Total Synthesis of (+)-Lactacystin

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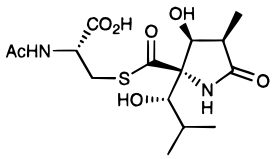
Abstract: A total synthesis of the novel neurotrophic agent (+)-lactacystin (**1**) has been achieved in 11 steps and 14% overall yield from (2*R*,3*S*)-3-hydroxyleucine [(+)-**16**]. The construction and bioassay of several active analogs are also described. A new asymmetric approach furnished the four stereoisomers of 3-hydroxyleucine, as required starting materials in high overall yield and enantiomeric purity.

Neurotrophic factors (NTFs)[1] are proteins essential for the survival and function of nerve cells. Decreased availability of NTFs is thought to cause various nerve disorders including Alzheimer's disease, leading to speculation that NTF-like substances might be therapeutically useful.[2] To this end, ca. 6000 soil isolates (containing predominantly actinomycetes and fungi) were screened for differentiation of the mouse neuroblastoma cell line Neuro 2a. During this process (+)-lactacystin (**1**) was isolated from a culture broth of *Streptomyces* sp. OM-

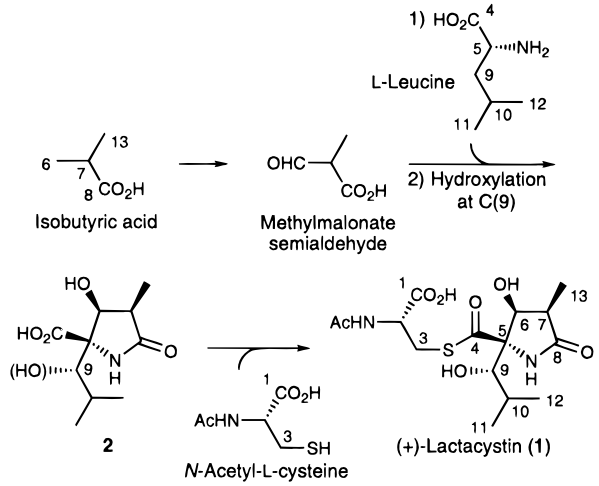


(+)-Lactacystin (**1**)

6519[3a] and the novel γ-lactam thioester structure elucidated via ^{1}H and ^{13}C NMR;[3b] single-crystal X-ray analysis subsequently revealed the absolute stereochemistry.[3b] Lactacystin induces neuritogenesis with a characteristic parallel array of microtubules and neurofilaments, and also causes transient increases in intracellular cAMP levels as well as acetylcholine (Ach) esterase activity in the Neuro 2a neuroblastoma cells.[4] Its mode of action appears to be inhibition of the 20*S* proteasome peptidase activity via acylation of the amino-terminal threonine.[5]

The biosynthesis of lactacystin was investigated with L-[2-^{13}C]leucine, [1-^{13}C]isobutyrate, [1-^{13}C]propionate, and L,L-[1,1′-$^{13}C_2$]cystine (Scheme 1).[6] These studies established that the γ-lactam **2** is assembled via an aldol condensation of methyl-

Scheme 1



malonate semialdehyde, derived from isobutyric acid, with L-leucine, followed by intramolecular cyclization and C(9) hydroxylation. Coupling with *N*-acetylcysteine then affords lactacystin.

Our continuing studies have focused on both the total synthesis and further biological analysis of lactacystin and analogs thereof.[7] The intriguing structures and significant pharmacological potential of these substances have stimulated considerable interest; three other routes to **1** have now been reported.[8−10] In this paper,[7] we describe a concise approach to lactacystin, designed to afford easy access to the natural product and a variety of analogs. Our retrosynthetic analysis is outlined in Scheme 2. The γ-lactam moiety and C(9−12) side chain, containing four stereocenters, were envisioned to arise via two key reactions: stereoselective hydroxymethylation of oxazoline

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(1) (a) Perez-Polo, J. R. In *Cell Culture in the Neurosciences*; Bottenstein, J. E., Sato, G., Eds.; Plenum Press: New York, 1985; pp 95−123. (b) Hefti, F.; Weinen, W. J. *Ann. Neurol.* **1986**, *20*, 275−281. (c) Barde, Y.-A. *Neuron* **1989**, *2*, 1525−1534.

(2) Dohty, P.; Dickson, J. G.; Flanigan, T. P.; Walsh, F. S. *J. Neurochem.* **1985**, *44*, 1259−1265; see also: Sabei, B. A.; Gottlieb, J.; Schneider, G. E. *Brain Res.* **1988**, *459*, 373−380.

(3) (a) Ōmura, S.; Fujimoto, T.; Otoguro, K.; Matsuzaki, K.; Moriguchi, R.; Tanaka, H.; Sasaki, Y. *J. Antibiot.* **1991**, *44*, 113−116. (b) Ōmura, S.; Matsuzaki, K.; Fujimoto, T.; Kosuge, K.; Furuya, T.; Fujita, S.; Nakagawa, A. *J. Antibiot.* **1991**, *44*, 117−118.

(4) Tanaka, H.; Katagiri, M.; Arinie, S.; Matsuzaki, K.; Inokoshi, J.; Ōmura, S. *Biochem. Biophys. Res. Commun.* **1995**, *216*, 291−297.

(5) Fenteany, G.; Standaert, R. F.; Lane, W. S.; Choi, S.; Corey, E. J.; Schreiber, S. L. *Science* **1995**, *268*, 726−731.

(6) Nakagawa, A.; Takahashi, S.; Uchida, K.; Matsuzaki, K.; Ōmura, S.; Nakamura, A.; Kurihara, N.; Nakamatsu, T.; Miyake, Y.; Take, K.; Kainosho, M. *Tetrahedron Lett.* **1994**, *35*, 5009−5012.

(7) Preliminary communication: Sunazuka, T.; Nagamitsu, T.; Matsuzaki, K.; Tanaka, H.; Ōmura, S.; Smith, A. B., III. *J. Am. Chem. Soc.* **1993**, *115*, 5302.

(8) (a) Corey, E. J.; Reichard, G. A. *J. Am. Chem. Soc.* **1992**, *114*, 10677−10678. (b) Corey, E. J.; Reichard, G. A.; Kania, R. *Tetrahedron Lett.* **1993**, *34*, 6977−6980.

(9) Uno, H.; Baldwin, J. E.; Russell, A. *J. Am. Chem. Soc.* **1994**, *116*, 2139−2140.

(10) Chida, N.; Takeoka, J.; Tsutsumi, N.; Ogawa, S. *J. Chem. Soc., Chem. Commun.* **1995**, 793−794.

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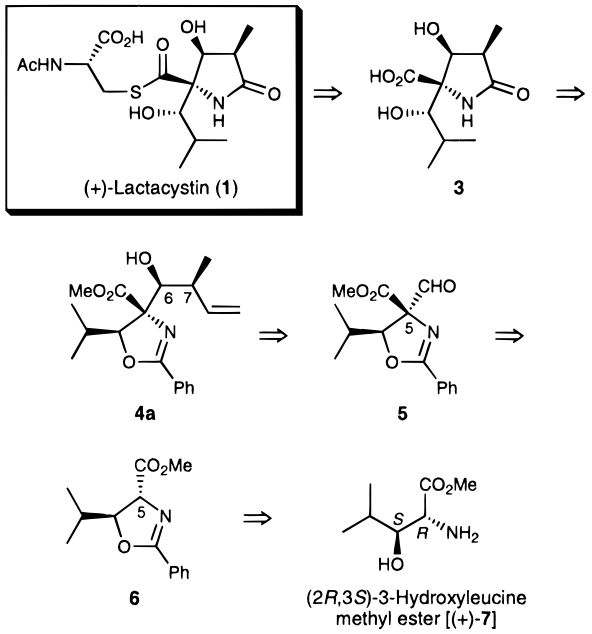
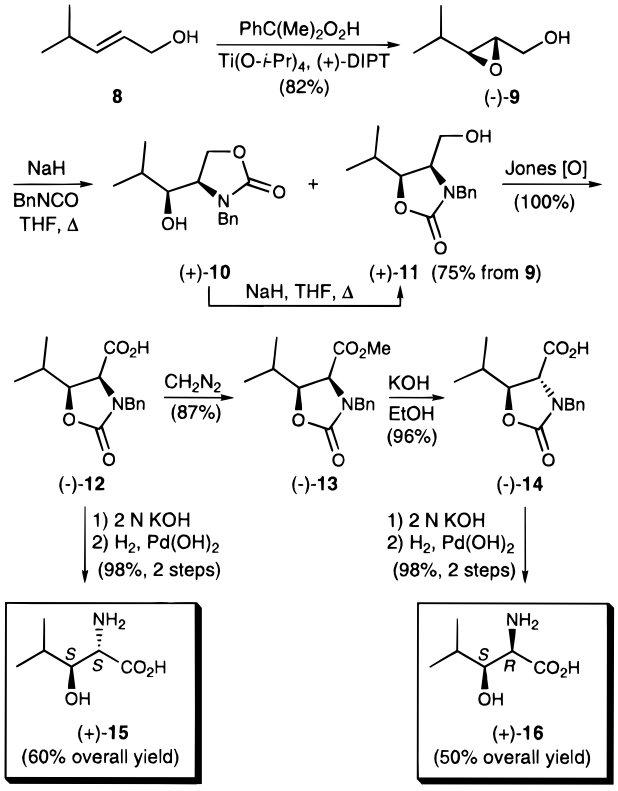
Scheme 2



Scheme 3



6 to introduce the C(5) quaternary carbon, and asymmetric allylboration of the corresponding aldehyde **5** to install the hydroxyl and methyl substituents at C(6) and C(7), respectively. The end game would then entail S-acylation of *N*-acetylcysteine and deprotection. Oxazoline **6** would derive from the known (2*R*,3*S*)-3-hydroxyleucine methyl ester [(+)-**7**].

Efficient Asymmetric Synthesis of the Four Stereoisomers of 3-Hydroxyleucine. To construct lactacystin and its analogs in the quantities required for biological analysis, we had to produce multigram amounts of all four stereoisomers of 3-hydroxyleucine. Several meritorious approaches have been reported previously, although they either lack generality (i.e., yield only one of the four possible diastereomers) or required the preparation of a scalemic catalyst.[11] We developed a simple, concise strategy employing commercially available materials, exploiting a catalytic Sharpless epoxidation,[12] benzyl isocyanate-induced epoxide opening,[13] and epimerization of an oxazolidinone ester.[14]

For the synthesis of (2*S*,3*S*) and (2*R*,3*S*) diastereomers (+)-**15** and (+)-**16** (Scheme 3), *(E)*-4-methyl-2-penten-1-ol (**8**) was prepared in quantity via condensation of isobutyraldehyde with triethyl phosphonoacetate, followed by DIBAL reduction and distillation.[11b] Sharpless epoxidation[12] with cumene hydroperoxide, diisopropyl L-tartrate [(+)-DIPT], and titanium(IV) isopropoxide smoothly afforded epoxide (−)-**9**[11b] in 82% yield and greater than 97% enantiomeric excess (ee), as determined by ^{1}H NMR analysis of the derived (+)-MPTA ester.[15] Treatment of epoxy alcohol **9** with benzyl isocyanate (1.5 equiv) and sodium hydride (2.15 equiv) in THF at reflux for 2 h initially furnished a 5:1 ratio of regioisomeric oxazolidinones (+)-**10** and (+)-**11**; reexposure of the mixture to NaH (0.85 equiv, THF, reflux, 1 h) then produced the rearranged heterocycle (+)-**11** in 75% yield after chromatography.[16] Jones oxidation[17] gave the corresponding carboxylic acid (−)-**12** quantitatively. Finally, hydrolysis of the urethane (2 N KOH, reflux) and debenzylation [H_2, $Pd(OH)_2$, MeOH] generated (2*S*,3*S*)-3-hydroxyleucine [(+)-**15**] in 98% yield (60% overall from **8**).

The synthesis of the (2*R*,3*S*) epimer (+)-**16** entailed epimerization of the *cis*-oxazolidinone (−)-**12** (Scheme 3). To this end, (−)-**13** was prepared via diazomethane esterification (87%); exposure to ethanolic KOH (reflux, 30 min) effected both equilibration and saponification, affording the *trans*-acid (−)-**14** in 96% yield.[18] In the ^{1}H NMR spectrum of (−)-**14**, the 5.1-Hz $J_{4,5}$ coupling was fully in accord with the trans configuration [cf. 7.6 Hz for (−)-**13**].[19] Urethane cleavage and hydrogenolysis as before then gave (+)-**16** in 98% yield (50% overall from **8**).

The enantiomers (−)-**15** and (−)-**16** were generated via analogous sequences, simply by employing (−)-DIPT in the asymmetric epoxidation of **8**. In this fashion, multigram quantities of the four stereoisomers of 3-hydroxyleucine can be readily prepared in high overall yield and enantiomeric purity. Importantly, only **9** and **11** require chromatographic isolation; all of the other intermediates are carried forward without purification, greatly enhancing the practicality of the approach.

(11) See, for example: (a) Corey, E. J.; Lee, D.-H.; Choi, S. *Tetrahedron Lett.* **1992**, *33*, 6735−6738. (b) Caldwell, C. G.; Bondy, S. S. *Synthesis* **1990**, 34−36. (c) Jung, M. E.; Jung, Y. H. *Tetrahedron Lett.* **1989**, *48*, 6637−6640. The following authors proposed a sequence embodying elements of our approach: (d) Evans, D. A.; Sjogren, E. B.; Weber, A. E.; Conn, R. E. *Tetrahedron Lett.* **1987**, *28*, 39−43. (e) Seebach, D.; Juaristi, E.; Miller, D. D.; Schaicbli, C.; Weber, T. *Helv. Chim. Acta* **1987**, *70*, 1194−1215.

(12) Gao, Y.; Hanson, R.; Klunder, J. M.; Ko, S. Y.; Masamune, H.; Sharpless, K. B. *J. Am. Chem. Soc*. **1987**, *109*, 5765−5780.

(13) Knapp, S.; Kukkola, P. J.; Sharma, S.; Peitranico, S. *Tetrahedron Lett.* **1987**, *39*, 5399−5402.

(14) Preliminary communication: Sunazuka, T.; Nagamitsu, T.; Tanaka, H.; Sprengeler, P. A.; Smith, A. B., III; Ōmura, S. *Tetrahedron Lett.* **1993**, *34*, 4447−4448.

(15) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543−2549.

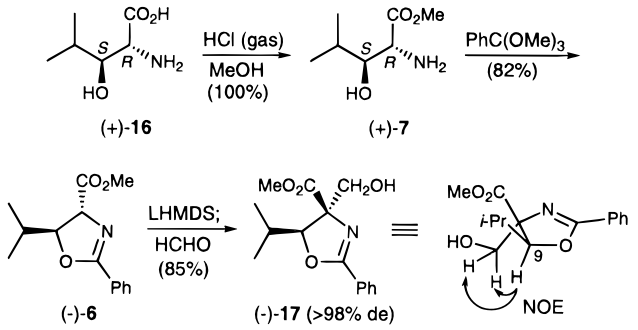
(16) Related amine- and isocyanate-induced epoxide openings have been employed previously (ref 11). Also see: Roush, W. R.; Adam, M. A. *J. Org. Chem.* **1985**, *50*, 3752−3757 and literature cited therein.

(17) Bowden, K.; Heilbron, I. M.; Jones, E. R. H.; Weedon, B. C. L. *J. Chem. Soc.* **1946**, 39. Also see, Meinwald, J.; Crandall, J.; Hymans, W. E. *Org. Synth.* **1965**, *45*, 77.

(18) Shanzer, A.; Somekh, L.; Butina, D. *J. Org. Chem.* **1979**, *44*, 3967−3969, and references cited therein.

(19) Jaime, C.; Ortuno, R. M.; Font, J. *J. Org. Chem.* **1986**, *51*, 3946−3951.

Scheme 4



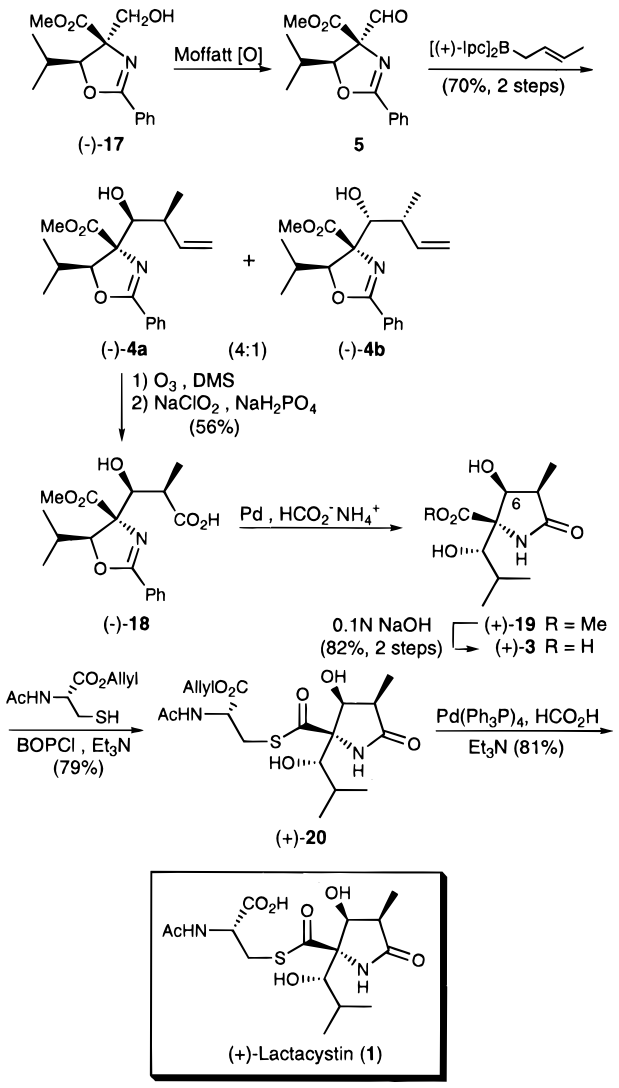
These amino acids were employed in the synthesis of lactacystin and analogs thereof.

Total Synthesis of (+)-Lactacystin (1). As our point of departure we required (+)-**16**, the *(2R,3S)* diastereomer of 3-hydroxyleucine, which quantitatively furnished the known methyl ester (+)-**7** upon acidic methanolysis.[11c] Treatment with methyl orthobenzoate in the presence of *p*-toluenesulfonic acid as catalyst yielded the *trans*-oxazoline (−)-**6**[20] in 82% yield. Aldol condensation with formaldehyde via the Seebach protocol[21] then gave (−)-**17** exclusively (85% yield, >98% de). The stereochemistry was assigned by ^{1}H NOE, as irradiation of the C(9) proton (lactacystin numbering) led to 1.7% and 1.4% enhancements of the C(6) methylene protons in **17** (Scheme 4).

Oxidation of primary alcohol (−)-**17** proved troublesome under a variety of conditions (e.g., Swern,[22] PCC,[23] Dess−Martin,[24] and Parikh−Doering[25]). Fortunately, Moffatt oxidation[26] did provide the requisite aldehyde **5** (Scheme 5). Deformylation to oxazoline **6** (syn/anti mixture) occurred quite readily during extraction and silica gel chromatography, so the aldehyde was isolated via nonaqueous workup and subjected without purification to Brown asymmetric allylboration with *(E)*-crotyl(diisopinocampheyl)borane.[27] This sequence afforded a 4:1 mixture of the desired homoallylic alcohol (−)-**4a** and the diastereomer (−)-**4b** in 70% overall yield from alcohol (−)-**17**. Other crotylation procedures gave lower selectivities: the Hiyama *(E)*-crotylchromium(II) reagent[28] and Roush *(E)*-crotylpinacol borane[29] gave 2:1 mixtures of **4a** and **4b**, and the *(R,R)-(E)*-crotyl tartrate borane of Roush[30] gave a 3:1 ratio. All of these results presumably reflect the steric congestion of the aldehyde moiety in **5**.

One-step cleavage of the vinyl group in (−)-**4a** with ruthenium(III) chloride and sodium periodate[31] furnished carboxylic

Scheme 5

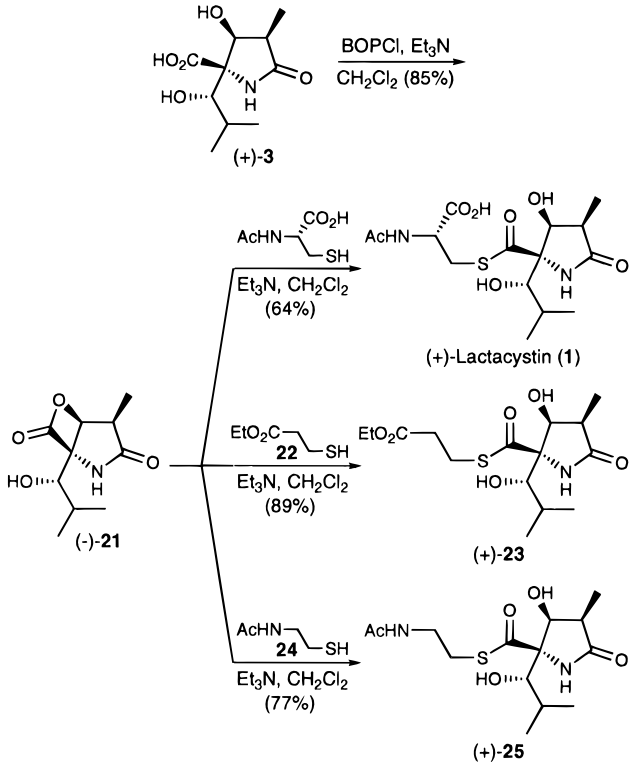


acid (−)-**18** (Scheme 5) in only 11% yield; a stepwise alternative employing osmium tetroxide/sodium periodate[32] and $NaClO_2$/H_2O_2[33] likewise proved unsatisfactory (19%). Fortunately, ozonolysis (O_3; DMS) and subsequent chlorite oxidation ($NaClO_2$, NaH_2PO_4, 2-methyl-2-butene)[34] gave the requisite acid (−)-**18** in 56% yield from (−)-**4a**. Attempted hydrogenolysis [Pd/C, $Pd(OH)_2$, or Pd black; MeOH, H_2] and acidic hydrolysis (6 N HCl, reflux) of the oxazoline moiety proved unsuccessful, but catalytic transfer hydrogenation[35] (Pd black, ammonium formate, AcOH, reflux) afforded γ-lactam ester (+)-**19** in 91% yield after ring closure. Without purification, **19** was saponified under mild conditions (0.1 N NaOH) to give acid (+)-**3** (90% yield). Exposure of **19** to 1 N NaOH resulted in epimerization at C(6).

To complete the synthesis, we employed the two-step sequence devised by Corey.[8a] Following thioesterification of (+)-**3** with bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOPCl)[36] and *N*-acetyl-L-cysteine allyl ester (79% yield), deallylation of (+)-**20** [$Pd(PPh_3)_4$, HCOOH, Et_3N][37] provided (+)-lactacystin (**1**) in 81% yield as colorless needles, indistin-

(20) Moss, R. A.; Lee, T. B. K. *J. Chem. Soc., Perkin Trans. 1* **1973**, 2778−2781.
(21) Seebach, D.; Aebi, J. D. *Tetrahedron Lett.* **1983**, *24*, 3311−3314.
(22) (a) Omura, K.; Swern, D. *Tetrahedron* **1987**, *34*, 1651−1660. (b) Mancuso, A. J.; Huang, S. L.; Swern, D. *J. Org. Chem.* **1978**, *43*, 2480−2482.
(23) Corey, E. J.; Suggs, J. W. *Tetrahedron Lett.* **1975**, *16*, 2647−2650.
(24) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155−4156.
(25) Parikh, J. R.; Doering, W. v. E. *J. Am. Chem. Soc*. **1967**, *89*, 5505−5507.
(26) Pfitzner, K. E.; Moffatt, J. G. *J. Am. Chem. Soc.* **1965**, *87*, 5661−5669.
(27) (a) Brown, H. C.; Bhat, K. S. *J. Am. Chem. Soc.* **1986**, *108*, 293−294. (b) Brown, H. C.; Bhat, K. S. *J. Am. Chem. Soc.* **1986**, *108*, 5919−5923.
(28) (a) Okuda, Y.; Hirano, S.; Hiyama, T.; Nozaki, H. *J. Am. Chem. Soc*. **1977**, *99*, 3179−3181. (b) Hiyama, T.; Kimura, K.; Nozaki, H. *Tetrahedron Lett.* **1981**, *22*, 1037−1040.
(29) Roush, W. R.; Adam, M. A.; Walts, A. E.; Harris, D. J. *J. Am. Chem. Soc*. **1986**, *108*, 3422−3434.
(30) Roush, W. R.; Ando, K.; Powers, D. B.; Palkowitz, A. D.; Halterman, R. L. *J. Am. Chem. Soc*. **1990**, *112*, 6339−6348.
(31) Carlsen, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem*. **1981**, *46*, 3936−3938.
(32) Shue, Y.-K.; Carrera, G. M., Jr.; Nadzan, A. M. *Tetrahedron Lett.* **1987**, *28*, 3225−3228.
(33) Dalcanale, E.; Montanari, F. *J. Org. Chem.* **1986**, *51*, 567−569.
(34) Corey, E. J.; Meyers, A. G. *J. Am. Chem. Soc.* **1985**, *107*, 5574−5576.
(35) Bieg, T.; Szeja, W. *Synthesis* **1985**, 76−77.
(36) Tung, R. D.; Rich, D. H. *J. Am. Chem. Soc.* **1985**, *107*, 4342−4343.

Scheme 6

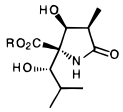
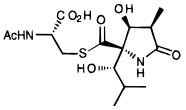
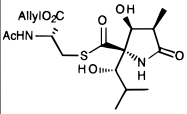
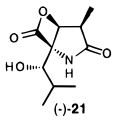
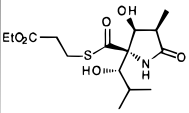
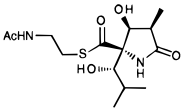


guishable from the natural product (400-MHz ^{1}H and 100-MHz ^{13}C NMR, IR, HRMS, optical rotation, melting and mixed melting points, and TLC in four solvent systems).

Construction of Bioactive Lactacystin Analogs. To facilitate the elaboration of side-chain analogs, we explored an improved end game exploiting the β-lactone (−)-**21** first employed by Corey in his second-generation lactacystin synthesis;[8b] the latter was readily generated by treatment of (+)-**3** with BOPCl and triethylamine (85% yield, Scheme 6).[8b] We found, however, that coupling of (−)-**21** could be achieved with *unprotected N*-acetylcysteine (Et_3N, CH_2Cl_2) to afford (+)-lactacystin (**1**) in 64% yield, thereby eliminating the need for a deprotection step. Similarly, acylation of thiols **22** and **24** afforded the novel lactacystin congeners (+)-**23** and (+)-**25**.

Because the mechanism of action of lactacystin apparently involves amine acylation,[5] we envisioned that related active esters could also induce neuritogenesis. In addition, we have sought to develop analogs with lower cytotoxicity indices vis-à-vis lactacystin (**1**) itself. As expected, synthetic precursors (+)-**19** and (+)-**3**, which are not reactive acylating agents, showed no activity (Table 1).[38] Interestingly, β-lactone (−)-**21** proved to be as active as lactacystin, and analogs (+)-**20**, (+)-**23**, and (+)-**25** all proved to be significantly more potent than **1** in the neurite outgrowth bioassay.[38] Moreover, we were delighted to discover that the descarboxy analog (+)-**25**[38] displayed a specificity (i.e., cytotoxicity/minimum effective dose) of 125. As such, (+)-**25** represents a significantly more potent non-protein neurotrophic agent than lactacystin.

Table 1. Minimum Effective *in Vitro* Doses[a] for Neuritogenesis, Cytotoxicity Data, and Specificities

Compound	Minimum[b] effective dose (mM)	Cytotoxicity[c] (mM)	Specificity[d]
 (+)-**3** R = H (+)-**19** R = Me	Not effective	–	–
 (+)-Lactacystin (**1**)	1.56	12.5	8
 (+)-**20**	0.20	6.25	31
 (-)-**21**	1.56	12.5	8
 (+)-**23**	0.20	3.13	16
 (+)-**25**	0.10	12.5	125

[a] Neuro 2A cells were plated at a density of 1×10^4 cells/cm^2 and grown for 24 h in MEM-H with 10% FBS prior to any treatment. The number of cells with neurites was microscopically counted 24 h after addition of the drug. [b] Approximately 20% cells with neurites were observed when the drug was added to the culture medium at the indicated concentrations. [c] No attached Neuro 2A cells were observed when the drug was added to the culture medium at the indicated concentrations. [d] Cytotoxicity/minimum effective dose.

Summary

An economic total synthesis of (+)-lactacystin (**1**) from (2*R*,3*S*)-3-hydroxyleucine [(+)-**16**] has been completed (11 steps, 14% overall yield). The versatile strategy has also furnished several bioactive analogs. Preliminary pharmacological studies, now in progress, reveal a significant increase in the specificity of the descarboxy analog (+)-**25**. Finally, the new highly stereocontrolled asymmetric approach to the four stereoisomers of 3-hydroxyleucine sets the stage for the preparation of additional bioactive congeners of lactacystin.

Experimental Section[39]

(Hydroxymethyl)oxazolidinone (+)-11. A suspension of 60% sodium hydride (5.50 g, 138 mmol) in THF (300 mL) was cooled to 0 °C, and a solution of epoxy alcohol (−)-**9**[11b] (7.44 g, 64.1 mmol) in THF (20 mL) was added dropwise over 15 min. The reaction was

(37) Jeffrey, P. D.; McCombie, S. W. *J. Org. Chem.* **1982**, *47*, 587−590.

(38) Nagamitsu, T.; Sunazuka, T.; Stump, H.; Obata, R.; Arima, S.; Matsuzaki, K.; Tanaka, H.; Ōmura, S. *J. Antibiot.* **1995**, *48*, 747−748.

(39) Except as stated otherwise, reactions were carried out under an argon atmosphere with freshly distilled solvents, magnetic stirring, and monitoring by thin layer chromatography (TLC) with 0.25-mm precoated silica gel plates (E. Merck). Column chromatography was performed with silica gel (particle size 0.040−0.063 mm, E. Merck). Melting points were measured using a Yanagimoto micro melting point apparatus and are uncorrected. Infrared spectra were recorded on a Horiba FT-210 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on JEOL-EX270 and Varian XL-400 spectrometers. Chemical shifts for $CDCl_3$ or pyridine solutions are reported relative to TMS. ^{1}H NMR data collected in methanol-d_4 and deuterium oxide are reported relative to the methanol peak at 3.31 ppm and the deuterium peak at 4.76 ppm, respectively. Mass spectra were obtained with a JMS-D100 or JMS-D300 instrument. Elemental analyses were performed by YANACO CHN Corder MT-5. Optical rotations were measured with a JASCO DIP-370 digital polarimeter.

stirred for 10 min further, and neat benzyl isocyanate (11.9 mL, 96.3 mmol) was introduced dropwise over 30 min. The resultant mixture was heated at reflux for 2 h, cooled to 0 °C, treated with 60% sodium hydride (2.18 g, 54.5 mmol), and heated at reflux for an additional 1 h. The solution was then cooled to 0 °C, quenched with saturated aqueous NH_4Cl (150 mL), and extracted with $CHCl_3$ (3 × 150 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, filtered, and concentrated. Flash chromatography (2:1 hexane/EtOAc) afforded (+)-**11** (8.85 g, 75% yield) as a yellow powder: mp 134−135 °C (EtOAc); $[\alpha]_D^{25}$ +9.4° (*c* 1.0, $CHCl_3$); IR (CCl_4) 3700−3200 (w), 1730 (s), 1420 (m) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.82 (d, J = 6.6 Hz, 3 H), 1.03 (d, J = 6.6 Hz, 3 H), 2.08 (m, 1 H), 2.09 (br s, 1 H), 3.43 (m, 1 H), 3.74 (t, J = 3.3 Hz, 2 H), 3.94 (dd, J = 10.4, 7.1 Hz, 1 H), 4.17 (d, J = 15.2 Hz, 1 H), 4.79 (d, J = 15.2 Hz, 1 H), 7.25 (m, 5 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 18.9, 19.7, 27.5, 46.5, 58.0, 58.4, 83.0, 127.9, 128.0, 128.9, 136.6, 158.8; high-resolution mass spectrum (EI, 70 eV) *m/z* 249.1367 (M^+; calcd for $C_{14}H_{19}NO_3$: 249.1365). Anal. Calcd for $C_{14}H_{19}NO_3$: C, 67.43; H, 7.69; N, 5.62. Found: C, 67.06; H, 7.76; N, 5.30.

(Hydroxymethyl)oxazolidinone (−)-11. Following the procedure described above for (+)-**11**, reaction of epoxy alcohol (+)-**9**[11b] with NaH and benzyl isocyanate furnished (−)-**11** as a yellow powder: mp 110−113 °C (EtOAc); $[\alpha]_D^{26}$ −9.4° (*c* 1.0, $CHCl_3$).

Carboxylic Acid (−)-12. A solution of alcohol (+)-**11** (5.17 g, 20.8 mmol) in acetone (200 mL) was cooled to 0 °C and treated with Jones reagent [20 mL; prepared by dissolving CrO_3 (5.34 g, 53.4 mmol) dropwise over 10 min in concentrated H_2SO_4 (4.6 mL) and diluting with water to 20 mL]. The resultant mixture was stirred for 1 h at room temperature, and excess Jones reagent was decomposed by adding 2-propanol until the color changed from brown to green blue. The supernatant was decanted and concentrated, and the residue was dissolved in $CHCl_3$ (100 mL). The solids were taken up in a minimum of saturated aqueous NaCl and extracted with the above $CHCl_3$ solution and with additional $CHCl_3$ (2 × 100 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated, affording (−)-**12** (4.55 g, 100% yield) as a white powder which was carried forward without purification: mp 183−185 °C ($CHCl_3$); $[\alpha]_D^{27}$ −30° (*c* 1.0, MeOH); IR (KBr) 3700−3300 (m), 1740 (s), 1695 (s) cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 0.93 (d, J = 6.6 Hz, 3 H), 0.99 (d, J = 6.6 Hz, 3 H), 1.98 (m, 1 H), 3.91 (d, J = 15.2 Hz, 1 H), 3.93 (d, J = 7.6 Hz, 1 H), 4.12 (dd, J = 9.4, 7.6 Hz, 1 H), 4.79 (d, J = 15.2 Hz, 1 H), 7.28 (m, 5 H); ^{13}C NMR (67.5 MHz, CD_3OD) δ 19.0, 19.3, 30.5, 48.4, 50.0, 83.5, 129.0, 129.4, 129.9, 137.1, 160.7, 172.0; high-resolution mass spectrum (EI, 70 eV) *m/z* 263.1144 (M^+; calcd for $C_{14}H_{17}NO_4$: 263.1157). Anal. Calcd for $C_{14}H_{17}NO_4$: C, 63.85; H, 6.51; N, 5.32. Found: C, 63.75; H, 6.49; N, 5.04.

Carboxylic Acid (+)-12. Following the procedure described above for (−)-**12**, Jones oxidation of alcohol (−)-**11** gave (+)-**12** as a white powder which was carried forward without purification: mp 183−187 °C ($CHCl_3$); $[\alpha]_D^{27}$ +36° (*c* 0.5, MeOH).

Methyl Ester (−)-13. Carboxylic acid (−)-**12** (4.55 g, 17.3 mmol) was suspended in Et_2O (300 mL) at room temperature, and CH_2N_2 gas [generated by adding *N*-methyl-*N*-nitrosourea (8 mL) in several portions to 40% aqueous KOH (50 mL)] was bubbled through the stirring mixture until the white powder disappeared. The resultant solution was stirred overnight and concentrated, furnishing (−)-**13** (5.00 g, 87% yield) as a white powder which was used without purification: mp 75−76 °C (Et_2O); $[\alpha]_D^{24}$ −39° (*c* 1.0, $CHCl_3$); IR (CCl_4) 1750 (s), 1400 (m) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.89 (d, J = 6.6 Hz, 3 H), 1.04 (d, J = 6.6 Hz, 3 H), 1.73 (m, 1 H), 3.70 (s, 3 H), 3.92 (d, J = 14.9 Hz, 1 H), 4.08 (dd, J = 9.0, 7.6 Hz, 1 H), 4.09 (d, J = 7.6 Hz, 1 H), 4.84 (d, J = 14.9 Hz, 1 H), 7.28 (m, 5 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 18.3, 19.0, 29.2, 47.5, 52.3, 60.9, 81.5, 128.1, 128.5, 128.8, 135.2, 157.8, 168.8; high-resolution mass spectrum (EI, 70 eV) *m/z* 277.1314 (M^+; calcd for $C_{15}H_{19}NO_4$: 277.1314). Anal. Calcd for $C_{15}H_{19}NO_4$: C, 64.95; H, 6.91; N, 5.05. Found: C, 64.89; H, 6.95; N, 4.96.

Methyl Ester (+)-13. Following the procedure described above for (−)-**13**, diazomethane esterification of acid (+)-**12** afforded (+)-**13** as a white powder which was used without purification: mp 75−76 °C (Et_2O); $[\alpha]_D^{24}$ +42° (*c* 0.5, $CHCl_3$).

Carboxylic Acid (−)-14. A solution of methyl ester (−)-**13** (4.83 g, 18.4 mmol) in anhydrous EtOH (98.2 mL) was treated with solid KOH (5.7 g, 87 mmol) and the reaction mixture heated at reflux for 30 min, cooled, concentrated, acidified to pH 1 with 1 N HCl, and extracted with $CHCl_3$ (3 × 200 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated, providing (−)-**14** (4.40 g, 96% yield) as a white powder, which was used without purification in the next step: mp 95−96 °C ($CHCl_3$); $[\alpha]_D^{24}$ −20° (*c* 0.5, MeOH); IR (CCl_4) 3200−2400 (m), 1740 (s), 1400 (m) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.87 (d, J = 6.5 Hz, 6 H), 1.85 (m, 1 H), 3.76 (d, J = 5.0 Hz, 1 H), 4.17 (d, J = 14.5 Hz, 1 H), 4.33 (apparent t, J = 5.0 Hz, 1 H), 5.00 (d, J = 14.5 Hz, 1 H), 7.32 (m, 5 H), 8.37 (br s, 1 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 16.2, 17.0, 32.5, 47.1, 58.1, 81.1, 128.3, 128.6, 128.9, 134.6, 157.9, 173.3; high-resolution mass spectrum (EI, 70 eV) *m/z* 263.1163 (M^+; calcd for $C_{14}H_{17}NO_4$: 263.1157). Anal. Calcd for $C_{14}H_{17}NO_4$: C, 63.85; H, 6.51; N, 5.32. Found: C, 63.65; H, 6.51; N, 5.04.

Carboxylic Acid (+)-14. Following the procedure described above for (−)-**14**, methyl ester (+)-**13** was transformed to acid (+)-**14**, a white powder which was used without purification in the next step: mp 102−103 °C ($CHCl_3$); $[\alpha]_D^{26}$ +23° (*c* 0.5, MeOH).

(2*S*,3*S*)-3-Hydroxyleucine [(+)-15]. A solution of oxazolidinone (−)-**12** (2.65 g, 10.1 mmol) in 2 N aqueous KOH (100.8 mL) was heated at reflux for 5 h, cooled to 0 °C, and acidified to pH 5 with 6 N HCl. The resultant mixture was stirred for 30 min at 0 °C and passed through a 3G4 glass filter, and the precipitate was then washed with ice-cold water (20 mL), ice-cold acetone (20 mL), and Et_2O (20 mL) and dried under reduced pressure, affording crude (2*S*,3*S*)-*N*-benzyl-3-hydroxyleucine.

At room temperature a mixture of $Pd(OH)_2$ (350 mg) and MeOH (70 mL) was stirred for 30 min under H_2, and a solution of the benzyl derivative in MeOH (70 mL) was added. The mixture was stirred under H_2 for 5 h, filtered, and concentrated, furnishing (+)-**15** (1.1 g, 98% yield) as a white powder: mp 219−222 °C (MeOH); $[\alpha]_D^{28}$ +37° (*c* 1.0, H_2O) {lit.[11b] mp 220−223 °C; $[\alpha]_D$ +37° (*c* 0.99, 1 N aqueous HCl)}; IR (KBr) 3600−3300 (w), 3290 (m), 1620 (s) cm^{-1}; ^1H NMR (270 MHz, D_2O) δ 0.87 (d, J = 6.6 Hz, 3 H), 0.88 (d, J = 6.6 Hz, 3 H), 1.84 (m, 1 H), 3.42 (dd, J = 8.7, 3.0 Hz, 1 H), 3.74 (d, J = 3.0 Hz, 1 H); ^{13}C NMR (67.5 MHz, D_2O) δ 19.3, 19.6, 31.1, 58.1, 77.4, 173.9; high-resolution mass spectrum (FAB, glycerol matrix) *m/z* 148.0968 [(M + H)$^+$; calcd for $C_6H_{14}NO_3$: 148.0974]. Anal. Calcd for $C_6H_{13}NO_3 \cdot {}^3/_2H_2O$: C, 41.35; H, 9.26; N, 8.04. Found: C, 41.25; H, 8.96; N, 8.09.

(2*R*,3*R*)-3-Hydroxyleucine [(−)-15]. Following the procedure described above for (+)-**15**, oxazolidinone (+)-**12** was converted to (−)-**15**, a white powder: mp 218−220 °C (MeOH); $[\alpha]_D^{26}$ −37° (*c* 1.0, H_2O).

(2*R*,3*S*)-3-Hydroxyleucine [(+)-16]. Following the procedure described above for (+)-**15**, (−)-**14** was converted to (+)-**16**, a white powder: mp 213−214 °C (MeOH); $[\alpha]_D^{27}$ +3.5° (*c* 1.0, H_2O); IR (KBr) 3310 (s), 1635 (s), 1510 (s) cm^{-1}; ^1H NMR (270 MHz, D_2O) δ 0.92 (d, J = 6.8 Hz, 3 H), 0.96 (d, J = 6.8 Hz, 3 H), 1.70 (m, 1 H), 3.72 (dd, J = 7.8, 4.0 Hz, 1 H), 3.78 (d, J = 4.0 Hz, 1 H); ^{13}C NMR (67.5 MHz, D_2O) δ 18.4, 19.4, 31.2, 57.9, 76.1, 172.2.

(2*S*,3*R*)-3-Hydroxyleucine [(−)-16]. Following the procedure described above for (+)-**15**, (+)-**14** was converted to (−)-**16**, a white powder: mp 213−216 °C (MeOH); $[\alpha]_D^{27}$ −3.5° (*c* 1.0, H_2O) {lit.[11c] mp 213−217 °C; $[\alpha]_D$ −3.5° (*c* 2.2, H_2O).

Methyl Ester (+)-7. Dry hydrogen chloride gas was passed rapidly into a stirred suspension of acid (+)-**16** (1.5 g, 10.2 mmol) in MeOH (45 mL) until the solution boiled (ca. 15 min). The introduction of HCl was terminated, and the solution was cooled to room temperature, stirred for 24 h, and concentrated. Exposure to high vacuum overnight gave (+)-**7** (2.02 g, 100% yield) as a yellow solid which was carried forward without purification: $[\alpha]_D^{24}$ +26° (*c* = 1.0, $CHCl_3$); IR (CCl_4) 3300 (w), 1720 (s), 1440 (w) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.78 (d, J = 6.6 Hz, 3 H), 0.84 (d, J = 6.6 Hz, 3 H), 1.59 (m, 1 H), 3.69 (s, 3 H), 3.71 (apparent t, J = 3.3 Hz, 1 H), 4.16 (d, J = 3.3 Hz, 1 H).

Oxazoline (−)-6. A solution of methyl ester hydrochloride (+)-**7** (1.96 g, 9.87 mmol) in dimethoxyethane (20 mL) was treated with *p*-TsOH·H_2O (1.88 g, 9.87 mmol) and trimethyl orthobenzoate (5.1

mL, 29.6 mmol) and heated at reflux for 3.5 h. The reaction mixture was cooled to room temperature and quenched with H_2O (10 mL), the layers were separated, and the aqueous phase was extracted with $CHCl_3$ (3 × 30 mL). The combined organic solutions were dried over Na_2SO_4, filtered, and concentrated. Flash chromatography (8:1 hexane/EtOAc) afforded (−)-**6** (2.01 g, 82% yield) as a yellow oil: $[\alpha]_D^{23}$ −124° (*c* 1.0, $CHCl_3$); IR (CCl_4) 2880 (m), 1740 (s), 1640 (s) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.98 (d, J = 6.6 Hz, 3 H), 1.02 (d, J = 6.6 Hz, 3 H), 1.96 (m, 1 H), 3.79 (s, 3 H), 4.56 (d, J = 7.3 Hz, 1 H), 4.67 (apparent t, J = 6.6 Hz, 1 H), 7.44 (m, 3 H), 7.99 (d, J = 7.3 Hz, 2 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 17.2, 17.3, 29.5, 52.3, 66.8, 80.1, 89.2, 126.8, 128.3, 128.6, 131.9, 166.4, 171.5; high-resolution mass spectrum (EI, 70 eV) *m/z* 247.1208 (M^+; calcd for $C_{14}H_{17}NO_3$: 247.1208). Anal. Calcd for $C_{14}H_{17}NO_3$: C, 67.98; H, 6.93; N, 5.67. Found: C, 67.70; H, 6.86; N, 5.55.

Primary Alcohol (−)-17. A solution of lithium bis(trimethylsilyl)-amide (1.0 M in hexane, 6.6 mL, 6.6 mmol) in THF (55 mL) was cooled to −78 °C and treated dropwise with a solution of oxazoline (−)-**6** (1.49 g, 6.03 mmol) in THF (5 mL). After 1 h at −78 °C, a freshly prepared solution of monomeric formaldehyde in Et_2O (24 mL, ca. 32 mmol of HCHO, maintained at −78 °C) was added via cannula. The mixture was stirred for 10 min further and then rapidly quenched with water (50 mL) at −78 °C. The cooling bath was removed and the mixture allowed to warm to room temperature. The aqueous layer was extracted with Et_2O (3 × 50 mL), and the combined organic phases were dried over Na_2SO_4, filtered, and concentrated. Flash chromatography (2:1 hexane/EtOAc) furnished (−)-**17** (1.42 g, 85% yield) as a white powder: mp 76−77 °C (EtOAc); $[\alpha]_D^{23}$ −1.8° (*c* 1.0, $CHCl_3$); IR (KBr) 3500−3100 (w), 1730 (s), 1640 (s) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.12 (d, J = 6.6 Hz, 3 H), 1.13 (d, J = 6.6 Hz, 3 H), 2.13 (m, 1 H), 2.30 (t, J = 7.3 Hz, 1 H), 3.88 (s, 3 H), 3.95 (dd, J = 11.5, 7.3 Hz, 1 H), 4.11 (dd, J = 11.5, 7.3 Hz, 1 H), 4.56 (d, J = 7.3 Hz, 1 H), 7.55 (m, 3 H), 8.07 (m, 2 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 18.6, 19.4, 29.5, 52.3, 66.8, 80.1, 89.2, 126.8, 128.3, 128.6, 131.9, 166.4, 171.5; high-resolution mass spectrum (EI, 70 eV) *m/z* 278.1392 (M^+; calcd for $C_{15}H_{19}NO_4$: 278.1392). Anal. Calcd for $C_{15}H_{19}NO_4$: C, 64.72; H, 7.25; N, 5.03. Found: C, 65.00; H, 6.99; N, 5.16.

Aldehyde 5. A solution of alcohol (−)-**17** (1.23 g, 4.44 mmol) in benzene (24.2 mL) and DMSO (6.67 mL) was treated with DCC (2.75 g, 13.3 mmol), pyridine (0.36 mL, 4.4 mmol), and trifluoroacetic acid (0.17 mL, 2.2 mmol). The mixture was stirred overnight at room temperature and then concentrated, and the residue was dissolved in *n*-pentane (20 mL). Following suction filtration through a fritted glass funnel, under N_2 into an oven-dried flask, concentration and exposure to high vacuum for 15 h furnished **5**, a yellow oil which was not purified: ^1H NMR (270 MHz, $CDCl_3$) δ 0.88 (d, J = 6.6 Hz, 3 H), 1.03 (d, J = 6.6 Hz, 3 H), 2.00 (m, 1 H), 3.77 (s, 3 H), 4.85 (d, J = 8.2 Hz, 1 H), 7.42 (m, 3 H), 7.92 (d, J = 9.9 Hz, 2 H), 9.73 (s, 1 H).

Homoallylic Alcohols (−)-4a and (+)-4b. A mixture of *trans*-2-butene (2.00 mL, 22.2 mmol), 90% potassium *tert*-butoxide (1.32 g, 10.6 mmol), and THF (6.35 mL) was cooled to −78 °C, and *n*-butyllithium (2.5 M in THF, 4.22 mL, 10.6 mmol) was added dropwise. The resultant bright yellow solution was stirred at −50 °C for 10 min and recooled to −78 °C. A solution of (−)-*B*-methoxy-(diisopinocampheyl)borane (4.00 g, 12.7 mmol) in Et_2O (12.7 mL) was then introduced dropwise, and the mixture was stirred for 30 min further. After dropwise treatment with neat boron trifluoride etherate (1.74 mL, 14.2 mmol), aldehyde **5** (1.22 g, 4.44 mmol maximum) in THF (11 mL) was immediately added dropwise, and the mixture was allowed to stir overnight at −78 °C. The reaction mixture was warmed to 0 °C, and 10% NaOH (7.80 mL, 19.5 mmol) followed by 35% H_2O_2 (2.72 mL, 28.0 mmol) was added very slowly. The solution was heated at reflux for 1 h and cooled to room temperature. The aqueous layer was extracted with Et_2O (3 × 20 mL), and the combined organic phases were dried over Na_2SO_4, filtered, and concentrated. Flash chromatography (50:1 hexane/EtOAc) afforded (−)-**4a** (1.03 g, 70% yield) and (−)-**4b** (275 mg, 17%) as white powders. Data for (−)-**4a**: more polar; mp 98−99 °C (EtOAc); $[\alpha]_D^{25}$ −4° (*c* 0.5, $CHCl_3$); IR (KBr) 3550 (w), 1720 (s), 1640 (s) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.82 (d, J = 6.6 Hz, 3 H), 1.01 (d, J = 6.9 Hz, 3 H), 1.09 (d, J = 6.9 Hz, 3 H), 1.88 (m, 1 H), 2.56 (m, 1 H), 3.71 (s, 3 H), 3.87 (dd, J = 9.1, 2.8 Hz, 1 H), 4.70 (d, J = 5.0 Hz, 1 H), 4.95 (dd, J = 15.8, 1.7 Hz, 1 H), 5.07 (dd, J = 10.6, 1.7 Hz, 1 H), 6.17 (m, 1 H), 7.33 (m, 3 H), 7.93 (m, 2 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 16.9, 18.1, 20.7, 29.7, 39.5, 52.3, 78.4, 83.6, 89.2, 116.2, 128.3, 128.6, 128.8, 131.7, 139.1, 165.5, 172.7; high-resolution mass spectrum (EI, 70 eV) *m/z* 331.1785 (M^+; calcd for $C_{19}H_{25}NO_4$: 331.1783). Anal. Calcd for $C_{19}H_{25}NO_4$: C, 68.85; H, 7.60; N, 4.23. Found: C, 68.99; H, 7.88; N, 4.40. Data for (−)-**4b** less polar; mp 102−104 °C (EtOAc); $[\alpha]_D^{28}$ −84° (*c* 0.5, $CHCl_3$); IR (KBr) 3500 (m), 1710 (s), 1650 (s) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.79 (d, J = 6.6 Hz, 3 H), 1.11 (d, J = 7.3 Hz, 3 H), 1.13 (d, J = 7.3 Hz, 3 H), 1.99 (m, 1 H), 2.82 (m, 1 H), 3.36 (d, J = 11.6 Hz, 1 H), 3.41 (dd, J = 11.2, 2.0 Hz, 1 H), 3.73 (s, 3 H), 4.83 (d, J = 3.0 Hz, 1 H), 5.00 (dd, J = 10.2, 1.7 Hz, 1 H), 5.11 (dd, J = 17.3, 1.7 Hz, 1 H), 5.77 (m, 1 H), 7.33 (m, 3 H), 7.93 (m, 2 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 15.3, 19.9, 21.2, 29.6, 39.6, 52.1, 77.5, 81.7, 90.1, 115.9, 127.2, 128.3, 128.7, 131.7, 138.2, 165.1, 173.1; high-resolution mass spectrum (EI, 70 eV) *m/z* 331.1780 (M^+; calcd for $C_{19}H_{25}NO_4$: 331.1783). Anal. Calcd for $C_{19}H_{25}NO_4$: C, 68.85; H, 7.60; N, 4.23. Found: C, 68.90; H, 7.54; N, 4.39.

Carboxylic Acid (−)-18. A solution of olefin (−)-**4a** (717 mg, 2.16 mmol) in absolute MeOH (20 mL) and CH_2Cl_2 (10 mL) was cooled to −78 °C and treated with a stream of O_3 in O_2 until TLC analysis indicated the complete consumption of starting material. The solution was purged with O_2 to remove excess O_3, Me_2S (0.43 mL, 5.88 mmol) was added, and the mixture was stirred for 1 h at −78 °C and 1 h at room temperature. Concentration followed by exposure to high vacuum for 2 h afforded the aldehyde as a yellow oil. Without purification, a portion of aldehyde (12.2 mmol maximum) was dissolved in a mixture of *t*-BuOH (21.6 mL), and 2-methyl-2-butene (4.8 mL, 45.4 mmol) and treated with a solution of $NaClO_2$ (1.96 g, 21.6 mmol) and NaH_2PO_4 (4.49 g, 28.9 mmol) in H_2O (10.8 mL). The mixture was stirred vigorously at room temperature for 45 min, and then an adequate amount of Na_2SO_3 was added to consume the excess $NaClO_2$. After 10 min, the reaction mixture was concentrated and extracted with $CHCl_3$ (3 × 30 mL), and the combined organic phases were dried over Na_2SO_4, filtered, and concentrated. Flash chromatography (10:1 $CHCl_3$/MeOH) gave (−)-**18** (423 mg, 56% yield) as a white powder: mp 116−118 °C (MeOH); $[\alpha]_D^{25}$ −20° (*c* 0.1, MeOH); IR (KBr) 3300 (m), 1720 (s), 1630 (s) cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 0.83 (d, J = 6.6 Hz, 3 H), 0.99 (d, J = 6.9 Hz, 3 H), 1.19 (d, J = 7.3 Hz, 3 H), 1.89 (m, 1 H), 2.60 (m, 1 H), 3.68 (s, 3 H), 4.05 (d, J = 3.6 Hz, 1 H), 4.71 (d, J = 5.3 Hz, 1 H), 7.40 (m, 3 H), 7.90 (d, J = 7.3 Hz, 2 H); ^{13}C NMR (67.5 MHz, CD_3OD) δ 17.9, 18.3, 21.7, 31.8, 43.9, 53.5, 79.2, 85.6, 91.3, 108.7, 129.1, 130.3, 130.6, 134.0, 168.6, 173.7; high-resolution mass spectrum (FAB, glycerol matrix) *m/z* 350.1607 [$(M + H)^+$; calcd for $C_{18}H_{24}NO_6$: 350.1603]. Anal. Calcd for $C_{18}H_{23}NO_6$: C, 61.86; H, 6.64; N, 4.01. Found: C, 61.62; H, 6.89; N, 3.85.

γ-Lactam (+)-19. A mixture of carboxyoxazoline (−)-**18** (61.0 mg, 0.175 mmol), ammonium formate (55 mg, 0.88 mmol), palladium black (175 mg), and acetic acid (5.4 mL) was heated at reflux until TLC analysis showed complete consumption of the starting material (ca. 4 h). The catalyst was filtered off and washed with $CHCl_3$ (10 mL). The filtrate was partially concentrated to remove $CHCl_3$ and the remaining solution neutralized with 2 N NaOH. The resultant mixture was extracted with $CHCl_3$ (3 × 10 mL), and the combined organic phases were dried over Na_2SO_4, filtered, and concentrated. Flash chromatography (15:1 $CHCl_3$/MeOH) gave (+)-**19** (38.9 mg, 91% yield) as a colorless powder: $[\alpha]_D^{24}$ +65° (*c* = 0.3, MeOH); ^1H NMR (270 MHz, CD_3OD) δ 0.84 (d, J = 6.6 Hz, 3 H), 0.88 (d, J = 6.6 Hz, 3 H), 1.06 (d, J = 7.3 Hz, 3 H), 1.65 (m, 1 H), 2.94 (m, 1 H), 3.72 (s, 3 H), 3.90 (d, J = 7.3 Hz, 1 H), 4.43 (d, J = 5.9 Hz, 1 H); mass spectrum (FAB, glycol matrix) *m/z* 246 [$(M + H^+)$; calcd for $C_{11}H_{20}NO_5$: 246].

Carboxylic Acid (+)-3. A solution of lactam methyl ester (+)-**19** (14 mg, 0.06 mmol) in EtOH (0.57 mL) was treated with 0.1 N NaOH (1.14 mL), and the resultant mixture was stirred for 9 h at room temperature and then neutralized with 2 N HCl. Following partial concentration to remove EtOH, the solution was subjected to column chromatography on activated carbon (80% acetone), affording (+)-**3** (11.9 mg, 90% yield) as a colorless powder: mp 244 °C dec (acetone/H_2O); $[\alpha]_D^{23}$ +42° (*c* 0.5, MeOH); IR (KBr) 3600−3100 (s), 1660 (s), 1600 (s) cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 0.85 (d, J = 6.6 Hz, 3 H), 0.86 (d, J = 6.6 Hz, 3 H), 0.98 (d, J = 7.6 Hz, 3 H), 1.69 (m, 1 H), 2.86 (m, 1 H), 3.83 (d, J = 5.3 Hz, 1 H), 4.30 (d, J = 5.9 Hz,

1 H); ^{13}C NMR (67.5 MHz, CD_3OD) δ 9.2, 19.3, 19.8, 32.0, 42.5, 76.0, 76.3, 79.5, 172.5, 179.8; mass spectrum (FAB, glycerol matrix) *m/z* 232 [(M + H)$^+$; calcd for $C_{10}H_{18}NO_5$: 232], 254 [(M + Na)$^+$; calcd for $C_{10}H_{17}NO_5Na$: 254].

Lactacystin Allyl Ester [(+)-20]. A suspension of acid (+)-**3** (20 mg, 0.087 mmol) in CH_2Cl_2 (0.87 mL) was cooled to 0 °C and treated with *N*-acetyl-L-cysteine allyl ester (42.2 mg, 0.21 mmol), Et_3N (24.1 μL, 0.17 mmol), and bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOPCl; 26.4 mg, 0.10 mmol). The mixture was stirred for 10 min further at 0 °C, warmed to room temperature, and stirred for 5 h. The resultant solution was then quenched with H_2O (5 mL) and extracted with $CHCl_3$ (3 × 5 mL), and the combined organic phases were dried over Na_2SO_4, filtered, and concentrated. Preparative thin layer chromatography (0.5 mm, 10:1 $CHCl_3$/MeOH) gave (+)-**20** (28.5 mg, 79% yield) as a white solid: mp 182−184 °C dec (MeOH); $[\alpha]_D^{23}$ +34° (*c* 0.5, $CHCl_3$); IR (CCl_4) 3600 (w), 1710 (s), 1680 (s) cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.85 (d, *J* = 6.9 Hz, 3 H), 0.92 (d, *J* = 6.6 Hz, 3 H), 1.12 (d, *J* = 7.5 Hz, 3 H), 1.69 (m, 1 H), 1.94 (s, 3 H), 2.86 (m, 1 H), 3.17 (dd, *J* = 14.1, 6.3 Hz, 1 H), 3.55 (dd, *J* = 14.1, 4.3 Hz, 1 H), 4.00 (d, *J* = 5.9 Hz, 1 H), 4.51 (d, *J* = 5.6 Hz, 1 H), 4.57 (m, 2 H), 4.81 (m, 1 H), 5.25 (dd, *J* = 10.4, 1.5 Hz, 1 H), 5.31 (dd, *J* = 17.3, 1.5 Hz, 1H), 5.86 (m, 1 H), 6.20 (d, *J* = 7.6 Hz, 1 H), 6.28 (s, 1 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 7.8, 16.7, 20.9, 29.7, 30.2, 36.5, 40.3, 51.3, 66.6, 75.4, 78.1, 79.1, 119.4, 131.0, 170.0, 170.2, 179.6, 203.1; high-resolution mass spectrum (FAB, NBA matrix) *m/z* 439.1519 [(M + Na)$^+$; calcd for $C_{18}H_{28}N_2O_7SNa$: 439.1515].

(+)-Lactacystin (1). Allyl ester (+)-**20** (15 mg, 0.04 mmol) was dissolved in THF (0.72 mL), and $(Ph_3P)_4Pd(0)$ (4.2 mg, 3.60 μmol), triethylamine (50.3 μL, 0.36 mmol), and formic acid (13.6 μL, 0.36 mmol) were added. The resultant mixture was stirred for 1.5 h at room temperature and then concentrated, and the residue was dried under high vacuum for 3 h. Preparative thin layer chromatography (0.25 mm, 9:1 THF/H_2O) afforded (+)-**1** (11.0 mg, 81% yield) as a colorless powder: mp 236−238 °C dec {lit.[3] [natural (+)-**1**] mp 237−238 °C dec; mixed mp 236−238 °C dec (H_2O)}; $[\alpha]_D^{23}$ +73° (*c* 0.5, MeOH) {lit.[3] [natural (+)-**1**] $[\alpha]_D^{23}$ +71° (*c* 0.5, MeOH)}; IR (KBr) 3400 (s), 1680 (s), 1650 (s) cm^{-1}; ^{1}H NMR (400 MHz, pyridine-d_5) δ 1.07 (d, *J* = 6.7 Hz, 3 H), 1.14 (d, *J* = 6.5 Hz, 3 H), 1.50 (d, *J* = 7.6 Hz, 3 H), 1.94 (s, 3 H), 2.14 (m, 1 H), 3.36 (m, 1 H), 3.73 (dd, *J* = 13.5, 6.7 Hz, 1 H), 3.94 (dd, *J* = 13.5, 4.8 Hz, 1 H), 4.48 (d, *J* = 7.0 Hz, 1 H), 5.23 (d, *J* = 7.0 Hz, 1 H), 5.30 (m, 1 H), 8.71 (d, *J* = 8.3 Hz, 1 H), 9.78 (s, 1 H); ^{13}C NMR (100 MHz, pyridine-d_5) δ 10.4, 20.1, 21.6, 23.2, 31.5, 32.2, 42.0, 53.1, 76.2, 80.1, 81.5, 170.5, 173.8, 181.5, 203.1; high-resolution mass spectrum (FAB, NBA matrix) *m/z* 377.1390 [(M + H)$^+$; calcd for $C_{15}H_{25}N_2O_7S$: 377.1382]. Anal. Calcd for $C_{15}H_{24}N_2O_7S$: C, 47.87; H, 6.38; N, 7.45. Found: C, 47.51; H, 6.27; N, 7.11.

***β*-Lactone (−)-21.** A suspension of acid (+)-**3** (43.3 mg, 0.19 mmol) in CH_2Cl_2 (0.7 mL) was cooled to 0 °C and treated with Et_3N (85 μL, 0.61 mmol) and bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOPCl; 62 mg, 0.23 mmol). The cold bath was removed, and the reaction mixture was then stirred at room temperature for 1 h, quenched with H_2O (3 mL), and extracted with $CHCl_3$ (3 × 5 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated. Flash chromatography (50:1 $CHCl_3$/MeOH) furnished (−)-**21** (34 mg, 85% yield) as colorless needles: mp 185−187 °C dec (MeOH); $[\alpha]_D^{23}$ −92° (*c* 0.5, $CHCl_3$); IR ($CHCl_3$) 1840 (s), 1730 (s), 1640 (s) cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.84 (d, *J* = 6.8 Hz, 3 H), 0.99 (d, *J* = 6.8 Hz, 3 H), 1.27 (d, *J* = 7.6 Hz, 3 H), 1.82 (m, 1 H), 2.69 (m, 1 H), 3.91 (d, *J* = 6.9 Hz, 1 H), 5.15 (d, *J* = 6.3 Hz, 1 H), 6.25 (s, 1 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 8.1, 16.3, 20.0, 29.6, 38.1, 64.0, 71.8, 76.0, 171.1, 176.8; high-resolution mass spectrum (FAB, NBA matrix) *m/z* 214.1074 [(M + H)$^+$; calcd for $C_{10}H_{16}NO_4$: 214.1079].

(+)-Lactacystin (1). A solution of *β*-lactone (−)-**21** (19.8 mg, 0.094 mmol) in CH_2Cl_2 (1.0 mL) was treated with triethylamine (40 μL, 0.28 mmol) and *N*-acetylcysteine (22.8 mg, 0.14 mmol), and the resultant solution was stirred for 50 min at room temperature. After concentration the residue was dried under high vacuum for 3 h. Preparative thin layer chromatography (0.25 mm, 9:1 THF/H_2O) furnished (+)-**1** (23.7 mg, 64% yield) as a colorless powder.

Ethyl Ester Analog (+)-23. A solution of *β*-lactone (−)-**21** (13.0 mg, 0.060 mmol) in CH_2Cl_2 (0.87 mL) was treated with triethylamine (25 μL, 0.18 mmol) and ethyl 3-mercaptopropionate (12.1 μL, 0.09 mmol), and the resultant solution was stirred for 70 min at room temperature. After concentration the residue was dried under high vacuum for 3 h. Preparative thin layer chromatography (0.25 mm, 10:1 $CHCl_3$/MeOH) furnished (+)-**23** (18.8 mg, 89% yield) as a colorless powder: mp 159−162 °C (MeOH); $[\alpha]_D^{24}$ +120° (*c* 0.03, $CHCl_3$); IR (KBr) 3467 (s), 1693 (m), 1674 (s) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.96 (d, *J* = 7.0 Hz, 6H), 1.18 (d, *J* = 7.5 Hz, 3H), 1.26 (t, *J* = 7.0 Hz, 3 H), 1.58 (br s, 2 H), 1.78 (m, 1 H), 2.62 (t, *J* = 7.0 Hz, 2 H), 2.95 (m, 1 H), 3.19 (t, *J* = 7.0 Hz, 2 H), 4.05 (d, *J* = 5.0 Hz, 1 H), 4.15 (d, *J* = 7.0 Hz, 2 H), 4.66 (d, *J* = 7.0 Hz, 1 H), 6.07 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 8.8, 14.2, 17.2, 21.0, 24.4, 30.8, 33.8, 40.5, 61.0, 76.1, 78.7, 79.1, 128.8, 171.4, 179.7, 201.4; high-resolution mass spectrum (FAB, NBA matrix) *m/z* 347.1405 [(M + H)$^+$; calcd for $C_{15}H_{25}NO_6S$: 347.1402).

(+)-Descarboxylactacystin (25). A solution of *β*-lactone (−)-**21** (6.8 mg, 0.032 mmol) in CH_2Cl_2 (0.87 mL) was treated with triethylamine (13.4 μL, 0.09 mmol) and *N*-acetylcysteamine (5.4 μL, 0.048 mmol), and the resultant solution was stirred for 30 min at room temperature. After concentration the residue was dried under high vacuum for 3 h. Preparative thin layer chromatography (0.25 mm, 8:1 $CHCl_3$/MeOH) afforded (+)-**25** (8.1 mg, 77% yield) as a colorless powder: mp 167−169 °C (MeOH); $[\alpha]_D^{24}$ +84° (*c* 0.1, MeOH); IR (KBr) 3382 (s), 1688 (s), 1649 (s) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (d, *J* = 7.0 Hz, 3 H), 0.97 (d, *J* = 7.0 Hz, 3 H), 1.10 (d, *J* = 7.5 Hz, 3 H), 1.68 (m, 1 H), 1.92 (s, 3 H), 2.89 (m, 1 H), 3.10 (m, 2 H), 3.31 (m, 2 H), 3.95 (d, *J* = 7.0 Hz, 1 H), 4.54 (d, *J* = 7.0 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.1, 19.5, 21.3, 22.6, 29.2, 32.2, 39.8, 42.7, 76.6, 80.3, 81.5, 173.4, 183.2, 202.4; high-resolution mass spectrum (FAB, NBA matrix) m/z 333.1497 [(M + H)$^+$; calcd for $C_{14}H_{25}N_2O_5S$: 333.1484).

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