Total Synthesis of Dendroamide A, a Novel Cyclic Peptide That Reverses Multiple Drug Resistance

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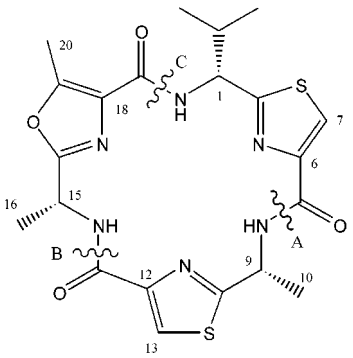
Dendroamide A (**1**) was isolated from a blue-green alga on the basis of its ability to reverse drug resistance in tumor cells that overexpress either of the transport proteins, P-glycoprotein or MRP1. Because of this activity, methods for the synthesis of analogues of this oxazole- and thiazole-containing cyclic peptide have been developed, and the total synthesis of **1** has been completed. Highlights of the synthetic strategy are as follows: (1) a dicyclohexylcarbodiimide coupling of D-Ala and L-Thr, followed by reaction with Burgess reagent and DBU-assisted oxidation to form D-Ala-oxazole; (2) formation of D-Val-thiazole and D-Ala-thiazole via modified Hantzsch reactions; and (3) use of molecular modeling to select the preferred precursor for the final cyclization of the peptide analogue. Synthetic **1** demonstrated spectral properties identical to those of the natural product and reversed P-glycoprotein-mediated drug resistance more effectively than MRP1-mediated resistance. Certain of the synthetic precursors had biological activity, indicating that cell permeability and peptide cyclization are necessary for optimal activity. Thus, the structure and the biological activities of the natural product are confirmed, and methods for the synthesis of analogues for further structure−activity explorations are defined.

Introduction

The development of multiple drug resistance (MDR) in cancer cells is believed to be one of the major obstacles to successful cancer chemotherapy.[1] MDR refers to the phenomenon whereby cancer cells undergoing chemotherapy simultaneously develop resistance to a number of drugs with diverse structures and mechanisms. One of the most important mechanisms of MDR is the overexpression of P-glycoprotein (Pgp) and related drug transporters in cancer cells. Pgp is a plasma membrane-associated, energy-dependent efflux pump that can effectively transport a variety of anticancer drugs out of the cell.[2] Overexpression of Pgp by tumor cells is thought to be an important cause of both intrinsic and acquired resistance to anticancer drugs. Therefore, Pgp is a promising target for cancer therapy, and great interest and effort have been focused on the development of effective inhibitors of Pgp-mediated MDR.[3]

In 1996, Dendroamide A (**1**) was isolated from the cyanobacterium *Stigonema dendroideum fremy* on the basis of its ability to reverse Pgp-mediated MDR.[4] This natural product demonstrated significant ability to antagonize Pgp and MRP1 at noncytotoxic doses and, therefore, has potential utility against tumors that have developed resistance. Because **1** is a modified cyclic peptide, it may serve as the starting point for the synthesis of a variety of analogues for structure−activity analyses. To facilitate its further evaluation, we have developed methods for the total synthesis of **1**.



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Results and Discussion

Retrosynthetic Analysis. The structure of **1** reveals that this cyclic natural product is composed of a methyl-oxazole- and two thiazole-containing subunits connected by peptide bonds. Recently, there has been interest in the synthesis of other cyclic compounds containing either oxazole or thiazole heterocycles, providing some methodological insights.[5] Typically, one of the most difficult reactions in the synthesis of such compounds is the final cyclization, especially for compounds built with multiple sterically hindered heterocyclic rings.[6] Therefore, an initial consideration in the synthesis of **1** involved select-

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Table 1. Estimation of Distances for the Amino Group and the Acyl Azide Group of the Cyclization Intermediates

precursor	centers of cyclization	MM2[a] (Å)	MOPAC[b] (Å)
A	C N	12.76	13.85
B	C N	3.56	6.14
C	C N	8.03	10.25

[a] MM2 is a molecular modeling method based on the laws of classical physics to molecular nuclei without explicit consideration of the electrons. [b] MOPAC is a molecular modeling method based on the quantum mechanical calculation that relies on the Schrödinger equation to describe a molecule with explicit treatment of electronic structure.

ing the cyclization precursor that might provide the best yield. To this end, the lowest energy conformation of each of the three possible acyl-azide precursors was determined using both MM2 and MOPAC modeling (Chem3D, CambridgeSoft). As indicated in Table 1, the distances between the N-terminal nitrogen and the C-terminal carbonyl carbon in those precursors were variable, ranging from approximately 3.5 Å to greater than 12 Å. Since the reaction is most likely to occur when the reaction centers are close, precursor B (**22**) was selected as being most promising for the final cyclization reaction. Formal testing of this optimization hypothesis would require completing the synthesis of **1** from all of the three precursors. As the purpose of the modeling studies was only to suggest a route, the additional wet chemistry was not conducted to validate this hypothesis. However, as detailed below, the yield for cyclization of **22** was considerably higher than that of a related compound bistramide A (56% versus 15−43%[6a]) suggesting that the modeling has utility.

Dendroamide A is characterized by three chiral centers at the 1, 9, and 15 positions (**1**) which are all *R* configurations, implying their derivation from D-amino acids. Therefore, the stereochemically correct precursor **20** could be built by coupling of (*R*)-2-[1-*N*-BOC-amino]-ethyl-5-methyloxazole-4-carboxylic acid (**18**) and (*R*)-2-[1-[(*R*)-2-(1-amino)isobutylthiazole-4-carbonyl]amino]-ethylthiazole-4-carboxylic acid ethyl ester (**19**). Similarly, the protected thiazole dimer **17** could be made by coupling of selectively deprotected (*R*)-2-(1-*N*-BOC-amino)ethylthiazole-4-carboxylic acid ethyl ester (**13**) and (*R*)-2-(1-*N*-BOC-amino)isobutylthiazole-4-carboxylic acid ethyl ester (**14**). Thus, the chiral oxazole **6** and thiazoles **13** and **14** provide the building blocks for the synthesis of **1**. The oxazole could be prepared by dehydrogenation of the corresponding oxazoline synthesized from the dipeptide, D-Ala-L-Thr, using Burgess reagent.[7] In parallel, the thiazoles could be prepared through a modified Hantzsch reaction[8] from thioamides made from the corresponding amides of D-alanine or D-valine using Lawesson's reagent.

Synthesis. As indicated in Scheme 1, BOC-D-alanine was coupled with L-threonine methyl ester using DCC (1,3-dicyclohexylcarbodiimide) to afford BOC-D-Ala-L-Thr methyl ester (**4**) in good yield. Treatment of **4** under an inert atmosphere with Burgess reagent (methyl (carboxysulfamoyl)triethylammonium hydroxide) provided the oxazoline amino acid mixture (4*S*,5*R*)-2-[(*R*)-1-*N*-BOC-amino]ethyl-5-methyloxazoline-4-carboxylic acid methyl ester (**5a**) and (4*S*,5*S*)-2-[(*R*)-1-*N*-BOC-amino]-ethyl-5-methyloxazoline-4-carboxylic acid methyl ester (**5b**) in high yield. The reaction likely proceeds by an E1cb mechanism. ^{1}H and ^{13}C NMR spectral analyses indicated that one stereoisomer in the mixture was dominant, presumably **5a** since steric hindrance makes **5b** less favorable. However, since both **5a** and **5b** will generate oxazole amino acid **6** after dehydrogenation, it was unnecessary to separate the isomers **5a** and **5b**. Attempts to use metal peroxide (either NiO_2[9] or MnO_2[10]) for dehydrogenation of the mixture of **5a** and **5b** produced only a low yield (∼30%) of oxazole amino acid **6**. Consequently, a more efficient method was adopted for this dehydrogenation. Using bromotrichloromethane (BTCM) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)[11] under mild conditions allowed **5** to be dehydrogenated to produce the fully protected oxazole amino acid **6** in good yield (69%).

(R)-2-(1-*N*-BOC-amino)ethylthiazole-4-carboxylic acid ethyl ester (**13**) and (R)-2-(1-*N*-BOC-amino)isobutylthiazole-4-carboxylic acid ethyl ester (**14**) were prepared according to the method of Bredenkamp et al.[8a] as indicated in Scheme 2. BOC-D-alanine (**7**) and BOC-D-valine (**8**) were used as the chiral starting materials and were converted to BOC-D-amino acid amides **9** and **10** in very good yields by a mixed anhydride method. This reaction was followed by thionation using Lawesson's reagent to form BOC-D-amino acid thioamides **11** and **12** in high yields. The thioamides **11** and **12** were then coupled with ethyl bromopyruvate to give intermediate hydroxythiazolines which were, without isolation, dehydrated using trifluoroacetic anhydride to afford fully protected thiazole amino acids **13** and **14** in high yields.

(*R*)-2-(1-*N*-BOC-amino)isobutylthiazole-4-carboxylic acid (**16**) was prepared in high to quantitative yield by hydrolysis of ester **14** using 3 equiv of NaOH at room temperature as indicated in Scheme 3. This reaction was carefully monitored by TLC to avoid overreaction that may lead to racemization. (*R*)-2-(1-*N*-BOC-amino)ethylthiazole-4-carboxylic acid ethyl ester (**13**) was deprotected readily to afford (*R*)-2-(1-amino)ethylthiazole-4-carboxylic acid ethyl ester (**15**) in quantitative yield using either trifluoroacetic acid or acetyl chloride in ethanol. The resolutions of the ^{1}H and ^{13}C NMR spectra for thiazole amino acid **15** trifluoroacetic acid salt were much better than those of the hydrochloric acid salt. Subsequent coupling of **15** and **16** using diisopropylcarbodiimide (DIC) and *N*-hydroxybenzotriazole (HOBt) afforded (*R*)-2-[1-[(*R*)-(1-*N*-BOC-amino)isobutylthiazole-4-carbonyl]amino]ethylthiazole-4-carboxylic acid ethyl ester (**17**) in good yield.

As shown in Scheme 4, the same strategies used to deprotect **15** and **16** were applied to the preparation of **18** from **6** and **19** from **17** in high to quantitative yields.

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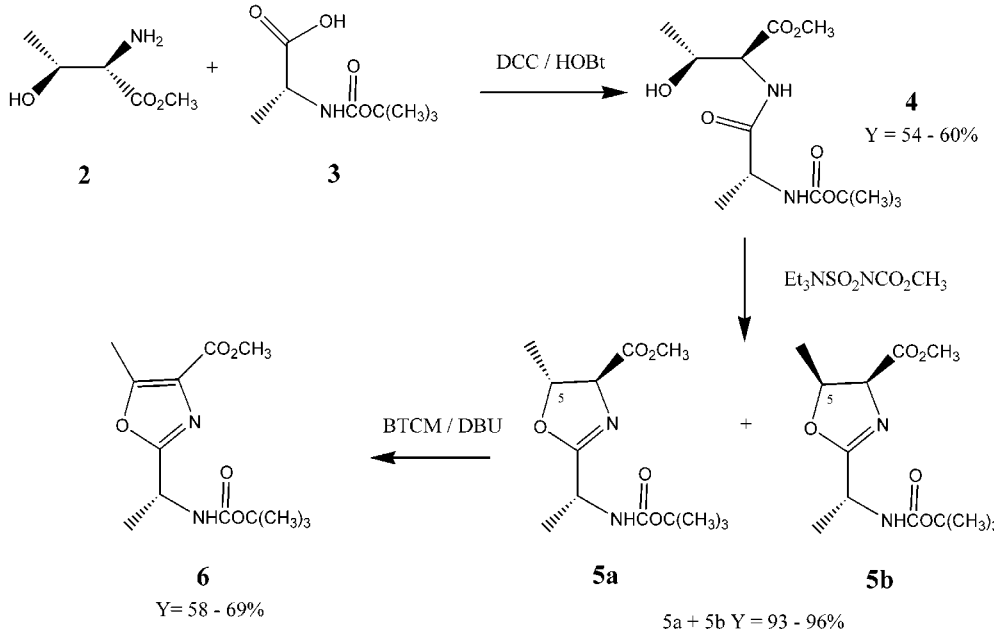
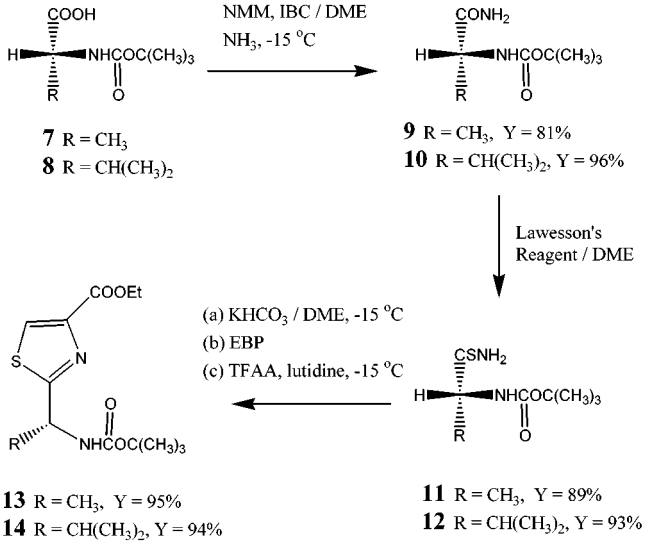
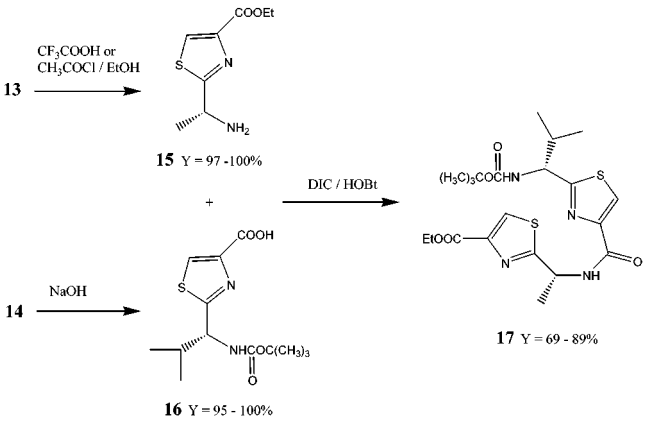
Scheme 1



Scheme 2

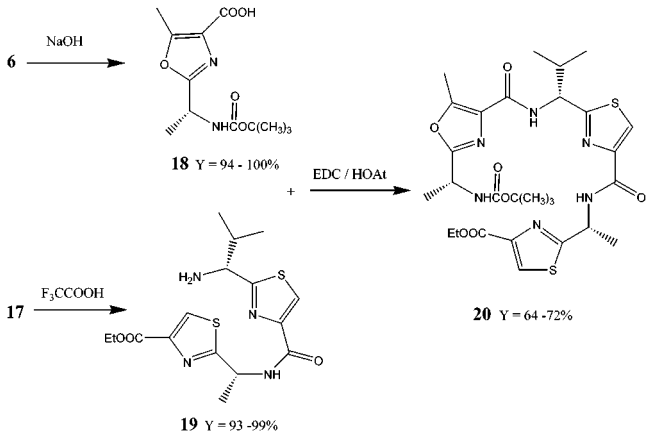


Scheme 3



However, the subsequent condensation of **18** and **19** using conventional coupling reagents, such as DIC/HOBt, gave the desired protected precursor B **20** in only a very low yield. This may reflect the stereo hindrance of the isobutyl group at the *N*-terminus of **19**. In addition, the workup of the reaction mixture was difficult due to the side-product *N*,*N*′-diisopropylurea. The use of 1-hydroxy-7-azabenzotriaole (HOAt) as a condensing additive instead of HOBt has been useful in the synthesis of peptides or amides with stereo hindered components,[12] perhaps at least partially due to the neighboring group effect of the HOAt.[13] Moreover, use of a water-soluble coupling agent such as ethyl (dimethylamino)propylcarbodiimide hydrochloride (EDC) often circumvents the contamination from the urea byproduct since the byproducts of this reagent can be readily removed by aqueous extraction. With this coupling reagent system (EDC/HOAt), **20** was prepared in very good yield (72%).

Scheme 4



Using the strategies described for the deprotection of **15** and **16**, compound **20** was sequentially deprotected at C-terminus to yield **21** and then at the N-terminus to afford **22** as shown in Scheme 5. Although several coupling reagents could be used for cyclization,[14] we chose diphenylphosphoryl azide (DPPA) because it is com-

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Scheme 5

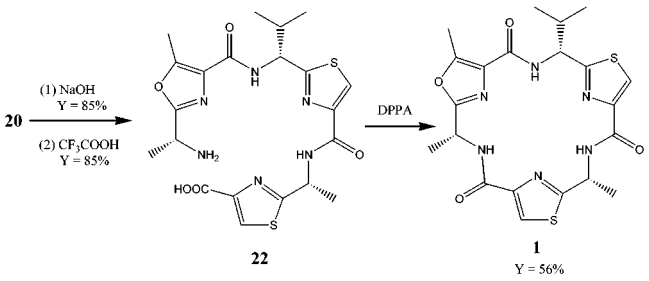

Table 2. ^{1}H and ^{13}C Chemical Shifts (δ/ppm) and ^{1}H−^{1}H Coupling Constants (mult, *J*/Hz) for Dendroamide A and Its Isomer[a]

	dendroamide A (**1**)		isomer of dendroamide A (**23**)	
position	δ (^{13}C)	δ (^{1}H)	δ (^{13}C)	δ (^{1}H)
N1		8.50 (br d; 8.2)		8.51 (br d; 8.5)
1	56.1	5.31 (dd; 8.0, 4.8)	56.4	5.22 (m)
2	35.1	2.33 (m)	34.7	2.32 (m)
3	18.3	1.08 (d; 6.8)	18.1	1.07 (d; 6.8)
4	18.4	0.98 (d; 6.7)	18.8	0.93 (d; 6.7)
5	168.4		168.1	
6	149.0		149.0	
7	123.8	8.15 (s)	123.5	8.20 (s)
8	159.6		159.9	
N3		8.56 (br d; 8.4)		8.55 (br d; 7.3)
9	47.1	5.72 (quintet)	48.1	5.58 (quintet)
10	25.0	1.74 (d; 5.8)	23.7	1.81 (d; 6.7)
11	171.3		171.9	
12	149.0		149.3	
13	123.6	8.14 (s)	124.2	8.15 (s)
14	159.9		160.0	
N5		8.65 (br d; 6.5)		8.61 (br d; 6.1)
15	44.4	5.21 (quintet, 6.5)	44.7	5.22 (m)
16	21.0	1.71 (d; 6.1)	19.8	1.66 (d; 6.7)
17	161.8		161.3	
18	153.8		154.1	
19	128.6		128.6	
20	11.6	2.68 (s)	11.6	2.69 (s)
21	160.6		160.7	

[a] The positions for the isomer are relative to **1**.

mercially available and has been proven to be an efficient cyclization reagent for cyclic peptides.[15−17] Using DPPA, the cyclization of **22** produced dendroamide A (**1**) in 56% yield. An additional product (**23**) of the mass expected for **1** was isolated in 18% yield and demonstrated spectral properties virtually identical to **1** (Table 2), indicating that it may be a stable conformational isomer of **1**. Compounds **1** and **23** were not interconverted by incubation at room temperature for at least 2 months. In the ^{1}H NMR spectrum of the isomer, the protons at positions 1, 4 and 9 were shifted about 0.1 ppm to higher fields compared to those of **1**. In contrast, the chemical shift of the proton at position 10 was downfield more than 0.1 ppm. In the ^{13}C NMR spectrum of the isomer, the chemical shifts of carbon at positions 10 and 16 were moved about 1 ppm to higher fields compared to those of **1**. In contrast, the carbon at position 9 was shifted about 1 ppm to lower field.

Biological Activities. Dendroamide A (**1**) was originally isolated from a blue-green alga because of its ability to overcome drug resistance in tumor cells that express the transport proteins Pgp and/or MRP1.[4] These activities were confirmed with the synthetic compounds **1** and **23** as follows. MCF-7 human breast carcinoma cells that are considered to be drug-sensitive and cells from two drug-resistant cell lines, NCI/ADR and MCF-7/VP, were treated with varying doses of the test compound alone or in the presence of a cytotoxic drug that is a substrate for Pgp or MRP1. Consequently, NCI/ADR cells were treated with the test compound alone or in the presence of 50 nM vinblastine, while MCF-7/VP cells were treated with the test compound alone or in the presence of 1 nM vincristine. If the test compound inhibits Pgp or MRP1, the toxicity of the concurrent cytotoxic drug is dramatically increased. After exposure to the drug combinations for 48 h, the number of surviving cells was determined using an established assay.[18] Verapamil was used as a positive control in all experiments and is known to inhibit the activities of both Pgp and MRP1. These analyses have been widely used by ourselves and others to evaluate the activity of new compounds as inhibitors of these transport proteins.[4,19]

Figure 1A demonstrates the effects of **1** and **23** on the survival of NCI/ADR cells. In the absence of vinblastine (open symbols), **1** and **23** had relatively low levels of toxicity toward these cells, with **23** being slightly more cytotoxic than **1**. Both compounds markedly enhanced the cytotoxicity of a concentration of vinblastine that was not by itself toxic to these cells (filled symbols). Sensitization was apparent at the lowest doses of **1** and **23** tested (0.4 μM), with the two compounds having essentially the same potency. Figure 1B demonstrates that both compounds sensitize MRP1-overexpressing MCF-7/VP cells to vincristine, albeit with lower potency and efficacy than their effects on Pgp. Neither NCI/ADR nor MCF-7/VP cells were resistant (compared with MCF-7 cells) to the direct cytotoxicity of **1** or **23**, indicating that these compounds are not substrates for the drug transporters. Therefore, **1** and **23** potently reverse MDR mediated by Pgp and have lower but significant activity against MRP1-mediated MDR.

To further explore structure−activity relationships of the newly synthesized compounds, several monomeric (**5a/5b**, **6**, **13**, **14**, and **18**), dimeric (**17** and **19**), and trimeric (**20**, **21**, and **22**) precursors were tested for their abilities to antagonize Pgp and MRP1. As indicated in Table 3, most of these compounds were relatively nontoxic toward MCF-7, as well as NCI/ADR and MCF-7/VP, cells. Of the monomeric compounds, only **14** had measurable activity against Pgp. This compound is the dually protected valine-thiazole and therefore is likely to penetrate the cells reasonably well. Interestingly, the dually protected alanine-thiazole congener had no activity, indicating that side-chain bulk is important for activity. The dually protected dimeric thiazole (**17**) had quite good activity against Pgp and modest activity against MRP1. Removal of the *N*-BOC protecting group to produce **19** decreased both activities, which is likely due to poorer cell permeability because of charge at the

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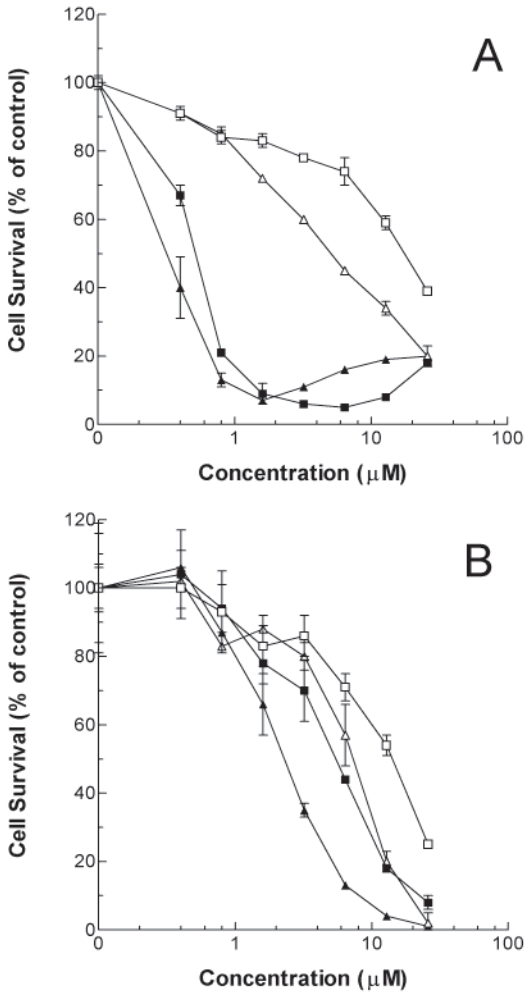

Figure 1. Reversal of Pgp- and MRP1-mediated drug resistance by dendroamide A and its isomer. Panel A: NCI/ADR cells were treated with the indicated concentrations of **1** (squares) or **23** (triangles) either alone (open symbols) or in the presence of 50 nM vinblastine (filled symbols). Panel B: MCF-7/VP cells were treated with the indicated concentrations of **1** (squares) or **23** (triangles) either alone (open symbols) or in the presence of 1 nM vincristine (filled symbols). After 48 h, the percentage of cells surviving was determined as indicated in the Experimental Section. Values represent the mean ± sd survival in one of three similar experiments.

N-terminus. Similarly, the dually protected trimeric compound **20** had good activity against both Pgp and MRP1, whereas deprotection at either the C-terminus (**21**) or both termini (**22**) dramatically decreased activity.

Conclusion

The total synthesis of **1** in satisfactory yield has been accomplished by modifying several peptide synthesis protocols and by using molecular modeling to select the precursor for the final cyclization reaction. These methods can now be applied to the synthesis of further analogues of these unusual thiazole- and oxazole-containing cyclic peptides. This may be useful since the biological activities of these compounds appear to be highly dependent on the substitution present at the side-chains of the initial amino acids, e.g., **13** versus **14**. The cyclic nature of the target compounds is likely to overcome two common limitations with peptide-based therapeutics. First, their small size, hydrophobicity, and lack of charge make them cell permeable. This is supported by the dramatic loss of biological activity in compounds containing charged termini. Second, the incorporation of the thiazole and oxazole moieties is likely to protect these compounds from proteases that efficiently destroy traditional peptides. Furthermore, the amino acids used in the synthesis of these compounds are in the unnatural D-stereochemistry, which will also prevent their proteolytic cleavage. Therefore, this class of MDR modulator has interesting potential for both clinical application and structural characterization of the substrate-binding sites on these important drug transport proteins.

Table 3. Biological Activities of Selected Compounds

compd	toxicity[a]	antagonism of Pgp[b]	antagonism of MRP1[c]
1	18 ± 5	14.3 ± 2.9	3.1 ± 0.2
5a/b	>87	1.0 ± 0	0.9 ± 0.3
6	>88	1.1 ± 0.1	1.2 ± 0
13	>83	1.1 ± 0	0.7 ± 0.1
14	>76	2.0 ± 0.2	1.2 ± 0
17	105 ± 30	7.9 ± 1.8	2.1 ± 0.1
18	>93	0.9 ± 0	1.1 ± 0
19	>65	2.8 ± 0.5	1.4 ± 0
20	28 ± 2	6.5 ± 2.7	3.2 ± 0.2
21	>41	1.2 ± 0	1.0 ± 0.3
22	>49	1.0 ± 0	0.9 ± 0.1
23	11 ± 2	9.9 ± 0	2.3 ± 0.1
verapamil	17 ± 2	15.3 ± 0	7.2 ± 0.9

[a] Toxicity values (in μM) indicate the concentration of compound that kills 50% of MCF-7 cells (IC_{50}). For several compounds, the IC_{50} was not reached at the indicated highest concentration tested. [b] Antagonism of Pgp is calculated as the percentage of NCI/ADR cells that survive in the presence of the test compound alone/the percentage of NCI/ADR cells that survive in the presence of test compound and 50 nM vinblastine. Consequently, a high value indicates good inhibition of Pgp while a value of 1 indicates no activity. The indicated values are the maximum ratio ± standard deviation at doses of test compound lower than its IC_{50}. [c] Antagonism of MRP1 is calculated as the percentage of MCF-7/VP cells that survive in the presence of the test compound alone/the percentage of MCF-7/VP cells that survive in the presence of test compound and 1 nM vincristine. Consequently, a high value indicates good inhibition of MRP1 while a value of 1 indicates no activity. The indicated values are the maximum ratio ± standard deviation at doses of test compound lower than its IC_{50}.

Experimental Section

General Procedures. All reagents used were of reagent grade or better. Anhydrous organic solvents were obtained either from Aldrich or distilled from the drying agents: Na or CaH_2. Melting points are expressed uncorrected. High-resolution mass spectra (MALDI or FAB mode) were obtained from the Scripps Research Institute or University of California at Berkeley. The purities of intermediates and products were analyzed by HPLC on a C18 column eluted with CH_3CN/0.1% TFA-water (7:3) at 1 mL/min. The eluant was monitored at multiple wavelengths to ensure compound purity. Nuclear magnetic resonance spectra (^1H NMR and ^{13}C NMR) were acquired using a 200 MHz instrument. Chemical shifts are relative to either the internal standard tetramethylsilane or the deuterium solvent itself, and exchangeable protons (O*H* or N*H*) were confirmed by addition of D_2O. The purity of all synthetic compounds was high as evidenced by homogeneity during HPLC and low-noise NMR spectra. Spectra of compounds used for SAR analyses (Table 3) are presented as Supporting Information.

Human breast carcinoma MCF-7 cells and Pgp-overexpressing NCI/ADR cells[20] were obtained from the Division of Cancer Treatment of the National Cancer Institute, while MRP-

overexpressing MCF-7/VP cells[21] were kindly provided by Dr. Erasmus Schneider (Division of Molecular Medicine, Wadsworth Center, NY). Both cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum at 37 °C in a humidified 5% CO_2 atmosphere.

Synthesis of BOC-D-Ala-L-Thr-OMe (4). *N*-Ethylmorpholine (1.28 mL, 10 mmol) was added to a solution of BOC-D-alanine (**3**) (1.89 g, 10 mmol) in DMF (5 mL). The solution was cooled to 0 °C, and a solution of DCC (2.41 g, 12 mmol) in DMF (5 mL) was added. After the solution was stirred at 0 °C for 1 h, a solution of L-threonine methyl ester (**2**) (1.70 g, 10 mmol) in DMF (5 mL) was added. The reaction mixture was allowed to warm from 0 °C to rt and incubated for 12 h. After filtration and removal of the solvent in vacuo, the residue was dissolved in EtOAc (100 mL) and washed with 10% citric acid (50 mL), saturated $NaHCO_3$ (50 mL), and water (50 mL) sequentially. The EtOAc solution was dried over $MgSO_4$. After removal of solvent, the residue was chromatographed on a silica gel column with an elution gradient of 1−10% MeOH in $CHCl_3$ to afford **4** (1.81 g, Y = 60%) as a colorless syrup that became a white solid upon storage at −20 °C: purity (HPLC, UV 214 nm) 97%; R_f 0.39 (MeOH/$CHCl_3$ 1:9); mp 86−87 °C; $[\alpha]_D$ = 23.4 (*c* 1.06, MeOH, 23 °C); ^{1}H NMR ($CDCl_3$) δ 7.19 (d, *J* = 8.9 Hz, 1 H), 5.42 (br s, 1 H), 4.58 (dd, *J* = 8.9 Hz, 2.3 Hz, 1 H), 4.10−4.45 (m, 2 H), 3.76 (s, 3 H), 2.66 (br s, 1 H), 1.45 (s, 9 H), 1.41 (d, *J* = 7.2 Hz, 3 H), 1.22 (d, *J* = 6.5 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 173.9, 171.0, 155.2, 79.4, 66.9, 57.3, 51.8, 50.2, 28.2 (3 C), 19.4, 18.0; HRMS (MALDI) *m*/*z* calcd for $C_{13}H_{24}N_2O_6$-Na (M + Na) 327.1527, found 327.1526.

Synthesis of (4*S*,5*R*)-2-[(2*R*)-1-*N*-BOC-amino]ethyl-5-methyloxazoline-4-carboxylic Acid Methyl Ester (5a) and (4*S*,5*S*)-2-[(2*R*)-1-*N*-BOC-amino]ethyl-5-methyloxazoline-4-carboxylic Acid Methyl Ester (5b). Under a flow of dry N_2, a solution of Burgess reagent (413 mg, 1.73 mmol) in anhydrous THF (20 mL) was added dropwise to a solution of **4** (418 mg, 1.37 mmol) in anhydrous THF (20 mL) containing molecular sieves. The reaction mixture flask was then capped and stirred at 85 °C for 3 h. After cooling and removal of the solvent in vacuo, the residue was purified by radial chromatography on silica eluted with 3% MeOH in $CHCl_3$ to afford a mixture of oxazolines **5a** and **5b** as a yellowish syrup (377 mg, Y = 96%), which was used immediately in the preparation of oxazole **6**: purity (**5a** + **5b**) (HPLC, UV 220 nm) 96%; R_f (**5a** + **5b**) 0.32 (MeOH/$CHCl_3$ 3:97); $[\alpha]_D$ = 46.2 (**5a** + **5b**, *c* 2.32, MeOH, 23 °C); ^{1}H NMR ($CDCl_3$) of **5a** δ 5.31 (d, *J* = 14.4 Hz, 1 H), 4.95 (m, 1 H), 4.79 (d, *J* = 10.1 Hz, 1 H), 4.45 (dq, *J* = 14.4, 7.2 Hz, 1 H), 3.76 (s, 3 H), 1.45 (s, 9 H), 1.41 (d, *J* = 7.2 Hz, 3 H), 1.30 (d, *J* = 6.3 Hz, 3 H); ^{1}H NMR ($CDCl_3$) of **5b** δ 5.31 (d, *J* = 14.4 Hz, 1 H), 4.96 (m, 1 H), 4.79 (d, *J* = 10.1 Hz, 1 H), 4.45 (dq, *J* = 14.4, 7.2 Hz, 1 H), 3.76 (s, 3 H), 1.44 (s, 9 H), 1.41 (d, *J* = 7.2 Hz, 3 H), 1.28 (d, *J* = 6.3 Hz, 3 H); ^{13}C NMR ($CDCl_3$) of **5a** δ 170.7, 170.0, 155.0, 79.8, 78.6, 71.0, 52.1, 45.1, 28.4 (3 C), 19.5, 16.1; ^{13}C NMR ($CDCl_3$) of **5b** δ 170.9, 170.0, 155.0, 79.8, 78.5, 70.9, 52.1, 45.1, 28.4 (3 C), 19.5, 16.0; HRMS (FAB) *m*/*z* calcd for $C_{13}H_{23}N_2O_5$ (M + H) 287.1601, found 287.1604.

Synthesis of (*R*)-2-[1-*N*-BOC-amino]ethyl-5-methyloxazole-4-carboxylic Acid Methyl Ester (6). DBU (85 μL, 0.57 mmol) was added to a solution of the mixture of **5a** and **5b** (143 mg, 0.50 mmol) in CH_2Cl_2 (5 mL) at 0 °C, followed by addition of BTCM (54 μL, 0.55 mmol) within 10 min, and the reaction mixture was incubated at 0 °C for 6 h. After dilution with CH_2Cl_2 (5 mL), the reaction mixture solution was washed with saturated NH_4Cl (2 × 5 mL). The water solution was extracted with EtOAc (2 × 5 mL), and the organic solution was dried over $MgSO_4$. After removal of the solvent in vacuo, the residue was purified by radial chromatography on silica eluted with 3% MeOH in $CHCl_3$ to afford oxazole **6** as a yellow oil that became a yellow solid upon storage at −20 °C (98 mg, *Y* = 69%): purity (HPLC, UV 254 nm) 99%; R_f 0.60 (MeOH/$CHCl_3$ 3:97); mp 69−77 °C (no sharp melting point observed); $[\alpha]_D$ = 31.5 (*c* 0.85, THF, 26 °C); ^{1}H NMR ($CDCl_3$) δ 5.38 (d, *J* = 7.0 Hz, 1 H), 4.94 (quintet, *J* = 7.0 Hz, 1 H), 3.91 (s, 3 H), 2.62 (s, 3 H), 1.54 (d, *J* = 7.0 Hz, 3 H), 1.44 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 163.0, 162.6, 156.5, 154.9, 127.4, 80.1, 51.9, 44.8, 28.3 (3 C), 20.2, 12.0; HRMS (MALDI) *m*/*z* calcd for $C_{13}H_{20}N_2O_5$-Na (M + Na) 307.1264, found 307.1251.

Synthesis of BOC-D-alanine Amide (9). *N*-Methylmorpholine (NMM) (1.66 mL, 15.1 mmol) and isobutyl chloroformate (IBC) (1.96 mL, 15.1 mmol) were added to a solution of BOC-D-alanine (**7**) (2.86 g, 15.1 mmol) in ethylene glycol dimethyl ether (DME) (40 mL) at −15 °C. The solution was bubbled with NH_3 through a fritted gas dispersion tube at −15 °C for 15 min and then at rt for 15 min. After the addition of water (80 mL), the mixture was extracted with $CHCl_3$ (3 × 40 mL). The $CHCl_3$ solution was dried over $MgSO_4$ and evaporated in vacuo to yield **9** as a white solid (2.28 g, *Y* = 81%): purity (HPLC, UV 214 nm) 99%; mp 120−121 °C; $[\alpha]_D$ = 6.6 (*c* 1.44, MeOH, 24 °C); ^{1}H NMR (DMSO-d_6) δ 7.23 (br s, 1 H), 6.93 (br s, 1 H), 6.78 (d, *J* = 7.2 Hz, 1 H), 3.89 (quintet, *J* = 7.2 Hz, 1 H), 1.37 (s, 9 H), 1.16 (d, *J* = 7.2 Hz, 3 H); ^{13}C NMR (DMSO-d_6) δ 174.6, 154.8, 77.8, 49.5, 28.0 (3 C), 18.2; HRMS (MALDI) *m*/*z* calcd for $C_8H_{16}N_2O_3Na$ (M + Na) 211.1053, found 211.1046.

Synthesis of BOC-D-valine Amide (10). NMM (1.65 mL, 15.0 mmol) and IBC (1.95 mL, 15.0 mmol) were added to a solution of BOC-D-valine (**8**) (3.26 g, 15.0 mmol) in DME (40 mL) at −15 °C. The solution was bubbled with NH_3 and worked up as described for **9**. After removal of the solvent in vacuo, **10** was obtained as a white solid (3.13 g, *Y* = 96%): purity (HPLC, UV 214 nm) 100%; mp 156−157 °C; $[\alpha]_D$ = 5.2 (*c* 1.36, MeOH, 25 °C); ^{1}H NMR (DMSO-d_6) δ 7.29 (br s, 1 H), 7.03 (br s, 1 H), 6.54 (d, *J* = 8.9 Hz, 1 H), 3.73 (dd, *J* = 8.9, 6.6 Hz, 1 H), 1.90 (m, 1 H), 1.38 (s, 9 H), 0.85 (d, *J* = 7.1 Hz, 3 H), 0.81 (d, *J* = 7.1 Hz, 3 H); ^{13}C NMR (DMSO-d_6) δ 173.2, 155.2, 77.8, 59.4, 30.2, 28.0 (3 C), 19.1, 17.7; HRMS (MALDI) *m*/*z* calcd for $C_{10}H_{20}N_2O_3Na$ (M + Na) 239.1366, found 239.1366.

Synthesis of BOC-D-alanine Thioamide (11). Lawsson's reagent (1.71 g, 4.23 mmol) was added to a solution of **9** (1.52 g, 8.09 mmol) in DME (40 mL), and the resulting suspension was stirred at rt for 4.5 h. After removal of the solvent, the residue was extracted with $CHCl_3$ (40 mL) and washed with 1% NaOH (80 mL). The water solution was extracted with $CHCl_3$ (2 × 40 mL), and the $CHCl_3$ phase was concentrated in vacuo and chromatographed on a silica gel column eluted with EtOAc/hexane (2:1) to afford **11** as a yellowish syrup (1.47 g, *Y* = 89%): purity (HPLC, UV 254 nm) 99%; R_f 0.32 (EtOAc/hexane 1:1); $[\alpha]_D$ = −5.1 (*c* 0.89, MeOH, 24 °C); ^{1}H NMR (CD_3-OD) δ 4.36 (q, *J* = 7.1 Hz, 1 H), 1.44 (s, 9 H), 1.38 (d, *J* = 7.1 Hz, 3 H); ^{13}C NMR (CD_3OD) δ 212.1, 157.1, 80.9, 57.3, 28.7 (3 C), 21.9; HRMS (MALDI) *m*/*z* calcd for $C_8H_{16}N_2O_2NaS$ (M + Na) 227.0825, found 227.0817.

Synthesis of BOC-D-valine Thioamide (12). Lawsson's reagent (2.09 g, 5.17 mmol) was added to a solution of **10** (2.17 g, 10.0 mmol) in DME (40 mL), and the resulting suspension was stirred at rt for 4 h. After removal of the solvent, the residue was extracted with $CHCl_3$ (100 mL) and washed with 1% NaOH (50 mL). The NaOH water solution was extracted with $CHCl_3$ (2 × 25 mL), and the $CHCl_3$ phase was concentrated in vacuo and chromatographed on a silica gel column eluted with EtOAc/hexane (1:1) to afford **12** as a yellow solid (2.15 g, Y = 93%): purity (HPLC, UV 254 nm) 97%; R_f 0.42 (EtOAc/hexane 1:1); mp 75−85 °C (no sharp melting point observed); $[\alpha]_D$ = 17.1 (*c* 1.81, MeOH, 25 °C); ^{1}H NMR ($CDCl_3$) δ 8.41 (br s, 1 H), 7.90 (br s, 1 H), 5.41 (d, *J* = 7.9 Hz, 1 H), 4.25 (dd, *J* = 8.6, 7.9 Hz, 1 H), 2.10 (m, 1 H), 1.43 (s, 9 H), 0.98 (d, *J* = 6.4 Hz, 6 H); ^{13}C NMR ($CDCl_3$) δ 209.4, 156.1, 80.5, 65.4, 33.3, 28.4 (3 C), 19.6, 18.1; HRMS (MALDI) *m*/*z* calcd for $C_{10}H_{20}N_2O_2NaS$ (M + Na) 255.1138, found 255.1137.

Synthesis of (*R*)-2-(1-*N*-BOC-amino)ethylthiazole-4-carboxylic Acid Ethyl Ester (13). A solution of **11** (1.17 g, 5.74 mmol) in DME (35 mL) was cooled to −13 °C, followed by addition of $KHCO_3$ (4.97 g, 49.7 mmol) under a nitrogen atmosphere. The suspension was vigorously stirred for 15 min,

(20) Fairchild, C. R.; Ivy, S. P.; Kao-Shan, C.-S.; Whang-Peng, J.; Rosen, N.; Isreal, M. A.; Melera, P. W.; Cowan, K.. H.; Goldsmith, M. E. *Cancer Res.* **1987**, *47*, 5141.

(21) Schneider, E.; Horton, J. K.; Yang, C. H.; Nakagawa, M.; Cowan, K. H. *Cancer Res.* **1994**, *54*, 152.

followed by addition of ethyl bromopyruvate (2.4 mL, 19.1 mmol) under the inert atmosphere at −13 °C. The reaction mixture was further incubated at −13 °C for 0.5 h and then at rt for 0.5 h. After the reactants were cooled to −13 °C again, a solution of trifluoroacetic anhydride (3.6 mL, 25.5 mmol) and lutidine (6.2 mL, 53.2 mmol) in DME (10 mL) was added dropwise to it. The reaction mixture was allowed to warm from 0 °C to rt and incubated for 12 h. After removal of the volatile components in vacuo and addition of water (100 mL), the solution was extracted with $CHCl_3$ (3 × 50 mL). The $CHCl_3$ phase was dried over Na_2SO_4, and following removal of the solvent, the residue was chromatographed on silica gel column eluted with $CHCl_3$/EtOAc (2:1) to afford **13** as a yellow semisolid (1.64 g, $Y = 95\%$): purity (HPLC, UV 254 nm) 99%; R_f 0.53 ($CHCl_3$/EtOAc 2:1); $[\alpha]_D = 10.7$ (c 1.17, MeOH, 23 °C); ^{1}H NMR ($CDCl_3$) δ 8.09 (s, 1 H), 5.26 (br s, 1 H), 5.11 (m, 1 H), 4.42 (q, $J = 7.2$ Hz, 2 H), 1.63 (d, $J = 6.8$ Hz, 3 H), 1.45 (s, 9H), 1.40 (t, $J = 7.2$ Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 169.6, 161.4, 154.9, 147.3, 127.1, 80.3, 61.4, 49.1, 28.3 (3 C), 21.8, 14.4; HRMS (MALDI) *m/z* calcd for $C_{13}H_{20}N_2O_4NaS$ (M + Na) 323.1036, found 323.1051.

Synthesis of (*R*)-2-(1-*N*-BOC-amino)isobutylthiazole-4-carboxylic Acid Ethyl Ester (14). A solution of **12** (514 mg, 2.21 mmol) in DME (20 mL) was cooled to −15 °C, followed by addition of $KHCO_3$ (1.77 g, 17.7 mmol) under a nitrogen atmosphere. The suspension was vigorously stirred for 15 min, followed by addition of ethyl bromopyruvate (0.84 mL, 6.69 mmol) under the inert atmosphere at −15 °C. The reaction was continued at −15 °C for 0.5 h and then at rt for 0.5 h. After the reactants were cooled to −15 °C again, a solution of trifluoroacetic anhydride (1.3 mL, 9.20 mmol) and lutidine (2.2 mL, 18.9 mmol) in DME (10 mL) was added dropwise to it. The reaction mixture was incubated and worked up as described for **13**. The residue was applied to a silica gel column eluted with EtOAc/hexane (3:4) to afford **14** as a yellow solid (683 mg, $Y = 94\%$): purity (HPLC, UV 254 nm) 100%; R_f 0.50 (EtOAc/hexane 3:4); mp 111−113 °C; $[\alpha]_D$ = 23.5 (*c* 2.02, MeOH, 21 °C); ^{1}H NMR ($CDCl_3$) δ 8.10 (s, 1 H), 5.41 (d, J = 8.5 Hz, 1 H), 4.91 (dd, J = 8.5, 5.5 Hz, 1 H), 4.42 (q, J = 7.1 Hz, 2 H) 2.45 (m, 1 H) 1.45 (s, 9 H), 1.40 (t, $J = 7.1$ Hz, 3 H), 0.99 (d, $J = 6.8$ Hz, 3 H), 0.92 (d, $J = 6.8$ Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 173.3, 161.3, 155.5, 147.4, 126.8, 80.1, 61.3, 58.2, 33.3, 28.3 (3 C), 19.4, 17.3, 14.4; HRMS (MALDI) *m/z* calcd for $C_{15}H_{24}N_2O_4NaS$ (M + Na) 351.1349, found 351.1356.

Synthesis of (*R*)-2-(1-Amino)ethylthiazole-4-carboxylic Acid Ethyl Ester (15). Method 1. A solution of CH_3COCl (1.2 mL, 16.9 mmol) in EtOH (6 mL) was added dropwise to a suspension of **13** (185 mg, 0.61 mmol) in EtOH (4 mL) at rt, and the mixture was stirred for 4 h. After removal of the solvent in vacuo, the residue was dissolved in methanol and filtered. The filtrate was dried in vacuo to afford **15** hydrochloric acid salt as a yellow semisolid (140 mg, $Y = 97\%$): purity (HPLC, UV 254 nm) 95%; $[\alpha]_D = 13.7$ (*c* 1.46, MeOH, 22 °C); ^{1}H NMR (CD_3OD) δ 8.50 (s, 1 H), 4.80−5.10 (q, J = 4.3 Hz, 1 H), 4.39 (q, $J = 7.0$ Hz, 2 H), 1.76 (d, $J = 4.3$ Hz, 3 H), 1.38 (t, $J = 7.0$ Hz, 3 H); ^{13}C NMR (CD_3OD) δ 168.6, 162.1, 147.6, 130.9, 62.7, 49.7, 20.5, 14.6; HRMS (MALDI) *m/z* calcd for $C_8H_{13}N_2O_2S$ (M + H) 201.0692, found 201.0700. **Method 2.** Trifluoroacetic acid (2 mL, 26.0 mmol) was added dropwise to a solution of **13** (185 mg, 0.61 mmol) in CH_2Cl_2 (8 mL) at 0 °C, and the solution was stirred at rt for 1.5 h. After removal of the solvent, the residue was coevaporated with toluene (3 × 5 mL) and dried in vacuo to afford **15** trifluoroacetic acid salt as a yellow syrup (192 mg, $Y = \sim 100\%$): ^{1}H NMR ($CDCl_3$) δ 9.09 (br s, 2 H), 8.20 (s, 1 H), 5.04 (q, $J = 6.8$ Hz, 1 H), 4.35 (q, $J = 7.1$ Hz, 2 H), 1.83 (d, $J = 6.8$ Hz, 3 H), 1.35 (t, $J = 7.1$ Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 168.3, 161.8, 146.5, 129.0, 62.1, 48.8, 19.8, 14.0.

Synthesis of (*R*)-2-(1-*N*-BOC-amino)isobutylthiazole-4-carboxylic Acid (16). Compound **14** (202 mg, 0.62 mmol) was dissolved in dioxane/water (9:1) (10 mL), and 1 N NaOH (1.8 mL) was added dropwise over 3 h using a syringe pump. The reaction was monitored by TLC until all the starting material was depleted. After removal of the dioxane in vacuo and dilution with water to a volume of 20 mL, the solution was washed with ether (2 × 10 mL). The water solution was acidified with 10% $KHSO_4$ to pH 2 at 0 °C and then extracted with EtOAc (6 × 25 mL). The EtOAc solution was dried over $MgSO_4$, and the solvent was removed in vacuo to afford **16** as a white foam (180 mg, $Y = 97\%$): purity (HPLC, UV 254 nm) 100%; $[\alpha]_D = 42.3$ (*c* 0.72, $CHCl_3$, 24 °C); ^{1}H NMR (CD_3OD) δ 8.31 (s, 1 H), 5.25 (br s, 1 H), 4.80 (d, $J = 6.1$ Hz, 1 H), 2.35 (m, 1 H), 1.45 (s, 9 H), 0.95 (d, $J = 6.7$ Hz, 6 H); ^{13}C NMR (CD_3OD) δ 175.8, 163.9, 157.6, 148.2, 128.6, 80.8, 59.9, 34.1, 28.7 (3 C), 19.8, 18.3; HRMS (MALDI) *m/z* calcd for $C_{13}H_{20}N_2O_4$-NaS (M + Na) 323.1036, found 323.1036.

Synthesis of (*R*)-2-[1-[(*R*)-(1-*N*-BOC-amino)isobutylthiazole-4-carbonyl]amino]ethylthiazole-4-carboxylic Acid Ethyl Ester (17). DIC (60 μL, 0.38 mmol) was added to a solution of HOBt (82 mg, 0.61 mmol) and **16** (91 mg, 0.30 mmol) in anhydrous THF (4 mL) at 0 °C. The solution was stirred at 0 °C for 1 h, allowed to warm to rt, incubated for 1 h, and then cooled to 0 °C again. To it was added a solution of NMM (40 μL, 0.36 mmol) and **15** hydrochloric acid salt (85 mg, 0.36 mmol) in anhydrous THF (4 mL). The reaction mixture was allowed to warm from 0 °C to rt and incubated for 24 h. After filtration and removal of the solvent, the residue was extracted with EtOAc (30 mL) and washed with 1 N HCl (10 mL), 5% $NaHCO_3$ (10 mL), and water (10 mL) in sequence. The EtOAc solution was dried over $MgSO_4$, and after removal of the solvent, the residue was purified by radial chromatography on silica eluted with 1% MeOH in $CHCl_3$ to afford **17** as a yellowish foam (129 mg, $Y = 89\%$): purity (HPLC, UV 254 nm) 98%; R_f 0.50 (MeOH/$CHCl_3$ 3:97); $[\alpha]_D = 29.4$ (*c* 1.01, $CHCl_3$, 24 °C); ^{1}H NMR ($CDCl_3$) δ 8.10 (s, 1 H), 8.04 (s, 1 H), 7.91 (d, $J = 8.1$ Hz, 1 H), 5.59 (dq, $J = 8.1$, 7.0 Hz, 1 H), 5.29 (d, $J = 8.3$ Hz, 1 H), 4.84 (dd, $J = 8.3$, 5.6 Hz, 1 H), 4.41 (q, J = 7.1 Hz, 2 H), 2.32 (m, 1 H), 1.80 (d, $J = 7.0$ Hz, 3 H), 1.44 (s, 9 H), 1.39 (t, $J = 7.1$ Hz, 3 H), 0.98 (d, $J = 6.8$ Hz, 3 H), 0.93 (d, $J = 6.8$ Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 173.1 (2 C), 161.3, 160.6, 155.5, 149.4, 147.3, 127.4, 123.5, 80.3, 61.4, 58.2, 47.3, 33.2, 28.4 (3 C), 21.1, 19.4, 17.6, 14.4; HRMS (MALDI) *m/z* calcd for $C_{21}H_{30}N_4O_5NaS_2$ (M + Na) 505.1550, found 505.1565.

Synthesis of (*R*)-2-[1-*N*-BOC-amino]ethyl-5-methyloxazole-4-carboxylic Acid (18). Oxazole **6** (123 mg, 0.43 mmol) was dissolved in dioxane/water (9:1) (4 mL), and a syringe pump was used to add 1 N NaOH (1.4 mL) at a rate of 15 μL/min at rt. The reaction was monitored by TLC until oxazole **6** was depleted. The solution was then cooled to 0 °C, followed by acidification with 10% $KHSO_4$ to pH 2. After removal of the organic solvent in vacuo and dilution with water to a total volume of 20 mL, the water solution was extracted with EtOAc (6 × 10 mL). The EtOAc solution was dried over Na_2SO_4 and concentrated. **18** was isolated by precipitation from EtOAc−hexane and appeared as a white solid (110 mg, $Y = 94\%$): purity (HPLC, UV 220 nm) 97%; R_f 0.26 (MeOH/$CHCl_3$ 1:9); mp 159−161 °C; $[\alpha]_D = 50.0$ (*c* 0.42, MeOH, 26 °C); ^{1}H NMR ($CDCl_3$) δ 10.90 (br s, 1 H), 6.41 (d, $J = 8.5$ Hz, 1 H), 5.01 (dq, $J = 8.5$, 7.1 Hz, 1 H), 2.62 (s, 3 H), 1.56 (d, $J = 7.1$ Hz, 3 H), 1.41 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 164.9, 164.2, 157.1, 155.5, 127.2, 80.1, 44.8, 28.3 (3 C), 20.1, 12.0; HRMS (MALDI) *m/z* calcd for $C_{12}H_{18}N_2O_5Na$ (M + Na) 293.1108, found 293.1102.

Synthesis of (*R*)-2-[1-[(*R*)-(1-Amino)isobutylthiazole-4-carbonyl]amino]ethylthiazole-4-carboxylic Acid Ethyl Ester (19). Trifluoroacetic acid (2 mL, 26.0 mmol) was added dropwise to a solution of **17** (122 mg, 0.25 mmol) in CH_2Cl_2 (8 mL) at 0 °C. The solution was stirred at rt for 1.5 h. After removal of the solvent, the residue was coevaporated with toluene (2 × 5 mL) and dried in vacuo to afford **19** trifluoroacetic acid salt as a yellow syrup (125 mg, $Y = \sim 100\%$): purity (HPLC, UV 254 nm) 98%; $[\alpha]_D = -30.4$ (*c* 1.37, MeOH, 23 °C); ^{1}H NMR ($CDCl_3$) δ 10.41 (br s, 1 H), 9.45 (d, $J = 5.2$ Hz, 1 H), 9.18 (br s, 2 H), 8.10 (s, 1 H), 8.08 (s, 1 H), 5.49 (qd, $J = 6.8$, 5.2 Hz, 1 H), 4.87 (br s, 1 H), 4.38 (q, $J = 7.0$ Hz, 2 H), 2.35 (m, 1 H), 1.91 (d, $J = 6.8$ Hz, 3 H), 1.37 (t, $J = 7.0$ Hz, 3 H), 1.05 (d, $J = 6.4$ Hz, 3 H), 0.84 (d, $J = 6.4$ Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 175.6, 164.1, 161.4 (2 C), 149.6, 146.3, 127.0, 125.7, 61.9, 58.1, 48.1, 32.3, 20.1, 19.7, 17.3, 14.2; HRMS (MALDI) *m/z* calcd for $C_{16}H_{23}N_4O_3S_2$ (M + H) 383.1206, found 383.1213.

Synthesis of (*R*)-2-[1-[(*R*)-2-[1-((*R*)-2-(1-*N*-BOC-amino)-

ethyl-5-methyloxazole-4-carbonyl)amino]isobutylthiazole-4-carbonyl]amino]ethylthiazole-4-carboxylic Acid Ethyl Ester (20). NMM (28 μL, 0.25 mmol) was added to a solution of HOAt (70 mg, 0.51 mmol), **18** (70 mg, 0.26 mmol), and **19** (125 mg, 0.25 mmol) in anhydrous CH_2Cl_2 (10 mL) at 0 °C. After the mixture was stirred for 15 min at 0 °C, a solution of EDC (73 mg, 0.38 mmol) in anhydrous CH_2Cl_2 (5 mL) was added. The reaction mixture was allowed to warm from 0 °C to rt and incubated for 24 h. After dilution with CH_2Cl_2 (15 mL), the reaction mixture was washed with 1 N HCl (2 × 15 mL), 5% $NaHCO_3$ (15 mL), and water (15 mL) in sequence. The CH_2Cl_2 solution was dried over $MgSO_4$, and after removal of the solvent, the residue was purified by radial chromatography on silica eluted with EtOAc/hexane (1:1) to afford **20** as a yellowish syrup (115 mg, Y = 72%): purity (HPLC, UV 254 nm) 96%; R_f 0.42 (EtOAc/hexane 4:1); $[\alpha]_D$ = 24.8 (*c* 1.15, $CHCl_3$, 24 °C); ^{1}H NMR ($CDCl_3$) δ 8.11 (s, 1 H), 8.06 (s, 1 H), 7.93 (d, J = 7.8 Hz, 1 H), 7.49 (d, J = 8.1 Hz, 1 H), 5.61 (m, 1 H), 5.28 (dd, J = 8.1, 6.3 Hz, 1 H), 5.20−5.30 (br s, 1 H), 4.92 (m, 1 H), 4.42 (q, J = 7.1 Hz, 2 H), 2.62 (s, 3 H), 2.51 (m, 1 H), 1.82 (d, J = 6.8 Hz, 3 H), 1.55 (d, J = 6.8 Hz, 3 H), 1.44 (s, 9 H), 1.40 (t, J = 7.1 Hz, 3 H), 1.03 (d, J = 5.7 Hz, 6 H); ^{13}C NMR ($CDCl_3$) δ 173.2, 172.1, 162.0, 161.7, 161.3, 160.5, 154.9, 153.8, 149.4, 147.2, 128.6, 127.4, 123.7, 80.2, 61.4, 56.1, 47.3, 44.9, 33.1, 28.4 (3 C), 21.1, 19.9, 19.6, 17.9, 14.4, 11.7; HRMS (MALDI) *m*/*z* calcd for $C_{28}H_{38}N_6O_7NaS_2$ (M + Na) 657.2136, found 657.2165.

Synthesis of (*R*)-2-[1-[(*R*)-2-[1-((*R*)-2-(1-*N*-BOC-amino)-ethyl-5-methyloxazole-4-carbonyl)amino]isobutylthiazole-4-carbonyl]amino]ethylthiazole-4-carboxylic Acid (21). Compound **20** (157 mg, 0.25 mmol) was dissolved in dioxane/water (9:1) (5 mL), and 1 N NaOH (0.75 mL) was added dropwise over 3 h using a syringe pump. The reaction was monitored by TLC until all the starting material was depleted. The solution was acidified with 10% $KHSO_4$ to pH 2 at 0 °C. After removal of the dioxane in vacuo and dilution with water to the volume of 15 mL, the solution was extracted with EtOAc (6 × 30 mL). The EtOAc solution was dried over $MgSO_4$ and then concentrated *in vacuo*, followed by precipitation from EtOAc/hexane. The precipitate was dissolved in methanol, filtered, and dried *in vacuo* to afford **21** as a white semisolid (129 mg, Y = 85%): purity (HPLC, UV 254 nm) 99%; $[\alpha]_D$ = 64.1 (*c* 0.99, $CHCl_3$, 24 °C); ^{1}H NMR (CD_3OD) δ ^{1}H NMR ($CDCl_3$) ä 8.20 (s, 1 H), 8.19 (s, 1 H), 7.62 (br s, 1 H), 7.32 (br s, 1 H), 5.63 (q, J = 5.7 Hz, 1 H), 5.52 (br s, 1 H), 5.27 (m, 1 H), 4.94 (m, 1 H), 2.62 (s, 3 H), 2.49 (m, 1 H), 1.80 (d, J = 5.7 Hz, 3 H), 1.54 (d, J = 6.5 Hz, 3 H), 1.45 (s, 9 H), 1.04 (d, J = 7.0 Hz, 3 H), 1.00 (d, J = 6.7 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 173.1, 171.6, 162.9, 161.9, 161.7, 160.7, 155.4, 153.8, 149.2, 146.7, 128.5, 128.1, 124.0, 80.5, 56.0, 47.2, 44.9, 33.1, 28.3 (3 C), 21.0, 19.7, 19.5, 18.1, 11.6; HRMS (MALDI) *m*/*z* calcd for $C_{26}H_{34}N_6O_7NaS_2$ (M + Na) 629.1823, found 629.1832.

Synthesis of (*R*)-2-[1-[(R)-2-[1-((*R*)-2-(1-amino)ethyl-5-methyloxazole-4-carbonyl)amino]isobutylthiazole-4-carbonyl]amino]ethylthiazole-4-carboxylic Acid (22). Trifluoroacetic acid (2 mL, 26.0 mmol) was added dropwise to a solution of **21** (110 mg, 0.18 mmol) in CH_2Cl_2 (10 mL) at 0 °C. The solution was allowed to warm to rt and stirred for 1.5 h. After removal of the solvent, the residue was precipitated from $CHCl_3$/hexane to afford **22** as a yellow semisolid (95 mg, Y = 85%): purity (HPLC, UV 254 nm) 98%; $[\alpha]_D$ = −12.6 (*c* 0.95, CH_3OH, 24 °C); ^{1}H NMR (CD_3OD) δ 8.28 (br s, 2 H), 4.40−6.05 (m, 3 H), 2.62 (s, 3 H), 2.40−2.70 (m, 1 H), 1.73 (br s, 6 H), 1.05 (s, 3 H), 1.00 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 173.3, 170.7, 163.5, 161.2 (2 C), 157.7, 155.4, 148.9, 146.5, 129.0 (2 C), 124.5, 56.1, 47.0, 45.3, 33.6, 21.4, 19.4, 18.6, 17.2, 11.5; HRMS (MALDI) *m*/*z* calcd for $C_{21}H_{26}N_6O_5NaS_2$ (M + Na) 529.1298, found 529.1281.

Synthesis of Dendroamide A (1) and Its Isomer (23). A solution of **22** (90 mg, 0.15 mmol) in anhydrous DMF (20 mL) was added by a syringe pump (10 μL/min) to a solution of DPPA (80 μL, 0.36 mmol) and triethylamine (45 μL, 0.32 mmol) in anhydrous DMF (100 mL) at 0 °C. The reaction mixture was allowed to warm from 0 °C to rt and incubated for 48 h. After removal of the solvent, the residue was purified by radial chromatography on silica eluted with EtOAc/hexane (1:1) to afford **1** as a colorless semisolid (40 mg, Y = 56%), and the isomer of Dendroamide A (**23**) as a white semisolid (13 mg, Y = 18%). Dendroamide A (**1**): purity (HPLC, UV 254 nm) 99%; R_f 0.38 (MeOH/$CHCl_3$ 3:97); $[\alpha]_D$ = 52.5 (*c* 0.33, CH_3Cl, 25 °C); ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) (See Table 2); HRMS (MALDI) *m*/*z* calcd for $C_{21}H_{24}N_6O_4NaS_2$ (M + Na) 511.1193, found 511.1203. The isomer of dendroamide A (**23**): purity (HPLC, UV 254 nm) 99%; R_f 0.41 (MeOH/$CHCl_3$ 3:97); ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) (see Table 2); HRMS (MALDI) *m*/*z* calcd for $C_{21}H_{25}N_6O_4S_2$ (M + H) 489.1373, found 489.1454.

Assay for Reversal of MDR. All test compounds and cytotoxic drugs were dissolved in EtOH and stored at 4 °C. To test the effects of the compounds and cytotoxic drugs on growth, cells were seeded into 96-well tissue culture plates at approximately 15% of confluency and were allowed to attach and recover for 24 h. Varying concentrations of the test compounds alone or in combination with vinblastine or vincristine as indicated in the Results and Discussion section were added to each well, and the plates were incubated for an additional 48 h. The number of surviving cells was then determined by staining with sulforhodamine B[18] as previously described.[19] The percentage of cells killed was calculated as the percentage decrease in sulforhodamine B binding compared with control cultures. Control cultures received equivalent amounts of ethanol, which does not modulate the growth or drug-sensitivity of these cells. Reversal of Pgp-mediated MDR is defined as the ability of the test compound to potentiate the cytotoxicity of 50 nM vinblastine toward NCI/ADR cells. Similarly, reversal of MRP1-mediated MDR is defined as the ability of the test compound to potentiate the cytotoxicity of 1 nM vincristine toward MCF-7/VP cells.

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