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Stereospecific Total Syntheses of Proteasome Inhibitors Omuralide and Lactacystin

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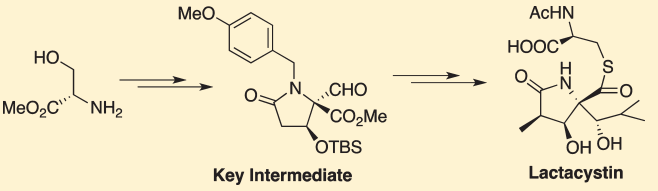
Supporting Information

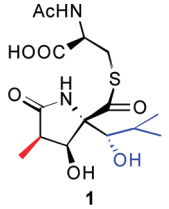
ABSTRACT: Omuralide, a transformation product of the microbial metabolite lactacystin, was the first molecule discovered as a specific inhibitor of the proteasome and is unique in that it specifically inhibits the proteolytic activity of the 20S subunit of the proteasome without inhibiting any other protease activities of the cell. The total syntheses of omuralide and (+)-lactacystin are reported. An important key intermediate is synthesized at an early stage, which allows analogues of these two natural products to be made readily.



INTRODUCTION

The proteasome, a high-molecular-weight, multicatalytic protease complex responsible for most nonlysosomal intracellular protein degradation, is emerging as an important target for cancer chemotherapy, because small-molecule inhibitors of its catalytic activity induce apoptosis in both in vitro and in vivo models of human malignancies and are proving to have efficacy in early clinical trials.[1] A role for the proteasome in programmed cell death was uncovered using small-molecular-weight, cell-permeable inhibitors, which induce apoptosis in a variety of tumor-derived cell lines.[2] Given the importance of the proteasome to normal cellular homeostasis, however, it is likely that inhibitors induce programmed cell death by affecting many apoptosis-associated pathways. Eukaryotic intracellular protein degradation occurs predominantly through the ubiquitin proteasome pathway (UPP) composed of the Ub-conjugating system and the 26S proteasome.[3]

Lactacystin (**1**), a microbial natural product that inhibits cell proliferation and induces neurite outgrowth in a murine neuroblastoma cell line, has become a widely used reagent in functional studies of the proteasome.[4] The proteasome is composed of a 20S catalytic core particle and additional subunits that are thought to be involved in the recognition and unfolding of ubiquitinated proteins; the composite structure has a sedimentation coefficient of 26S. Lactacystin binds to certain catalytic subunits of the 20S proteasome and inhibits the three best characterized peptidase activities of the proteasome, two irreversibly and all at different rates.[5]



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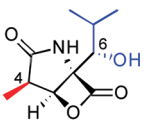

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Omuralide (**2**; also called *clasto*-lactacystin-β-lactone), a transformation product of the microbial metabolite lactacystin,[6] was originally isolated by Omura and co-workers from cultures of a terrestrial *Streptomyces* sp.[7] Omuralide, remarkable because it was the first molecule discovered to be a truly specific inhibitor of the proteasome, is unique in that it specifically inhibits the proteolytic activity of the 20S subunit of the proteasome without inhibiting any other protease activities of the cell.[8] This is an important attribute, which can be utilized in future potential drug design.

Stimulated by the unusual structure of lactacystin, its remarkable biological activity, and the scarcity of natural material, several research groups undertook total syntheses.[9−15] The first total synthesis of lactacystin in 1992[9] made lactacystin (and radiolabeled lactacystin) available and was instrumental in research, which demonstrated that the biological activity of lactacystin results from its potent, highly selective, and irreversible inhibition of proteasome-mediated peptidase activity.[4] Syntheses of omuralide (**2**) also have been reported.[13,15,16] Conclusive evidence was obtained that lactacystin is converted in vivo to the equipotent β-lactone omuralide (**2**), which is the actual agent that acts by acylation of the amino terminal threonine residue of a proteasome subunit. This result was confirmed by X-ray crystallographic studies at 2.4 Å resolution.[17−19]

Many analogues of **1** were synthesized to discover the most potent agent.[20,21] There is an absolute requirement for the β-lactone ring, and the stereochemical fidelity is dictated by that of the natural product. Furthermore, methylation of the γ-lactam nitrogen abolishes activity. The one region of the molecule that supported chemical modification was the C^4 alkyl group. Replacement of the methyl group at C^7 with short aliphatic chains enhanced the potency of the lactone inhibitor. The best activities were recorded for ethyl, *n*-propyl, isopropyl, and

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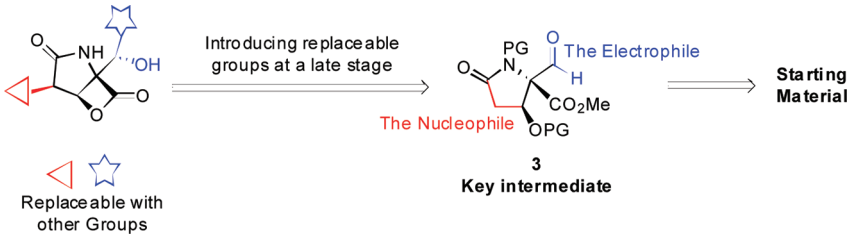
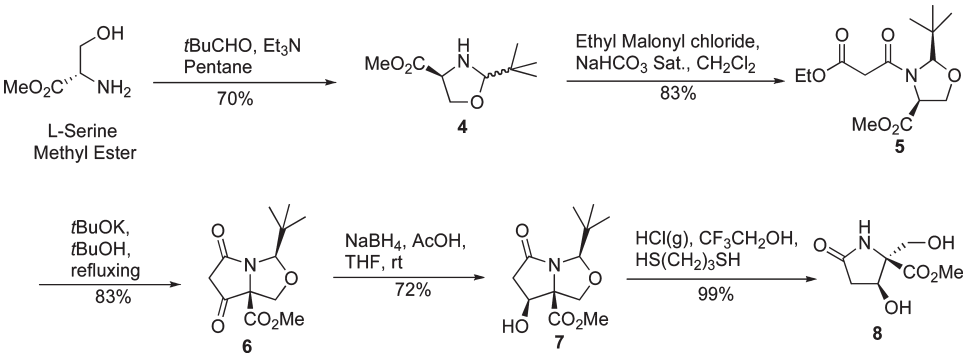
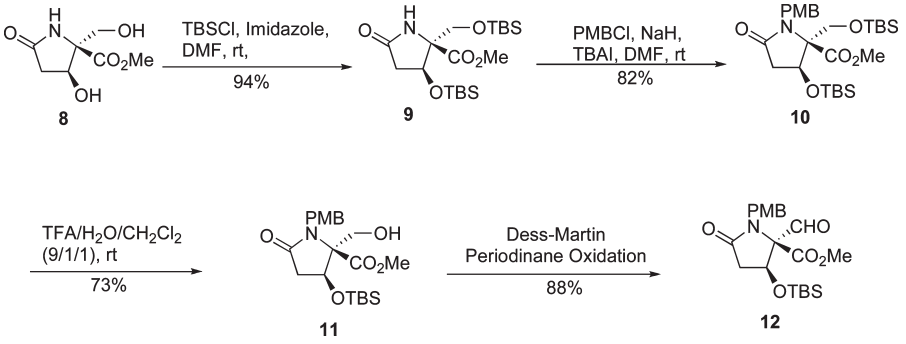


Figure 1. Retrosynthetic plan for lactacystin and omuralide.

Scheme 1. Synthesis of 8



Scheme 2. Synthesis of Key Intermediate 12



n-butyl, all possessing 2−3 times the potency of the corresponding (+)-lactacystin-β-lactone.

From the view of medicinal chemistry, an ideal synthesis of a target molecule is one in which a stable key intermediate can be used to efficiently assemble a diverse library of analogues of the target on a large scale. Here we report a synthesis of lactacystin (**1**) and omuralide (**2**) via an important intermediate (**3**, Figure 1), leading to the efficient preparation of analogues in which the moieties of **3** can readily be replaced by other groups.

RESULTS AND DISCUSSION

With L-serine methyl ester as the starting material, β-hydroxylated lactam **8** was prepared in five steps with very high stereoselectivity by the elegant method of Maloney and coworkers for diastereoselectively controlled hydroxylated lactams[22] (Scheme 1). Thus, serine-derived oxazolidine **4** was acylated with ethyl malonyl chloride to give the N-acyl derivative **5**. In our hands, the acylation of **4** in a two-phase reaction medium of saturated aqueous $NaHCO_3$ in methylene chloride gave the best results and made the workup easier than that reported. A Dieckmann condensation followed by reduction generated alcohol **7** and a minor amount of the epimer, which was removed by recrystallization to generate the desired isomer in a 60% overall yield for the two steps. Protection of the β-hydroxyl group of alcohol **7** also was explored. The TBS ether, benzyl ether, and acetate were attempted, all of which, however, resulted in production of an unsaturated lactam side product as a result of in situ elimination of the protected alcohol of **7**. This suggests that cis substitution at the β position of this bicyclic lactam is unstable and favors elimination. Diol **8** was obtained in a quantitative yield by reaction of **7** in a mixture of propane-1,3-dithiol and acidic trifluoroethanol.

Key intermediate **12** was prepared as shown in Scheme 2. The two hydroxyl groups of **8** were protected as TBS ethers by treatment with TBS chloride in DMF with imidazole as base.

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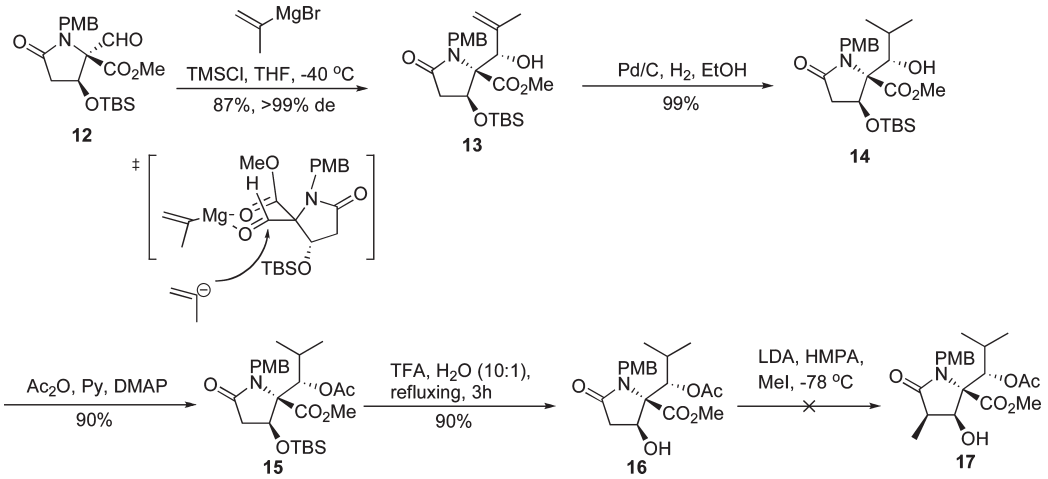
Scheme 3. Synthesis of 17



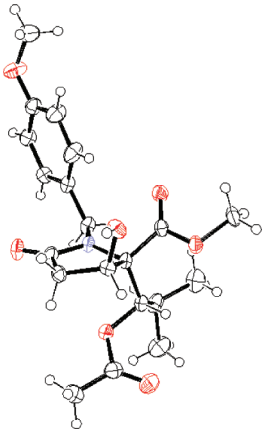


Figure 2. X-ray crystal structure of **16**.

The lactam of the resulting bis-TBS ether **9** was protected as its PMB ether (**10**). Initially, Boc was used as the protecting group for **9**; however, in the next step the Boc group migrated from the lactam to the adjacent hydroxyl group after its TBS ether was removed. The TBS ether at the primary alcohol in **10** was then regioselectively deprotected. After we screened various acidic and fluoride reagent conditions, TFA/H_2O/CH_2Cl_2 (9/1/1) was found to be the most efficient conditions to prepare alcohol **11**, which was Dess−Martin periodinane oxidized to aldehyde **12**, the key intermediate.

Next, the two hydrophobic subunits of the β-hydroxy lactam skeleton were installed (Scheme 3). Aldehyde **12** was treated with 2-propenyl Grignard reagent and trimethylchlorosilane, as was reported earlier for addition to aldehydes with high stereoslectivity,[18a] giving only one stereoisomer of adduct **13**. A six-membered-ring transition state, with Mg^{2+} coordinating to the carbonyl groups of the ester and aldehyde, was proposed to account for the stereoselectivity of the addition.[18a] Hydrogenation of **13** followed by acylation and deprotection of the TBS ether of **15** gave **16**; the absolute stereochemistry of **16** was confirmed by X-ray crystallography (Figure 2). Unfortunately, various bases, including LDA and potassium and sodium HMDS, followed by methyl iodide, failed to methylate the α-position of the lactam, and only a trace amount of the *O*-methylated enolate was observed. It was previously reported that Boc protection of a lactam amide nitrogen assisted in stabilization of the α-anion by formation of a cyclic transition state with the lactam carbonyl group and the lithium cation.[10]

Therefore, the PMB group of **15** was exchanged for a Boc protecting group (**19**) in two steps (Scheme 4). The TBS ether was removed with TBAF in acetic acid; the acetic acid is important, as decomposition was observed when only TBAF was utilized. Methylation of **20** was performed by the method of Donohoe et al.[11] with LDA and HMPA, generating **21** in 60% yield with very high stereoselectivity. After Boc deprotection and hydrolysis of the methyl ester and the acetic ester of **22**, the β-lactone was formed by the reported coupling procedure using BOPCl,[18a] yielding omuralide (**2**) in a total of 19 steps. Further treatment of **2** with *N*-acetyl L-cysteine by the reported procedure[18a] gave (+)-lactacystin (**1**).

CONCLUSION

We have completed total stereospecific syntheses of omuralide (**2**) and (+)-lactacystin (**1**) in 3% (19 steps) and 1.5% (20 steps) overall yields, respectively. Key intermediate **12** was prepared at an early stage of the synthesis; then the two alkyl substituents were introduced at a later stage. The introduction of these substituents permits analogous modifications of these natural products. The PMB protecting group was found to be important for the introduction of the isopropyl substituent, and the Boc protecting group was critical for the installation of the methyl group.

EXPERIMENTAL SECTION

(2*R*,3*S*)-Methyl 3-(*tert*-Butyldimethylsilyloxy)-2-((*tert*-butyldimethylsilyloxy)methyl)-5-oxopyrrolidine-2-carboxylate (9). To a solution of **8** (5 g, 26.43 mmol) in DMF (200 mL) was added TBSCl (8.76 g, 58.15 mmol) followed by imidazole (7.20 g, 106 mmol) at room temperature. The reaction mixture was stirred overnight. The reaction was quenched by the addition of saturated aqueous NH_4Cl solution and then was extracted with EtOAc (3 × 500 mL). The combined organic phases were washed with brine, dried with Na_2SO_4, and concentrated. The residue was purified by flash column

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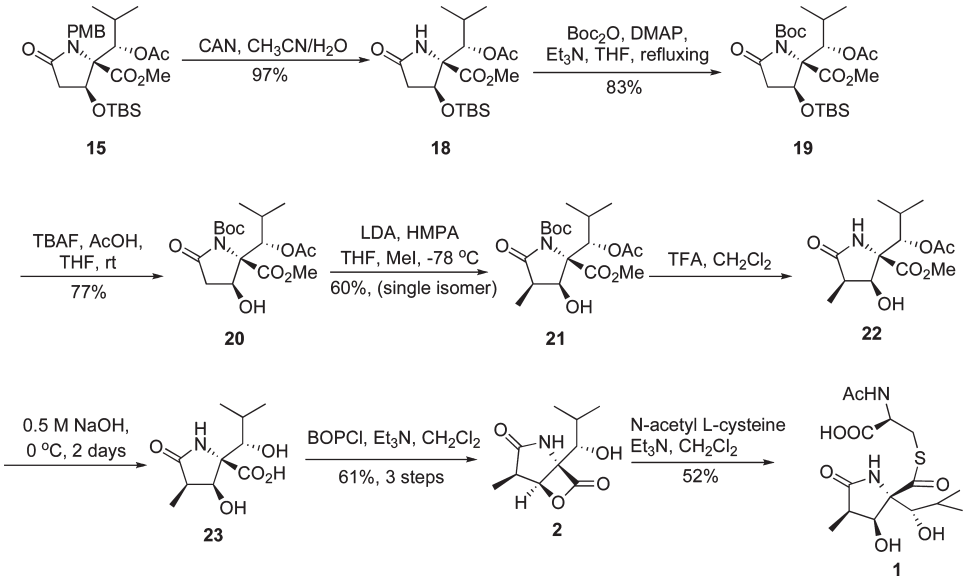
Scheme 4. Final Steps in the Synthesis of Omularide (2) and Lactacystin (1)



chromatography (hexanes/EtOAc 100/0 to 0/100) to afford **9** (10.4 g, 94%) as a colorless oil: $[\alpha]^{25}_{D}$ = +31.19° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.30 (s, 1H), 4.30 (dd, *J* = 6.4, 3.6 Hz, 1H), 4.00 (d, *J* = 9.8 Hz, 1H), 3.70 (s, 3H), 3.61 (d, *J* = 9.8 Hz, 1H), 2.60 (dd, *J* = 16.8, 6.5 Hz, 1H), 2.31 (dd, *J* = 16.8, 3.6 Hz, 1H), 0.83 (s, 9H), 0.82 (s, 9H), 0.03 (s, 3H), 0.02 (s, 3H), 0.02 (s, 3H), 0.01 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.9, 170.0, 73.7, 71.2, 66.4, 52.4, 40.6, 26.3, 26.1, 25.9, 25.8, 25.7, 25.6, 25.4, 25.2, 18.3, 17.9, −4.3, −4.7, −5.1, −5.5, −5.6, −6.0; HRMS (ESI) calcd for $[(C_{19}H_{39}NO_5Si_2) + H]^+$ 418.2421, found 418.2424.

(2*R*,3*S*)-Methyl 3-(*tert*-Butyldimethylsilyloxy)-2-((*tert*-butyldimethylsilyloxy)methyl)-1-(4-methoxybenzyl)-5-oxopyrrolidine-2-carboxylate (10). NaH (60% in mineral oil, 1.05 g, 26.33 mmol) was prewashed with hexanes and then was suspended in DMF (300 mL). To the mixture was carefully added a solution of **9** (10 g, 23.94 mmol) in DMF (100 mL) at 0 °C. The resulting mixture was stirred for 1 h before PMBCl (4.5 g, 3.90 mL, 28.73 mmol) was added dropwise, followed by TBAI (1.77 g, 4.79 mmol). The reaction mixture was stirred at room temperature overnight and then was quenched by adding saturated aqueous NH_4Cl solution and extracted with EtOAc (3 × 500 mL). The combined organic phases were washed with brine (30 mL), dried with Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (hexanes/EtOAc 100/0 to 0/100) to afford **10** (10.6 g, 82%) as a colorless oil: $[\alpha]^{25}_{D}$ = +55.82° (*c* 0.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.19 (d, *J* = 8.5 Hz, 2 H), 6.78 (d, *J* = 8.5 Hz, 2 H), 4.94 (d, *J* = 15 Hz, 1 H), 4.56 (t, *J* = 8.5 Hz, 1 H), 4.98 (dd, *J* = 12, 50 Hz, 1H), 3.84 (d, *J* = 12 Hz, 1H), 3.77 (s, 3H), 3.27 (s, 3H), 2.63 (d, *J* = 8.5 Hz, 1H), 0.89 (s, 9H), 0.81 (s, 9H), 0.10 (s, 6H), 0.09 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.9, 170.1, 159.1, 130.6, 128.5, 113.7, 95.0, 73.8, 68.4, 59.0, 55.5, 51.6, 43.5, 39.1, 26.0, 25.9, 25.7, 25.6, 18.3, 17.9, −5.0, −5.4, −5.8; HRMS (ESI) calcd for $[(C_{27}H_{47}NO_6Si_2) + H]^+$ 538.3013, found 538.3020.

(2*R*,3*S*)-Methyl 3-(*tert*-Butyldimethylsilyloxy)-2-(hydroxymethyl)-1-(4-methoxybenzyl)-5-oxopyrrolidine-2-carboxylate (11). To a stirred solution of TFA/H_2O (9/1, 500 mL) was added a solution of **10** (10 g, 18.59 mmol) in CH_2Cl_2 (50 mL) at room temperature. The reaction was monitored by TLC until the starting material was consumed. Most of the solvent and TFA was evaporated, and the crude product was extracted with EtOAc (3 × 500 mL). The combined organic phases were washed with brine (30 mL), dried with Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (hexanes/EtOAc 100/0 to 0/100) to afford **11** (5.8 g, 73%) as a colorless oil, which gradually changed to a white solid: $[\alpha]^{25}_{D}$ = +10.99° (*c* 1.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27 (d, *J* = 8.5 Hz, 2H), 6.84 (d, *J* = 8.5 Hz, 2H), 4.73 (d, *J* = 15.0 Hz, 1H), 4.49 (t, *J* = 8.2 Hz, 1H), 4.09 (d, *J* = 15.0 Hz, 1H), 3.95 − 3.71 (m, 4H), 3.55 (s, 3H), 2.65 (ddd, *J* = 51.3, 16.5, 8.2 Hz, 1H), 0.84 (s, 9H), 0.05 (d, *J* = 4.8 Hz, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.3, 170.4, 159.4, 130.0, 129.3, 114.3, 77.5, 77.2, 77.0, 75.1, 68.8, 61.4, 55.4, 52.2, 44.2, 39.0, 25.6, 17.9; HRMS (ESI) calcd for $[(C_{21}H_{33}NO_6Si) + H]^+$ 424.2153, found 424.2155.

(2*S*,3*S*)-Methyl 3-(*tert*-Butyldimethylsilyloxy)-2-formyl-1-(4-methoxybenzyl)-5-oxopyrrolidine-2-carboxylate (12). To a solution of **11** (5.0 g, 11.80 mmol) in CH_2Cl_2 (500 mL) was added Dess−Martin periodinane (3.75 g, 8.85 mmol) in several portions at 0 °C. The resulting mixture was stirred at room temperature for 2 h. Then an aqueous solution of $Na_2S_2SO_3$ was added very carefully to quench the reaction. To the resulting mixture was added saturated aqueous $NaHCO_3$ solution, which was extracted with CH_2Cl_2 (3 × 500 mL). The combined organic phases were washed with brine (50 mL), dried with Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (hexanes/EtOAc 100/0 to 0/100) to afford **12** (4.4 g, 88%): $[\alpha]^{25}_{D}$ = +56.99° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.76 (s, 1H), 7.02 (d, *J* = 8.2 Hz, 2H), 6.73 (d, *J* = 8.1 Hz, 2H), 4.65 (d, *J* = 14.6 Hz, 1H), 4.46 (t, *J* = 7.1 Hz, 1H), 4.31 (d, *J* = 14.6 Hz, 1H), 3.69 (s, 3H), 3.47 (s, 3H), 2.58 (ddd, *J* = 23.1, 16.4, 7.2 Hz, 2H), 0.76 (s, 9H), −0.04 (d, *J* = 8.1 Hz, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.6, 167.6, 159.4, 131.0, 127.2, 113.8, 77.8, 77.5, 77.2, 77.0, 70.7, 55.2, 52.3, 44.4, 39.0, 25.8, 25.4, 25.3, 24.9, 17.7; HRMS (ESI) calcd for $[(C_{21}H_{31}NO_6Si) + H]^+$ 422.1999, found 422.1997.

(2*S*,3*S*)-Methyl 3-(*tert*-Butyldimethylsilyloxy)-2-((*S*)-1-hydroxy-2-methylallyl)-1-(4-methoxybenzyl)-5-oxopyrrolidine-2-carboxylate (13). To a stirred solution of aldehyde **12** (4.0 g, 9.49 mmol) in THF (500 mL) at −40 °C was added TMSCl (5.15 g, 6.0 mL, 47.5 mmol). Isopropenylmagnesium bromide (0.5 M in THF, 57 mL, 28.47 mmol) was then added dropwise at −40 °C. After the mixture was stirred for 2 h at −40 °C, the reaction was quenched with saturated aqueous NH_4Cl at that temperature and the mixture stirred

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for an additional 0.5 h. The resulting mixture was extracted with EtOAc (3 × 500 mL). The combined organic phases were washed with brine (30 mL), dried with Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (hexanes/EtOAc 100/0 to 0/100) to afford **13** (3.8 g, 87%) as a colorless glass: $[\alpha]^{25}_D$ = +50.00° (*c* 0.9, $CHCl_3$); ^{1}H NMR (499 MHz, $CDCl_3$) δ 7.17 (d, *J* = 8.1 Hz, 2H), 6.71 (d, *J* = 8.1 Hz, 2H), 5.02 (d, *J* = 51.3 Hz, 2H), 4.78 (d, *J* = 15.1 Hz, 1H), 4.67 (d, *J* = 4.7 Hz, 1H), 4.63 − 4.55 (m, 1H), 4.08 (d, *J* = 5.0 Hz, 1H), 4.01 (d, *J* = 14.9 Hz, 1H), 3.66 (s, 3H), 3.20 (s, 3H), 2.74 (dd, *J* = 16.7, 7.5 Hz, 1H), 2.33 (dd, *J* = 16.8, 4.6 Hz, 1H), 1.70 (s, 3H), 0.74 (s, 9H), −0.03 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.1, 171.2, 158.4, 142.7, 129.7, 129.0, 116.6, 113.2, 76.5, 76.3, 69.6, 55.0, 51.4, 45.4, 40.2, 25.3, 20.4, 17.5, −4.7, −5.4; HRMS (ESI) calcd for $[(C_{24}H_{37}NO_6Si) + H]^+$ 464.2444, found 464.2446.

(2*S*,3*S*)-Methyl 3-(*tert*-butyldimethylsilyloxy)-2-((*S*)-1-hydroxy-2-methylpropyl)-1-(4-methoxybenzyl)-5-oxopyrrolidine-2-carboxylate (14). To a stirred solution of **13** (3.0 g, 6.47 mmol) in EtOH (50 mL) in a single-necked flask was added Pd/C (10%, 300 mg) very carefully at room temperature. The flask was evacuated and connected to a hydrogen balloon through a three-way stopcock adaptor. The solution was evacuated under vacuum for 3 min with stirring, and was filled with H_2. This cycle was repeated three times. Then the reaction was stirred overnight under H_2. The reaction mixture was filtered through an EtOAc prewashed Celite layer to give a clear filtrate. The Celite layer was was washed with EtOAc again. The filtrate was evaporated to give **14** (3.0 g, 99%) as a clear colorless oil: $[\alpha]^{25}_D$ = +21.63° (*c* 0.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.21 (d, *J* = 8.5 Hz, 2H), 6.77 (d, *J* = 8.6 Hz, 2H), 4.73 (dd, *J* = 8.2, 6.0 Hz, 1H), 4.67 (d, *J* = 14.9 Hz, 1H), 4.08 (dd, *J* = 4.7, 3.3 Hz, 1H), 4.04 (d, *J* = 14.8 Hz, 1H), 3.73 (s, 3H), 3.41 (d, *J* = 4.8 Hz, 1H), 3.27 (s, 3H), 2.76 (dd, *J* = 17.1, 8.4 Hz, 1H), 2.43 (dd, *J* = 17.1, 5.9 Hz, 1H), 1.78 − 1.60 (m, 1H), 0.92 (t, *J* = 7.1 Hz, 6H), 0.78 (s, 9H), 0.02 (d, *J* = 4.7 Hz, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.5, 172.5, 158.9, 130.4, 128.8, 113.6, 76.8, 76.2, 68.2, 55.3, 51.7, 45.4, 40.0, 28.7, 25.5, 25.4, 22.4, 17.7, 17.4, −4.6, −5.2; HRMS (ESI) calcd for $[(C_{24}H_{39}NO_6Si) + H]^+$ 466.2632, found 466.2634.

(2*S*,3*S*)-Methyl 2-((*S*)-1-Acetoxy-2-methylpropyl)-3-(tert-butyldimethylsilyloxy)-1-(4-methoxybenzyl)-5-oxopyrrolidine-2-carboxylate (15). To a solution of **14** (2.4 g, 5.15 mmol) in pyridine (20 mL) at 0 °C was added Ac_2O (789 mg, 729 μL, 7.73 mmol), followed by DMAP (126 mg, 1.03 mmol). The reaction mixture was stirred overnight at room temperature, and the solvent was evaporated. To the resulting residue was added saturated NH_4Cl solution, and the aqueous phase was extracted with EtOAc (3 × 300 mL). The combined organic phases were washed with brine (30 mL), dried with Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (hexanes/EtOAc 100/0 to 0/100) to afford **15** (2.36 g, 90%) as a colorless oil: $[\alpha]^{25}_D$ = +2.31° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25 (d, *J* = 8.5 Hz, 2H), 6.81 (d, *J* = 8.5 Hz, 2H), 5.49 (d, *J* = 5.5 Hz, 1H), 4.67 (d, *J* = 15.5 Hz, 1H), 4.56 (dd, *J* = 7.0, 3.5 Hz, 1H), 4.35 (d, *J* = 15.5 Hz, 1H), 3.77 (s, 3H), 3.47 (s, 3H), 2.71 (dd, *J* = 17.0, 7.0 Hz, 1H), 2.41 (dd, *J* = 17.0, 3.5 Hz, 1H), 2.09 (s, 3H), 1.89 (m, 1H), 0.94 (d, *J* = 7.0 Hz, 3H), 0.84 (d, *J* = 7.0 Hz, 3H), 0.85 (s, 9 H), 0.08 (s, 3 H), 0.06 (s, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.6, 170.4, 169.0, 158.8, 129.2, 129.1, 113.7, 77.0, 70.0, 55.4, 51.9, 46.6, 40.6, 29.3, 25.6, 21.6, 21.2, 19.0, 17.8, −4.3, −5.1; HRMS (ESI) calcd for $[(C_{26}H_{41}NO_7Si) + H]^+$ 508.2762, found 508.2752.

(2*R*,3*S*)-Methyl 2-((*S*)-1-Acetoxy-2-methylpropyl)-3-hydroxy-1-(4-methoxybenzyl)-5-oxopyrrolidine-2-carboxylate (16). **15** (2.0 g, 3.94 mmol) was added to a solution of TFA/H_2O (10/1, 50 mL), and the resulting reaction mixture was heated to reflux for 3 h. Most of the solvent was evaporated, and the crude product was purified directly by flash column chromatography (hexanes/EtOAc 100/0 to 0/100) to afford **19** (1.4 g, 90%): $[\alpha]^{25}_D$ = −11.81° (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23 (d, *J* = 8.5 Hz, 2H), 6.84 (d, *J* = 8.5 Hz, 2H), 5.54 (d, *J* = 5.8 Hz, 1H), 4.79 (d, *J* = 15.3 Hz, 1H), 4.68 (dd, *J* = 7.5, 4.8 Hz, 1H), 4.33 (d, *J* = 15.3 Hz, 1H), 3.79 (s, 3H), 3.48 (s, 3H), 2.93 (dd, *J* = 17.7, 7.7 Hz, 1H), 2.62 (dd, *J* = 17.7, 4.6 Hz, 1H), 2.13 (s, 3H), 1.89 (td, *J* = 13.1, 6.5 Hz, 1H), 0.97 (d, *J* = 6.9 Hz, 3H), 0.87 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.5, 170.0, 159.0, 129.7, 128.7, 113.9, 75.8, 69.3, 55.4, 52.3, 46.1, 38.6, 28.9, 21.1; HRMS (ESI) calcd for $[(C_{20}H_{27}NO_7) + H]^+$ 394.1842, found 394.1850.

(2*R*,3*S*)-Methyl 2-((*S*)-1-Acetoxy-2-methylpropyl)-3-(*tert*-butyldimethylsilyloxy)-5-oxopyrrolidine-2-carboxylate (18). To a solution of **15** (1.4 g, 2.76 mmol) in CH_3CN/H_2O (1/1, 100 mL), was added ceric ammonium nitrate (4 g, 7.30 mmol) at room temperature. The resulting solution was stirred until the TLC showed no more starting material. The solution was extracted with EtOAc (3 × 300 mL), and the combined organic phases were washed with brine (30 mL), dried with Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (hexanes/EtOAc 100/0 to 0/100) to afford **18** (1.04 g, 97%) as a colorless oil: $[\alpha]^{25}_D$ = +16.00° (*c* 0.5, $CHCl_3$); ^{1}H NMR (499 MHz, $CDCl_3$) δ 7.74 (s, 1H), 5.36 (d, *J* = 5.6 Hz, 1H), 4.23 (s, 1H), 3.68 (s, 3H), 2.61 − 2.35 (m, 1H), 2.10 (d, *J* = 17.0 Hz, 1H), 2.03 (s, 3H), 1.80 (m, 1H), 0.80 (d, *J* = 4.6 Hz, 6H), 0.75 (s, 9H), −0.04 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 176.4, 170.3, 169.3, 78.9, 75.5, 73.0, 52.4, 41.2, 29.9, 25.5, 20.9, 19.7, 18.6, 17.7, −5.0, −5.3; HRMS (ESI) calcd for $[(C_{18}H_{33}NO_6Si) + H]^+$ 388.2155, found 388.2162.

(2*R*,3*S*)-1-*tert*-Butyl 2-Methyl 2-((*S*)-1-Acetoxy-2-methylpropyl)-3-(*tert*-butyldimethylsilyloxy)-5-oxopyrrolidine-1,2-dicarboxylate (19). To a solution of **18** (900 mg, 2.32 mmol) in THF (20 mL) was added Boc_2O (760 mg, 3.48 mmol) at room temperature. Then Et_3N (470 mg, 647 μL, 4.64 mmol) was added dropwise, followed by DMAP (57 mg, 0.46 mmol). The reaction mixture was then refluxed overnight under N_2. The solvent was evaporated, and saturated NH_4Cl solution (50 mL) was added. The mixture was extracted with EtOAc (3 × 200 mL). The combined organic phases were washed with brine (30 mL), dried with Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (hexanes/EtOAc 100/0 to 0/100) to afford **19** (950 mg, 83%) as a colorless oil: $[\alpha]^{25}_D$ = −41.82° (*c* 0.6, $CHCl_3$); ^{1}H NMR (499 MHz, $CDCl_3$) δ 5.85 (d, *J* = 3.6 Hz, 1H), 4.72 (dd, *J* = 8.3, 6.2 Hz, 1H), 3.60 (s, 3H), 2.83 (dd, *J* = 18.1, 8.6 Hz, 1H), 2.56 (dd, *J* = 18.1, 5.9 Hz, 1H), 2.05 (s, 3H), 1.71 (m, 1H), 1.41 (s, 9H), 0.86 (d, *J* = 6.8 Hz, 6H), 0.77 (s, 9H), 0.04 (s, 3H), −0.00 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.1, 169.8, 168.8, 148.4, 74.9, 74.2, 65.3, 52.1, 41.3, 29.2, 27.7, 25.4, 21.6, 21.2, 17.6, 17.3, −4.2, −5.3; HRMS (ESI) calcd for $[(C_{23}H_{41}NO_8Si) + Na]^+$ 510.2499, found 510.2502.

(2*R*,3*S*)-1-*tert*-Butyl 2-Methyl 2-((*S*)-1-Acetoxy-2-methylpropyl)-3-hydroxy-5-oxopyrrolidine-1,2-dicarboxylate (20). To a solution of TBAF (400 mg, 453 μL, 1.54 mmol) in THF (5 mL) was added AcOH (92 mg, 87 μL, 1.54 mmol) at room temperature. Then a solution of **19** (500 mg, 1.03 mmol) in THF (5 mL) was added dropwise to the reaction mixture. The resulting reaction mixture was stirred overnight. The solution was added to saturated NH_4Cl solution (50 mL) and extracted with EtOAc (3 × 100 mL). The combined organic phases were washed with brine (30 mL), dried with Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (hexanes/EtOAc 100/0 to 0/100) to afford **20** (295 mg, 77%) as a colorless oil: $[\alpha]^{25}_D$ = +31.19° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.91 (d, *J* = 3.6 Hz, 1H), 4.89 (td, *J* = 8.8, 3.7 Hz, 1H), 3.75 (s, 3H), 2.90 (dd, *J* = 17.9, 9.0 Hz, 1H), 2.76 (dd, *J* = 17.9, 8.7 Hz, 1H), 2.22 (t, *J* = 8.2 Hz, 1H), 2.16 (s, 3H), 1.84 (m, 1H), 1.50 (s, 9H), 0.97 (d, *J* = 6.9 Hz, 3H), 0.89 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.1, 169.7, 169.7, 148.6, 84.8, 75.4, 74.2, 65.5, 52.6, 38.4, 29.2, 27.8, 21.5, 21.1, 17.3; HRMS (ESI) calcd for $[(C_{17}H_{27}NO_8) + Na]^+$ 396.1634, found 396.1638.

(2*R*,3*S*,4*R*)-Methyl 2-((*S*)-1-Acetoxy-2-methylpropyl)-3-hydroxy-4-methyl-5-oxopyrrolidine-2-carboxylate (21). To a solution of diisopropylamine (52 mg, 72 μL, 0.52 mmol) in THF (5 mL)

was added butyllithium in hexane (200 μL of 2.5 M, 0.52 mmol) at 0 °C. After being stirred for 10 min, the solution was cooled to −78 °C and stirred for 10 min. Iodomethane (487 mg, 213 μL, 3.43 mmol) was added, followed by the dropwise addition of a solution of **20** (160 mg, 0.43 mmol) and HMPA (0.5 mL) in THF (3 mL). After this mixture was stirred at −78 °C for 1 h, additional HMPA (0.5 mL) was added. The reaction mixture was stirred at −78 °C for 3 h and quenched with saturated NH_4Cl (20 mL). After the mixture was poured into water (30 mL), the products were extracted with EtOAc (3 × 50 mL). The combined organic phases were washed with brine (30 mL), dried with Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (hexanes/EtOAc 100/0 to 0/100) to afford **21** (100 mg, 60%) as a colorless oil: $[\alpha]^{25}_D = -16.76°$ (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.89 (d, *J* = 3.8 Hz, 1H), 4.89 (dd, *J* = 9.8, 5.6 Hz, 1H), 3.73 (s, 3H), 2.91 (dq, *J* = 15.4, 7.7 Hz, 1H), 2.16 (s, 3H), 2.00 (d, *J* = 5.6 Hz, 1H), 1.92 − 1.73 (m, 1H), 1.51 (s, 9H), 1.32 (d, *J* = 7.7 Hz, 3H), 0.98 (d, *J* = 6.9 Hz, 3H), 0.86 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.2, 170.3, 169.8, 149.0, 84.9, 75.4, 72.4, 67.1, 52.7, 40.9, 29.4, 28.0, 21.8, 21.3, 17.6, 10.7; HRMS (ESI) calcd for $[(C_{18}H_{29}NO_8) + Na]^+$ 410.1791, found 410.1789.

Omuralide (2). Trifluoroacetic acid (5 mL) was added to a solution of **21** (50 mg, 0.13 mmol) in CH_2Cl_2 (5 mL) at 4 °C. The reaction mixture was stirred at room temperature for another 2 h and concentrated. The crude product was purified by flash column chromatography (hexanes/EtOAc 100/0 to 0/100) to afford **22** (30 mg, 81%): $[\alpha]^{25}_D = -18.25°$ (*c* 0.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.34 (s, 1H), 5.53 (d, *J* = 6.3 Hz, 1H), 4.32 (d, *J* = 5.4 Hz, 1H), 3.88 (s, 3H), 2.88 − 2.53 (m, 1H), 2.12 (s, 3H), 1.91 (dt, *J* = 13.2, 6.6 Hz, 1H), 1.26 (s, 1H), 1.18 (d, *J* = 7.3 Hz, 3H), 0.92 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.8, 171.4, 170.5, 78.5, 76.0, 75.5, 53.5, 41.3, 30.5, 21.1, 19.6, 18.4, 8.2; HRMS (ESI) calcd for $[(C_{13}H_{21}NO_6) + Na]^+$ 310.1267, found 310.1274.

A solution of 0.5 M aqueous NaOH (2 mL of 0.5 M, 1.0 mmol) was cooled to 4 °C, and **22** (30 mg) was added, and this mixture was stirred at 4 °C for 64 h. HCl (1 N) was added dropwise to the reaction mixture until pH 1. The resulting solution was extracted with EtOAc (3 × 50 mL), and the combined organic phases were dried over Na_2SO_4. The solid was filtered, and the filtrate was concentrated in vacuo to provide **23** as a white solid. Compound **23** was suspended in CH_2Cl_2 at room temperature. Triethylamine (39 mg, 54 μL, 0.39 mmol) and bis(2-oxo-3-oxazolidinyl)phosphinic chloride (19 mg, 0.19 mmol) were added. After being stirred for 2 h, the reaction mixture was poured into water (50 mL). The resulting solution was extracted with EtOAc (3 × 50 mL), and the combined organic phases were dried over Na_2SO_4. The solid was filtered, and the filtrate was concentrated. The crude product was purified by flash column chromatography (hexanes/EtOAc 100/0 to 0/100) to afford a solid, which was recrystallized from EtOAc to give the β-lactone omuralide **2** (17 mg): $[\alpha]^{23}_D$ −93.7° (*c* 0.25, CH_3CN); ^{1}H NMR (499 MHz, pyridine) δ 10.47 (s, 1H), 7.88 (s, 1H), 5.70 (d, *J* = 6.1 Hz, 1H), 4.37 (s, 1H), 3.26 − 2.88 (m, 1H), 2.13 (ddd, *J* = 13.5, 10.5, 6.7 Hz, 1H), 1.48 (d, *J* = 7.4 Hz, 3H), 1.13 (d, *J* = 6.9 Hz, 3H), 1.02 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, pyridine) δ 177.8, 172.9, 81.0, 77.5, 71.0, 39.3, 30.3, 20.9, 16.9, 9.3; HRMS (ESI) calcd for $[(C_{10}H_{15}NO_4) + NH_4]^+$ 231.1344, found 231.1347.

Lactacystin (1). The conversion of **2** to **1** was conducted by following the literature procedure.[23] A suspension of **2** (3 mg, 0.014 mmol) in CH_2Cl_2 was treated with *N*-acetyl-L-cysteine (2.296 mg, 0.014 mmol) and Et_3N (4.27 mg, 6 μL, 0.042 mmol) at 23 °C. The resulting reaction mixture was stirred for 4 h at room temperature and concentrated in vacuo. The solid residue was dissolved in dry pyridine (5 mL) and evaporated under reduced pressure. Regeneration of the free acid by azeotropic distillation with a THF/HOAc mixture (v/v 5/1) was followed by trituration with a EtOAc/HOAc mixture (v/v 20/1) to afford lactacystin (2.8 mg) as a colorless glass: ^{1}H NMR (300 MHz, pyridine) δ 9.94 (s, 1H), 8.79 (d, *J* = 8.0 Hz, 1H), 5.41 (dd, *J* = 13.0, 6.5 Hz, 2H), 5.34 (d, *J* = 7.1 Hz, 1H), 4.59 (d, *J* = 7.0 Hz, 1H), 4.06 (dd, *J* = 13.5, 4.8 Hz, 1H), 3.85 (dd, *J* = 13.5, 6.7 Hz, 1H), 3.54 − 3.41 (m, 1H), 2.24 (dd, *J* = 13.5, 6.8 Hz, 1H), 2.02 (s, 3H), 1.56 (d, *J* = 7.6 Hz, 3H), 1.25 (d, *J* = 6.6 Hz, 3H), 1.17 (d, *J* = 6.8 Hz, 3H).

■ ASSOCIATED CONTENT

Supporting Information. Figures giving ^{1}H and ^{13}C NMR spectra of all new compounds and ^{1}H NMR spectra of all known compounds and a CIF file giving crystallographic data for **16**. This material is available free of charge via the Internet at http://pubs.acs.org.

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