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## SYNTHESIS AND EVALUATION OF DENDROAMIDE A AND THREE REGIOISOMERIC ANALOGS HAVING A REVERSED AZOLE RING AS P-GLYCOPROTEIN INHIBITORS

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**Abstract** – We have synthesized dendroamide A and three new analogs having a differently oriented azole moiety to reveal the effect of the orientation of the azole rings on the P-glycoprotein inhibitory activity. The appropriate assembly of modified azole units and dimers from natural-type azole units is examined. Although linear trimers including a modified thiazole unit were hard to cyclize compared with a derivative containing a modified oxazole unit, the yields of cyclization were successfully improved by changing the reaction conditions or the position of ring closure. Evaluation of P-glycoprotein inhibitory activity also reported.

### INTRODUCTION

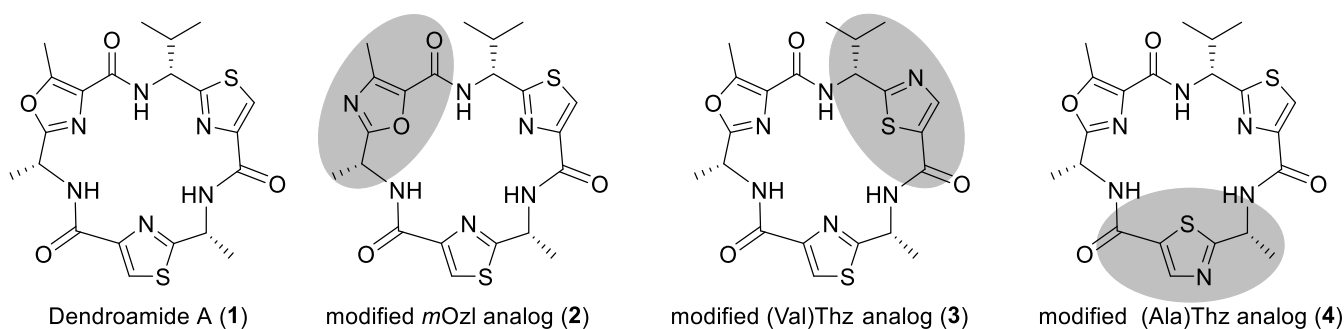
P-Glycoprotein (P-gp) is a transporter belonging to the adenosine triphosphate-binding cassette superfamily, which expels foreign substrates from the cell membrane. Overexpressing of P-gp in cancer cells is a major form of multidrug resistance (MDR).<sup>1</sup> This P-gp-mediated MDR can be reversed using dendroamide A (**1**), a cyclic hexapeptide isolated from terrestrial blue-green algae (cyanobacterium) in 1996 that exhibits inhibitory activity against P-gp.<sup>2</sup> This natural cyclic peptide is characterized by three kinds of azole amino acid subunits with (*R*)-absolute configuration, i.e., an alanine-based 5-methyloxazole and valine- and alanine-based thiazoles.

Because of its interesting biological activity, various total syntheses of dendroamide A have been accomplished.<sup>3</sup> Meanwhile, the synthesis of dendroamide A analogs has also been examined enthusiastically. These research efforts resulted in several developments. For instance, Smith filed a patent disclosing the synthesis of cyclic trimers comprising azole units having various side chains.<sup>4</sup>

Matsugi and coworkers synthesized all stereoisomers of dendroamide A.<sup>5</sup> Chang and coworkers synthesized a cyclic trimer of 1,3-selenazole bearing an isopropyl side chain and its enantiomer, reporting their cocrystal structures with P-gp. Interestingly, the analog having the same stereochemistry as dendroamide A was less potent than its enantiomer.<sup>1</sup> Talele and coworkers found that the thiazole isostere (cyclic trimer, QZ59S-SSS) are equipotent to the corresponding linear trimer, and they developed a novel thiazole-containing P-gp modulator.<sup>6</sup> These results indicate that the naturally occurring chemical structure is not always optimized, leaving room for improvement.<sup>7</sup>

The thiazole and oxazole rings of dendroamide A are produced by intramolecular condensation of the neighboring amide carbonyl group with a thiol or a hydroxy group in the amino acid side chain, respectively. Therefore, natural azole peptides possess azole rings bearing substituents at the C2 and C4 positions, thereby orienting all nitrogen atoms of the azole rings toward the inside of the macrocyclic ring. Thus, 2,5-substituted positional isomers of the thiazole amino acids have not been isolated from nature. Moreover, to the best of our knowledge, no research has reported the effect of the azole ring orientation on the P-gp inhibitory activity of dendroamide A.<sup>8</sup> This prompted us to synthesize isomers of dendroamide A having a 2,5-substituted azole ring to examine the effect of the orientation of the heterocyclic rings on the P-gp inhibitory activity. As the contiguous array of the hydrogen atom of the amides and the azole nitrogen with high electron density is considered to govern the rigidity and planarity of the molecules by intramolecular hydrogen bond,<sup>9</sup> the modification of the orientation of the azole ring would distort the macrocyclic ring that would affect the rigidity and planarity of the molecule.

Herein, we report a synthesis of dendroamide A (**1**) and three regioisomeric analogs **2–4**, which maintain all functionalities of dendroamide A except for a modified azole ring and evaluation of their P-gp inhibitory activities (Figure 1).

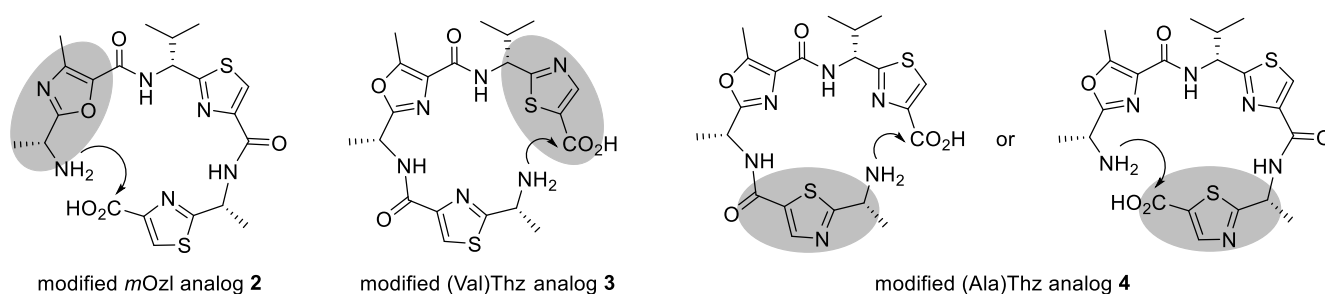


**Figure 1.** Dendroamide A and three kinds of analogs with a reversed azole ring

## RESULTS AND DISCUSSION

Following our previous work on the synthesis of 2,5-substituted oxazole<sup>10</sup> and 2,5-substituted thiazole<sup>11</sup> subunits, we then focused on assembling these units to synthesize the three analogs **2–4**, in which one of the azole amino acid units in dendroamide A was replaced with the corresponding 2,5-substituted positional isomer, respectively.

Figure 2 outlines our synthetic plan. After condensing two 2,4-disubstituted azole units (natural-type units), a 2,5-substituted azole unit (unnatural-type unit) was connected to the resulting dimer to avoid cyclization at the amino residue bearing a bulky isopropyl side chain.



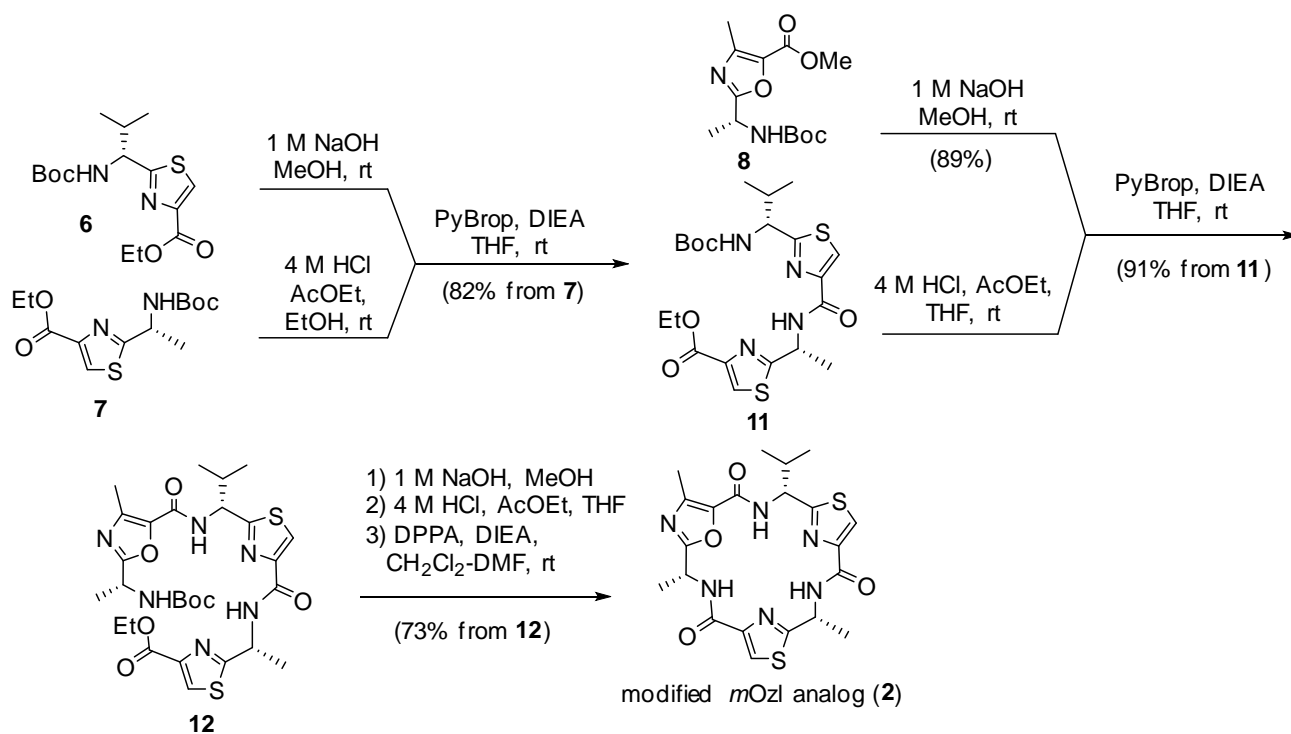
**Figure 2.** Macrocyclization precursors of dendroamide A analogs

2,4-Disubstituted oxazole unit **5**<sup>9,12</sup> and thiazole units **6** and **7**<sup>3e</sup> were synthesized according to reported procedures. 2,5-Disubstituted oxazole amino acid unit **8**<sup>10</sup> and thiazole units **9**,<sup>11a</sup> **10a**,<sup>11a</sup> and **10b**<sup>11b</sup> were synthesized following our previous reports.

### Chemistry

#### (1) Synthesis of dendroamide A analog **2** having a modified 5-methyloxazole (*mOzl*) moiety.

Thiazole units **6** and **7** were condensed with bromo-tris-pyrrolidinophosphonium hexafluorophosphate (PyBrop<sup>®</sup>) in the presence of 10 equiv of *N,N*-diisopropylethylamine (DIEA) after deprotecting the ethyl ester of **6** and the Boc group of **7** to give dimer **11**<sup>3e</sup> in 82% yield over two steps. Then, a screening of conditions for the condensation of dimer **11** and the modified oxazole unit **8** revealed that PyBrop<sup>®</sup> exhibited the best results among the condensing agents evaluated (Table 1).



**Scheme 1.** Synthesis of dendroamide A analog **2**

Thus, the modified oxazole unit **8** was hydrolyzed (89%) and coupled with the deprotected dimer to give linear trimer **12** in 91% yield over two steps (entry 1, Table 1).

The reaction using (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP<sup>®</sup>) afforded **12** in moderate yield (entry 2, Table 1), whereas the yields using 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate (HATU) or 1-hydroxy-7-azabenzotriazole (HOAt)/1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) were poor (entries 3 and 4, Table 1).

**Table 1.** Synthesis of trimer **12** containing the modified *mOzI* moiety

| Entry | Conditions <sup>a</sup>  | Yield (%) <sup>b</sup> |
|-------|--|------------------------|
| 1     | PyBroP (1.5 eq), DIEA (10 eq), THF   | 91                     |
| 2     | PyBOP (1.5 eq), DIEA (10 eq), THF  | 56                     |
| 3     | HATU (1.5 eq), DIEA (10 eq), THF   | 4                      |
| 4     | HOAt (1.5 eq), EDC·HCl (1.5 eq), DIEA (10 eq), CH <sub>2</sub> Cl <sub>2</sub> | 39                     |

<sup>a</sup> The reactions were conducted by adding DIEA and the condensing agent to a mixture of deprotected **8** and **11** with stirring at 0 °C. Then, the reaction mixture was stirred at room temperature (rt) for 2 h.

<sup>b</sup> Yields from dimer **11**.

The final macrolactamization of linear trimer **12** containing the modified oxazole moiety was then examined (Table 2). A high dilution method using a 2:1 mixture of CH<sub>2</sub>Cl<sub>2</sub> and DMF was applied. In this case, PyBrop<sup>®</sup> was not an effective agent for the macrolactamization (entry 1, Table 2). By contrast, PyBOP<sup>®</sup> and diphenylphosphoryl azide (DPPA) exhibited better results (entries 2 and 3, Table 2). Although their yields were comparable, DPPA was superior in terms of the purification of the product. Finally, the modified oxazole analog **2** was obtained with DPPA in CH<sub>2</sub>Cl<sub>2</sub>/DMF (3:1) in 73% yield over three steps (entry 4, Table 2).

**Table 2.** Synthesis of dendroamide A analog **2** containing the modified *mOzl* moiety

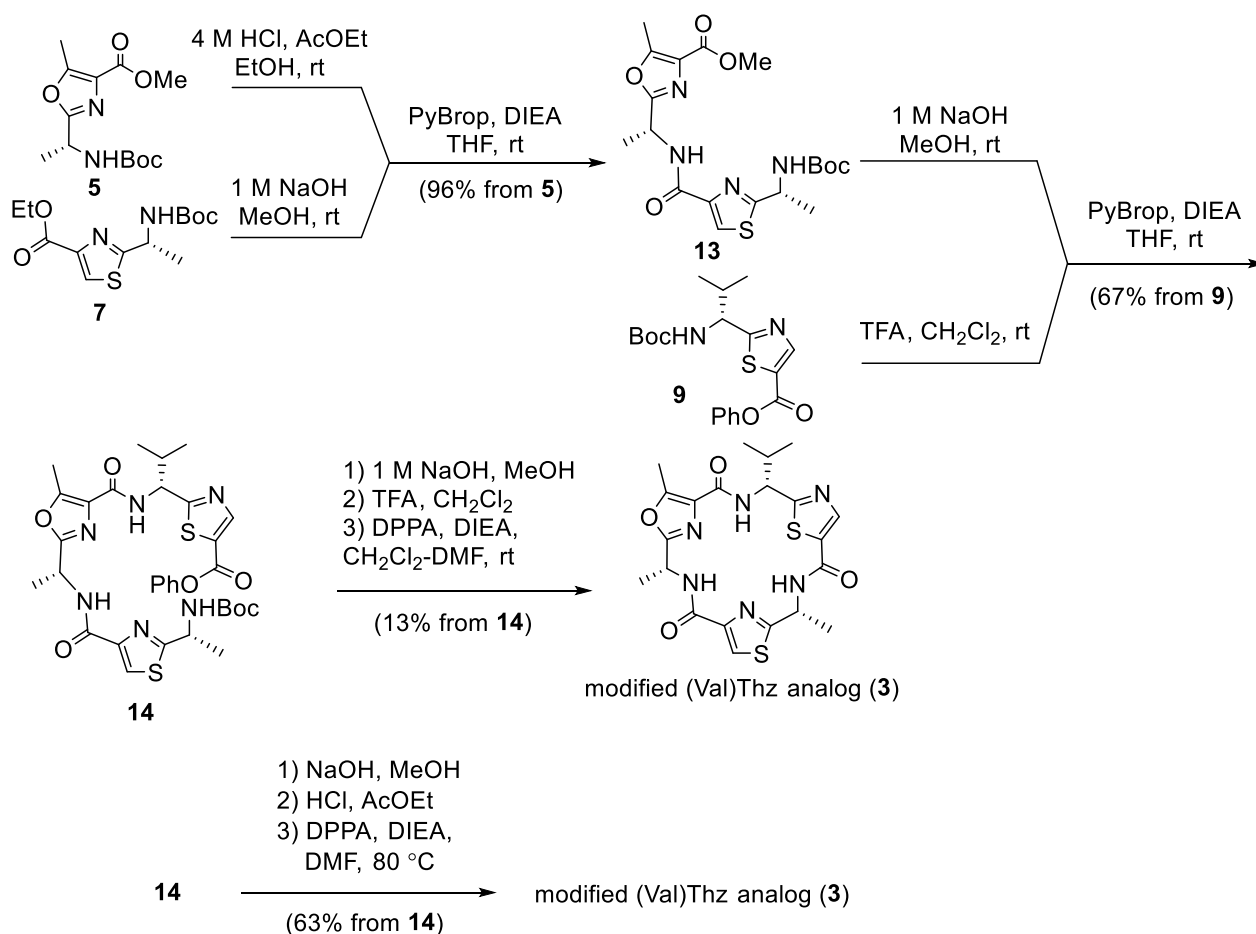
| Entry | Conditions <sup>a</sup>  | Yield (%) <sup>b</sup> |
|-------|--|------------------------|
| 1     | PyBroP (6.0 eq), DIEA (6.0 eq), CH <sub>2</sub> Cl <sub>2</sub> /DMF (2:1) | 20                     |
| 2     | PyBOP (6.0 eq), DIEA (6.0 eq), CH <sub>2</sub> Cl <sub>2</sub> /DMF (2:1)  | 63                     |
| 3     | DPPA (6.0 eq), DIEA (6.0 eq), CH <sub>2</sub> Cl <sub>2</sub> /DMF (2:1)   | 62                     |
| 4     | DPPA (6.0 eq), DIEA (6.0 eq), CH <sub>2</sub> Cl <sub>2</sub> /DMF (3:1)   | 73                     |

<sup>a</sup> The reactions were conducted by slow addition (3 h) of a solution of deprotected **12** in a CH<sub>2</sub>Cl<sub>2</sub>/DMF mixture to a solution containing the condensing agent and a base using a syringe pump. The reaction mixture was then stirred at rt for 2 h.

<sup>b</sup> Yields from trimer **12**.

## (2) Synthesis of dendroamide A analog **3** having a modified (Val)Thz moiety.

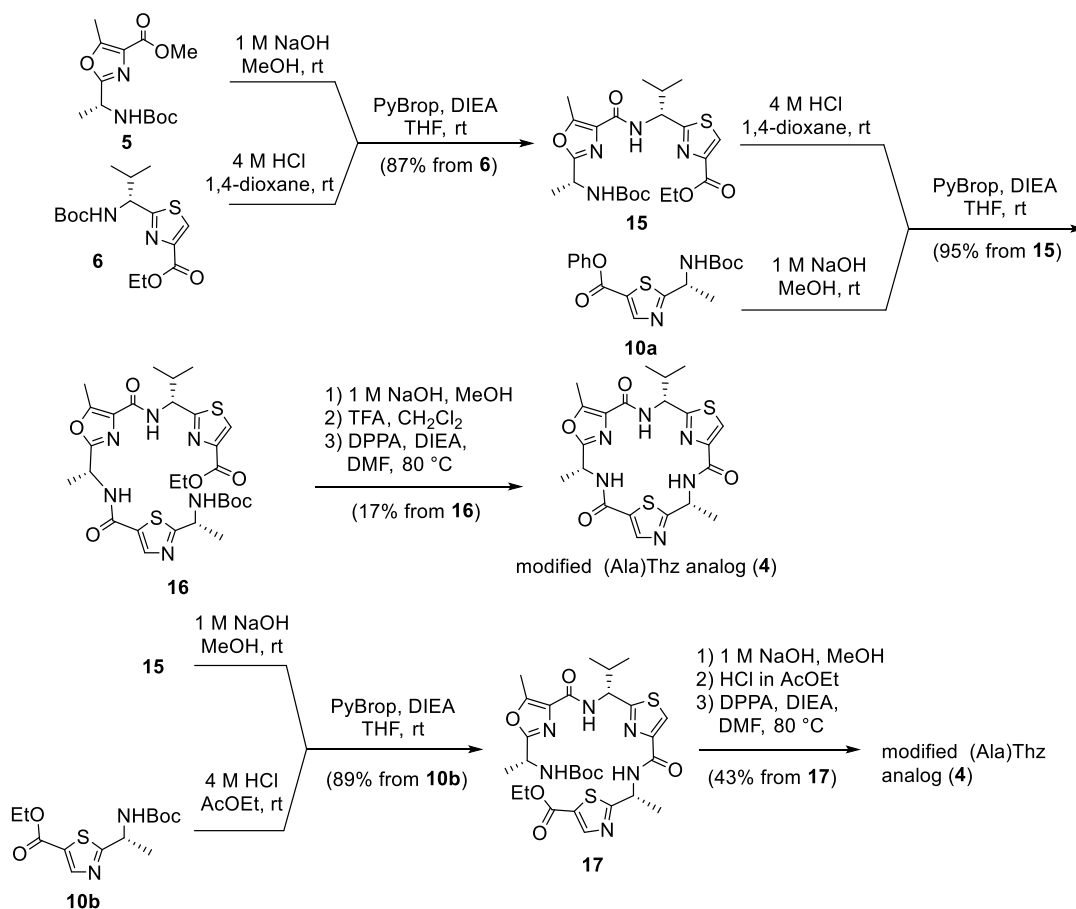
Next, we examined the synthesis of dendroamide A analog **3** containing a modified (Val)Thz unit (Scheme 2). After the modified thiazole unit **9** was deprotected with trifluoroacetic acid (TFA) in CH<sub>2</sub>Cl<sub>2</sub>, dimer **13**, which was prepared from oxazole unit **5** and thiazole unit **7** in 96% yield, was converted into trimer **14** in 67% yield from **9**. Then, deprotected trimer **14** was cyclized to the modified (Val)Thz analog **3** with DPPA in CH<sub>2</sub>Cl<sub>2</sub>/DMF (3:1) under high dilution conditions; however, the yield was low (13%). Presumably, the modified thiazole orientation bearing long S–C bonds inside the ring hinders the ring closure. This problem was overcome by increasing the reaction temperature to 80 °C in DMF, which afforded **3** in a yield of up to 63% in three steps.



**Scheme 2.** Synthesis of dendroamide A analogue 3

**(3) Synthesis of dendroamide A analog 4 having a modified (Ala)Thz moiety.**

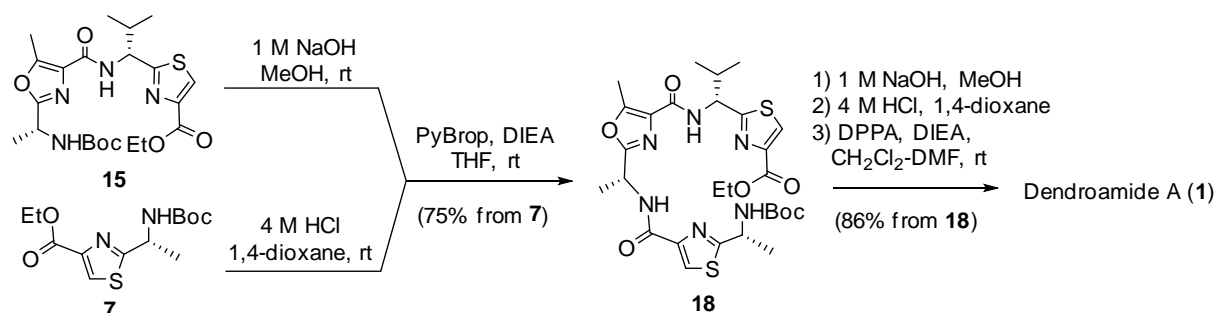
According to the synthetic route of analog 3, natural-type dimer 15, which was prepared from 5 and 6 in 87% yield, was assembled with the modified (Ala)Thz unit 10a<sup>11a</sup> in 95% yield (Scheme 3). Although the resulting trimer 16 was used for cyclization, the reaction was sluggish, giving the modified (Ala)Thz analog 4 in low yield (17%) despite using the optimized conditions for analog 3. To circumvent this issue, we decided to conduct the ring closure at a different site. Thus, deprotected (Ala)Thz unit 10b<sup>11b</sup> was joined at the *N*-terminus with the thiazole carboxylic acid of hydrolyzed 15 in 89% yield over two steps. The cyclization of the resulting trimer 17 was conducted after deprotection. Fortunately, the yield of analog 4 was improved up to 43% in three steps.



**Scheme 3.** Synthesis of dendroamide A analog **4**

#### (4) Synthesis of dendroamide A (**1**).

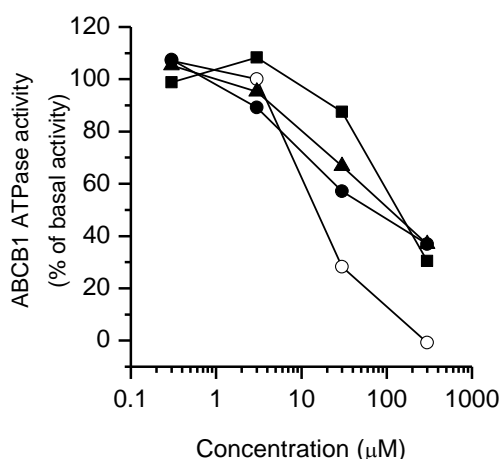
Finally, we synthesized dendroamide A (**1**) as a positive control for the evaluation of the P-gp inhibitory activity. In their report on a total synthesis of dendroamide A, Xia and Smith predicted that the best final cyclization position was the peptide bond between the *m*Ozl moiety and the (Ala)Thz moiety according to the calculated distances between the amino group and the acyl azide group in the cyclization intermediates.<sup>3e</sup> Later, a calculation reported by Shioiri and coworkers revealed that the difference of the distance between the *N*- and *C*-terminal of the cyclization precursors is sufficiently small to allow cyclization at the other peptide bonds.<sup>3a</sup> We planned to synthesize dendroamide A via cyclization at the worst cyclization position according to Smith and coworkers prediction using the same condensing agent that they applied.<sup>13</sup> Therefore, we synthesized linear trimer **18** by introducing *N*-Boc deprotected **7** into dimer **15** at the *C*-terminus in 75% yield over two steps (Scheme 4). The cyclization of deprotected **18** afforded dendroamide A in 86% yield in three steps. These results agree with those reported by Shioiri. The specific rotation of our synthetic dendroamide A matched that reported in the literature; **1**:  $[\alpha]_D^{20} +77.7$  (*c* 0.46, CHCl<sub>3</sub>); lit.<sup>3c</sup>:  $[\alpha]_D^{25} +83.8$  (*c* 0.76, CHCl<sub>3</sub>), lit.<sup>3b</sup>:  $[\alpha]_D^{26} +69.1$  (*c* 0.33, CHCl<sub>3</sub>). The <sup>1</sup>H and <sup>13</sup>C NMR spectra of **1** were identical to those previously reported.<sup>2,3</sup>



**Scheme 4.** Synthesis of dendroamide A (1)

### Inhibition of P-gp

The activity of ABCB1-ATPase was measured by the PREDEASY™ ATPase Kit Assay (SOLVO Biotechnology), according to the manufacturer's instructions. As shown in Figure 3, dendroamide A (1) and the analogs 2–4 inhibited the ATP hydrolysis in a concentration-dependent manner with the half-maximal inhibitory concentration ( $IC_{50}$ ) values of 14.9, 67.8, 110, and 136  $\mu$ M, respectively. The inhibitory activities of the three analogs decreased compared to that of dendroamide A. These results suggest that the orientation of eachazole ring in dendroamide A is important for satisfying the inhibitory activity. The lower inhibitory activity of the analogs compared to dendroamide A is presumably because of the change in theazole ring orientation, which would weaken the intramolecular hydrogen bond network inside the ring, and thus, decrease the rigidity or change the conformation of the molecules.



**Figure 3.** Effects of dendroamide A and the analogs on the ABCB1-ATPase activity; concentration of dendroamide A (open circle), analog 2 (filled circle), analog 3 (filled triangle), and analog 4 (filled square). Data are the average of the duplicate determinations expressed as a percentage of basal control activity.



## CONCLUSIONS

In conclusion, we have synthesized dendroamide A and three kinds of analogs, in which one of the three 2,4-substituted azole rings was replaced with the corresponding 2,5-substituted positional isomer, respectively. A screening of condensing agents revealed that PyBrop<sup>®</sup> is suitable for the synthesis of linear trimers, and DPPA is appropriate for the cyclization into cyclic trimers. The introduction of the modified oxazole unit did not affect the cyclization. Conversely, the modified thiazole units hindered the cyclization. However, the yields could be improved by optimizing the condensation conditions and the position of cyclization. The ATPase assay of dendroamide A (**1**) and the analogs **2–4** elucidated that orientation of all the azole rings is important for P-gp inhibitory activity.

## EXPERIMENTAL

**General Information** Optical rotations were measured with a JASCO P-1020 digital polarimeter. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded in CDCl<sub>3</sub> solution at 400 and 100 MHz, respectively, using a JNM-ECZ-400S spectrometer. Chemical shifts of <sup>1</sup>H NMR are expressed in ppm downfield from tetramethylsilane as an internal standard ( $\delta = 0$ ). Chemical shifts of <sup>13</sup>C NMR are expressed as ppm using CDCl<sub>3</sub> as an internal standard ( $\delta = 77$ ). The following abbreviations are used: broad = br, singlet = s, doublet = d, triplet = t, quartet = q, and multiplet = m. IR absorption spectra (FT: diffuse reflectance spectroscopy) were recorded with KBr powder on a JASCO FT-6300 IR spectrophotometer, and only noteworthy absorptions (cm<sup>-1</sup>) are listed. Mass spectra were obtained with a JEOL AccuTOF LC-plus 4G mass spectrometer. The purification of the crude products was conducted by flash column chromatography. Fuji Silysia Silica Gel BW-300 was used as an adsorbent for column chromatography. For preparative TLC (PTLC), silica gel 60F<sub>254</sub> (Merck) was used. All air- or moisture-sensitive reactions were conducted in flame-dried glassware in an atmosphere of Ar or N<sub>2</sub>. All organic extracts were dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated under reduced pressure with a rotary evaporator, unless otherwise stated.

General procedures and characterization data for representative compounds are described. The data for other compounds are provided in the supplementary material, which can be found as an attachment.

### Ethyl

**2-[(R)-1-[2-[(R)-1-[(*tert*-butoxycarbonyl)amino]-2-methylpropyl]thiazole-4-carboxamido]ethyl]thiazole-4-carboxylate (**11**).** To a solution of thiazole unit **6** (65.7 mg, 0.20 mmol) in MeOH (1.0 mL) was added 1 M NaOH (1.0 mL) with stirring at 0 °C. After the mixture was stirred at rt for 30 min, most of the solvent was evaporated under reduced pressure. The resulting residue was diluted with AcOEt (7 mL) and acidified with ice-cooled 1 M HCl (1.2 mL). After the organic layer was

separated, the aqueous layer was extracted twice with AcOEt (7 mL). The combined organic layers were washed with brine (7 mL) and dried over MgSO<sub>4</sub>. After removal of the solvent *in vacuo*, the obtained carboxylic acid was used for further reaction without purification. To a solution of thiazole unit **7** (60 mg, 0.20 mmol) in EtOH (2.0 mL) was added 4 M HCl in AcOEt (2.0 mL) with stirring at 0 °C. After stirring at rt for 1 h, the solvent was evaporated under reduced pressure. The amine salt and the carboxylic acid were dissolved in THF (10 mL). PyBrop<sup>®</sup> (140 mg, 0.30 mmol) and DIEA (0.35 mL, 2.00 mmol) were added to the solution with stirring at 0 °C under Ar. After stirring at rt for 2 h, the mixture was diluted with AcOEt (20 mL) and washed with 5% KHSO<sub>4</sub> aq. (10 mL), saturated NaHCO<sub>3</sub> aq. (10 mL), and brine (10 mL) before drying over MgSO<sub>4</sub> and solvent evaporation. The residue was chromatographed on silica gel with hexane/AcOEt (3:2) to give **11** (79 mg, 82%) as a pale yellow foam;  $[\alpha]_D^{17} +27.9$  (*c* 0.89, CHCl<sub>3</sub>) (lit. 3e  $[\alpha]_D^{24} +29.4$  (*c* 1.01, CHCl<sub>3</sub>)); <sup>1</sup>H NMR  $\delta$ : 0.94 (d, *J* = 6.9 Hz, 3H), 1.00 (d, *J* = 6.9 Hz, 3H), 1.40 (t, *J* = 7.1 Hz, 3H), 1.46 (s, 9H), 1.80 (d, *J* = 6.9 Hz, 3H), 2.36 (octet, *J* = 6.4 Hz, 1H), 4.42 (q, *J* = 7.1 Hz, 2H), 4.86 (dd, *J* = 8.5, 5.9 Hz, 1H), 5.15 (d, *J* = 8.5 Hz, 1H), 5.60 (qn, *J* = 8.0 Hz, 1H), 7.82 (d, *J* = 8.0 Hz, 1H), 8.04 (s, 1H), 8.10 (s, 1H); <sup>13</sup>C NMR  $\delta$ : 14.22, 17.41, 19.28, 20.84, 28.19 (3C), 32.94, 47.03, 57.87, 61.29, 80.07, 123.39, 127.33, 147.01, 149.14, 155.29, 160.42, 161.16 (C=O<sub>2</sub>Et), 172.85, 172.97; IR (KBr) cm<sup>-1</sup>: 3254, 3117, 1713, 1668, 1639, 1538, 1487; MS (TOF) *m/z*: 505 [*M*+Na]<sup>+</sup>; HRMS (TOF) *m/z*: calcd for C<sub>21</sub>H<sub>30</sub>N<sub>4</sub>O<sub>5</sub>NaS<sub>2</sub>: 505.15498, found: 505.15548 [*M*+Na]<sup>+</sup>.

**Ethyl 2-[(*R*)-1-[2-[(*R*)-1-[2-[(*R*)-1-[(*tert*-butoxycarbonyl)amino]ethyl]-4-methyloxazole-5-carboxamido]-2-methylpropyl]thiazole-4-carboxamido]ethyl]thiazole-4-carboxylate (**12**).** To a solution of oxazole unit **8** (63 mg, 0.22 mmol) in MeOH (1.1 mL) was added 1 M NaOH (1.1 mL) with stirring at 0 °C. After stirring at rt for 30 min, most of the solvent was evaporated under reduced pressure. The residue was then diluted with AcOEt (10 mL) and acidified with ice-cooled 1 M HCl (1.3 mL). After the organic layer was separated, the aqueous layer was extracted three times with AcOEt (5 mL). The combined organic layers were washed with brine (1 mL) and dried over MgSO<sub>4</sub>. After the removal of the solvent *in vacuo*, the resulting carboxylic acid was used for further reaction without purification. Dimer **11** (97 mg, 0.20 mmol) was deprotected with 4 M HCl in AcOEt (2.0 mL, 8.00 mmol) with stirring at 0 °C. After stirring at rt for 1 h, the solvent was evaporated under reduced pressure. The amine salt and the carboxylic acid were dissolved in THF (10 mL). PyBrop<sup>®</sup> (140 mg, 0.30 mmol) and DIEA (0.35 mL, 2.00 mmol) were then added to the solution with stirring at 0 °C under Ar. After stirring at rt for 2 h, the mixture was diluted with AcOEt (20 mL) and washed with 5% KHSO<sub>4</sub> aq. (10 mL), saturated NaHCO<sub>3</sub> aq. (10 mL), and brine (10 mL), and then dried over MgSO<sub>4</sub>. The residue obtained after evaporation of the solvent was purified by PTLC with AcOEt to give **12** (116 mg, 91%) as a colorless foam;  $[\alpha]_D^{21}$

–21.0 (*c* 1.44, acetone);  $^1\text{H NMR } \delta$ : 1.00 (d,  $J = 6.9$  Hz, 3H), 1.03 (d,  $J = 6.9$  Hz, 3H), 1.40 (t,  $J = 7.3$  Hz, 3H), 1.42 (s, 9H), 1.56 (d,  $J = 6.9$  Hz, 3H), 1.82 (d,  $J = 6.9$  Hz, 3H), 2.42 (octet,  $J = 6.9$  Hz, 1H), 2.48 (s, 3H), 4.41 (q,  $J = 7.0$  Hz, 2H), 4.93 (qn,  $J = 6.9$  Hz, 1H), 5.21 (d,  $J = 8.7$  Hz, 1H), 5.27 (dd,  $J = 8.7, 6.9$  Hz, 1H), 5.61 (qn,  $J = 7.5$  Hz, 1H), 6.84 (d,  $J = 8.7$  Hz, 1H), 7.96 (d,  $J = 7.8$  Hz, 1H), 8.04 (s, 1H), 8.10 (s, 1H);  $^{13}\text{C NMR } \delta$ : 12.88, 14.25, 18.14, 19.57, 19.77, 20.94, 28.20 (3C), 33.02, 44.57, 47.23, 55.74, 61.46, 80.14, 123.80, 127.37, 138.66, 143.39, 146.98, 149.24, 154.88, 157.78, 160.43, 161.27, 164.18, 170.83, 173.16; IR (KBr)  $\text{cm}^{-1}$ : 3349, 1720, 1616, 1519; MS (TOF)  $m/z$ : 657 [ $M+\text{Na}$ ] $^+$ ; HRMS (TOF)  $m/z$ : calcd for  $\text{C}_{28}\text{H}_{38}\text{N}_6\text{O}_7\text{NaS}_2$ : 657.21356, found: 657.21350 [ $M+\text{Na}$ ] $^+$ .

**(5<sup>2</sup>Z,9<sup>2</sup>Z,2R,6R,10R)-10-Isopropyl-1<sup>4</sup>,2,6-trimethyl-3,7,11-triaza-1(2,5)-oxazola-5,9(4,2)-dithiazolacyclododecaphane-4,8,12-trione [modified *mOzl* analog] (2).** To a solution of trimer **12** (100 mg, 0.16 mmol) in MeOH (0.8 mL) was added 1 M NaOH (0.8 mL, 0.80 mmol) with stirring at 0 °C. After the mixture was stirred at rt for 45 min, the solvent was almost completely evaporated under reduced pressure. The carboxylic acid was diluted with AcOEt (10 mL) and acidified with ice-cooled 1 M HCl (5 mL). After the organic layer was separated, the aqueous layer was extracted three times with AcOEt (10 mL). The combined organic layers were washed with brine (5 mL) and dried over MgSO<sub>4</sub>. After removal of the solvent *in vacuo*, the carboxylic acid was dissolved in EtOH (1.6 mL), and 4 M HCl in AcOEt (1.6 mL, 6.40 mmol) was added to the solution with stirring at 0 °C for 15 min and then at rt for 1 h. After removal of the solvent *in vacuo*, the amine salt was dissolved in a 3:1 mixture of CH<sub>2</sub>Cl<sub>2</sub>/DMF (8 mL), and the resulting solution was added over 3 h to a solution of DPPA (0.21 mL, 0.96 mmol) and DIEA (0.17 mL, 0.96 mmol) in CH<sub>2</sub>Cl<sub>2</sub>/DMF (3:1, 32 mL) using a syringe pump with stirring at rt under Ar. After further stirring at rt for 2 h, the mixture was diluted with AcOEt (100 mL) and washed with water (40 mL) and brine (40 mL) before drying over MgSO<sub>4</sub> and solvent evaporation. The residue was purified by PTLC (SiO<sub>2</sub>) with AcOEt, affording the modified *mOzl* analog **2** (57.3 mg, 73%) as a colorless powder; mp 194–195 °C; [ $\alpha$ ]<sub>D</sub><sup>28</sup> +100.4 (*c* 0.59, CHCl<sub>3</sub>);  $^1\text{H NMR } \delta$ : 1.03 (d,  $J = 6.9$  Hz, 3H), 1.08 (d,  $J = 6.9$  Hz, 3H), 1.72 (d,  $J = 6.9$  Hz, 3H), 1.74 (d,  $J = 6.9$  Hz, 3H), 2.29 (m, 1H), 2.52 (s, 3H), 5.44 (dd,  $J = 8.9, 4.8$  Hz, 1H), 5.62 (qn,  $J = 7.3$  Hz, 1H), 5.65 (qn,  $J = 6.9$  Hz, 1H), 7.44 (d,  $J = 8.7$  Hz), 8.01 (d,  $J = 9.1$  Hz, 1H), 8.146 (s, 1H), 8.154 (s, 1H), 8.47 (d,  $J = 8.2$  Hz, 1H);  $^{13}\text{C NMR } \delta$ : 12.89, 18.11, 18.77, 22.03, 24.66, 35.41, 44.04, 47.06, 55.37, 124.01, 124.06, 138.76, 143.64, 148.36, 148.76, 157.00, 159.20, 159.39, 161.94, 168.62, 171.28; IR (KBr)  $\text{cm}^{-1}$ : 3336, 1711, 1681, 1630, 1496; MS (TOF)  $m/z$ : 511 [ $M+\text{Na}$ ] $^+$ ; HRMS (TOF)  $m/z$ : calcd for  $\text{C}_{21}\text{H}_{24}\text{N}_6\text{O}_4\text{NaS}_2$ : 511.11927, found: 511.11971 [ $M+\text{Na}$ ] $^+$ .

### Methyl

**2-[(R)-1-[2-[(R)-1-[(*tert*-butoxycarbonyl)amino]ethyl]thiazole-4-carboxamido]ethyl]-5-methyloxazole-4-carboxylate (13).** To a solution of thiazole unit **7** (300 mg, 1.00

mmol) in MeOH (5 mL) was added 1 M NaOH (5 mL) with stirring at 0 °C. After the mixture was stirred at rt for 30 min, most of the solvent was evaporated under reduced pressure. The residue was diluted with AcOEt (20 mL) and acidified with ice-cooled 1 M HCl (6 mL). After the organic layer was separated, the aqueous layer was extracted three times with AcOEt (10 mL). The combined organic layers were dried over MgSO<sub>4</sub>. After removal of the solvent *in vacuo*, the carboxylic acid was used for further reaction without purification. Oxazole unit **5** (284 mg, 1.00 mmol) was deprotected with 4 M HCl in AcOEt (10 mL) and EtOH (10 mL) with stirring at 0 °C. After stirring at rt for 30 min, the solvent was evaporated under reduced pressure. The amine salt and the carboxylic acid were dissolved in THF (50 mL). PyBrop<sup>®</sup> (699 mg, 1.50 mmol) and DIEA (1.74 mL, 10.0 mmol) were added to the solution with stirring at 0 °C under Ar. After the stirring was continued at rt for 2 h, the mixture was diluted with AcOEt (75 mL) and washed with 5% KHSO<sub>4</sub> (60 mL) and saturated NaHCO<sub>3</sub> (60 mL) before drying over MgSO<sub>4</sub> and evaporating the solvent. The residue was chromatographed on silica gel with hexane/AcOEt (2:3) to give **13** (420 mg, 96%) as a pale yellow foam;  $[\alpha]_D^{25} +12.3$  (c 0.59, acetone); <sup>1</sup>H NMR (acetone-*d*<sub>6</sub>)  $\delta$ : 1.41 (s, 9H), 1.54 (d, *J* = 7.3 Hz, 3H), 1.65 (d, *J* = 6.9 Hz, 3H), 2.57 (s, 3H), 3.82 (s, 3H), 5.00 (qn, *J* = 7.3 Hz, 1H), 5.38 (qn, *J* = 7.5 Hz, 1H), 6.88 (d, *J* = 6.4 Hz, 1H), 8.13 (s, 1H), 8.45 (d, *J* = 8.2 Hz, 1H); <sup>13</sup>C NMR (acetone-*d*<sub>6</sub>)  $\delta$ : 11.46, 18.52, 20.41, 27.99 (3C), 43.27, 49.21, 51.38, 79.18, 123.00, 127.50, 149.69, 155.52, 156.60, 160.71, 162.70, 162.86, 176.61; IR (KBr) cm<sup>-1</sup>: 3314, 3114, 1713, 1661, 1623, 1539; MS (TOF) *m/z*: 461 [M+Na]<sup>+</sup>; HRMS (TOF) *m/z*: calcd for C<sub>19</sub>H<sub>26</sub>N<sub>4</sub>O<sub>6</sub>NaS: 461.14653, found: 461.14684 [M+Na]<sup>+</sup>.

**Phenyl**                            **2-[(R)-1-[2-[(R)-1-[2-[(R)-1-[(*tert*-butoxycarbonyl)amino]ethyl]thiazole-4-carboxamido]ethyl]-5-methyloxazole-4-carboxamido]-2-methylpropyl]thiazole-5-carboxylate (14).**

To a solution of dimer **13** (44 mg, 0.10 mmol) in MeOH (0.5 mL) was added 1 M NaOH (0.5 mL) with stirring at 0 °C. After the mixture was stirred at rt for 30 min, most of the solvent was evaporated under reduced pressure. The residue was diluted with AcOEt (50 mL) and acidified with ice-cooled 1 M HCl (1.5 mL). After the organic layer was separated, the aqueous layer was extracted three times with AcOEt (20 mL). The combined organic layers were washed with brine (6 mL) and dried over MgSO<sub>4</sub>. After removal of the solvent *in vacuo*, the carboxylic acid was used for further reaction without purification. To a solution of thiazole unit **9** (38 mg, 0.10 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (2.0 mL) was added CF<sub>3</sub>CO<sub>2</sub>H (0.38 mL, 5.00 mmol) with stirring at 0 °C. After stirring at rt for 1 h, the solvent was evaporated under reduced pressure. Then, the acid was azeotropically removed with toluene (0.5 mL). A solution of HCl in MeOH (0.5 mL) was added to the residue, and the solvent was evaporated. This operation was repeated four times. Then, toluene (0.5 mL) was added and evaporated to give the amine salt. The carboxylic acid was dissolved in

THF (4.0 mL). PyBrop<sup>®</sup> (70 mg, 0.15 mmol) and DIEA (0.17 mL, 1.00 mmol) were added to the solution with stirring at 0 °C under Ar. After stirring at rt for 30 min, a solution of the amine salt in THF (4 mL) was added to the mixture, and the whole was further stirred at rt for 2 h. The mixture was diluted with AcOEt (30 mL) and washed with 5% KHSO<sub>4</sub> aq. (15 mL), saturated NaHCO<sub>3</sub> aq. (30 mL), and brine (15 mL) before drying over MgSO<sub>4</sub> and evaporating the solvent. The residue was purified by PTLC (SiO<sub>2</sub>) with hexane/AcOEt (1:1) to give **14** (45.7 mg, 67%) as a colorless foam;  $[\alpha]_{\text{D}}^{25} +9.9$  (*c* 0.56, acetone); <sup>1</sup>H NMR  $\delta$ : 1.05 (d, *J* = 6.9 Hz, 3H), 1.06 (d, *J* = 6.9 Hz, 3H), 1.45 (s, 9H), 1.59 (d, *J* = 6.4 Hz, 3H), 1.68 (d, *J* = 6.9 Hz, 3H), 2.53 (m, 1H), 2.63 (s, 3H), 5.08 (qn, *J* = 7.1 Hz, 1H), 5.22 (br s, 1H), 5.34 (dd, *J* = 8.9, 5.7 Hz, 1H), 5.44 (qn, *J* = 8.2 Hz, 1H), 7.18 (d, *J* = 8.2 Hz, 2H), 7.28 (d, *J* = 6.9 Hz, 1H), 7.42 (t, *J* = 7.5 Hz, 2H), 7.61 (d, *J* = 9.1 Hz, 1H), 7.83 (d, *J* = 8.7 Hz, 1H), 8.06 (s, 1H), 8.50 (s, 1H); <sup>13</sup>C NMR  $\delta$ : 11.71, 17.79, 19.42, 19.53, 21.32, 28.25 (3C), 33.09, 42.95, 48.74, 56.14, 80.39, 121.43 (2C), 123.76, 126.24, 128.07, 128.45, 129.51 (2C), 149.09, 149.68, 150.15, 154.12, 154.88, 159.57, 160.29, 161.21, 161.59, 174.74, 178.21; IR (KBr) cm<sup>-1</sup>: 3300, 1708, 1665, 1494; MS (TOF) *m/z*: 705 [M+Na]<sup>+</sup>; HRMS (TOF) *m/z*: calcd for C<sub>32</sub>H<sub>38</sub>N<sub>6</sub>O<sub>7</sub>NaS<sub>2</sub>: 705.21356, found: 705.21364 [M+Na]<sup>+</sup>.

**(1<sup>2</sup>Z,5<sup>2</sup>Z,2R,6R,10R)-10-Isopropyl-1<sup>5</sup>,2,6-trimethyl-3,7,11-triaza-1(2,4)-oxazola-5(4,2),9(5,2)-dithiazolacyclododecaphane-4,8,12-trione [modified (Val)Thz analog] (3)**. To a solution of trimer **14** (27 mg, 0.04 mmol) in MeOH (0.40 mL) was added 1 M NaOH (0.40 mL) with stirring at 0 °C. After the mixture was stirred at rt for 30 min, the solvent was almost completely evaporated under reduced pressure. The residue was diluted with AcOEt (10 mL) and acidified with ice-cooled 0.33 M HCl (6.0 mL). After the organic layer was separated, the aqueous layer was extracted three times with AcOEt (10 mL). The combined organic layers were dried over MgSO<sub>4</sub>. After removal of the solvent *in vacuo*, the carboxylic acid was dissolved in MeOH (0.75 mL), and 4 M HCl in AcOEt (0.75 mL, 3.00 mmol) was added to the solution with stirring at 0 °C. The resulting mixture was further stirred at rt for 1 h. After removal of the solvent *in vacuo*, the residue was dissolved in MeOH (3 mL), and the solvent was evaporated. This operation was repeated two times. Finally, the residue was dissolved in toluene (1 mL), and the solvent was evaporated. The amine salt was dissolved in DMF (2 mL), and the resulting solution was added over 2 h to a solution of DPPA (0.052 mL, 0.24 mmol) and DIEA (0.042 mL, 0.24 mmol) in DMF (8 mL) using a syringe pump with stirring at 80 °C under Ar. After stirring at rt for 5 h, the mixture was diluted with AcOEt (50 mL) and washed with water (20 mL) and brine (20 mL) before drying over MgSO<sub>4</sub> and evaporating the solvent. The residue was purified by PTLC (SiO<sub>2</sub>) with AcOEt to produce the modified (Val)Thz analog **3** (12.1 mg, 63%) as a colorless foam;  $[\alpha]_{\text{D}}^{21} +108.6$  (*c* 0.50, CHCl<sub>3</sub>); <sup>1</sup>H NMR  $\delta$ : 0.92 (d, *J* = 6.9 Hz, 3H), 1.19 (d, *J* = 6.9 Hz, 3H), 1.72 (d, *J* = 6.9 Hz, 3H), 1.74 (d, *J* = 6.9 Hz, 3H), 2.65 (s,

3H), 2.73 (m, 1H), 5.32 (qn,  $J = 6.2$  Hz, 1H), 5.40 (qn,  $J = 7.3$  Hz, 1H), 5.45 (dd,  $J = 10.5, 3.7$  Hz, 1H), 7.20 (d,  $J = 10.5$  Hz, 1H), 7.69 (d,  $J = 5.9$  Hz, 1H), 7.90 (d,  $J = 7.8$  Hz, 1H), 8.21 (s, 1H), 8.33 (s, 1H);  $^{13}\text{C}$  NMR  $\delta$ : 11.49, 16.33, 19.69, 21.55, 24.18, 32.37, 43.81, 48.88, 57.14, 125.24, 129.72, 131.23, 147.12, 147.57, 153.42, 159.03, 159.24, 161.98, 162.49, 171.36, 177.57; IR (KBr)  $\text{cm}^{-1}$ : 3385, 3008, 1667, 1534; MS (TOF)  $m/z$ : 511 [ $M+\text{Na}$ ] $^{+}$ ; HRMS (TOF)  $m/z$ : calcd for  $\text{C}_{21}\text{H}_{24}\text{N}_6\text{O}_4\text{NaS}_2$ : 511.11927, found: 511.11961 [ $M+\text{Na}$ ] $^{+}$ .

**Ethyl** **2-[(R)-1-[2-[(R)-1-[(tert-butoxycarbonyl)amino]ethyl]-5-methyloxazole-4-carboxamido]-2-methylpropyl]thiazole-4-carboxylate (15)**. To a solution of oxazole unit **5** (180 mg, 0.63 mmol) in MeOH (3.0 mL) was added 1 M NaOH (3.0 mL) with stirring at 0 °C. After the mixture was stirred at rt for 30 min, most of the solvent was evaporated under reduced pressure. The residue was diluted with AcOEt (50 mL) and acidified with ice-cooled 1 M HCl (4.0 mL). After the organic layer was separated, the aqueous layer was extracted three times with AcOEt (20 mL). The combined organic layers were dried over  $\text{MgSO}_4$ . After removal of the solvent *in vacuo*, the obtained carboxylic acid was used for further reaction without purification. Thiazole unit **6** (198 mg, 0.60 mmol) was deprotected with 4 M HCl in 1,4-dioxane (3.0 mL) with stirring at 0 °C. After stirring at rt for 30 min, the solvent was evaporated under reduced pressure. The amine salt and the carboxylic acid were dissolved in THF (30 mL). PyBrop<sup>®</sup> (420 mg, 0.90 mmol) and DIEA (1.05 mL, 6.00 mmol) were added to the solution with stirring at 0 °C under Ar. After stirring at rt for 4 h, the mixture was diluted with AcOEt (75 mL) and washed with 5%  $\text{KHSO}_4$  aq. (15 mL), saturated  $\text{NaHCO}_3$  aq. (15 mL), and brine (15 mL) before drying over  $\text{MgSO}_4$  and evaporating the solvent. The residue was chromatographed on silica gel with hexane/AcOEt (3:2) to give **15** (251 mg, 87%) as a pale yellow foam;  $[\alpha]_{\text{D}}^{18} +59.3$  ( $c$  0.71, acetone);  $^1\text{H}$  NMR  $\delta$ : 1.00 (d,  $J = 6.9$  Hz, 3H), 1.03 (d,  $J = 6.9$  Hz, 3H), 1.40 (t,  $J = 7.1$  Hz, 3H), 1.46 (s, 9H), 1.54 (d,  $J = 6.9$  Hz, 3H), 2.61 (s, 3H), 2.62 (octet,  $J = 6.7$  Hz, 1H), 4.42 (q,  $J = 7.2$  Hz, 2H), 4.92 (qn,  $J = 6.6$  Hz, 1H), 5.14 (d,  $J = 6.9$  Hz, 1H), 5.29 (dd,  $J = 9.1, 6.9$  Hz, 1H), 7.54 (d,  $J = 9.1$  Hz, 1H), 8.08 (s, 1H);  $^{13}\text{C}$  NMR  $\delta$ : 11.61, 14.29, 17.88, 19.62, 20.02, 28.25 (3C), 32.86, 44.59, 55.88, 61.32, 80.07, 126.80, 128.40, 147.48, 153.61, 154.81, 161.23, 161.57, 161.75, 172.01; IR (KBr)  $\text{cm}^{-1}$ : 3321, 1713, 1669, 1634, 1505; MS (TOF)  $m/z$ : 503 [ $M+\text{Na}$ ] $^{+}$ ; HRMS (TOF)  $m/z$ : calcd for  $\text{C}_{22}\text{H}_{32}\text{N}_4\text{O}_6\text{NaS}$ : 503.19348, found: 503.19329 [ $M+\text{Na}$ ] $^{+}$ .

**Ethyl** **2-[(R)-1-[2-[(R)-1-[2-[(R)-1-[(tert-butoxycarbonyl)amino]ethyl]thiazole-5-carboxamido]ethyl]-5-methyloxazole-4-carboxamido]-2-methylpropyl]thiazole-4-carboxylate (16)**. To a solution of thiazole unit **10a** (35 mg, 0.10 mmol) in MeOH (0.5 mL) was added 1 M NaOH (0.5 mL) with stirring at 0 °C. After the mixture was stirred at rt for 30 min, most of the solvent was evaporated under reduced pressure. The residue was diluted with AcOEt (50 mL) and acidified with ice-cooled 1 M

HCl (6 mL). After the organic layer was separated, the aqueous layer was extracted two times with AcOEt (25 mL). The combined organic layers were dried over MgSO<sub>4</sub>. After removal of the solvent *in vacuo*, the resulting carboxylic acid was used for further reaction without purification. Dimer **15** (48 mg, 0.10 mmol) was deprotected with 4 M HCl in 1,4-dioxane (0.5 mL) with stirring at 0 °C. After stirring at rt for 30 min, the solvent was evaporated under reduced pressure. The amine salt and the carboxylic acid were dissolved in THF (5 mL). PyBrop<sup>®</sup> (70 mg, 0.15 mmol) and DIEA (0.26 mL, 1.50 mmol) were added to the solution with stirring at 0 °C under Ar. After stirring at rt for 2 h, the mixture was diluted with AcOEt (20 mL) and washed with 5% KHSO<sub>4</sub> aq. (5 mL), saturated NaHCO<sub>3</sub> aq. (5 mL), and brine (5 mL) before drying over MgSO<sub>4</sub> and solvent evaporation. The residue was purified by PTLC (SiO<sub>2</sub>) with hexane/AcOEt (1:3), affording **16** (60.6 mg, 95%) as a colorless foam;  $[\alpha]_D^{20} +45.4$  (*c* 0.61, acetone); <sup>1</sup>H NMR (acetone-*d*<sub>6</sub>)  $\delta$ : 0.83 (d, *J* = 7.3 Hz, 3H), 0.90 (d, *J* = 7.3 Hz, 3H), 1.21 (t, *J* = 7.1 Hz, 3H), 1.29 (s, 9H), 1.44 (d, *J* = 6.9 Hz, 3H), 1.48 (d, *J* = 7.3 Hz, 3H), 2.38 (octet, *J* = 6.9 Hz, 1H), 2.42 (s, 3H), 4.22 (q, *J* = 7.0 Hz, 2H), 4.87 (qn, *J* = 7.3 Hz, 1H), 5.12 (dd, *J* = 8.7, 7.3 Hz, 1H), 5.23 (qn, *J* = 7.5 Hz, 1H), 6.76 (d, *J* = 7.3 Hz, 1H), 7.80 (d, *J* = 9.1 Hz, 1H), 8.12 (s, 1H), 8.24 (s, 1H), 8.24 (d, *J* = 6.4 Hz, 1H); <sup>13</sup>C NMR (acetone-*d*<sub>6</sub>)  $\delta$ : 11.54, 14.51, 18.63, 18.81, 19.86, 20.96, 28.48 (3C), 33.42, 44.08, 50.04, 56.97, 61.68, 79.59, 128.73, 129.41, 135.02, 144.24, 147.53, 154.27, 155.96, 160.65, 161.83, 162.04, 162.45, 173.01, 181.04; IR (KBr) cm<sup>-1</sup>: 3311, 1716, 1638, 1510; MS (TOF) *m/z*: 657 [*M*+Na]<sup>+</sup>; HRMS (TOF) *m/z*: calcd for C<sub>28</sub>H<sub>38</sub>N<sub>6</sub>O<sub>7</sub>NaS<sub>2</sub>: 657.21356, found: 657.21346 [*M*+Na]<sup>+</sup>.

**Ethyl 2-[(*R*)-1-[2-[(*R*)-1-[2-[(*R*)-1-[(*tert*-butoxycarbonyl)amino]ethyl]-5-methyloxazole-4-carboxamido]-2-methylpropyl]thiazole-4-carboxamido]ethyl]thiazole-5-carboxylate (**17**).** To a solution of dimer **15** (154 mg, 0.32 mmol) in MeOH (2.6 mL) was added 1 M NaOH (1.6 mL) with stirring at 0 °C. After the mixture was stirred at rt for 30 min, the solvent was almost completely evaporated under reduced pressure. The residue was diluted with AcOEt (120 mL) and acidified with ice-cooled 1 M HCl (4.8 mL). After the organic layer was separated, the aqueous layer was extracted two times with AcOEt (120 mL). The combined organic layers were dried over MgSO<sub>4</sub>. After removal of the solvent *in vacuo*, the carboxylic acid was used for further reaction without purification. Thiazole unit **10b** (95 mg, 0.32 mmol) was deprotected with 4 M HCl in AcOEt (1.6 mL, 6.40 mmol) with stirring at 0 °C. After stirring at rt for 30 min, the solvent was evaporated under reduced pressure. The amine salt and the carboxylic acid were dissolved in THF (16 mL). PyBrop<sup>®</sup> (224 mg, 0.48 mmol) and DIEA (0.56 mL, 3.20 mmol) were added to the solution with stirring at 0 °C under Ar. After stirring at rt for 1 h, the mixture was diluted with AcOEt (60 mL) and washed with 5% KHSO<sub>4</sub> aq. (15 mL), saturated NaHCO<sub>3</sub> aq. (15 mL), and brine (15 mL) and then dried over MgSO<sub>4</sub> and evaporated to dryness. The residue was

chromatographed on silica gel with hexane/AcOEt (1:1) to give **17** (181 mg, 89%) as a colorless foam;  $[\alpha]_D^{26} -1.47$  (*c* 0.51, acetone);  $^1\text{H NMR } \delta$ : 1.03 (d, *J* = 6.4 Hz, 3H), 1.04 (d, *J* = 6.9 Hz, 3H), 1.35 (t, *J* = 7.3 Hz, 3H), 1.45 (s, 9H), 1.53 (d, *J* = 6.9 Hz, 3H), 1.78 (d, *J* = 6.9 Hz, 3H), 2.49 (octet, *J* = 6.9 Hz, 1H), 2.62 (s, 3H), 4.34 (q, *J* = 7.0 Hz, 2H), 4.93 (m, 1H), 5.11 (d, *J* = 6.9 Hz, 1H), 5.28 (dd, *J* = 8.9, 6.2 Hz, 1H), 5.58 (qn, *J* = 7.3 Hz, 1H), 7.47 (d, *J* = 9.1 Hz, 1H), 7.88 (d, *J* = 7.8 Hz, 1H), 8.07 (s, 1H), 8.31 (s, 1H);  $^{13}\text{C NMR } \delta$ : 11.68, 14.22, 17.86, 19.58, 19.89, 21.04, 28.27 (3C), 33.03, 44.63, 47.34, 55.95, 61.57, 80.20, 123.71, 128.39, 129.50, 148.14, 149.18, 153.82, 154.83, 160.44, 161.24, 161.67, 161.78, 172.04, 178.17; IR (neat)  $\text{cm}^{-1}$ : 3308, 3117, 1712, 1664, 1509; MS (TOF) *m/z*: 657 [*M*+Na] $^+$ ; HRMS (TOF) *m/z*: calcd for  $\text{C}_{28}\text{H}_{38}\text{N}_6\text{O}_7\text{NaS}_2$ : 657.21356, found: 657.21326 [*M*+Na] $^+$ .

**(1<sup>2</sup>Z,5<sup>2</sup>Z,4R,8R,12R)-4-Isopropyl-1<sup>5</sup>,8,12-trimethyl-3,7,11-triaza-1(4,2)-oxazola-5(2,4),9(2,5)-dithiazolacyclododecaphane-2,6,10-trione [modified (Ala)Thz analog] (4) from 16.** To a solution of trimer **16** (13 mg, 0.02 mmol) in MeOH (0.20 mL) was added 1 M NaOH (0.20 mL, 0.20 mmol) with stirring at 0 °C. The mixture was stirred at rt for 30 min and then taken almost to dryness under reduced pressure. The carboxylic acid was diluted with AcOEt (10 mL) and acidified with ice-cooled 0.33 M HCl (6 mL). After the organic layer was separated, the aqueous layer was extracted three times with AcOEt (10 mL). The combined organic layers were washed with brine (10 mL) and dried over  $\text{MgSO}_4$ . After removal of the solvent *in vacuo*, the carboxylic acid was dissolved in MeOH (0.38 mL), and 4 M HCl in AcOEt (0.38 mL, 1.50 mmol) was added to the solution with stirring at 0 °C, followed by further stirring at rt for 1 h. After removal of the solvent *in vacuo*, the amine salt was dissolved in MeOH (3 mL), and the solvent was evaporated. This operation was repeated two times. The amine salt was dissolved in DMF (1 mL), and the solution was added over 2 h to a solution of DPPA (0.026 mL, 0.12 mmol) and DIEA (0.020 mL, 0.12 mmol) in DMF (4 mL) using a syringe pump with stirring at 80 °C under Ar. After stirring at this temperature for 5 h, the mixture was diluted with AcOEt (50 mL) and washed with water (20 mL) and brine (20 mL) before drying over  $\text{MgSO}_4$  and solvent evaporation. The residue was purified by PTLC with hexane/AcOEt (1:5) and then with  $\text{CHCl}_3/\text{AcOEt}/\text{MeOH}$  (5:10:1) to give **4** (1.6 mg, 17%) as a colorless foam.

**(1<sup>2</sup>Z,5<sup>2</sup>Z,4R,8R,12R)-4-Isopropyl-1<sup>5</sup>,8,12-trimethyl-3,7,11-triaza-1(4,2)-oxazola-5(2,4),9(2,5)-dithiazolacyclododecaphane-2,6,10-trione [modified (Ala)Thz analog] (4) from 17.** To a solution of trimer **17** (57 mg, 0.09 mmol) in MeOH (1.0 mL) was added 1 M NaOH (1.0 mL, 0.10 mmol) with stirring at 0 °C. After the mixture was stirred at rt for 30 min, most of the solvent was evaporated under reduced pressure. The carboxylic acid was dissolved with AcOEt (60 mL) and acidified with ice-cooled 0.33 M (18 mL). After the organic layer was separated, the aqueous layer was extracted three times with AcOEt



(40 mL). The combined organic layers were dried over MgSO<sub>4</sub>. After removal of the solvent *in vacuo*, the residue was used for further reaction without purification. To a solution of the carboxylic acid in MeOH (2.0 mL) was added 4 M HCl in AcOEt (1.88 mL, 7.50 mmol) with stirring at 0 °C. After stirring at rt for 1 h, the solvent was evaporated under reduced pressure. The residue was dissolved with MeOH (6 mL), and the solvent was evaporated. This operation was repeated two times. Then the amine salt was dissolved in toluene (1 mL), and the solvent was evaporated. The amine salt was dissolved in DMF (5.4 mL), and the solution was added over 4 h to a solution of DPPA (0.12 mL, 0.54 mmol) and DIEA (0.095 mL, 0.54 mmol) in DMF (21.6 mL) using a syringe pump with stirring at 80 °C under Ar. After stirring at rt for 10 h, the mixture was diluted with AcOEt (150 mL) and washed with water (50 mL) and brine (50 mL) before drying over MgSO<sub>4</sub> and evaporating to dryness. The residue was purified by short column on silica gel with AcOEt. Then, the crude was purified by PTLC with CHCl<sub>3</sub>/AcOEt /MeOH (5:10:1) to afford **4** (19.1 mg, 43%) as a colorless foam;  $[\alpha]_D^{27} +86.1$  (*c* 0.55, CHCl<sub>3</sub>); <sup>1</sup>H NMR  $\delta$ : 0.99 (d, *J* = 7.3 Hz, 3H), 1.14 (d, *J* = 6.9 Hz, 3H), 1.65 (d, *J* = 6.9 Hz, 3H), 1.78 (d, *J* = 6.9 Hz, 3H), 2.32 (m, 1H), 2.66 (s, 3H), 5.03 (qn, *J* = 6.9 Hz, 1H), 5.21 (dd, *J* = 9.1, 8.2 Hz, 1H), 5.73 (dq, *J* = 10.3, 7.1 Hz, 1H), 7.30 (d, *J* = 6.9 Hz, 1H), 7.64 (d, *J* = 10.5 Hz, 1H), 7.79 (d, *J* = 9.6 Hz, 1H), 8.09 (s, 1H), 8.23 (s, 1H); <sup>13</sup>C NMR  $\delta$ : 11.70, 19.09, 19.24, 20.39, 21.00, 35.01, 45.52, 47.82, 55.48, 123.84, 127.90, 123.16, 145.98, 149.95, 155.07, 159.67, 160.18, 160.21, 161.75, 168.92, 177.89; IR (neat) cm<sup>-1</sup>: 3494, 3404, 3295, 3096, 1664, 1541; MS (TOF) *m/z*: 511 [M+Na]<sup>+</sup>; HRMS (TOF) *m/z*: calcd for C<sub>21</sub>H<sub>24</sub>N<sub>6</sub>O<sub>4</sub>NaS<sub>2</sub>: 511.11927, found: 511.11953 [M+Na]<sup>+</sup>.

**Ethyl 2-[(R)-1-[2-[(R)-1-[2-[(R)-1-[(tert-butoxycarbonyl)amino]ethyl]thiazole-4-carboxamido]ethyl]-5-methyloxazole-4-carboxamido]-2-methylpropyl)thiazole-4-carboxylate (18).**

To a solution of thiazole unit **7** (90 mg, 0.30 mmol) in MeOH (1.5 mL) was added 1 M NaOH (1.5 mL) with stirring at 0 °C. After the mixture was stirred at rt for 30 min, most of the solvent was evaporated under reduced pressure. The residue was diluted with AcOEt (100 mL) and acidified with ice-cooled 1 M HCl (3.0 mL). After the organic layer was separated, the aqueous layer was extracted twice with AcOEt (50 mL). The combined organic layers were dried over MgSO<sub>4</sub>. After removal of the solvent *in vacuo*, the obtained carboxylic acid was used for further reaction without purification. A solution of **15** (144 mg, 0.30 mmol) in 1,4-dioxane (0.5 mL) was deprotected with 4 M HCl in 1,4-dioxane (1.5 mL, 6.0 mmol) with stirring at 0 °C. After stirring at rt for 50 min, the solvent was evaporated under reduced pressure. The amine salt and the carboxylic acid were dissolved in THF (15 mL). PyBrop<sup>®</sup> (210 mg, 0.45 mmol) and DIEA (0.52 mL, 3.00 mmol) were added to the solution with stirring at 0 °C under Ar. After stirring at rt for 1.5 h, the mixture was diluted with AcOEt (60 mL) and washed with 5% KHSO<sub>4</sub> aq. (15 mL),

saturated NaHCO<sub>3</sub> aq. (15 mL), and brine (15 mL) before drying over MgSO<sub>4</sub> and solvent evaporation. The residue was chromatographed on silica gel with hexane/AcOEt (1:1→2:3) to give **18** (142 mg, 75%) as a colorless foam;  $[\alpha]_{\text{D}}^{21} +39.4$  (*c* 0.62, CHCl<sub>3</sub>); <sup>1</sup>H NMR  $\delta$ : 0.99 (d, *J* = 6.9 Hz, 3H), 1.03 (d, *J* = 6.4 Hz, 3H), 1.39 (t, *J* = 7.5 Hz, 3H), 1.45 (s, 9H), 1.63 (d, *J* = 7.3 Hz, 3H), 1.68 (d, *J* = 7.3 Hz, 3H), 2.59 (octet, *J* = 6.4 Hz, 1H), 2.62 (s, 3H), 4.41 (q, *J* = 7.0 Hz, 2H), 5.09 (qn, *J* = 6.4 Hz, 1H), 5.29 (dd, *J* = 8.9, 6.6 Hz, 1H), 5.31 (m, 1H), 5.42 (qn, *J* = 7.8 Hz, 1H), 7.60 (d, *J* = 9.1 Hz, 1H), 7.80 (d, *J* = 8.7 Hz, 1H), 8.06 (s, 2H); <sup>13</sup>C NMR  $\delta$ : 11.69, 14.28, 17.87, 19.53, 19.61, 21.14, 28.24 (3C), 32.93, 42.91, 48.71, 55.84, 61.35, 80.28, 123.77, 126.83, 128.49, 147.41, 149.01, 153.96, 154.93, 160.29, 161.13, 161.22, 161.53, 171.88, 174.69; IR (neat) cm<sup>-1</sup>: 3313, 3114, 1713, 1661, 1510; MS (TOF) *m/z*: 657 [*M*+Na]<sup>+</sup>; HRMS (TOF) *m/z*: calcd for C<sub>28</sub>H<sub>38</sub>N<sub>6</sub>O<sub>7</sub>NaS<sub>2</sub>: 657.21356, found: 657.21388 [*M*+Na]<sup>+</sup>.

**(1<sup>2</sup>Z,5<sup>2</sup>Z,9<sup>2</sup>Z,2R,6R,10R)-10-Isopropyl-1<sup>5</sup>,2,6-trimethyl-3,7,11-triaza-1(2,4)-oxazola-5,9(4,2)-dithiazolacyclododecaphane-4,8,12-trione (Dendroamide A) (1)**. To a solution of trimer **18** (55 mg, 0.087 mmol) in MeOH (0.44 mL) was added 1 M NaOH (0.44 mL, 0.44 mmol) with stirring at 0 °C. After the mixture was stirred at rt for 30 min, the solvent was almost completely evaporated under reduced pressure. The residue was diluted with AcOEt (10 mL) and acidified with ice-cooled 0.33 M HCl (6 mL). After the organic layer was separated, the aqueous layer was extracted twice with AcOEt (10 mL). The combined organic layers were washed with brine (10 mL) and dried over MgSO<sub>4</sub>. After removal of the solvent *in vacuo*, the carboxylic acid was dissolved in 1,4-dioxane (1.7 mL), and 4 M HCl in 1,4-dioxane (1.7 mL, 6.80 mmol) was then added to the solution with stirring at 0 °C. After stirring at rt for 50 min, the solvent was evaporated under reduced pressure. After removal of the solvent *in vacuo*, the amine salt was dissolved in CH<sub>2</sub>Cl<sub>2</sub>/DMF (3:1, 4.4 mL), and the solution was added over 3.5 h to a solution of DPPA (0.11 mL, 0.52 mmol) and DIEA (0.091 mL, 0.52 mmol) in a 3:1 mixture of CH<sub>2</sub>Cl<sub>2</sub>/DMF (17.4 mL) using a syringe pump with stirring at rt under Ar. After further stirring at rt for 4 h, the mixture was diluted with AcOEt (50 mL) and washed with water (10 mL) and brine (10 mL) before drying over MgSO<sub>4</sub> and evaporating the solvent. The residue was purified by PTLC with AcOEt to give dendroamide A (**1**) (36.4 mg, 86%) as a colorless foam;  $[\alpha]_{\text{D}}^{20} +77.7$  (*c* 0.46, CHCl<sub>3</sub>); <sup>1</sup>H NMR  $\delta$ : 0.98 (d, *J* = 6.8 Hz, 3H), 1.08 (d, *J* = 7.2 Hz, 3H), 1.71 (d, *J* = 6.8 Hz, 3H), 1.74 (d, *J* = 7.2 Hz, 3H), 2.33 (m, 1H), 2.68 (s, 3H), 5.21 (qn, *J* = 6.6 Hz, 1H), 5.31 (dd, *J* = 8.0, 4.8 Hz), 5.72 (qn, *J* = 7.6 Hz, 1H), 8.14 (s, 1H), 8.15 (s, 1H), 8.49 (br d, *J* = 8.4 Hz, 1H), 8.55 (br d, *J* = 8.0 Hz), 8.65 (br d, *J* = 6.4 Hz, 1H); <sup>13</sup>C NMR  $\delta$ : 11.60, 18.27, 18.35, 20.94, 24.92, 35.06, 44.27, 47.02, 55.94, 123.65, 123.83, 128.39, 148.75, 148.78, 153.75, 159.55, 159.81, 160.54, 161.67, 168.23, 171.12; IR (neat) cm<sup>-1</sup>: 3494, 3395, 3120, 1668, 1638, 1540, 1509; MS (TOF) *m/z*: 511 [*M*+Na]<sup>+</sup>; HRMS (TOF) *m/z*: calcd for C<sub>21</sub>H<sub>24</sub>N<sub>6</sub>O<sub>4</sub>S<sub>2</sub>Na: 511.11927, found:

511.11980 [M+Na]<sup>+</sup>.

### ATPase Assay Procedure<sup>14</sup>

The SB-MDR1 PREDEASY™ ATPase Kit from SOLVO Biotechnology (Budapest, Hungary) was used for the determination of the ABCB1-ATPase activity, according to the manufacturer's suggestions. MDR1-Sf9 membrane vesicles (4 μg) were incubated in 50 μL ATPase assay buffer with 2 mM ATP and 0, 0.3, 3, 30, and 300 μM of the test drugs for 10 min at 37 °C. ATPase activities were determined by the difference of the inorganic phosphate liberation measured with and without the presence of 1.2 mM sodium orthovanadate (vanadate-sensitive ATPase activity). The assay was performed in duplicate.

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