastereomeric carboxylates (**1a–d**) used in the DFT calculations.

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the combination MPW1PW91[28]/6-311+G(2d,p)-polarizable continuum model was used for proton and carbon chemical shift calculations.[29] The sets of ^{1}H and ^{13}C chemical shifts were compared by mean absolute error (MAE), R^2 of $\delta_{calcd}/\delta_{expt}$, by the linear regression of calculated (δ_{scaled}) and by the statistical DP4+ parameter developed by Sarotti and co-workers.[30] A 100% probability DP4+ value for both carbon and proton chemical shifts in favor to **1d** was in agreement with the synthetic 3,5-*epi*-**1** configuration.

Comparison of the optical rotation of (−)-thelepamide and 3,5-*epi*-**1** showed them to be equal in sign and magnitude further confirming the configuration to be 3*S*,5*R*,2′*R*. From the NMR data, we could not make a direct assignment of the C2″-configuration of the major thelepamide diastereomer. However, DFT calculations suggest the configuration to be 2″*R* (**1d**).

In order to probe the reported biological activity for thelepamide, the three synthetic diastereomers were tested against the CCRF-CEM cell line. Unfortunately, no cytotoxicity for concentrations up to 100 μM could be detected.

In conclusion, we have completed a short and efficient synthesis of (−)-thelepamide. Key steps include a catalyst controlled thia-Michael addition and a sonication-assisted oxazolidinone synthesis. Comparison of the spectra of three synthetic diastereomers with those of isolated material led to a revision of the structure of thelepamide from **1** to **1d**, the sodium carboxylate of 3,5-*epi*-**1**. In addition, DFT calculations support the revised structure by including the correct protonation state of pH-sensitive functional groups (e.g., carboxylic acid−carboxylate) in the computational analysis.[31]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b03706.

Procedures and spectroscopic data (PDF)

Accession Codes

CCDC 1587470 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare no competing financial interest.

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