A Novel and Efficient Synthesis of a Highly Active Analogue of *clasto*-Lactacystin β-Lactone

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Abstract: Herein, we describe a new convergent synthesis of a more potent analogue of *clasto*-lactacystin β-lactone (**2**), PS-519 compound **4**, which is currently in preclinical development for the treatment of ischemia−reperfusion injury in stroke and myocardial infarction. The synthetic strategy relies on building two intermediates (an oxazoline and an aldehyde) which are joined through a doubly diastereoselective aldol reaction, setting up the requisite unichiral centers in the final product (**4**). The facial selectivity and ultimate stereocontrol are achieved by employing a trivalent aluminum Lewis acid, Me_2AlCl, in a chelation-induced reaction which yields a single aldol adduct. The efficiency of the synthetic approach has allowed for the preparation of multigram quantities of clinical grade material, which will support Phase I studies.

Introduction

The 20S proteasome is a large (700 kDa) and highly conserved multicatalytic proteinase complex that constitutes the catalytic core of the 26S proteasome, present in all eukaryotic organisms.[1] It is essential for the normal turnover of cellular proteins and for removal of damaged or misfolded proteins, and it also plays a role in processing and degrading regulatory proteins that control cell growth, transcriptional activation, and metabolism.[2] The barrel-shaped 20S proteasome is an abundant particle, comprising about 1% of cell proteins. It is composed of four stacked oligomeric rings, two outer (α) rings and two inner (β) rings (Figure 1), wherein each ring contains seven distinct 20−30-kDa subunits. The four rings enclose a central chamber, where proteolysis occurs. While serving an essential physiological role, the proteasome is also implicated in a number of pathophysiological conditions where inappropriate or accelerated protein degradation occurs as a result or cause of normal cellular processes becoming disregulated.

One notable example relates to the activation of the transcriptional factor NF-κB.[3] NF-κB activation is generally associated with the mounting of an inflammatory response in ischemic tissue, or in response to autoantigens in various autoimmune diseases. It is normally sequestered outside the nucleus, interacting with the inhibitory protein IκBα, which masks the nuclear localization sequences, and maintaining transcriptional silence. When cells become activated in response to tissue injury or antigenic activation, IκBα is degraded by the proteasome, releasing NF-κB, which translocates to the nucleus, where it binds to promoter regions of proinflammatory genes (e.g., IL-1, TNF-α, COX2, iNOS, I-CAM, V-CAM, E-Selectin). Proteasome inhibitors impede this activation by blocking the degradation of IκBα. Selective proteasome inhibitors have demonstrated efficacy in animal models of myocardial infarction, stroke, asthma, and arthritis.[4] We have sought to explore the structure−activity relationship of proteasome inhibitors based on the lactacystin core structure (vide infra). Herein we describe the efficient synthesis of an active analogue of *clasto*-lactacystin β-lactone.

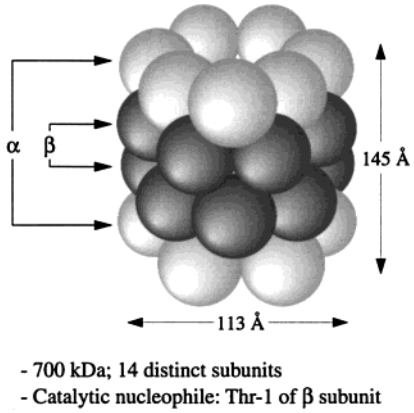

Figure 1. Structure of the 20S proteasome.

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(1) Reviews: (a) Goldberg, A. L.; Stein, R. L.; Adams, J. *Chem. Biol.* **1995**, *2*, 503. (b) Coux, O.; Tanaka, K.; Goldberg, A. L. *Annu. Rev. Biochem*. **1996**, *65*, 801. (c) Deshaies, R. J. *Trends Cell Biol.* **1995**, *5*, 428. (d) Rubin, D.; Finley, D. M. *Curr. Biol.* **1995**, *3*, 854. (e) Tanaka, K. *Mol. Biol. Rep.* **1995**, *21*, 21. (f) Ciechanover, A. *Cell* **1994**, *79*, 13. (g) Peters, J. M. *Trends Biochem. Sci*. **1994**, *19*, 377. (h) Wilk, S. *Enzyme Protein* **1993**, *47*, 187. (i) Rivett, A. J. *Biochem. J.* **1993**, *291*, 1.

(2) (a) Rock, K. L.; Gramm, C.; Rothstein, L.; Clark, K.; Stein, R.; Dick, L.; Hwang, D.; Goldberg, A. L. *Cell* **1994**, *78*, 761. (b) King, R. W.; Deshaies, R. J.; Peters, J.-M.; Kirschner, M. W. *Science* **1996**, *78*, 761.

(3) (a) Barnes, P. J.; Karin, M. *News Eng. J. Med*. **1997**, *336*, 1066. (b) Baldwin, A. S. *Annu. Rev. Immunol.* **1996**, *14*, 649. (c) Thanos, D.; Maniatis, T. *Cell* **1995**, *80*, 529. (d) Grilli, M.; Chiu, J. J.-S.; Leonardo, M. J. *Int. Rev. Cytol*. **1993**, *143*, 1. (e) Baeuerle, P. A.; Baltimore, D. *Cell* **1996**, *87*, 13.

(4) (a) Palombella, V. J.; Conner, E. M.; Fuseler, J. W.; Destree, A. D.; Davis, J. M.; Laroux, F. S.; Wolf, R. E.; Huang, J.; Brand, S.; Elliott, P. E.; Lazarus, D.; McCormack, T.; Parent, L.; Stein, R.; Adams, J.; Grisham, M. B. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 15671. (b) Campbell, B.; Adams, J.; Shin, Y. K.; Lefer, A. M. *J. Mol. Cell Cardiol.* **1999**, *31*, 467 (c) Elliott, P. J.; Pien, C. S.; McCormack, T. A.; Chapman, I. D.; Adams, J. *J. Allergy Clin. Immunol.*, in press. (d) Vanderlugt, C. L.; Rahbe, S. M.; Elliott, P. J.; Dal Canto, M. C.; Miller, S. D. *J. Neuroimmunol*., in press. (e) Phillips, J. B.; Williams, A. J.; Elliott, P. E.; Tortella, F. C. *FASEB* **1999**, *13*, A1099 (abstract no. 817.3). (f) Phillips, J. B.; Williams, A. J.; Elliott, P. E.; Tortella, F. C. *J. Neurol.* **1998**, *24*, 215 (abstract no. 86.11).

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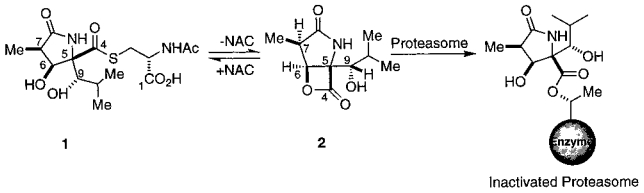
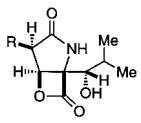
Scheme 1



In 1991, Ōmura and his colleagues reported the discovery of lactacystin (**1**), a natural product that could induce differentiation in cultured neuronal cells.[5,6] Lactacystin was isolated from the cultured broth of *Streptomices i* sp. OM-6519, and its structure and absolute configuration were confirmed by NMR and X-ray crystallographic analyses.[5] Subsequent work[7] demonstrated that the biological effects of lactacystin result from its ability to inhibit mammalian 20S and 26S proteasomes over a wide range of concentrations.[5a,7b,8] As part of our program to develop proteasome inhibitors into novel therapeutic agents, we have undertaken detailed studies of the mechanism of proteasome inactivation by lactacystin and have recently shown that lactacystin itself does not inhibit the proteasome. Rather, it acts as a proinhibitor, eliminating *N*-acetyl-L-cysteine to form *clasto*-lactacystin β-lactone (**2**), which in turn is able to directly inhibit the proteasome.[9] The derived β-lactone **2**, unlike lactacystin, is capable of permeating cell membranes, where it forms a covalent complex with glutathione, which acts as a reservoir for the β-lactone, which is the sole species inhibiting the proteasome. Inhibition occurs through acylation of the amino-terminal threonine residues of β-type proteasome subunits (Scheme 1).[7] This was confirmed by X-ray crystallographic studies at 2.4-Å resolution of the lactacystin-inactivated proteasome.[10]

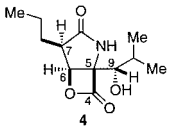
The biological uniqueness and the compact array of four contiguous stereocenters have rendered (+)-lactacystin **1** and the derived lactone **2** significant targets for synthesis. A number of syntheses,[11] as well as analogues[7,12] of **1** and **2**, have been reported. The most thorough treatment to date has been that of Corey and co-workers, who have not only described the first synthesis of the natural product but also defined strict structure−activity requirements for the substituents at C-7 and C-9. Our own efforts are both confirmatory and complementary to the analogues prepared by the Corey group. We have prepared a considerable number of structural variants and stereoisomers of **2** and have discovered, as others did,[12] that most of the structural features of **2**, including absolute and relative configuration of the chiral centers, are essential for its activity.[13] We found, however, that although the presence and absolute configuration of the C-7 methyl group is critical to the activity of **2**, replacing it with a bulky alkyl group can lead to a 2-fold increase in activity (Table 1).[13,14] One such active molecule is the C-7 *n*-propyl analogue PS-519 (**4**), which is both a better inactivator of the chymotrypsin-like activity of PA28-activated 20S proteasome than the natural product derived lactone **2**[9b] and a better inhibitor of intracellular protein degradation. In this article, we detail a new total synthetic route to **4** that minimizes the number of linear steps and chromatographic purifications, and thus greatly facilitates the preparation of multigram amounts of **4**. The process relies on a new doubly diastereoselective aldol coupling for the selective installation of two of the four stereogenic centers.[15] We also describe the synthesis of **2** using our new approach, which has also been used to prepare other C-7 analogues.

Table 1. Kinetics of Inhibition of 20S Proteasome and Inhibition of Intracellular Protein Degradation



compd	R	K_{obs}/[1] (M^{-1} s^{-1})[a]	IC_{50} (μM)[b]
2	Me	20 000	0.7−1.1
3	Et	39 000	0.32
4	*n*-Pr	46 500	0.29
5	n-Bu	38 000	0.33
6	*i*-Bu	17 000	0.51
7	CH_2Ph	6 400	

[a] Chymotrypsin-like activity with PA28-activated 20S proteasome. [b] Inhibition of intracellular protein degradation in C2C12 cells.



4

Results and Discussion

Our retrosynthetic analysis of the skeleton of **4** is outlined in Scheme 2. The γ-lactam intermediate **8** was envisioned to arise

(5) (a) Ōmura, S.; Fujimoto, T.; Otoguro, K.; Matsuzaki, K.; Moriguchi, R.; Tanaka, H.; Sasaki, Y. *J. Antibiot.* **1991**, *44*, 113. (b) Ōmura, S.; Matsuzaki, K.; Fujimoto, T.; Kosuge, K.; Furuya, T.; Fujita, S.; Nakagawa, A. *J. Antibiot.* **1991**, *44*, 117. Also see: (c) 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.

(6) For a review on microbial metabolites, see: Ōmura, S. *Gene* **1992**, *115*, 141.

(7) (a) Fenteany, G.; Standaert, R. F.; Reichard, G. A.; Corey, E. J.; Schreiber, S. L. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 3358. (b) Fenteany, G.; Standaert, R. F.; Lane, W. S.; Choi, S.; Corey, E. J.; Schreiber, S. L. *Science* **1995**, *268*, 726.

(8) Craiu, A.; Gaczynska, M.; Akopian, T.; Gramm, C. F.; Fenteany, G.; Goldberg, A. L.; Rock, K. L. *J. Biol. Chem.* **1997**, *272*, 13437.

(9) (a) Dick, L. R.; Cruikshank, A. A.; Grenier, L.; Melandri, F. D.; Nunes, S. L.; Palombella, V. J.; Parent, L. A.; Plamondon, L.; Stein, R. L. *J. Biol. Chem.* **1997**, *272*, 182. (b) Dick, L. R.; Cruikshank, A. A.; Destree, A. T.; Grenier, L.; McCormack, T. A.; Melandri, F. D.; Nunes, S. L.; Stein, R. L. *J. Biol. Chem.* **1996**, *271*, 7273.

(10) Groll, M.; Ditzel, L.; Löwe, J.; Stock, D.; Bochtler, M.; Bartunik, H. D.; Huber, R. *Nature* **1997**, *386*, 463.

(11) (a) Corey, E. J.; Reichard, G. A. *J. Am. Chem. Soc.* **1992**, *114*, 10677. (b) Corey, E. J.; Reichard, G. A.; Kania, R. *Tetrahedron Lett.* **1993**, *34*, 6977. (c) Corey, E. J.; Li, W.; Reichard, G. A. *J. Am. Chem. Soc.* **1998**, *120*, 2330. (d) Sunazuka, T.; Nagamitsu, K.; Matsuzaki, K.; Tanaka, H.; Ōmura, S.; Smith, A. B., III. *J. Am. Chem. Soc.* **1993**, *115*, 5302. (e) Nagamitsu, K.; Sunazuka, T.; Tanaka, H.; Ōmura, S.; Sprengeler, P. A.; Smith, A. B., III. *J. Am. Chem. Soc.* **1996**, *118*, 3584. (f) Uno, H.; Baldwin, J. E.; Russell, A. T. *J. Am. Chem. Soc.* **1994**, *116*, 2139. (g) Chida, N.; Takeoka, J.; Tsunimi, N.; Ogaa, S. *J. Chem. Soc., Chem. Commun.* **1995**, 793. (h) Corey, E. J.; Li, W.; Nagamitsu, T. *Angew. Chem., Int. Ed.* **1998**, *37*, 1676. (i) Kang, S. H.; Jun, H.-S. *J. Chem. Soc., Chem. Commun.* **1998**, 1929. (j) Corey, E. J.; Reichard, G. A. *Tetrahedron Lett.* **1993**, *34*, 6973.

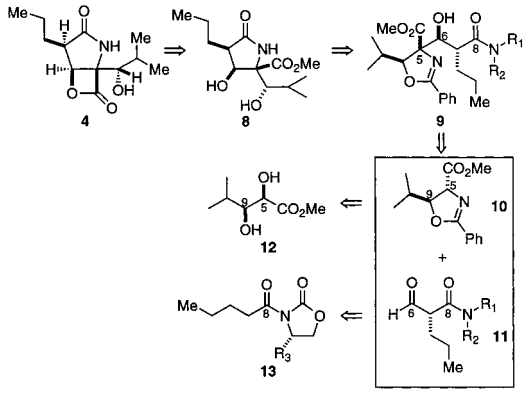
(12) For enantioselective syntheses of analogues of lactacystin and lactacystin β-lactone, see: (a) Corey, E. J.; Li, W.-D. Z.; Nagamitsu, T.; Fenteany, G. *Tetrahedron* **1999**, *55*, 3305. (b) Corey, E. J.; Li, W.-D. Z. *Tetrahedron Lett.* **1998**, *39*, 7475. (c) Corey, E. J.; Li, W.-D. Z. *Tetrahedron Lett.* **1998**, *39*, 8043. (d) Corey, E. J.; Reichard, G. A. *Tetrahedron Lett.* **1993**, *34*, 6973. (e) Corey, E. J.; Choi, S. *Tetrahedron Lett.* **1993**, *34*, 6969. Also see ref 11b.

(13) Those findings will be reported elsewhere. For a review on the biological activity of analogues of lactacystin and the related β-lactone, see: Corey, E. J.; Li, W.-D. Z. *Chem. Pharm. Bull.* **1999**, *47*, 1.

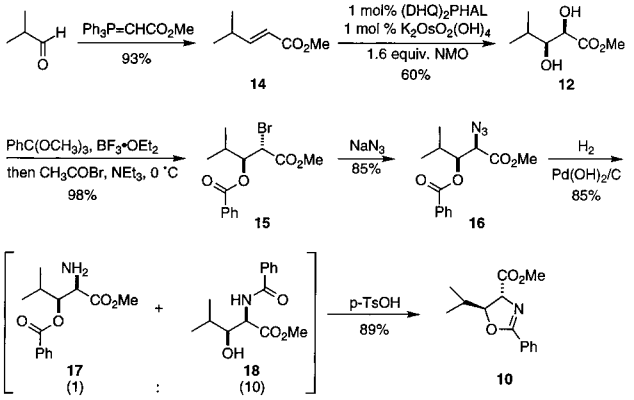
(14) Similar findings have been reported by Corey et al.; see refs 12a and 12b.

(15) An elegant Mukaiyama aldol coupling strategy has recently been reported by Corey in which an anti aldol product is formed in ca. 90% de and transformed in seven steps into lactacystin **1** (ref 11c). Corey and Choi have also used a syn-selective aldol route to prepare the inactive 6*R* diastereomer of lactacystin, which is incapable of forming the β-lactone (ref 12b).

Scheme 2



Scheme 3



from two key operations: (a) the selective aldol coupling of the known oxazoline **10** and chiral aldehyde **11** to give **9**, in which the C-5 quartenary carbon and C-6 stereocenter are installed selectively, and (b) the hydrogenolysis of the oxazoline moiety of **9** followed by ring closure in situ. Having the aldolate **9** with the correct oxidation state at C-8 allows us to avoid protection/deprotection steps and/or oxidation-state adjustments. The main strength of this strategy is that it allows rapid assembly of **9**, which can then be converted in only a few steps to the target molecule **4**. Crucial to the success of the face-selective aldol reaction is the configurational stability of aldehyde **11** (with $R_1 = R_2$ or $R_1 \neq R_2$), which can be prepared from readily available acyloxazolidinones **13**. We have also developed a concise and practical route to *trans*-oxazoline **10** from optically active diol **12**.[16,17]

Our new route to **10** starts with readily available isobutyraldehyde and requires only six steps (Scheme 3). Wittig condensation between isobutyraldehyde and methyl (triphenylphosphoranylidene)acetate to afford olefin **14** as a single geometric isomer occurs in 93% yield. That the expected (*E*)-olefin geometry was obtained was clear from the ^{1}H NMR spectrum of **14**, showing a large coupling constant ($J = 15.8$ Hz) between the two vinylic protons. Sharpless catalytic asymmetric dihydroxylation[18] of olefin **14** with AD-mix-α in the presence of methanesulfonamide proceeded smoothly to give diol **12** in 94% yield, as anticipated from the Sharpless face selection rule. The reaction could be performed conveniently on a small scale, but the potassium ferricyanide/potassium carbonate system was not practical on a larger scale because of the large amount of salts employed. Additionally, the low concentration (0.1 M) and throughput of the reaction resulted in a tedious workup procedure. This problem was circumvented by following the procedure of Ahrgren and Sutin,[19] where *N*-methylmorpholine *N*-oxide (NMO) is used as the internal reoxidant and reactions are performed at higher concentrations (0.4−0.5 M). By adding the neat olefin **14** slowly and continuously (over 48 h) to a solution of 1 mol % of $K_2OsO_2(OH)_4$, 1 mol % of hydroquinine 1,4-phthalazinediyl diether [$(DHQ)_2PHAL$], and 1.6 equiv of NMO, we were able to obtain the desired diol **12** with 100% yield and 70% ee (as determined by ^{1}H NMR of a 1:1 molar solution of diol and europium tris[3(heptafluoropropylhydroxymethylene)-(−)-camphorate] in C_6D_6). Recrystallization from 35−60 °C petroleum ether afforded pure diol **12** as white crystals in 60% yield and >99% ee (mp 32−34 °C). A solution of the diol in CH_2Cl_2 was treated at room temperature with a slight excess of trimethyl orthobenzoate in the presence of a catalytic amount of $BF_3{\cdot}OEt_2$. The solution was then evaporated to remove most of the methanol formed during the reaction, and the crude cyclic orthoester was taken up in CH_2Cl_2 and treated with acetyl bromide, providing the protected bromohydrin **15** in 98% yield (correcting for the presence of a small amount of methyl benzoate). The requisite α-amino group was then introduced by nuclophilic azide displacement. The nucleophilic displacement of the secondary bromide proved to be unexpectedly challenging, as the bromoester **15** was prone to elimination.[20] The amount of elimination product formed could be minimized by dissolving sodium azide in DMSO prior to adding the bromoester. Under those conditions, we obtained about 8% of α,β-unsaturated ester and 3% of unreacted starting material. A bulky ester protecting group proved important, as attempting the reaction with a bromoacetate (instead of a bromobenzoate) resulted in the exclusive formation of the dehydrohalogenated ester formed by loss of HBr. The desired azoester **16**, obtained in an estimated isolated yield of 85%, was used as such in the following step. Hydrogenation of **16** proceeded readily in methanol at atmospheric pressure using Pearlman's catalyst [20% $Pd(OH)_2/C$].[21] Careful choice of the reaction conditions was required in order to get rid of the byproducts from the previous step. Performing the reduction in the presence of 2 equiv of HCl provided the amine hydrochloride salt, which is soluble in water. The aqueous solution was simply

(16) It should be noted that the absolute configuration of unichiral center C5 in oxazoline **6** is a priori unimportant because this center undergoes pyramidalization during enolization.

(17) For the preparation of enantiomerically pure (2*S*,3*S*)- or (2*R*,3*R*)-3-hydroxyleucine, see: (a) Hale, K. J.; Manaviazar, S.; Delisser, V. M. *Tetrahedron* **1994**, *50*, 9181. (b) Caldwell, G. C.; Bondy, S. S. *Synthesis* **1990**, 34. Also see: (c) Sunazuka, T.; Nagamitsu, T.; Tanaka, H.; Sprengeler, P. A.; Smith, A. B., III; Ōmura, S. *Tetrahedron Lett.* **1993**, *34*, 4447. (d) Jung, M. E.; Jung, Y. H. *Tetrahedron* Lett. **1989**, *30*, 6637. (e) Hughes, P. F.; Smith, S. H.; Olson, J. T. *J. Org. Chem.* **1994**, *59*, 5799. (f) Fleming, P. R.; Sharpless, K. B. *J. Org. Chem.* **1991**, *56*, 2869. (g) Blaser, D.; Seebach, D. *Liebigs Ann. Chem.* **1991**, 1067. (h) Seebach, D.; Juaristi, E.; Miller, D. D.; Schickli, C.; Webber, T. *Helv. Chim. Acta* **1987**, *70*, 2037. (i) Evans, D. A.; Sjogren, E. B.; Weber, A. E.; Conn, R. E. *Tetrahedron Lett.* **1987**, *28*, 39. (j) Reno, D. S.; Lotz, B. T.; Miller, M. J. *Tetrahedron Lett.* **1990**, *31*, 827. (k) Williams, R. M.; Im, M.-N.; Cao, J. *J. Am. Chem. Soc.* **1991**, *113*, 6976. (l) Guanti, G.; Banfi, L.; Narisano, E. *Tetrahedron* **1988**, *44*, 5553. (m) Genet, J. P.; Juge, S.; Mallart, S. *Tetrahedron Lett.* **1988**, *29*, 6765. (n) Evans, D. A.; Weber, A. E. *J. Am. Chem. Soc.* **1987**, *109*, 7151. (o) Suga, H.; Shi, X.; Ibata, T. *J. Org. Chem.* **1993**, *58*, 7397. (p) Horikawa, M. H.; Busch-Petersen, J.; Corey, E. J. *Tetrahedron Lett.* **1999**, *40*, 3843.

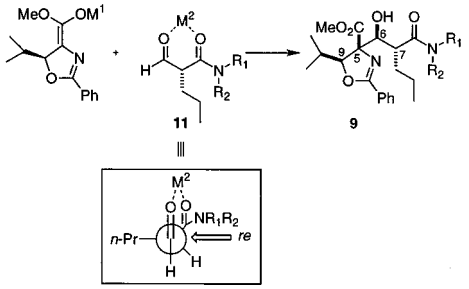
(18) (a) Sharpless, K. B.; Amberg, W.; Bennani, Y. L.; Crispino, G. A.; Hartung, J.; Jeong, K.-S.; Kwong, H.-L.; Morikawa, K.; Wang, Z.-W.; Xu, D.; Zhang, X.-L. *J. Org. Chem.* **1992**, *57*, 2768. For a review on catalytic asymmetric dihydroxylation and synthetic applications for 1,2-diols, see: (b) Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483. Also see: (c) Shao, H.; Goodman *J. Org. Chem.* **1996**, 61, *2582*.

(19) Ahrgren, L.; Sutin, L. *Org. Process Res. Dev.* **1997**, *1*, 425.

(20) The formation of such a dehydrohalogenated compound during a nucleophilic displacement by sodium azide has been reported before. See: Evans, D. A.; Weber, A. E. *J. Am. Chem. Soc.* **1987**, *109*, 7151.

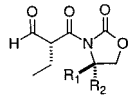
(21) Pearlman, W. M. *Tetrahedron Lett.* **1967**, 1663.

Scheme 4



washed with ethyl acetate to get rid of all byproducts and subsequently basified with sodium carbonate, providing a 1:10 mixture (85% yield) of benzoate **17** and benzamide **18**.[22] Finally, heating to reflux a solution of **17** and **18** in toluene containing *p*-toluenesulfonic acid resulted in cyclization with retention of configuration at C-9, affording, after purification of the mixture on a pad of silica gel, pure oxazoline **10** in 89% yield: $[\alpha]^{25}_D = -119.1°$ (*c* 1.15, $CHCl_3$); lit.[11e] $[\alpha]^{25}_D = -124°$ (*c* 1.0, $CHCl_3$). Interestingly, the treatment of pure benzamide **18** with thionyl chloride in methylene chloride afforded a *cis*-oxazoline through inversion of the C-9 hydroxyl group, which could be transformed to a *trans*-oxazoline enantiomeric to **10** under equilibrating conditions (MeONa/MeOH or H^+).[23] This short synthetic sequence and the fact that a single purification on silica gel is needed has allowed us to prepared multigram amounts (>100 g) of oxazoline **10**. The cumulative yield for the six-step sequence was 35%.

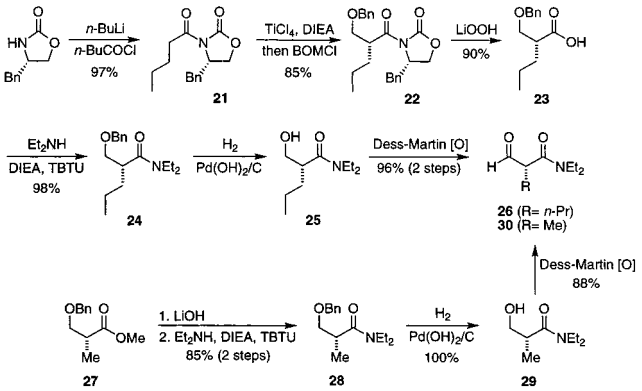
As previewed in our retrosynthetic analysis (Scheme 2), we decided to use a α-alkyl-*β*-formyl amide **(11)** in the key aldol coupling, hoping to get a highly or completely diastereoselective reaction. We reasoned that selectivity at C-6 could be induced by the use of a Lewis acid (M_2 in Scheme 4) that would chelate both carbonyl groups in **11**, thus blocking the *si* facial approach and leaving the *re* face open to attack in an anti-Felkin−Anh−Eisenstein fashion,[24] while the face selectivity at C-5 is clearly determined by the steric bias of the isopropyl group on the oxazoline ring. Our initial investigation of the aldol reaction using the diastereomeric and configurationally stable[25] aldehydes **19** and **20**, bearing an α-ethyl group and prepared by standard methods, was not promising. Varying temperature and solvent



19 (R_1= Bn, R_2= H)
20 (R_1= H, R_2= Bn)

as well as counterion M^1 (Li, K, Mg, Zn,) and Lewis acid M^2 led to either no reaction (recovering epimerized aldehyde) or low yields and/or diastereomeric excesses. The chiral auxiliary on the aldehyde clearly offers no particular advantage. It may be too far to influence face selectivity (open transition-state model) and may even impede reactivity.

Scheme 5



Recent reports by Fuji et al.[26] on the stereoselective addition of alkylaluminum dichloride to α-alkyl-*β*-formyl amides, and by Ooi et al.[27] on the effective chelate formation of Me_3Al and alkoxy-substituted carbonyl compounds, prompted us to prepare a simpler optically active aldehyde. We decided on preparing an *N*,*N*-diethyl amide, hoping the *N*-ethyl groups would impart good configurational stability to the aldehyde and knowing that the volatility of diethylamine would simplify the purification of *γ*-lactam after the cyclization of **9** to **8** (Scheme 2, $R_1 = R_2$ = Et). The six-step sequence (Scheme 5) begins with acylation of the lithium anion of the (*S*)-phenylalanine-derived oxazolidinone[28] with valeryl chloride to provide acyloxazolidinone **21** in 97% yield. Subsequent benzyloxymethylation[29] gave the benzyl ether **22** in 85% yield (after recrystallization from EtOAc/hexanes), provided benzyl chloromethyl ether is freshly prepared.[30] Peroxide mediated hydrolysis[31] of **22** (2.0 equiv of LiOOH at 4 °C) afforded a 90% yield of carboxylic acid **23**, which was coupled with diethylamine using 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) in the presence of triethylamine in CH_2Cl_2, yielding the *N*,*N*-diethylamide **24** in 98% yield. Hydrogenolysis of the benzyl group was accomplished using Pearlman's catalyst and afforded the primary alcohol **25**, while the final oxidation step was best accomplished using the periodinane reported by Dess and Martin,[32] providing the aldehyde **26** in 96% yield over two steps. The six-step sequence provided aldehyde **26** with a cumulative yield of 70%, and no chromatographic purification was required at any stage of the sequence. The aldehyde **26** was shown to be enantiomerically pure by reducing it with $NaBH_4$ and forming the corresponding Mosher ester using *R*-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride.[33] ^1H NMR analysis at 300 MHz revealed a single diastereomer, and remarkably, the chemically and configurationally stable aldehyde showed no signs of deterioration even after 3 months when stored at −20 °C. For making *clasto*-lactacystin *β*-lactone (**2**), we have

(22) For a similar benzoyl group migration, see: Wang, Z.-M.; Kolb, H. C.; Sharpless, K. B. *J. Org. Chem.* **1994**, *59*, 5014.

(23) An identical equilibration has been reported: Panek, J. S.; Masse, C. E. *J. Org. Chem.* **1998**, *63*, 2382−2384.

(24) (a) Anh, N. T.; Einsenstein, O. *Nouv. J. Chim.* **1977**, *1*, 61. (b) Anh, N. T. *Topics in Current Chemistry*; Springer-Verlag: New York, 1980; Vol. 88, p 145.

(25) (a) Evans, D. A.; Ennis, M. E.; Le, T.; Mandel, N.; Mandel, G. *J. Am. Chem. Soc.* **1984**, *106*, 1154. (b) Evans, D. A.; Sjogren, E. B. *Tetrahedron Lett.* **1986**, *27*, 4961. (c) Roush, W. R.; Palkowitz, A. D. *J. Org. Chem.* **1989**, *54*, 3009.

(26) Fuji, H.; Taniguchi, M.; Oshima, K.; Utimoto, K. *Tetrahedron Lett.* **1992**, *33*, 4579.

(27) Ooi, T.; Uraguchi, D.; Kagoshima, N.; Maruoka, K. *J. Am. Chem. Soc.* **1998**, *120*, 5327.

(28) Gage, U. R.; Evans, D. A. *Organic Syntheses*; Wiley: New York, 1989; Collect. Vol. VIII, 339.

(29) Evans, D. A.; Urpi, F.; Somers, T. C.; Clark, J. S.; Bilodeau, M. T. *J. Am. Chem. Soc.* **1990**, *112*, 8215.

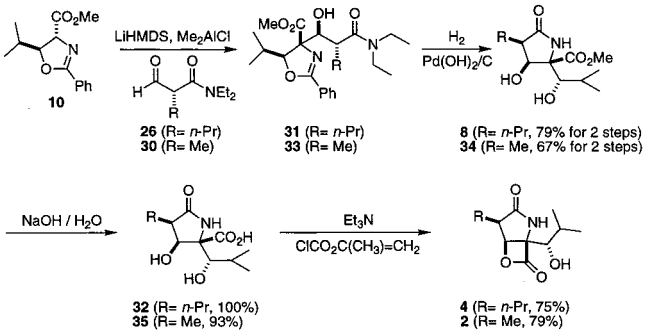
(30) Benzyl chloromethyl ether was prepared by a slight variation of the method described in the following: Coonor, D. S.; Klein, G. W.; Taylor, G. N. *Org. Synth.* **1972**, *52*, 16.

(31) Evans, D. A.; Britton, T. C.; Ellman, J. A.; Dorow, R. L. *J. Am. Chem. Soc.* **1990**, *112*, 4011.

(32) (a) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4156. (b) Ireland, R. E.; Liu, L. *J. Org. Chem.* **1993**, *58*, 2899.

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

Scheme 6



prepared the 3-hydroxy-2*R*-methylpropionamide **30** in an analogous way, but starting from the known ester **27**.[34] The four-step sequence provided the aldehyde **30** in a 75% overall yield. The stage was now set for the union of oxazoline **10** and aldehyde **26** (Scheme 6).

After considerable experimentation, we found that the treatment of the lithium enolate of **10** with 2.2−2.4 equiv of dimethylaluminum chloride, prior to introducing the aldehyde **26**, afforded the desired 6*S* alcohol **31** exclusively. The reaction worked well in THF and diethyl ether, and both the stoichiometry and the nature of the dialkylaluminum chloride were found to be crucial to the success of the reaction. Using less than 2 equiv of organometallic reagent or using a higher homologue such as diethylaluminum chloride resulted in deterioration of the 6*S* selectivity. Temperature also proved to be important. Conducting the reaction at −80 °C gave exclusively the desired isomer, while running the reaction at temperatures close to −70 °C gave lower diastereomeric excesses. The excess aldehyde could be removed by washing the crude mixture with 0.35 M aqueous $NaHSO_3$, and the aldol adduct was pure enough to be used directly in the subsequent step. This is fortunate as **31** tends to undergo retro aldol cleavage on attempted purification by silica gel chromatography.

The precise mechanism in this aldol reaction is not yet entirely clear. The fact that 2 equiv or more of the Lewis acid (one of which may be consumed by the lithium enolate of **10**) is needed for the reaction to be completely selective does suggest that this is a chelation-induced selective reaction. The high affinity of aluminum for oxygen is evident from the reported Al−O bond strengths (511 kJ/mol).[27,35] This is further reinforced by the presence of one chloride atom, which may lead to the formation of a chelate-type pentacoordinated complex.[27] Such a complex could accelerate the rate of reaction by effective activation of the aldehyde moiety (nonchelation leads to more recovered unreacted aldehyde) and promote the high selectivity observed at the 6*S* unichiral center through discrimination of the *re* and *si* faces of the complexed aldehyde. Although this type of Lewis acid behavior is not commonly recognized or documented (dialkyl aluminum chlorides are normally thought of as monodentate Lewis acids), there is precedent for its occurrence.[27,36] The mechanism is under continued investigation.

We now had a highly efficient access to the requisite aldol adduct **31**, in which two of the four stereogenic centers are installed with the correct relative and absolute configurations. With all four contiguous chiral centers in place, the only task remaining was the three-step conversion of **31** to lactone analogue **4**. Hydrogenolysis of oxazoline **31** in 1:10 AcOH/MeOH was performed under 55 psi H_2 and ambient temperature. Under those conditions, we achieved not only the hydrogenolysis of the oxazoline moeity but also the cyclization of the intermediate aminodiol to γ-lactam **8**. With only volatile byproducts formed (toluene and diethylamine), the virtually pure lactam was recrystallized from THF/hexanes and isolated as a stable, colorless, and crystalline solid (79% yield for two steps, mp 195−197 °C dec), the structure of which was confirmed by X-ray diffraction analysis (Figure 2).[37] Saponification of **8** under mild conditions ($NaOH/H_2O$ at 4 °C) followed by lactonization of dihydroxy acid **32** with isopropenyl chloroformate in the presence of triethylamine in THF afforded a 75% yield (two steps) of desired lactone **4**, after recrystallization from THF/CH_2Cl_2/hexanes [mp 181−183 °C, $[\alpha]^{23}_D = -130.8°$ (*c* 0.25, MeCN)]. From the *trans*-oxazoline **10**, only four linear steps were needed to complete the synthesis of **4** and with an overall yield of 59%. The cumulative yield of β-lactone **4** (PS-519) for the 10-step sequence (starting from oxazoline **10**) is > 20%. The *clasto*-lactacystin β-lactone (**2**) itself was prepared in an identical four-step sequence, but using aldehyde **30** (Scheme 6). The final product obtained using this route was indistinguishable from an authentic sample (mp, $[\alpha]_D$, ^1H and ^{13}C NMR, FAB-MS, and combustion analysis).

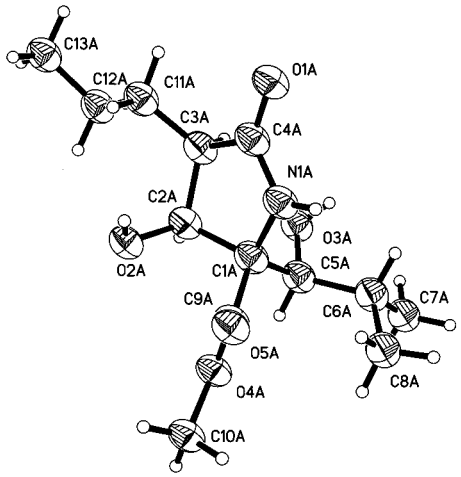


Figure 2. ORTEP drawing and labeling scheme for γ-lactam **8**.

Comparison in cell culture assays for the different lactone analogues (Table 1) involved a pulse-chase analysis of protein turnover using ^{35}S-labeled protein. Mouse myoblast cells, C2C12, were incubated for a period of 4 h, and the half-maximal inhibition for total protein turnover was recorded. PS-519 was consistently 2-fold more potent than the natural product-derived β-lactone. Additional experiments with PS-519 (**4**) indicated that it was also a potent inhibitor of NF-κB activation, by stabilizing IκBα, with an $IC_{50} = 1-5$ μM. Our own efforts to explore the pharmacology of PS-519 have led to the demonstration of efficacy in a number of animal models of acute ischemia−reperfusion injury. PS-519 was potently active in a rat model of myocardial infarction, as well as in a model of stroke.[4b,e,f,13]

In summary, we have achieved a short and highly selective synthesis of a novel and active analogue of *clasto*-lactacystin β-lactone (**4**) with an overall yield exceeding 20%. The key

(34) Ester **23** was prepared by benzylation of commercially available methyl (*R*)-(−)-3-hydroxy-2-methylpropionate. See: (a) Wessel, H.-P.; Iversen, T.; Bundle, D. R. *J. Chem. Soc., Perkin Trans. 1* **1985**, 2247. (b) Widmer, U. *Synthesis* **1987**, 568.

(35) Lide, D. R. *CRC Handbook of Chemistry and Physics*, 78th ed.; CRC Press: New York, 1997−1998.

(36) (a) Evans, D. A.; Chapman, K. T.; Bisaha, J. *J. Am. Chem. Soc.* **1998**, *110*, 1238. (b) Lehmkuhl, H.; Kobs, H.-D. *Liebigs Ann. Chem.* **1968**, *719*, 11.

(37) Crystal structure data for **8**: $C_{13}H_{23}NO_5$, orthorombic; $P2_12_12_1$, $a = 10.536(2)$ Å, $b = 20.451(4)$ Å, $c = 27.557(6)$ Å; $\alpha = \beta = \gamma = 90°$, $Z = 16$, $R1[I > 2\sigma(I)] = 0.0736$.

step of the synthesis is a novel and highly selective doubly diastereoselective aldol coupling between chiral fragments **10** and **26**. The longest linear sequence of only 10 synthetic steps (starting from isobutyraldehyde) has allowed the preparation of multigram amounts of lactone **4**. The potential of this viable synthetic route has allowed us to prepare clinical grade material and has further been demonstrated by synthesizing *clasto*-lactacystin β-lactone **2**. It should prove applicable to preparing many other C-7 analogues. The biological action and utility of lactonic inhibitor **4** (PS-519) in ischemia−reperfusion injury will be described in due course. Clinical trials in normal volunteers, with the ultimate intention to treat stroke patients, are to begin in the fall of 1999.

Experimental Section

General Methods. All solvents and reagents were obtained from commercial suppliers and were used without purification. All moisture-sensitive reactions were performed in flame-dried glassware under an atmosphere of nitrogen. ^{1}H and ^{13}C NMR spectra of $CDCl_3$, MeOD, pyridine-d_5, or DMSO-d_6 solutions were obtained at 300 and 75 MHz, respectively. High-resolution mass spectral analyses were performed by M-Scan, Inc. (West Chester, PA), and all elemental analyses were performed by Galbraith Laboratories, Inc. (Knoxville, TN). Melting points are uncorrected. Optical rotations were measured at ambient temperature using a 1-mL cell. Merck silica gel 60 F_{254} precoated plates (2.5 × 7.5 cm) were used for analytical thin-layer chromatography. Analytical HPLC was carried out on a reverse-phase column [4.6 mm i.d. × 25 cm length C18 Hichrom column (KR100-5C18-4847) from Richards Scientific], which was eluted with a mixture of H_2O containing 0.1% v/v TFA and MeOH containing 0.05% v/v TFA, using a flow rate of 1.0 mL/min, and monitoring products with an in-line UV absorbance detector set at λ = 202 nM.

(*E*)-4-Methylpent-2-enoic Acid Methyl Ester (14). A cooled (4 °C) solution of methyl (triphenylphosphoranylidene)acetate (100.12 g, 0.30 mol) in anhydrous CH_2Cl_2 (300 mL) at 0 °C was treated dropwise with isobutyraldehyde (30.0 mL, 0.33 mol). After 5 min, the reaction mixture was allowed to warm to ambient temperature and stirred for 24 h. The solvent was removed in vacuo, and pentane (500 mL) was added to the white oily solid to precipitate triphenylphosphine oxide. The solid was filtered off, and the filtrate was concentrated in vacuo. The procedure was repeated one more time, and the crude olefin was distilled under vacuum (45−46 °C at 10 mmHg), affording olefin **14** (35.85 g, 93%) as a clear, colorless liquid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.95 (dd, J = 15.7, 6.6 Hz, 1H), 5.77 (dd, J = 15.7, 1.5 Hz), 3.72 (s, 3H), 2.44 (m, 1H), 1.06 (d, J = 6.7 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.2, 155.5, 118.1, 51.1, 30.8, 21.0.

(2*R*,3*S*)-2,3-Dihydroxy-4-methylpentanoic Acid Methyl Ester (12). To a clear yellow solution of $K_2OsO_2(OH)_4$ (246.1 mg, 0.67 mmol, 0.95 mol %), hydroquinine 1,4-phthalazinediyl diether (555.1 mg, 0.71 mmol, 1.01 mol %), *N*-methylmorpholine *N*-oxide (50 wt % in water, 25.0 mL, 0.106 mol, 1.51 equiv), *t*-BuOH (84 mL), and H_2O (58 mL) was added the neat olefin **14** (9.0 g, 70.2 mmol) via a syringe pump over a period of 48 h (the syringe was connected to tubing, whose tip was immersed in the solution throughout the reaction time). The resulting clear orange solution was then stirred for another 60 min at ambient temperature, diluted with ethyl acetate (200 mL), and treated with a solution of Na_2SO_3 (15.0 g) in H_2O (150 mL). After the mixture was stirred for 4 h, the phases were separated, and the aqueous layer was extracted with ethyl acetate (2×). The organic layers were then combined, and the chiral ligand was extracted from the organic phase with a solution of 0.3 M H_2SO_4 in saturated aqueous Na_2SO_4 (2 × 100 mL). The phases were once again separated, and the aqueous layer was extracted with more ethyl acetate (1×). The organic layers were then combined, dried over Na_2SO_4, filtered, and concentrated in vacuo. This afforded 11.4 g (ca. 100%) of a the diol as a white oily solid, which was shown to have a 70% ee (determined by ^{1}H NMR from a 1:1 molar solution of diol and europium tris[3(heptafluoropropylhydroxymethylene)-(−)-camphorate] in C_6D_6). Recrystallization from 35−60 °C petroleum ether afforded 6.8 g (60%) of pure **12**, obtained as white crystals and with >99% ee: mp 32−34 °C; $[\alpha]^{23}_D$ = −10.7° (*c* 1.00, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.28 (dd, J = 5.6, 1.8 Hz, 1H), 3.80 (s, 3H), 3.48 (m, 1H), 3.28 (m, 1H), 2.33 (d, J = 9.3 Hz, 1H), 1.87 (m, 1H), 1.02 (d, J = 6.7 Hz, 3H), 0.95 (d, J = 6.7 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.5, 77.8, 71.3, 52.6, 30.9, 18.9, 18.8; FAB-MS, *m*/*z* (relative intensity) 163 [$(M + H)^+$, 100], 185 [$(M + Na)^+$, 45]. Anal. Calcd for $C_7H_{14}O_4$: C, 51.84; H, 8.70. Found: C, 52.18; H, 8.77.

(2*S*,3*S*)-2-Bromo-4-methyl-3-benzoyloxypentanoic Acid Methyl Ester (15). Compound **12** (30.0 g, 0.18 mol) was dissolved in CH_2Cl_2 (400 mL) and treated sequentially with trimethylorthobenzoate (41.3 mL, 0.24 mol) and BF_3•OEt_2 (1.16 mL, 9.15 mmol) . After the solution was stirred for 110 min at ambient temperature, triethylamine (1.82 mL, 13.1 mmol) was added, and the mixture was concentrated in vacuo and placed under high vacuum (0.05 mmHg) for 130 min. The yellow oil obtained was dissolved in CH_2Cl_2 (400 mL), and the solution was cooled to 5 °C. Acetyl bromide (14.3 mL, 0.19 mol) was added dropwise, and the mixture was then allowed to reach ambient temperature, stirred for 2 h, and treated with more acetyl bromide (0.68 mL, 9.2 mmol). After being stirred for another 30 min, the reaction mixture was treated with a saturated aqueous $NaHCO_3$ solution (500 mL) and stirred vigorously at ambient temperature for 10 min. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo, affording crude **15** (66.23 g, >100%) as a clear, colorless oil, contaminated by some methyl benzoate (ca. 9.7 wt % by ^{1}H NMR; estimated yield of **15** is ca. 60 g or 98%) but pure enough to be carried as such to the following step. A sample was purified by flash chromatography (hexanes/AcOEt 19:1) to afford pure bromoester **15** as a white solid: mp 35.5−37.0 °C; $[\alpha]^{25}_D$ = +31.1° (*c* 1.01, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05−8.00 (m, 2H), 7.47−7.40 (m, 3H), 5.57 (dd, J = 8.8, 3.9 Hz, 1H), 4.47 (d, J = 8.8 Hz, 1H), 3.67 (s, 3H), 2.45 (m, 1H), 1.01 (d, J = 6.8 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.1, 165.1, 133.2, 129.6, 129.4, 128.4, 77.0, 53.0, 44.3, 29.3, 19.4, 15.6 ; FAB-MS, *m*/*z* (relative intensity) 329 for $[C_{14}H_{17}{}^{79}BrO_4 + H]^+$ (18), 331 for $[C_{14}H_{17}{}^{81}BrO_4 + H]^+$ (16), 351 for $[C_{14}H_{17}{}^{79}BrO_4 + Na]^+$ (4), 353 for $[C_{14}H_{17}{}^{81}BrO_4 + Na]^+$ (4), 105 [$(PhCO)^+$, 100]; HRMS calcd for $[C_{14}H_{17}BrO_4 + H]^+$ 329.0389, found 329.0375.

(2*R*,3*S*)-2-Azo-4-methyl-3-benzoyloxypentanoic Acid Methyl Ester (16). Sodium azide (24.0 g, 0.37 mol) was added to 230 mL of DMSO, and the mixture was stirred at ambient temperature overnight. To the heterogeneous mixture was then added **15** (61.1 g, ca. 0.17 mol) along with 20 mL of DMSO. After being stirred for 11 h at ambient temperature, the mixture was partitioned between H_2O (1.5 L) and ether (300 mL). After the layers were separated, the aqueous layer was extracted with ether (2 × 100 mL), and the combined organic layers were washed with water (2 × 100 mL) and brine (100 mL) and then dried over $MgSO_4$ and concentrated in vacuo, affording the desired α-azo β-benzoate **16** as a yellow oil (57.54 g, >100%; contained ca. 3% starting material and 8% elimination product by ^{1}H NMR, estimated yield of **16** is ca. 85%). The product **16** was pure enough to be carried as such to the following step. A sample was purified by flash chromatography (hexanes/AcOEt 12:1) to afford pure azoester **16** as a clear, colorless oil: $[\alpha]^{25}_D$ = −21.4° (*c* 1.01, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07−8.02 (m, 2H), 7.55−7.43 (m, 3H), 5.40 (dd, J = 8.8, 2.8 Hz, 1H), 3.73 (s, 3H), 2.24 (m, 1H), 1.04 (d, J = 5.8 Hz, 3H), 0.98 (d, J = 5.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.2, 165.5, 133.3, 129.9, 129.0, 128.4, 78.9, 61.7, 52.8, 30.0, 18.7, 18.6; FAB-MS, *m*/*z* (relative intensity) 292 [$(M + H)^+$, 23], 105 [$(PhCO)^+$, 100]; HRMS calcd for $[C_{14}H_{17}O_4N_3 + H]^+$ 292.1297, found 292.1286.

(2*R*,3*S*)-2-Amino-3-benzoyloxy-4-methylpentanoic Acid Methyl Ester (17) and (2*R*,3*S*)-2-Benzoylamino-3-hydroxy-4-methylpentanoic Acid Methyl Ester (18). To a cooled (5 °C) solution of crude **16** (55 g, ca. 0.17 mol) in MeOH (300 mL) containing 2.75 g of 20% $Pd(OH)_2$/C was added 94.0 mL of a 4 M solution of HCl in 1,4-dioxane (0.38 mol). The suspension was allowed to warm to ambient temperature and was stirred vigorously in a H_2 atmosphere under balloon pressure. After 4 h, more 20% $Pd(OH)_2$/C (1.30 g) was added, and the mixture was stirred under H_2 for another 4 h. It was then filtered and concentrated in vacuo, affording a solid residue which was dissolved

in H_2O and washed with ethyl acetate (3 × 100 mL). The combined organic layers were extracted with H_2O (2 × 25 mL), and the combined aqueous extracts were basified with Na_2CO_3 (30 g) and extracted with ethyl acetate (200 mL and then 2 × 100 mL). The combined organic extracts were finally washed with brine and dried over Na_2SO_4. Filtration followed by concentration in vacuo afforded 38.4 g of thick yellow oil. ^{1}H NMR analysis (300 MHz, $CDCl_3$) showed the mixture to be a ca. 1:10 mixture of benzoate **17** and benzamide **18**, which was used as such in the following step. The estimated yield for the reduction was ca. 85%. Refluxing an ethyl acetate solution of benzoate **17** and benzamide **18** for 4 h results in complete migration of the benzoyl group, affording benzamide **18** exclusively. A sample was purified by flash chromatography (hexanes/AcOEt 1:1) to afford pure benzamide **18** as a clear and colorless gum: $[\alpha]^{25}_D = -22.8°$ (*c* 1.00, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85−7.83 (m, 2H), 7.46−7.40 (m, 3H), 6.99 (bd, J = 9.1 Hz, 1H), 5.05 (dd, J = 9.1, 1.9 Hz, 1H), 3.77 (s, 3H), 1.79 (m, 1H), 1.03 (d, J = 6.7 Hz, 3H), 0.99 (d, J = 6.7 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.2, 167.6, 133.7, 131.8, 128.5, 127.1, 77.5, 54.5, 52.5, 31.0, 18.9, 18.8; FAB-MS, *m*/*z* (relative intensity) 266 [$(M + H)^+$, 100]; HRMS calcd for $[C_{14}H_{19}N O_4 + H]^+$ 266.1392, found 266.1408.

Preparation of (4*R*,5*S*)-5-Isopropyl-2-phenyl-4,5-dihydrooxazole-4-carboxylic Acid Methyl Ester Oxazoline (10). A 1:10 mixture of benzoate **17** and benzamide **18** (37.3 g, 0.14 mol) in toluene (350 mL) containing *p*-TsOH·H_2O (2.68 g, 14.1 mmol, 0.10 equiv) was refluxed for 2.75 h while the water formed was removed with a Dean−Stark trap. The mixture was then cooled to ambient temperature, diluted with AcOEt (100 mL), washed successively with saturated aqueous $NaHCO_3$ (2 × 100 mL) and brine (100 mL), dried (Na_2SO_4), and concentrated in vacuo, affording a yellow oil. Purification was performed using a silica gel pad (7.5 × 13 cm SiO_2), eluting the product with 1:3 hexanes/AcOEt followed by 1:2 hexanes/AcOEt and collecting ca. 300-mL fractions. The five fractions containing the pure product were combined and concentrated in vacuo, affording the desired pure oxazoline **10** (30.8 g, 89%) as a pale yellow oil: $[\alpha]^{25}_D = -119.1°$ (*c* 1.15, $CHCl_3$); lit.[11e] $[\alpha]^{25}_D = -124°$ (*c* 1.0, $CHCl_3$); FTIR (film) 2962, 1744, 1644 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01−7.97 (m, 2H), 7.52−7.38 (m, 3H), 4.94 (d, J = 9.8 Hz, 1H), 4.53 (dd, J = 9.8, 7.8 Hz, 1H), 3.76 (s, 3H), 2.09 (m, 1H), 1.05 (d, J = 6.5 Hz, 3H), 1.01 (d, J = 6.7 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.9, 165.6, 131.6, 128.4, 128.2, 127.1, 87.1, 71.2, 52.5, 32.3, 17.3, 17.2; FAB-MS, *m*/*z* (relative intensity) 248 [$(M + H)^+$, 100]; 188 [$(M - CO_2Me)^+$, 19]. Anal. Calcd for $C_{14}H_{17}NO_3$: C, 68.00; H, 6.93; N, 5.66. Found: C, 67.83; H, 6.93; N, 5.67.

3-Pentanoyl-4*S*-benzyloxazolidin-2-one (21). A cooled (−78 °C) solution of (*S*)-(−)-4-benzyl-2-oxazolidinone (52.7 g, 0.30 mol) in 360 mL of anhydrous THF was treated with a 2.5 M solution of *n*-BuLi in hexane (119 mL, 0.30 mol) over 20 min. After 20 min, neat valeryl chloride (37.0 mL, 0.31 mol) was added dropwise over 10 min, and the mixture was stirred for another 60 min at −78 °C. It was then allowed to reach ambient temperature and was stirred for another 90 min before being treated with 200 mL of saturated aqueous NH_4Cl. The mixture was stirred for 15 min at ambient temperature, and after dilution with 200 mL of H_2O, the layers were separated. The organic layer was concentrated in vacuo, and the crude material was dissolved in 500 mL of 5:1 hexane/AcOEt containing 1% MeOH. This solution was washed successively with saturated aqueous NH_4Cl (200 mL) and H_2O (100 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. This afforded 75.9 g (97%) of desired acyl oxazolidinone **21** as a pale yellow liquid. A sample was purified by flash chromatography (hexanes/AcOEt 6:1) to afford pure acyl oxazolidinone **21** as a clear, colorless liquid: $[\alpha]^{23}_D = +56.6°$ (*c* 1.05, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36−7.20 (m, 5H), 4.71−4.64 (m, 1H), 4.23−4.14 (m, 1H), 3.40 (dd, J = 13.3, 3.2 Hz, 1H), 3.04−2.84 (m, 2H), 2.77 (dd, J = 13.3, 9.6 Hz, 1H), 1.74−1.63 (m, 2H), 1.46−1.38 (m, 2H), 0.96 (t, J = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.3, 153.3, 135.3, 129.3, 128.8, 127.2, 66.0, 55.0, 37.8, 35.2, 26.3, 22.2, 13.8. FAB-MS, *m*/*z* (relative intensity) 262 [$(M + H)^+$, 100], 178 [$(M - CH_3(CH_2)_2CO + 2H)^+$, 63], [$CH_3(CH_2)_2CO^+$, 25]. Anal. Calcd for $C_{15}H_{19}NO_3$: C, 68.94; H, 7.33; N, 5.36. Found: C, 68.93; H, 7.47; N, 5.41.

Benzyl Chloromethyl Ether. Benzyl chloromethyl ether was freshly prepared by a slight variation of the method described by Coonor et al.[30] Gaseous HCl was intoduced as a slow stream into a cold (0−4 °C) mixture of benzyl acohol (60.0 mL, 0.80 mol) and 1,3,5-trioxane (17.6 g, 0.20 mol), maintaining the internal temperature below 15 °C for the first 2 h. The ice bath was then removed, and a gentle stream of HCl was maintained until two phases had formed and ^{1}H NMR analysis of an aliquot showed no remaining starting material. The layers were then separated, and the top organic layer was dried over $CaCl_2$, filtered, and placed under high vacuum for 1 h. This afforded 79.0 g (87%) of the desired benzyl chloromethyl ether as a clear, colorless liquid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 (s, 5H), 5.54 (s, 2H), 4.76 (s, 2H).

3-(3-Benzyloxymethyl-2*R*-*n*-propylpropionyl)-4*S*-benzyloxazolidin-2-one (22). A three-neck 3-L flask equipped with a mechanical stirrer was charged with a solution of acyl oxazolidinone **21** (75.9 g, 0.29 mol) in 300 mL of anhydrous CH_2Cl_2. The solution was cooled to 4 °C and was treated over 20 min with $TiCl_4$ (32.0 mL, 0.29 mol), resulting in the formation of an abundant yellow precipitate. After 10 min, diisopropylethylamine (52.0 mL, 0.30) was added over 10 min, and the resulting dark brown solution was stirred at 4 °C for 60 min. Freshly prepared benzyl chloromethyl ether (49.0 mL, 0.36 mol) was then added over 5 min. The mixture was stirred for 60 min at 4 °C, after which time CH_2Cl_2 (100 mL) was added to rinse the sides of the reaction vessel and the mixture was stirred for another 5 h at 0−4 °C. CH_2Cl_2 (200 mL), saturated aqueous NH_4Cl (200 mL), and H_2O (200 mL) were then added sequentially, and the suspension was vigorously stirred for 30 min while being allowed to reach ambient temperature. The layers were separated, and the organic layer was washed with saturated aqueous $NaHCO_3$ (2 × 200 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude solid material was recrystallized from EtOAc/hexane, affording 93.7 g (85%) of desired pure benzyl ether **22** as white crystals: mp 74−75 °C; $[\alpha]^{23}_D = +33.7°$ (*c* 1.02, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34−7.18 (m, 10H), 4.77−4.69 (m, 1H), 4.55 (s, 2H), 4.32−4.23 (m, 1H), 4.21−4.10 (m, 2H), 3.80 (t, J = 9.0 Hz, 1H), 3.65 (dd, J = 9.0, 5.0 Hz, 1H), 3.23 (dd, J = 13.5, 3.3 Hz, 1H), 2.69 (dd, J = 13.5, 9.3 Hz, 1H), 1.74−1.64 (m, 1H), 1.54−1.44 (m, 1H), 1.40−1.28 (m, 2H), 0.91 (t, J = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.2, 153.1, 138.1, 135.3, 129.4, 128.8, 128.2, 127.5, 127.4, 127.1, 73.0, 71.3, 65.7, 55.2, 43.1, 37.6, 31.1, 20.3, 14.1; FAB-MS, *m*/*z* (relative intensity) 382 [$(M + H)^+$, 48], 91 (Bn^+, 100]. Anal. Calcd for $C_{23}H_{27}NO_4$: C, 72.42; H, 7.13; N, 3.67. Found: C, 72.82; H, 7.17; N, 3.94.

2*R*-Benzyloxymethylpentanoic Acid (23). A cold (4 °C) solution of of benzyl ether **22** (92.0 g, 0.24 mol) in 660 mL of 2:1 THF/H_2O was treated successively with 92.0 mL of 30% aqueous H_2O_2 and lithium hydroxide monohydrate (20.0 g, 0.48 mol). The mixture was stirred at 4 °C for 48 h and was then treated carefully, first with a solution Na_2SO_3 (147 g, 1.14 mol) in 500 mL of H_2O and then with a solution of $NaHCO_3$ (59 g, 0.70 mol) in 500 mL of H_2O. The mixture was stirred for 60 min at ambient temperature and diluted with H_2O (500 mL). The top organic layer was separated from the aqueous layer and concentrated in vacuo, and the residue was poured back into the aqueous layer, which was then washed with CH_2Cl_2 (5 × 200 mL), cooled to 0 °C, acidified with 6 N aqueous HCl (400 mL), and extracted with CH_2Cl_2 (4 × 200 mL). The combined organic layers were then washed with brine (200 mL), dried over $MgSO_4$, and concentrated in vacuo, affording 48.5 g (90%) of the desired carboxylic acid **23** as a clear, colorless oil: $[\alpha]^{23}_D = -3.6°$ (*c* 1.00, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38−7.26 (m, 5H), 4.55 (s, 2H), 3.67 (m, 1H), 3.57 (dd, J = 9.2, 5.2 Hz, 1H), 2.75 (m, 1H), 1.72−1.31 (m, 4H), 0.93 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 180.9, 137.9, 128.3, 127.6, 127.5, 73.1, 70.5, 45.8, 30.7, 20.3, 13.9; FAB-MS, *m*/*z* (relative intensity) 223 [$(M + H)^+$, 55], 91 (Bn^+, 100]. Anal. Calcd for $C_{13}H_{18}O_3$: C, 70.25; H, 8.16. Found: C, 70.58; H, 8.35.

2*R*-Benzyloxymethylpentanoic Acid Diethylamide (24). A cooled (0−4 °C) solution of **23** (48.5 g, 0.22 mol) in MeCN (250 mL) containing diethylamine (27.0 mL, 0.26 mol) and TBTU (77.0 g, 0.24 mol) was treated with diisopropylethylamine (76.0 mL, 0.44 mol). The mixture was stirred at 0−4 °C for 2 h and then concentrated in vacuo and partitioned between ether (250 mL) and H_2O (1 L). The aqueous

layer was extracted with more ether (2 × 250 mL), and the combined organic layers were washed successively with aqueous 1 N HCl (2 × 100 mL), saturated aqueous $NaHCO_3$ (200 mL), and brine (200 mL) and then dried over $MgSO_4$ and concentrated in vacuo, affording 58.94 g (98%) of diethylamide **24** as a pale yellow liquid that was used as such in the following step. A sample was purified by flash chromatography (hexanes/AcOEt 4:1) to afford pure diethylamide **24** as a clear, colorless oil: $[\alpha]^{25}_D = +17.8°$ (*c* 1.14, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35−7.23 (m, 5H), 4.52 (d, J = 12.0 Hz, 1H), 4.44 (d, J = 12.0 Hz, 1H), 3.67 (t, J = 8.6 Hz, 1H), 3.51 (dd, J = 8.7, 5.5 Hz, 1H), 3.46−3.27 (m, 4H), 2.96 (m, 1H), 1.67−1.57 (m, 1H), 1.48−1.22 (m, 4H), 1.20−1.10 (m, 6H), 0.90 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.7, 138.5, 128.1, 127.3, 127.2, 73.1, 72.9, 42.1, 41.8, 40.5, 32.1, 20.6, 14.7, 14.2, 13.0; FAB-MS, *m*/*z* (relative intensity) 278 [(M + H)$^+$, 100], 91 (Bn^+, 73]. Anal. Calcd for $C_{17}H_{27}NO_2$: C, 73.61; H, 9.81; N, 5.05. Found: C, 73.59; H, 10.02; N, 5.14.

2*R*-Hydroxymethylpentanoic Acid Diethylamide (25). To a solution of diethylamide **24** (58.9 g, 0.21 mol) in 350 mL of MeOH was added 10% $Pd(OH)_2$/C (4.0 g), and the suspension was hydrogenated at atmospheric pressure and ambient temperature for 48 h. Filtration of the catalyst and concentration in vacuo afforded 40.4 g (>100%) of the desired primary alcohol **25**, used as such in the following oxidation step. A sample was purified by flash chromatography (hexanes/AcOEt 1:3) to afford pure primary alcohol **25** as a clear, colorless oil: $[\alpha]^{25}_D = -3.5°$ (*c* 1.15, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.74 (br d, J = 4.2 Hz, 1H), 3.61−3.15 (m, 5H), 2.71 (m, 1H), 1.69−1.24 (m, 4H), 1.20 (t, J = 7.1 Hz, 3H), 1.12 (t, J = 7.1 Hz, 3H), 0.92 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.2, 63.3, 42.4, 41.9, 40.1, 31.2, 20.4, 14.6, 14.1, 12.9; FAB-MS, *m*/*z* (relative intensity) 188 [(M + H)$^+$, 100]. Anal. Calcd for $C_{10}H_{21}NO_2$: C, 64.13; H, 11.13; N, 7.48. Found: C, 63.96; H, 11.43; N, 7.62.

2*R*-Formylpentanoic Acid Diethylamide (26). To a solution of alcohol **25** (22.0 g, 0.12 mol) in wet CH_2Cl_2 (392 mL, prepared by stirring CH_2Cl_2 with water and separating the organic layer) was added Dess−Martin periodinane[31] (75.0 g, 0.18 mol) portionwise. The mixture was stirred at ambient temperature for 60 min and then cooled to ca. 4 °C and treated with 1.2 L of a 0.64 M aqueous $Na_2S_2O_3$ solution (250 mL) containing 48.5 g of $NaHCO_3$. The mixture was stirred for 5 min, and then ether (800 mL) was added and the biphasic mixture was stirred vigorously for an additional 10 min at ambient temperature. The layers were separated, and the aqueous layer was extracted with 20% CH_2Cl_2/Et_2O (2 × 150 mL). The combined organic layers were then washed succesively with H_2O (2 × 100 mL) and brine (2 × 200 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo, affording 20.93 g (96%) of desired aldehyde **26** as a clear, colorless oil: $[\alpha]^{23}_D = -64.1°$ (*c* 1.05, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.60 (d, J = 3.5 Hz, 1H), 3.49−3.30 (m, 5H), 1.96−1.85 (m, 2H), 1.39−1.31 (m, 2H), 1.19 (t, J = 7.1 Hz, 3H), 1.13 (t, J = 7.1 Hz, 3H), 0.95 (t, J = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.9, 167.9, 55.7, 41.9, 40.4, 30.2, 20.2, 14.7, 13.8, 12.9; FAB-MS, *m*/*z* (relative intensity) 186 [(M + H)$^+$, 100].

3-Benzyloxy-*N*,*N*-diethyl-2*R*-methylpropionamide (28). Lithium hydroxide monohydrate (1.27 g, 30.3 mmol) was added portionwise (over 10 min) to a cold (4 °C) solution of 3-benzyloxy-2*R*-methylpropionoic acid methyl ester[34] (4.20 g, 20.2 mmol) in 1:1 THF/H_2O (100 mL), and the mixture was stirred at 4 °C overnight. $NaHCO_3$ (2.5 g) and H_2O (50 mL) were then added, and the THF was removed in vacuo. More H_2O was added to the mixture, which was then washed with CH_2Cl_2 (3 × 50 mL), cooled to 0−5 °C, acidified with 6 N aqueous HCl (22 mL), and extracted with CH_2Cl_2 (1 × 75 mL and then 2 × 50 mL). The combined organic layers were then washed with half-saturated brine (50 mL), dried over $MgSO_4$, and concentrated in vacuo, affording 3.57 g (91%) of the desired carboxylic acid as a clear, colorless oil: $[\alpha]^{23}_D = -12.3°$ (*c* 1.06, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38−7.30 (m, 5H), 4.56 (s, 2H), 3.67 (dd, J = 9.2, 7.4 Hz, 1H), 3.55 (dd, J = 9.2, 5.6 Hz, 1H), 2.85−2.78 (m, 1H), 1.23 (d, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 181.0, 137.9, 128.3, 127.7, 127.6, 73.1, 71.5, 40.1, 13.7; FAB-MS, *m*/*z* (relative intensity) 195 [(M + H)$^+$, 40], 91 (Bn^+, 100].

A cooled (0−5 °C) solution of 3-benzyloxy-2*R*-methylpropionoic acid (3.29 g, 16.9 mmol) and TBTU (5.72 g, 17.8 mmol) in 1:1 CH_2Cl_2/MeCN (250 mL) was treated sequentially with diethylamine (2.18 mL, 21.2 mmol) and diisopropylethylamine (6.0 mL, 34.4 mmol). The mixture was allowed to warm to ambient temperature, stirred for 2.5 h, and then concentrated in vacuo and suspended in 125 mL of H_2O. After being stirred for 10 min at ambient temperature, the suspension was extracted with ether (1 × 75 mL and then 2 × 50 mL), and the combined organic layers were washed successively with aqueous 1 N HCl (3 × 50 mL), H_2O (50 mL), 10% w/v aqueous Na_2CO_3 (2 × 50 mL), and brine (2 × 50 mL) and then dried over $MgSO_4$ and concentrated in vacuo, affording 3.97 g (94%) of diethylamide **28** as a pale yellow liquid that was used as such in the following step. A sample was purified by flash chromatography (hexanes/AcOEt 2:1) to afford pure diethylamide **28** as a clear, colorless oil: $[\alpha]^{25}_D = -5.8°$ (*c* 1.01, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35−7.25 (m, 5H), 4.54 (d, J = 12.0 Hz, 1H), 4.46 (d, J = 12.0 Hz, 1H), 3.71 (dd, J = 8.7, 8.1 Hz, 1H), 3.48−3.24 (m, 5H), 3.03−2.96 (m, 1H), 1.17 (t, J = 7.2 Hz, 3H), 1.12 (d, J = 6.8 Hz, 3H), 1.11 (t, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.1, 138.4, 128.2, 127.3, 73.5, 73.2, 41.8, 40.3, 36.4, 14.9, 14.8, 13.0; FAB-MS, *m*/*z* (relative intensity) 250 [(M + H)$^+$, 100], 91 (Bn^+, 71]; HRMS calcd for [$C_{15}H_{23}N\,O_2$ + H]$^+$ 250.1807, found 250.1791.

***N*,*N*-Diethyl-3-hydroxy-2*R*-methylpropionamide (29).** To a solution of diethylamide **28** (3.95 g, 15.9 mmol) in 55 mL of MeOH was added 10% $Pd(OH)_2$/C (400 mg), and the suspension was hydrogenated at atmospheric pressure and ambient temperature for 60 h. Filtration of the catalyst and concentration in vacuo afforded 2.55 g (100%) of the desired primary alcohol **29**, used as such in the following oxidation step. A sample was purified by flash chromatography (hexanes/AcOEt 1:2) to afford pure primary alcohol **29** as a clear, colorless oil: $[\alpha]^{25}_D = -33.5°$ (*c* 1.15, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.71−3.69 (m, 2H), 3.52−3.21 (m, 5H), 2.84−2.74 (m, 1H), 1.22−1.15 (m, 6H), 1.11 (t, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.5, 65.0, 41.7, 39.9, 37.3, 14.6, 14.2, 12.9; FAB-MS, *m*/*z* (relative intensity) 160 [(M + H)$^+$, 100]; HRMS calcd for [$C_8H_{17}N\,O_2$ + H]$^+$ 160.1337, found 160.1344.

***N*,*N*-Diethyl-2*R*-methyl-3-oxopropionamide (30).** To a solution of alcohol **29** (2.35 g, 14.8 mmol) in wet CH_2Cl_2 (74 mL, prepared by stirring CH_2Cl_2 with water and separating the organic layer) was added Dess−Martin periodinane[30] (9.40 g, 22.2 mmol) portionwise. The mixture was stirred at ambient temperature for 60 min and then cooled to 0−5 °C and treated with a solution of $Na_2S_2O_3$ (15.8 g) and $NaHCO_3$ (6.2 g) in 160 mL of H_2O. The mixture was stirred for 5 min, and then ether (140 mL) was added and the biphasic mixture was stirred vigorously for an additional 10 min at ambient temperature. The layers were separated, and the aqueous layer was extracted with 1:2 CH_2Cl_2/Et_2O (2 × 35 mL). The combined organic layers were then washed succesively with brine (2 × 50 mL), dried over Na_2SO_4/$MgSO_4$, filtered, and concentrated in vacuo, affording 2.03 g (88%) of the desired aldehyde **30** as a pale yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.64 (d, J = 2.2 Hz, 1H), 3.51−3.24 (m, 5H), 1.40 (d, J = 7.0 Hz, 3H), 1.21 (apparent t, J = 7.0, 7.4 Hz, 3H), 1.14 (apparent t, J = 7.0, 7.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.3, 169.0, 49.6, 42.0, 40.3, 14.6, 12.9, 12.4; FAB-MS, *m*/*z* (relative intensity) 158 [(M + H)$^+$, 48].

3-(5*S*-Isopropyl-4*S*-methoxycarbonyl-2-phenyl-4,5-dihydrooxazol-4-yl)-3*S*-hydroxy-2*R*-*n*-propyl-*N*,*N*-diethylpropionamide (31). To a cold (−76.5 °C, internal temperature) solution of oxazolidine **10** (20.74 g, 83.9 mmol) in anhydrous THF (280 mL) was added lithium bis(trimethylsilyl)amide (92.4 mL of a 1 M solution in hexane, 92.4 mmol) over 1.25 h. After 30 min, the orange solution was treated over 2.5 h with a 1 M solution of dimethylaluminum chloride in hexane (202 mL, 202 mmol), and the mixture was stirred for another 40 min before being cooled to −81.5 °C (internal temperature, liquid N_2 was added to the dry ice/acetone bath). A solution of aldehyde **26** (19.43 g, 105 mmol) in THF (50 mL) was then added over 45 min along the side of the flask (internal temperature ≤−79 °C). The mixture was then allowed to warm to −20 °C over 65 min, cooled again to −78 °C, and then cautiously quenched by addition of saturated aqueous NH_4Cl (40 mL). The mixture was then poured into a mixture of saturated aqueous NH_4Cl (460 mL) and AcOEt (500 mL), and 6 N aqueous HCl (130 mL) was then slowly added. After the layers were separated, the aqueous

layer was extracted with AcOEt (2 × 200 mL), and the combined organic layers were washed successively with H_2O (2 × 200 mL), saturated aqueous $NaHCO_3$ (2 × 200 mL), and brine (2 × 300 mL) and then dried over $Na_2SO_4/MgSO_4$ and concentrated in vacuo, affording 41.55 g (>100%) of crude aldol **31** as a pale and thick yellow oil, which used as such in the subsequent step. A sample was purified by flash chromatography (hexanes/AcOEt 2:1), followed by recrystallization from hexanes to afford pure aldol adduct **31** as white needles: mp 55−57 °C; $[\alpha]^{25}_D = -124.0°$ (*c* 0.50, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02−7.97 and 7.53−7.39 (m, 5H), 6.58 (d, J = 9.9 Hz, 1H), 4.82 (d, J = 2.4 Hz, 1H), 3.73 (s, 3H), 3.69−3.61 (m, 2H), 3.49−3.39 (m, 2H), 3.24−3.16 (m, 1H), 3.05 (m, 1H), 2.89 (m, 1H), 2.28−2.23 (m, 1H), 1.98−1.91 (m, 1H), 1.37−1.20 (m, 6H), 1.19−1.06 (m, 6H), 0.87 (t, J = 7.1 Hz, 3H), 0.70 (d, J = 6.7 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.9, 170.5, 164.2, 131.6, 128.4, 128.2, 127.2, 89.5, 82.8, 78.6, 51.8, 42.4, 40.0, 37.2, 33.8, 29.6, 20.7, 20.3, 15.0, 13.9, 13.7, 12.8; FAB-MS, *m*/*z* (relative intensity) 433 [$(M + H)^+$, 100]. Anal. Calcd for $C_{24}H_{36}N_2O_5$: C, 66.64; H, 8.39; N, 6.48. Found: C, 66.76; H, 8.60; N, 6.40.

When some unreacted aldehyde **26** contaminates the aldol adduct **31**, it is conveniently removed by dissolving the material in hexanes and washing the solution successively with 0.35 M aqueous $NaHSO_3$ (made by dissolving 31 g of Na_2SO_3 in 380 mL of H_2O and 220 mL of 1 M aqueous HCl), H_2O, saturated aqueous $NaHCO_3$, and brine. The solution is then dried over Na_2SO_4, filtered, and concentrated in vacuo.

3*S*-Hydroxy-2*R*-(1*S*-hydroxy-2-methylpropyl)-4*R*-*n*-propyl-5-oxopyrrolidine-2-carboxylic Acid Methyl Ester (8). A solution of crude aldol adduct **31** (17.5 g, 40.5 mmol) in 100 mL of 1:9 AcOH/MeOH, to which was added 17.5 g of 20% $Pd(OH)_2/C$, was vigorously shaken under 55 psi H_2 for 75 h at ambient temperature. The mixture was then filtered and concentrated in vacuo. The solid obtained was taken up in a mixture of AcOEt (600 mL), CH_2Cl_2 (100 mL), and THF (100 mL), and the obtained solution was treated with a 4.5 M aqueous solution of K_2CO_3 (20 mL) and brine (30 mL). The layers were then separated, and the organic layer was washed with brine (50 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude solid (11.84 g) was then dissolved in 150 mL of boiling THF and slowly diluted with boiling hexane (300 mL). The cloudy solution was allowed to cool to ambient temperature for 2 h and was then placed in a 4 °C cold room for another 2 h. The crystals that had formed were then filtered and washed with 10% THF/hexane. This afforded 8.75 g of pure desired γ-lactam **8** (79%) as a white solid: mp 195−197 °C dec; $[\alpha]^{23}_D = -12.6°$ (*c* 0.54, MeCN); FTIR (KBr pellet) 3241, 2958, 1726, 1689 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (br s, 1H), 4.77 (br d, J = 11.5 Hz, 1H), 4.47 (dd, J = 11.5, 5.6 Hz, 1H), 4.08 (dd, J = 9.4, 5.0 Hz, 1H), 3.83 (s, 3H), 2.93 (m, 1H), 1.78−1.39 (m, 6H), 1.02−0.88 (m, 9H); ^{13}C NMR (75 MHz, pyridine-d_5) δ 179.7, 173.1, 79.9, 76.7, 75.9, 51.7, 47.1, 32.2, 27.0, 21.8, 20.3, 19.7, 14.6; FAB-MS, *m*/*z* (relative intensity) 274 ($M + H^+$, 100). Anal. Calcd for $C_{13}H_{23}NO_5$: C, 57.13; H, 8.48; N, 5.12. Found: C, 56.85; H, 8.58; N, 5.04.

3*S*-Hydroxy-2*R*-(1*S*-hydroxy-2-methylpropyl)-4*R*-*n*-propyl-5-oxopyrrolidine-2-carboxylic Acid (32). To a cold (0−5 °C) suspension of γ-lactam **8** (17.42 g, 63.7 mmol) in H_2O (100 mL) was added cold (0−5 °C) 0.6 N aqueous NaOH (213 mL, 127.8 mmol). The mixture was stirred at 4 °C for 34 h, acidified with 2 N aqueous HCl (75 mL), washed with CH_2Cl_2 (2 × 50 mL), frozen, and lyophilized for 4 days. The obtained solid was transferred into a desiccator containing P_2O_5 and dried under high vacuum for 30 h. It was then suspended in THF containing 6.0 g of Celite, and the suspension was filtered to get rid of sodium chloride. Concentration in vacuo afforded 16.68 g (100%) of the desired dihydroxy acid **32** as a white solid: $[\alpha]^{23}_D = +20.8°$ (*c* 0.50, MeCN); ^{1}H NMR (300 MHz, CD_3OD) δ 4.42 (d, J = 5.8 Hz, 1H), 3.90 (d, J = 6.5 Hz, 1H), 2.84 (m, 1H), 1.70−1.24 (m, 6H), 0.95−0.84 (m, 9H); ^{13}C NMR (75 MHz, pyridine-d_5) δ 179.9, 175.2, 79.3, 77.0, 76.4, 47.2, 32.2, 26.8, 21.6, 21.1, 18.7, 14.4; FAB-MS, *m*/*z* (relative intensity) 260 [$(M + H)^+$, 100], 282 {$(M + Na)^+$, 19]. Anal. Calcd for $C_{12}H_{21}NO_5$: C, 55.58; H, 8.16; N, 5.40. Found: C, 55.12.72; H, 8.39; N, 5.21.

[1*R*-[1*S*,4*R*,5*S*]]-1-(1-Hydroxy-2-methylpropyl)-4-*n*-propyl-6-oxa-2-azabicyclo[3.2.0]heptane-3,7-dione (4) [PS-519]. A cold (0−5 °C) solution of dihydroxy acid **32** (19.20 g, 74.0 mmol) in anhydrous THF (250 mL) containing triethylamine (15.9 mL, 114.1 mmol) was treated dropwise with isopropenyl chloroformate (8.65 mL, 79.2 mmol). After being stirred for 30 min at 0−5 °C, the mixture was allowed to reach ambient temperature, stirred for another 30 min, and diluted with diethyl ether (375 mL). The mixture was stirred for 10 min, filtered, and concentrated in vacuo. Purification on a SiO_2 pad, eluting with 2:3 AcOEt/hexane, afforded 15.71 g (86%) of desired β-lactone **4** as a white solid. It was further purified by dissolving it in 2:1 boiling THF/CH_2Cl_2 (145 mL) and slowly adding hot hexane (500 mL) to the solution. After the mixture was allowed to cool to ambient temperature, the solid formed was filtered and washed with 20% CH_2Cl_2/hexane (100 mL), and the last traces of solvent were removed under high vacuum. This afforded 13.7 g of pure β-lactone **4** (75% yield, 98.7% pure by reverse-phase HPLC) as a fluffy white solid: mp = 181−183 °C; $[\alpha]^{23}_D = -130.8°$ (*c* 0.25, MeCN); FTIR (KBr pellet) 1834, 1689 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.07 (br s, 1H), 5.26 (d, J = 6.1 Hz, 1H), 3.97 (dd, J = 6.4, 4.4 Hz, 1H), 2.70−2.63 (m, 1H), 2.03 (d, J = 6.4 Hz, 3H), 1.93−1.44 (m, 5H), 1.07 (d, J = 7.0 Hz, 3H), 0.99 (d, J = 7.3 Hz, 3H), 0.91 (d, J = 6.7 Hz, 3H); ^{1}H NMR (300 MHz, pyridine-d_5) δ 10.46 (br s, 1H), 7.86 (d, J = 6.8 Hz, 1H), 5.82 (d, J = 6.1 Hz, 1H), 4.98 (s, 1H), 4.37 (dd, J = 6.7, 3.8 Hz, 1H), 3.06−3.00 (m, 1H), 2.17−2.09 (m, 2H), 1.99−1.82 (m, 1H), 1.61−1.52 (m, 2H), 1.14 (d, J = 6.9 Hz, 3H), 1.04 (d, J = 6.7 Hz, 3H), 0.88 (t, J = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, pyridine-d_5) δ 177.03, 172.37, 80.60, 76.53, 70.74, 44.15, 29.91, 27.41, 21.45, 20.41, 16.64, 14.21; FAB LRMS, *m*/*z* 242 ($M + H^+$). Anal. Calcd for $C_{12}H_{19}NO_4$: C, 59.73; H, 7.94; N, 5.80. Found: C, 59.72; H, 8.00; N, 5.66.

3-(5*S*-Isopropyl-4*S*-methoxycarbonyl-2-phenyl-4,5-dihydro-oxazol-4-yl)-3*S*-hydroxy-2*R*-methyl-*N*,*N*-diethylpropionamide (33). To a cold (−78.8 °C, internal temperature) solution of oxazolidine **10** (2.00 g, 8.10 mmol) in anhydrous THF (40 mL) was added lithium bis(trimethylsilyl)amide (8.9 mL of a 1 M solution in hexane, 8.90 mmol) over 50 min. After 45 min, the orange solution was treated over 40 min with a 1 M solution of dimethylaluminum chloride in hexanes (19.4 mL, 19.4 mmol), and the mixture was stirred for another 60 min before being cooled to −85 °C (internal temperature, liquid N_2 was added to the dry ice/acetone bath). A solution of aldehyde **30** (1.61 g, 10.2 mmol) in THF (8 mL) was then added over 30 min, after which time the mixture was allowed to warm slowly to −20 °C and then cooled again to ca. −60 °C and cautiously quenched by addition of saturated aqueous NH_4Cl (2.0 mL). The mixture was cannulated into a mixture of saturated aqueous NH_4Cl (70 mL) and 1:1 AcOEt/hexanes (50 mL), and 6 N aqueous HCl (12 mL) was then added slowly. After the layers were separated, the aqueous layer was extracted with AcOEt/hexanes (2 × 30 mL), and the combined organic layers were washed successively with 0.5 N aqueous HCl (2 × 30 mL), H_2O (30 mL), 0.4 M $NaHSO_3$ (2 × 30 mL), saturated aqueous $NaHCO_3$ (2 × 30 mL), and brine (35 mL) and then dried over $Na_2SO_4/MgSO_4$ and concentrated in vacuo, affording 3.22 g of an off-white solid that was used as such in the following step. A pure sample was obtained by recrystallization and afforded pure aldol adduct **33** as white crystals: mp 91−92 °C; $[\alpha]^{23}_D = -151.8°$ (*c* 0.50, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02−7.99 (m, 2H), 7.54−7.39 (m, 3H), 6.51 (d, J = 9.9 Hz, 1H), 4.84 (d, J = 2.2 Hz, 1H), 3.74 (s, 3H), 3.67−3.54 (m, 2H), 3.47−3.35 (m, 1H), 3.26−3.12 (m, 2H), 2.97−2.85 (m, 1H), 2.34−2.24 (m, 1H), 1.36−1.29 (m, 6H), 1.12 (d, J = 7.0 Hz, 3H), 1.07 (t, J = 7.4 Hz, 3H), 0.68 (d, J = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.3, 170.4, 164.2, 131.7, 128.5, 128.2, 127.2, 89.4, 82.9, 81.8, 51.9, 42.3, 40.0, 32.4, 29.5, 20.7, 18.3, 14.9, 14.0, 12.8; FAB-MS, *m*/*z* (relative intensity) 405 [$(M + H)^+$, 100]. Anal. Calcd for $C_{22}H_{32}N_2O_5$: C, 65.32; H, 7.97; N, 6.93. Found: C, 65.53; H, 8.10; N, 6.83.

3*S*-Hydroxy-2*R*-(1*S*-hydroxy-2-methyl-propyl)-4*R*-methyl-5-oxopyrrolidine-2-carboxylic Acid Methyl Ester (34). A solution of crude aldol adduct **33** (3.12 g, 7.72 mmol) in 40 mL of 1:9 AcOH/MeOH, to which was added 3.10 g 20% $Pd(OH)_2/C$, was vigorously shaken under 50 psi H_2 for 75 h at ambient temperature. The mixture was then filtered and concentrated in vacuo. The solid obtained was taken up in 10:6:6:1 CH_2Cl_2/THF/AcOEt/MeOH and treated successively with brine (10 mL) and 6 N aqueous HCl (2 mL). The separated aqueous layer was then extracted with 2:2:1 CH_2Cl_2/THF/AcOEt (3 × 15 mL), and the

combined organic layers were treated with a 4.5 M aqueous solution of K_2CO_3 (3.5 mL) and brine (3.0 mL) and then washed with brine (5.0 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude solid was triturated with a mixture of 25 mL of CH_2Cl_2 and 30 mL of hexanes, and the suspended solid was filtered and washed with 30% CH_2Cl_2/hexanes. This afforded 1.27 g of pure desired γ-lactam **34** (67% for two steps) as a white solid: mp 220−221 °C; $[\alpha]^{23}_D$ = +46.6° (*c* 0.50, DMSO); ^{1}H NMR (300 MHz, MeOD) δ 4.47 (d, J = 6.0 Hz, 1H), 3.95 (d, J = 7.2 Hz, 1H), 3.77 (s, 3H), 3.03−2.94 (m, 1H), 1.75−1.63 (m, 6H), 1.10 (d, J = 7.5 Hz, 3H), 1.01 (d, J = 6.6 Hz, 3H), 0.88 (d, J = 6.8 Hz, 3H); ^{13}C NMR (75 MHz, pyridine-d_5) δ 182.2, 172.9, 80.0, 77.0, 76.9, 52.4, 42.5, 32.3, 20.1, 19.4, 8.7; FAB-MS, *m*/*z* (relative intensity) 246 [(M + H)$^+$, 100]; 491 [(2M + H)$^+$, 19]. Anal. Calcd for $C_{11}H_{19}NO_5$: C, 53.87; H, 7.81; N, 5.71. Found: C, 54.03; H, 8.00; N, 5.60.

3*S*-Hydroxy-2*R*-(1*S*-hydroxy-2-methylpropyl)-4*R*-methyl-5-oxopyrrolidine-2-carboxylic Acid (35). To γ-lactam **34** (1.60 g, 6.53 mmol) was added cold (0−5 °C) 0.6 N aqueous NaOH (20 mL, 12.0 mmol). The mixture was stirred at 4 °C for 20 h, acidified to pH = 1−2 with 6 N aqueous HCl, washed with CH_2Cl_2 (2 × 25 mL), frozen, and lyophilized. The obtained solid was transferred into a desiccator containing P_2O_5 and dried under high vacuum for 3 days. It was then suspended in THF, filtered to get rid of sodium chloride, concentrated in vacuo, and triturated with 1:1 CH_2Cl_2/hexanes, affording 1.41 g (93%) of the desired dihydroxy acid **35** as a white solid: $[\alpha]^{23}_D$ = +45.6° (*c* 0.50, DMSO); ^{1}H NMR (300 MHz, pyridine-d_5) δ 9.32 (s, 1H), 5.27 (d, J = 5.9 Hz, 1H), 4.77 (d, J = 6.1 Hz, 1H), 3.79−3.70 (m, 1H), 2.45−2.34 (m, 1H), 1.59 (d, J = 7.4 Hz, 3H), 1.36 (d, J = 6.7 Hz, 3H), 1.31 (d, J = 6.8 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 178.3, 172.6, 77.7, 74.5, 40.4, 30.4, 20.3, 19.0, 8.7; ^{13}C NMR (75 MHz, pyridine-d_5) δ 180.3, 175.1, 79.4, 77.4, 76.9, 42.1, 32.1, 21.0, 18.8, 9.4; FAB-MS, *m*/*z* (relative intensity) 232 [(M + H)$^+$, 100]. Anal. Calcd for $C_{10}H_{17}NO_5$: C, 51.94; H, 7.41; N, 6.06. Found: C, 51.83; H, 7.54; N, 5.97.

[1*R*-[1*S*,4*R*,5*S*]]-1-(1-Hydroxy-2-methylpropyl)-4-methyl-6-oxa-2-azabicyclo[3.2.0]heptane-3,7-dione (2) [*clasto*-Lactacystin β-Lactone). A cold (0−5 °C) solution of dihydroxy acid **35** (700 mg, 3.03 mmol) in anhydrous THF (15 mL) containing triethylamine (0.65 mL, 4.66 mmol) was treated dropwise with isopropenyl chloroformate (0.35 mL, 3.18 mmol). After being stirred for 5 min at 0−5 °C, the mixture was allowed to reach ambient temperature, stirred for another 70 min, and diluted with diethyl ether (20 mL). The mixture was stirred for 10 min, filtered, and concentrated in vacuo. Purification on a SiO_2 pad, eluting with AcOEt, afforded 640 mg of the lactone, which was further purified by trituration with 1:1 $CHCl_3$/hexanes. This afforded 508 mg (79%) of pure β-lactone **2** as a white solid: mp = 184−185 °C dec, lit.[11c] mp = 185 °CC; $[\alpha]^{23}_D$ = −93.8° (*c* 0.50, MeCN), lit.[11c] $[\alpha]^{23}_D$ = −93.9° (*c* 0.53, MeCN)];^{1}H NMR (300 MHz, pyridine-d_5) δ 10.04 (s, 1H), 7.85 (d, J = 6.8 Hz, 1H), 5.68 (d, J = 6.1 Hz, 1H), 4.97 (s, 1H), 4.35 (dd, J = 6.7, 3.7 Hz, 1H), 3.10−3.00 (m, 1H), 2.17−2.06 (m, 1H), 1.47 (d, J = 7.5 Hz, 3H), 1.12 (d, J = 6.9 Hz, 3H), 1.01 (d, J = 6.7 Hz, 3H); ^{13}C NMR (75 MHz, pyridine-d_5) δ 177.3, 172.3, 80.4, 76.9, 70.5, 38.8, 29.8, 20.3, 16.4, 8.7; FAB-MS, *m*/*z* (relative intensity) 214 [(M + H)$^+$, 100], 427 [(2M + H)$^+$, 17]. Anal. Calcd for $C_{10}H_{15}NO_4$: C, 56.33; H, 7.09; N, 6.57. Found: C, 56.22; H, 7.21; N, 6.38.

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Supporting Information Available: ^{1}H and ^{13}C spectra for compounds **4**, **8**, and **10** and X-ray crystallographic data of **8**, including tables of atomic coordinates, bond angles, and bond lengths (PDF). X-ray crystallographic data, in CIF format, are also available. This material is available free of charge via the Internet at http://pubs.acs.org.

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