Syntheses and Biological Evaluation of (+)-Lactacystin and Analogs

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Since its isolation in 1991, (+)-lactacystin (**1**) has attracted considerable attention among leading synthesis laboratories due to its highly selective and potent inhibition of the 20*S* proteasome. The syntheses of this molecule described herein demonstrate several important strategies in the area of acyclic stereocontrol including the use of chiral metal enolate and chiral allylmetal-based bond construction methods. Several analogs of **1** and of the related β-lactone **2** are also presented, which provide insight into the structure activity relationship relative to the molecule's inhibition of the 20*S* proteasome. Additionally, an analog of **2** is discussed regarding its clinical evaluation for the treatment of cerebral ischemia and stroke.

Biological Activity of Lactacystin

(+)-Lactacystin (**1**, Figure 1) is a secondary metabolite derived from a streptomyces bacterial strain (OM-6519) found in a Japanese soil sample. Isolation of the natural product from a culture broth was performed by Omura and colleagues at the Kitasato Institute.[1] The isolation procedure made use of standard chromatographic techniques. Fractions were screened for activity using a cellular bioassay. Differentiation of the mouse neuroblastoma cell line, Neuro 2A, was characterized by a morphological change in which the cells exhibited a bipolar extended growth pattern.

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Craig E. Masse was born in 1971 in Lowell, MA. He received his B. S. degree (summa cum laude) in chemistry in 1994 from the University of Massachusetts-Lowell where he did undergraduate research with Professor Sukant K. Tripathy. Since 1994, he has been pursuing a Ph. D. in synthetic organic chemistry under the direction of Professor James S. Panek. His research efforts have focused on the asymmetric synthesis of poly(propionate) and unusual amino acid-containing natural products utilizing chiral crotylsilane bond construction methodology. Upon graduation from Boston University, he will pursue postdoctoral studies in the laboratories of Professor David A. Evans at Harvard University.

Julian Adams received his bachelor's degree in science at McGill University and completed doctoral studies at the Massachusetts Institute of Technology in the field of synthetic organic chemistry. Following a post-doctoral fellowship with Professor Gilbert Stork at Columbia University, he joined the laboratories of Merck in Montreal, in 1982, where he worked in the area of lung diseases studying the leukotriene mediators in the arachidonic acid pathway. In 1987, Dr. Adams left Merck to become the associate director of medicinal chemistry at Boehringer Ingelheim Pharmaceuticals in Montreal where he set up the antiviral program looking specifically at herpes virus ribonucleotide reductase inhibition. Rising through the ranks, Dr. Adams was invited to become the director of medicinal chemistry at Boehringer's main laboratory headquartered in Ridgefield, CT. His responsibilities included antiviral programs concentrating on HIV and human rhinovirus, and inflammation-immunology, working in cell adhesion, T-cell regulation, and leukotriene biosynthesis. He was also responsible for the process chemistry division comprising of a GMP synthesis pilot-plant, as well as computational chemistry and X-ray crystallography. Significant contributions include the discovery and development of Nevirapine (VIRAMUNE), the first non-nucleoside reverse transcriptase inhibitor to be approved for treating HIV infection. He also championed the discovery of a novel leukotriene biosynthesis inhibitor, BI-RM-270, which is currently in Phase II clinical trails, for the treatment of asthma. In the spring of 1994, Dr. Adams joined ProScript, Inc (now part of Millennium Pharmaceuticals) where he currently serves as the Senior Vice President of Research and Development. In that capacity, Dr. Adams led the company to file an IND for proteasome inhibitor, PS-341 a novel anti-cancer drug, which began clinical trials in October 1998. A second clinical candidate, PS-519 for the treatment of stroke, began trials in November 1999. In September 1994 Dr. Adams was named Adjunct Professor of Chemistry at Boston University.

James S. Panek was born in Buffalo, NY in 1956. He received his B.S. degree from the State University of New York at Buffalo in 1979 and his Ph.D. in Medicinal Chemistry from the University of Kansas in 1984 under the supervision of Professor Dale Boger. After postdoctoral study with Professor Samuel Danishefsky at Yale University as an NIH Postdoctoral Fellow, he assumed the position of Assistant Professor of Chemistry at Boston University in 1986. His research interests are primarily concerned with the area of organic synthesis. His research group is engaged in the development of new reaction methodology concerned with acyclic stereocontrol as well as the application of these methods to the asymmetric synthesis of natural products and complex organic molecules.

Adam J. Morgan was born in 1978 in Phoenix, AZ. He is currently a senior at Boston University working toward a B. A. degree in chemistry where he is engaged in undergraduate research in the laboratories of Professor James S. Panek. For the past two summers, he has been the recipient of a Pfizer PREPARE fellowship. His research efforts include the asymmetric syntheses of natural products containing α-amino-β-hydroxyacid functionalities. Upon graduation, he plans on pursuing a career in the pharmaceutical industry.

MICROREVIEWS: This feature introduces the readers to the authors' research through a concise overview of the selected topic. Reference to important work from others in the field is included.

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This was potently enhanced by treatment with increasingly pure fractions containing (+)-lactacystin. Ultimately, the structure and absolute configuration of the natural product was determined by NMR spectroscopy and an X-ray crystallographic analysis.[2] (+)-Lactacystin was initially considered a neurotrophic agent, but has since found application in models of arthritis, ischemia, and asthma. As such, (+)-lactacystin possesses enormous potential as a therapeutic agent.

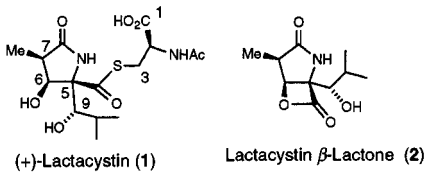


Figure 1. Structures of (+)-lactacystin and (+)-lactacystin-β-lactone

Further interpretation of the biological activity of **1** was to come with the discovery by Fentaeny and co-workers of the molecular target, the proteasome, which is responsible for intracellular protein degradation.[3] The ubiquitin proteasome pathway (UPP), present in all eukaryotic cells, consists of two separate biochemical steps which ultimately lead to protein degradation (Figure 2).[4]

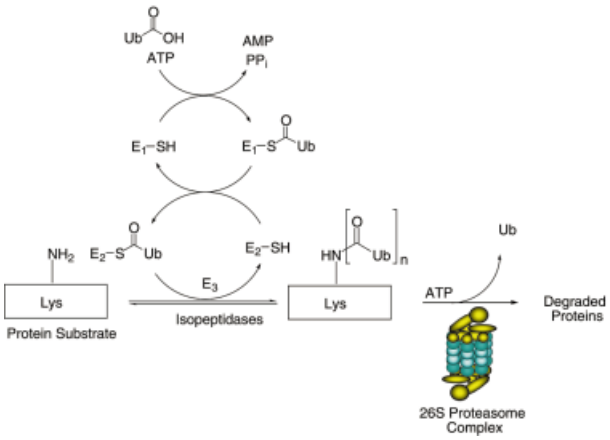


Figure 2. Ubiquitin-proteasome pathway

Through a regulated and coordinated series of enzymatic reactions, ubiquitin is covalently linked to the ε-amine of lysine residues in proteins in a processive manner producing multiubiquitinated chains. A protein once tagged with ubiquitin conjugates is destined for eventual degradation by the multi-catalytic protease, the 26*S* proteasome (Figure 3). The ubiquitin proteasome pathway thus regulates the level of intracellular proteins. Previously, the UPP was thought to be merely a disposal system for damaged intracellular proteins. However, more recent evidence indicates that the UPP is also exclusively responsible for the turnover of many

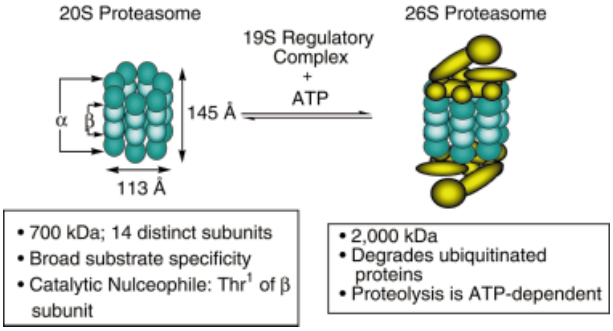


Figure 3. Structures of the 20*S* and 26*S* Proteasomes

regulatory proteins which govern a diverse array of cellular functions.

Thus, the biological activity first observed for (+)-lactacystin is probably due to an effect on the cell cycle machinery which is temporally regulated by the UPP. Inhibition of the proteasome leads to the stabilization of cyclins, cyclin-dependent kinase inhibitors (CDKIs), and tumor suppressor proteins (e.g. p 53). In Neuro 2A cells, which are terminally differentiated, the expected result is de-differentiation to the bipolar phenotype observed.

A host of additional biological activities have been reported for (+)-lactacystin.[5] Notably, numerous reports of selective proteasome inhibition using (+)-lactacystin confirmed an earlier finding that activation of the transcription factor, NF-κB, may be blocked by stabilizing the inhibitor protein IκB. Signal induced phosphorylation of IκB recruits the ubiquitination machinery which directs the IκB for degradation by the proteasome (Figure 4). NF-κB drives the transcription of many pro-inflammatory cytokines (IL-1, TNFα), enzymes (COX-2, iNOS), and the cell adhesion molecules, all of which conspire to mount inflammatory responses. The importance of this finding relates to the potential use of (+)-lactacystin and its analogs as anti-inflammatory agents to treat autoimmune disease and acute injury (vide infra).

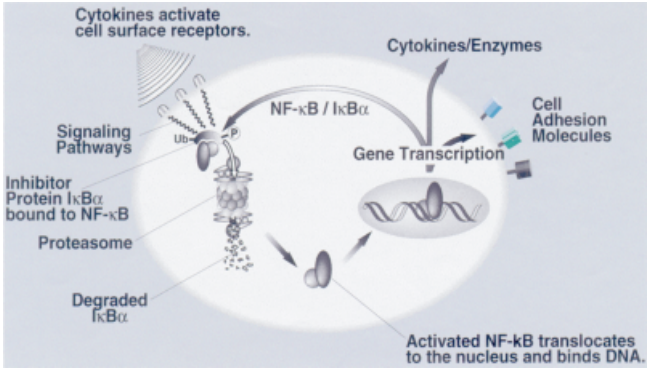


Figure 4. Mechanism of NF-κB activation

Seminal experiments by Fentaeny and co-workers as well as the elegant crystallographic work of Huber[6] clearly defined the mode of inhibition of (+)-lactacystin. A covalent bond in the form of an oxygen ester is formed between the C4 carbonyl and the Thr1-OH of the X subunit (chymotryptic site) of the proteasome. Huber had previously de-

fined the catalytic site of the proteasome as a threonine protease. Kinetic analysis shows that (+)-lactacystin and its synthetic precursor *clasto*-lactacystin-β-lactone (**2**) are time-dependent inactivators of the proteasome.

Further analysis of the (+)-lactacystin structure and detailed biochemical experiments by Dick and co-workers further elucidated that the natural product, a thiol ester, was in fact a pro-drug for the true inhibitor, (+)-lactacystin-β-lactone **2** (Figure 5).[7]

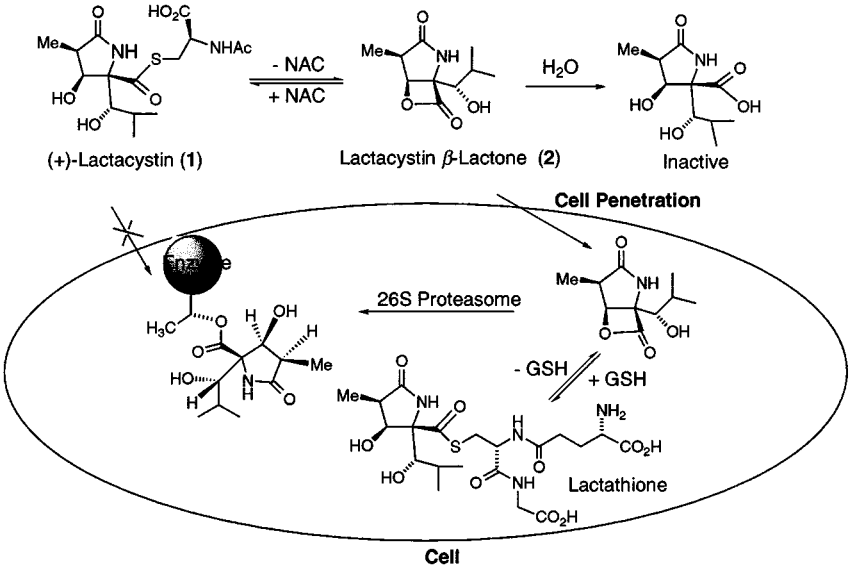


Figure 5. Mechanism of proteasome inactivation by lactacystin (in vivo)

This research showed that **1** spontaneously eliminates *N*-acetyl cysteine in a reversible manner to form **2**, which is the only species that penetrates the cell. Once inside the cell, **2** suffers one of three fates: i) inhibition of the proteasome; ii) formation of a thiol ester with glutathione (lactathione); iii) aqueous hydrolysis with water ($t_{1/2} \approx 15$ min). Owing to the high concentration of glutathione in cells (1−10 mM), the predominant species upon addition of (+)-lactacystin is, in fact, the glutathione adduct which functions as a reservoir for the drug. Paradoxically, although (+)-lactacystin forms a covalent ester bond via the proteasome threonine OH, this ester is subject to aqueous hydrolysis, and inhibition of the proteasome is temporary with full enzymatic activity restored in a matter of hours ($t_{1/2} \approx 30$ min). The unique biological activity of (+)-lactacystin and its structural complexity have made (+)-lactacystin and its analogs attractive targets for synthetic efforts. This review will focus on the synthetic efforts to date[8] and summarize the structure-activity relationships which have been elucidated for various analogs of (+)-lactacystin-β-lactone (**2**).

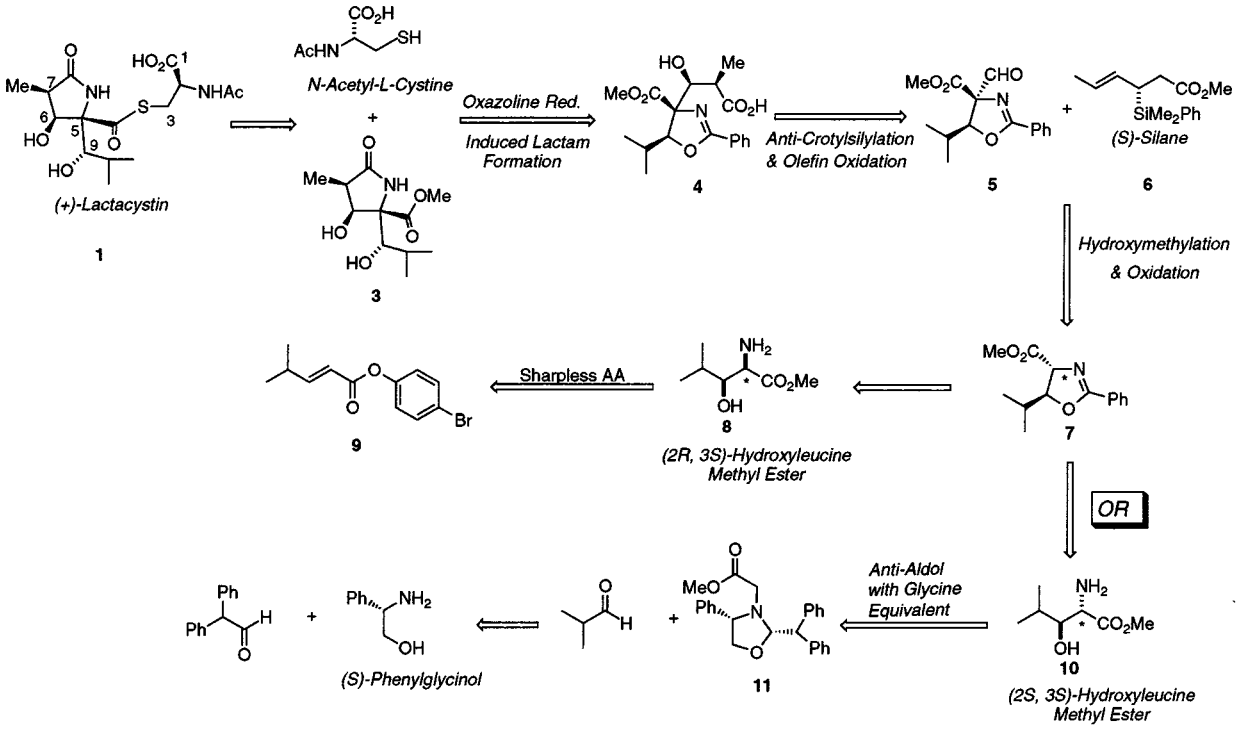
Studies on the Total Synthesis of Lactacystin

Panek Synthesis

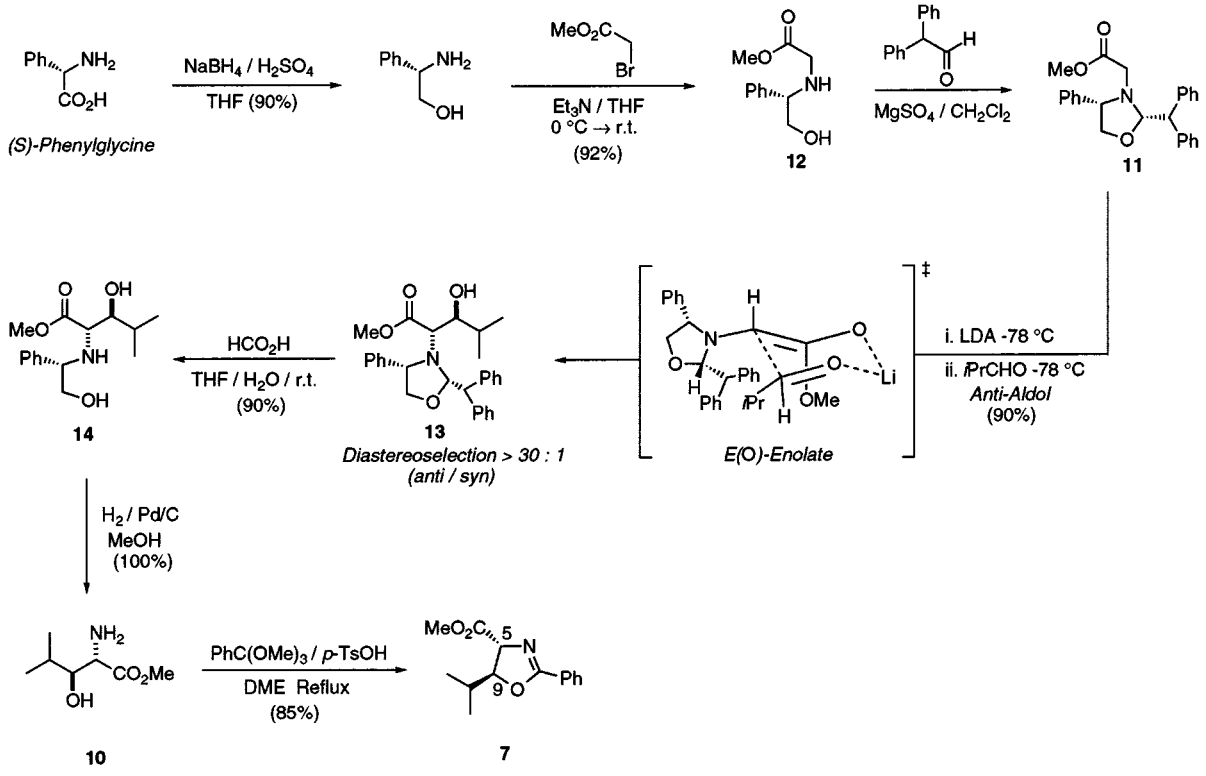
Studies from our laboratories directed at the synthesis of (+)-lactacystin (**1**) are outlined in retrosynthetic form in Scheme 1. Our approach involves the stereoselective generation of heterocyclic aldehyde **5** derived from 3-hydroxyleucine synthon **8** prepared via Sharpless asymmetric aminohydroxylation of olefin **9**. Generation of the critical C6−C7 stereocenters of **1** is accomplished by a highly stereoselective asymmetric crotylsilane addition to aldehyde **5**.[9] Our initial approach to the hydroxyleucine synthon centered around the preparation of the (2*S*,3*S*)-3-hydroxyleucine methyl ester (**10**). The construction of this synthon was envisioned to arise from a stereoselective *anti*-aldol reaction of a chiral oxazolidine (**11**) derived from (*S*)-phenylglycinol as outlined retrosynthetically in the bottom portion of Scheme 1.

Preparation of the oxazolidine auxiliary began with the *N*-alkylation of (*S*)-phenylglycinol with methyl bromoacetate to afford **12** (Scheme 2).[10] Condensation of this material with diphenylacetaldehyde and anhydrous magnesium sulfate at ambient temperature afforded 2,4-disubstituted oxazolidine **11** as a single diastereomer which served as the chiral glycine equivalent for the subsequent aldol.

The *anti*-selective aldol reaction between the lithium enolate of the phenylglycinol-derived oxazolidine **11** with isobutyraldehyde afforded the aldol product **13** as a single diastereomer ($dr > 30{:}1$ *anti*/*syn*). The aldol bond construction of such α-amino esters has been shown to exhibit high levels of *anti*-stereoselection (simple diastereoselectivity).[11] The stereochemical outcome of this aldol condensation is critically dependent on the geometry of the glycine enolate. However, there are conflicting literature precedents regarding the conformational preference of such glycine enolates.[12] The high levels of *anti*-stereoselection observed in this aldol reaction can be rationalized by a chair-like transition state via the *E*(O)-enolate as shown in Scheme 2. The amino alcohol **13** was then treated with formic acid to hydrolyze the oxazolidine and afford **14**. Subsequent heterogeneous hydrogenolysis to remove the phenylglycinol-de-



Scheme 1

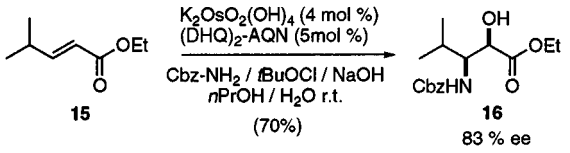


Scheme 2

rived amino protecting group afforded **10**, which was then cyclized by treatment with trimethylorthobenzoate and *p*-toluenesulfonic acid to give oxazoline **7**. While this enolate-based approach did provide access to useful amounts of the chiral oxazoline, we sought to develop a more streamlined approach to **7**.

In that regard, a more efficient synthesis of the 3-hydroxyleucine synthon was investigated, which was based on the Sharpless asymmetric aminohydroxylation (AA) of olefins.[13] This new approach was contingent upon the proper regiochemical outcome to furnish the α-amino-β-hydroxy ester, thereby establishing the C9 stereogenic center

of **1**. The correct stereochemistry at C5 would then be set at a later stage in the synthesis. Sharpless and co-workers have previously shown that cinnamates provided the desired β-hydroxy-α-amino esters when the $(DHQ)_2$-AQN ligand is utilized. At the time of our initial investigation, the regiochemical outcome of the AA process for aliphatic-substituted olefins of type **15** (Scheme 3) was unknown with the recently available $(DHQ)_2$-AQN ligand.



Scheme 3

The recent work of Janda and co-workers[14] with allylic alcohol substrates and the $(DHQD)_2$-PHAL ligand has shown that the steric bulk and the electronic properties of the substituents on either side of the olefinic substrate can be used to control the regiochemistry of the AA reaction. Despite the recent discovery of a method for obtaining β-hydroxy-α-aminocinnamate esters,[5b] the development of routes to enantiomerically pure β-alkyl-substituted β-hydroxy-α-amino acid derivatives using the AA process remains an active area of research. We decided to investigate if this trend of the reversal of regiochemistry was applicable to the required acrylate ester in an effort to further understand the nature of these electronic effects.

Our initial experiments with aliphatic substrates utilized alkyl esters (**15**, Scheme 3) with the $(DHQ)_2$-AQN ligand. However, this reaction afforded the undesired β-amino-α-hydroxy ester (**16**) as the sole regioisomeric product with high levels of enantioselection (83% *ee*), consistent with literature precedent for substrates of this type with the $(DHQ)_2$-PHAL ligand.[13a] With the presumption that the interactions of the aromatic group of the cinnamates and the $(DHQ)_2$-AQN alkaloid ligand used in the Sharpless investigation contributed to the regiochemical course of the reaction, a series of *p*-substituted aryl esters of type **9** were screened in the AA using the $(DHQ)_2$-AQN ligand.[15] These substrates proved to affect the regioselectivity pattern of the AA reaction to provide the β-hydroxy-α-amino ester as the major regioisomeric product. The turnover in regioselection for these aryl esters appears to rely on subtle changes in the substrate−ligand recognition event.

In order to more fully investigate the electronic contributions of the aryl esters on the aminohydroxylation reaction, a series of *p*-substituted aryl esters was surveyed for use in a Hammett-type analysis (Figure 6).[16]

These data suggested an empirical correlation between the electronic nature of the aryl ester and the enantioselection of the aminohydroxylation reaction. In general, electron-donating groups provided higher levels of enantioselection than electron-withdrawing groups, which, if sufficiently electron-withdrawing (e.g. *p*-NO_2), rendered the substrate unreactive. Interestingly, the *p*-halo-substituted esters were found to have a unique effect on the AA reaction providing higher levels of regio- and enantioselection than

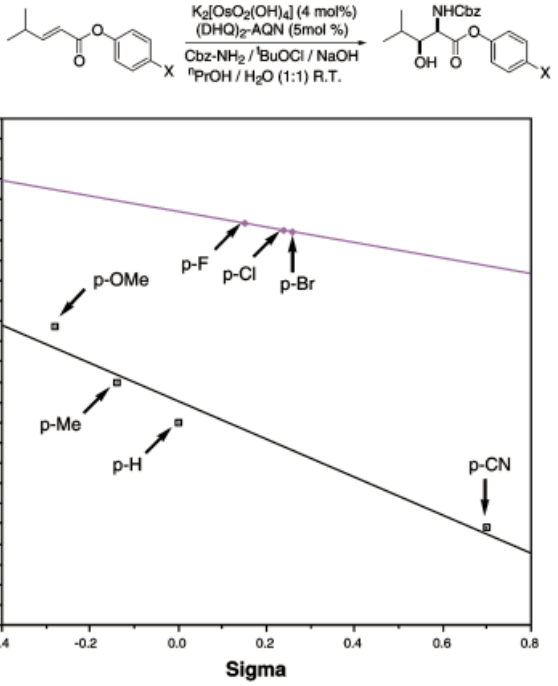


Figure 6. Hammett-type analysis for the aminohydroxylation process

other substituents. Indeed, the halogens defined a separate linear correlation distinct from that of the other functional groups. This may indicate a subtle change in the recognition of the substrate within the catalyst complex. The negative slope associated with both the halo-substituted esters and the other substituents surveyed indicates that electron-donating groups stabilize the developing partial positive charge on the olefinic carbons in the transition state, thus accelerating the reaction rates. The type of halogen atom utilized also seems to play a key role in the observed regioselection of the reaction, with the bromo-substituted aryl ester being the optimal substrate for the cases examined. An iodo-substituted aryl ester proved unreactive even after prolonged exposure (48 hours) to the reaction conditions. This may indicate a steric requirement for entry of the olefinic substrate into the active site of the catalyst.

The reversal of regioselection may arise from a conformational change induced by the aryl ester functionality. In order to determine the solution conformation of the aryl ester substrates, a series of NOE experiments was carried out with substrates **9** and **15**. These NOE experiments were performed in $[D_4]$methanol to mimic the alcoholic conditions of the AA reaction. Substrate **9** exhibited a 6% NOE between the β-olefinic proton and the *ortho* protons of the aryl ester (H^4 and H^1, respectively, see Figure 7).

The observation of this NOE confirms the population of an *s-trans*-type conformer which positions the *ortho* protons of the aryl ester (H^1) in close proximity to the β-olefinic proton (H^4).[17] Not surprisingly, NOE experiments on the ethyl ester substrate (**15**) showed no observable NOEs upon irradiation of either the β-olefinic proton or the methylene protons of the ethyl ester in agreement with the literature precedent of Wiberg and co-workers on the conformation

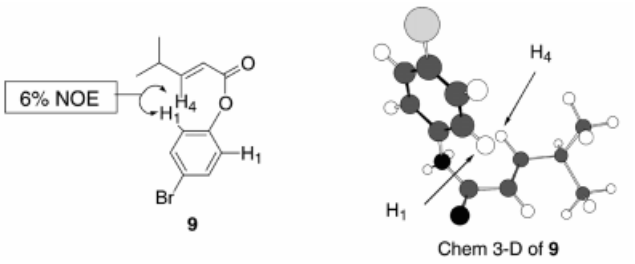


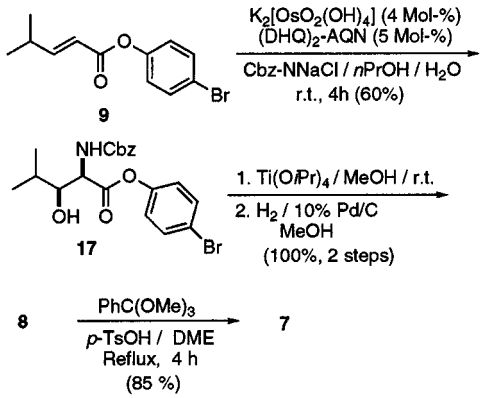
Figure 7. Solution conformation and 3-D minimized view of substrate **9**

of such alkyl esters.[18] Wiberg has shown that the alkyl ester substrates prefer an *s-cis* conformer with a *cis* orientation of the alkyl group relative to the carbonyl moiety. This conformer minimizes dipole−dipole interactions. The altered conformation of the alkyl ester substrates might allow for the assembly of a productive π-complex between the osmium bound to the alkaloid ligand, the aryl moiety of the ester, and the anthroquinone ring of the ligand.[19] This orientation of the substrate-catalyst complex may account for the turnover in regioselectivity of the AA leading to the formation of the β-hydroxy-α-amino ester.

This study of an Os^{VIII}/$(DHQ)_2$-AQN-promoted asymmetric aminohydroxylation of α,β-unsaturated aryl esters provides an advantageous approach to β-alkyl-β-hydroxy-α-amino acid derivatives using a convenient procedure and a readily available catalyst system. Presently, this AA process affords useful quantities of **8** with high levels of both regio- and stereoselection. Furthermore, our preliminary investigation clearly indicates a trend for the reversal of AA regioselection by simple variation of the ester moiety, which apparently alters the substrate-ligand recognition event. In this context, we have learned that subtle alterations of the substrate can be an effective alternative to catalyst modification. It has also been shown that the level of enantioselection is controlled, at least in part, by the electronic properties of the aryl ester functionality on the olefinic substrate. Most importantly, this AA protocol installs the C5 and C9 stereocenters of **1** in a single step with high levels of enantioselectivity. At this stage, we returned to the installation of the C6 and C7 stereogenic centers of **1** and the completion of the synthesis.

With a highly efficient synthesis of 3-hydroxyleucine synthon in hand, we next proceeded to the formation of *trans*-oxazoline **7** (Scheme 4). The aminohydroxylation of **9** with $(DHQ)_2$-AQN and the benzylcarbamate-based Sharpless AA gave **17** with good levels of regioselection (7:1) favoring the α-amino ester and high levels of enantioselection (87% *ee*). The ratio of regioisomers was determined by ^1H NMR analysis of the crude product and the initial *ee* of 87% could be raised to > 99% by a single recrystallization from EtOH/H_2O (1:1).

Subsequent transesterification of **17** to the methyl ester in the presence of $Ti(OiPr)_4$ and removal of the benzyloxycarbonyl group by hydrogenolysis afforded **8**. Treatment of **8** with trimethylorthobenzoate in the presence of *p*-toluenesulfonic acid provided the *trans*-oxazoline **7**. The preparation of the heterocyclic aldehyde **5** has been accomp-

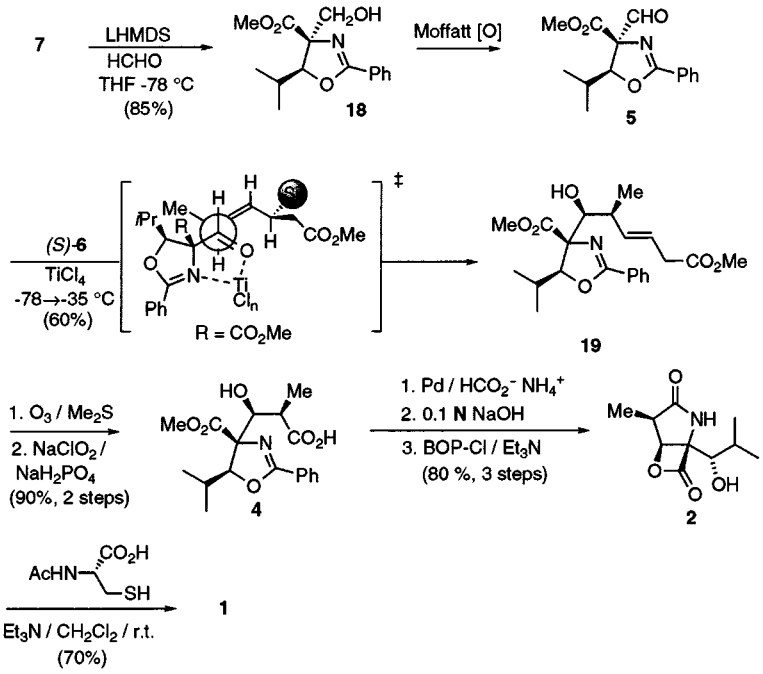


Scheme 4

lished according to literature precedent established by Smith.[20] Oxazoline **7** was subjected to an aldol condensation with formaldehyde according to the Seebach protocol to afford the primary alcohol **18** as a single diastereomer (Scheme 5). The topological bias for this ester enolate was presumably controlled by the chirality of the oxazoline where the bulky isopropyl group functions as the controller of diastereoselectivity. Oxidation of the primary alcohol using the Moffatt procedure (DCC, DMSO, pyridine, TFA) provided the desired heterocyclic aldehyde **5**, which was used without purification as any attempt to purify this product resulted in deformylation. With the requisite aldehyde in hand, the critical *anti*-selective crotylation reaction with (*S*)-**6** was carried out to establish the C6−C7 stereochemical relationship. This double stereodifferentiating[21] reaction was readily accomplished using $TiCl_4$ to afford the homoallylic alcohol **19** with high levels of diastereoselection (*dr* > 30:1 *anti*/*syn*) and in 50−60% yield. This *anti*-bond construction was presumably achieved through simultaneous coordination of the aldehyde carbonyl and the nitrogen of the oxazoline ring. The 1,3-relationship of the heteroatoms ideally predisposes the more Lewis basic nitrogen relative to the aldehyde carbonyl to generate a five-membered chelate with the bidentate Lewis acid $TiCl_4$ via the illustrated synclinal transition state (Scheme 5).[22] Oxidative cleavage of the (*E*)-olefin of **19** under standard ozonolysis conditions and subsequent oxidation with sodium chlorite furnished carboxylic acid **4**.

The completion of our synthesis of (+)-lactacystin was initiated by catalytic transfer hydrogenation of the oxazoline moiety of **4** using Pd-black to give the γ-lactam methyl ester after cyclization. Saponification of the methyl ester under mild conditions afforded the dihydroxyacid which was directly converted into β-lactone **2** by treatment with bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOPCl).

To attach the *N*-acetyl-L-cysteine side chain, we employed the lactone opening strategy developed by Corey.[23] Treatment of **2** with *N*-acetyl-L-cysteine/Et_3N furnished synthetic **1** in 13 steps with an overall yield of 13%. The virtues of this synthetic approach are apparent from the concise nature of the synthetic sequence leading to the critical hydroxyleucine synthon (**8**), the mild reaction conditions, and the fact that either enantiomer of **8** can be pre-



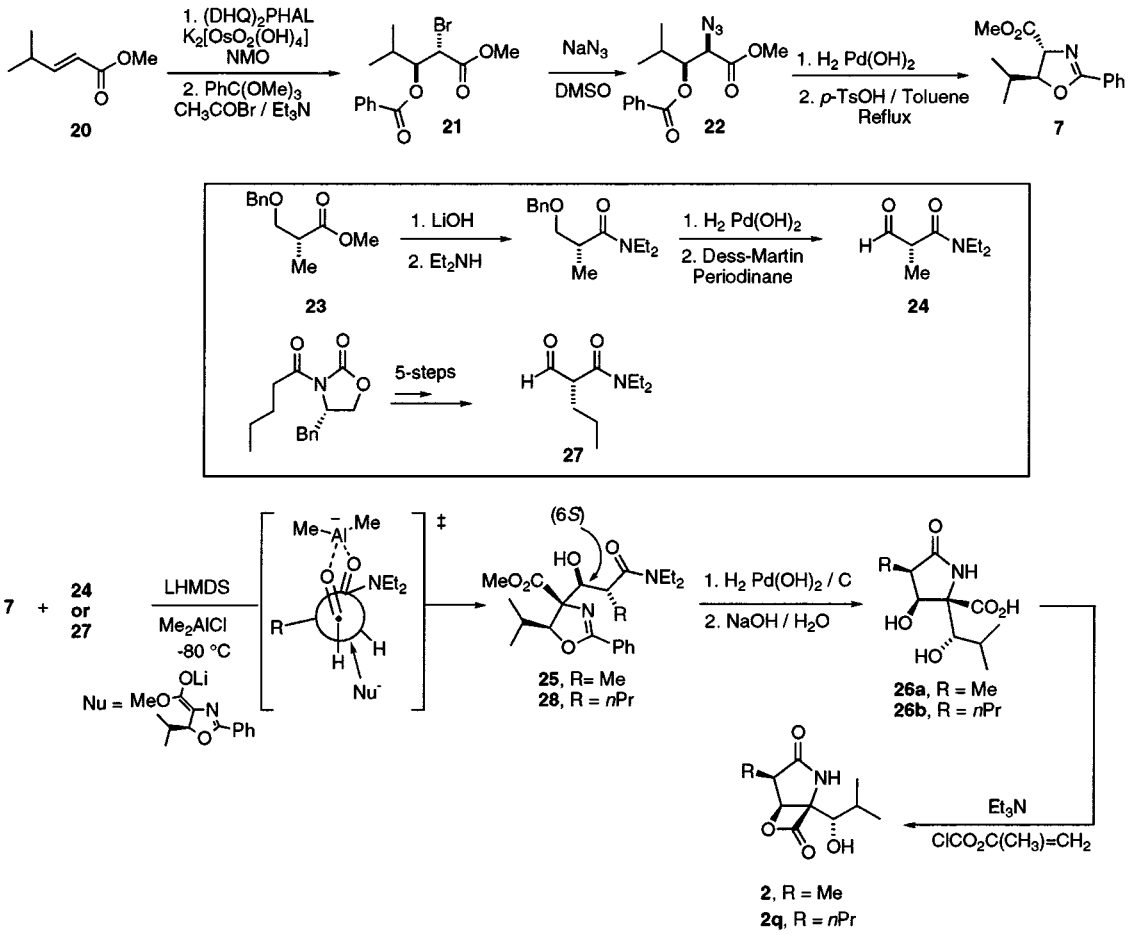
Scheme 5

pared by the proper choice of the alkaloid ligand. The synthesis also allows for a highly stereoselective approach to the construction of the C6−C7 aldol bond using the (*E*)-crotylsilane reagents. Additionally, structural modifications to the parent **1** are easily accessible using our chiral silane-based methodology. The C7 alkyl group may be controlled by the choice of the appropriate γ-substituted silane reagent, which can be readily accessed using the methodology developed in our laboratories.[24] The C9 isopropyl group may be altered by simply changing the olefin used in the AA reaction (Scheme 4) to provide the appropriately substituted oxazoline. Finally, the absolute configuration of the C6 hydroxyl group will be controlled by the choice of Lewis acid in the crotylsilane addition reaction (Scheme 5). The use of the monodentate Lewis acid $BF_3{\cdot}OEt_2$ in the addition reaction will provide the complimentary *syn* aldol bond construction to give the (6*R*)-derivative.

Millennium Synthesis

An interesting approach to **2** has recently been reported by researchers at Millennium, Inc (Cambridge, MA) utilizing a double stereodifferentiating aldol bond construction between the known oxazoline **7** and a β-amido aldehyde (Scheme 6).[25] One of the key features of the LeukoSite approach is the ability to preserve the correct oxidation state at C8, thereby avoiding protection/deprotection and/or oxidation state adjustments late in the synthesis.

The Millennium synthesis begins with oxazoline **7** which is prepared from methyl-4-methyl-(*E*)-pentenoate (**20**). Sharpless catalytic asymmetric dihydroxylation of olefin **20** with AD-mix-β afforded the chiral diol (70% *ee*) which was subsequently treated with trimethylorthobenzoate to form an intermediate cyclic orthoester which, in turn, is reacted with acetyl bromide to form bromohydrin **21**. The required α-amino moiety was then introduced via nucleophilic displacement with sodium azide to afford the azido benzoate **22**. Hydrogenation of **22** with Pearlman's catalyst afforded the (2*R*,3*R*)-hydroxyleucine derivative which underwent acid-catalyzed cyclization in refluxing toluene to afford oxazoline **7**. The required β-amido aldehyde **24**, needed for aldol coupling to oxazoline **7**, was prepared in a straightforward four-step sequence from the known ester **23**.[26] The critical aldol coupling of **7** and **24** was accomplished by treatment of the lithium enolate of **7** with dimethylaluminum chloride (Me_2AlCl) followed by addition of **24** to provide aldolate **25** with high levels of diastereoselection favoring the (6*S*)-isomer. The observed stereochemistry has been explained in terms of a chelate-organized transition state where the aluminum serves as a bidentate Lewis acid coordinating both the aldehyde and amido-carbonyl groups. Nucleophilic addition of the enolate from the less hindered *re*-face predicts a (6*S*)-stereochemistry (Scheme 6). Furthermore, the C5 stereochemistry is controlled by the topological bias of the substrate, where the bulky isopropyl group at C9 directs the newly forming C5−C6 bond to the bottom face of the oxazoline. Unmasking of the oxazoline under hydrogenolyisis conditions and cyclization of the resultant aminoamide gives the γ-lactam which is subjected to saponification conditions to afford the dihydroxy acid **26a**. β-Lactonization of **26a** with isopropenyl chloroformate afforded **2**. The Millennium researchers have utilized the synthetic sequence outlined in Scheme 6 to prepare a C7-analog of **2** via the aldolate **28**. In particular, they have prepared a C7 *n*-propyl analog (**2q**) by utilizing aldehyde **27** in the diastereoselective aldol bond construction. Aldehyde **27** was prepared in a five-step sequence using an Evan′s asymmetric alkylation of an *N*-butanoyloxazolidinone with benzyloxymethyl chloride (BOM-Cl) as the key step. The *n*-



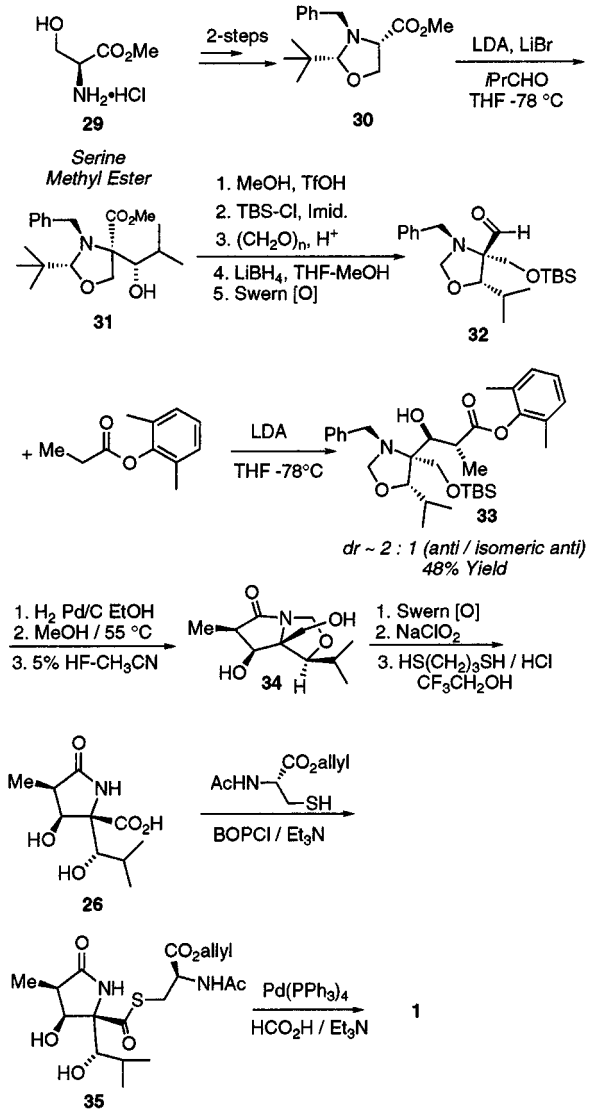
Scheme 6

propyl analog (**2q**, PS-519) proved to be four times more effective at proteasome inhibition than the parent lactone **2**. PS-519 has been tested in a variety of animal models for inflammation and demonstrated good efficacy. PS-519 is cardioprotective following ischemia and reperfusion injury.[25] The same finding was observed for cerebral ischemia. In models of immune-elicited inflammation, PS-519 blocks leukocyte infiltration in a rat model of asthma and has been shown to act synergistically with cortico-steroids to block the inflammatory response. In a model of multiple sclerosis in mice, PS-519 reduced the severity and increased the relapse time in experimental autoimmune encephalomyelitis. In several of these models, it was clearly shown that the action of the proteasome inhibitor is probably mediated through the inhibition of NF-κB activation. The unique characteristics of PS-519 have prompted extensive pre-clinical investigations including toxicology testing and formulation. The compound is currently in Phase I clinical trials for the treatment of cerebral ischemia and stroke.

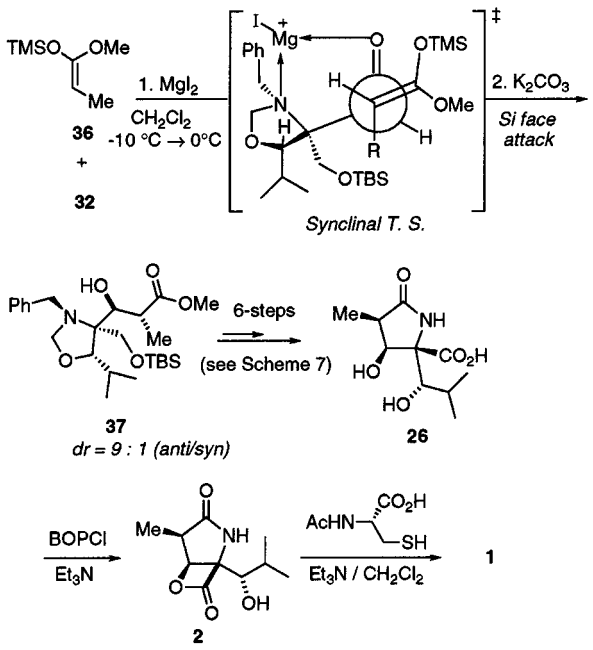
Corey Syntheses

Prior to our efforts in this area, a small number of research groups had been engaged in synthetic programs aimed at the synthesis of (+)-lactacystin and its analogs. The first total synthesis of (+)-lactacystin (**1**) was achieved by Corey and Reichard in 1992;[27] subsequent refinements to this approach have since been reported by the Corey group. Indeed, the first published synthetic efforts have come from the laboratories of Professor Corey and coworkers and are clearly illustrative of the degree of difficulty associated with this stereochemically dense natural product. The original synthetic approach (Scheme 7) utilized the *cis*-oxazolidine derivative (**30**) which is derived from the (*S*)-serine methyl ester (**29**) in a three-step sequence.

Oxazolidine **30** undergoes a highly stereoselective aldol reaction via the lithium enolate−lithium bromide complex with isobutyraldehyde to afford, after recrystallization, the diastereomerically pure aldolate **31**. This stereoselective aldol installs the C9 stereocenter of (+)-lactacystin. Methanolysis of the aminal moiety in **31**, protection of the resulting primary hydroxyl group as its TBS ether, formation of a topologically different methylene *N,O*-acetal by an acid-catalyzed condensation with formaldehyde, and conversion of the methyl ester to an aldehyde (i. $LiBH_4$; ii. Swern [O]) affords oxazolidine **32**. The *anti*-aldol reaction with aldehyde **32** to establish the C6 and C7 stereogenic centers of **1** was originally accomplished using the Pirrung−Heathcock *anti*-aldol conditions (lithium enolate of 2,6-dimethylpropionate).[28] This transformation provided the *anti*-aldol product **33** in moderate yields but with



Scheme 7



Scheme 8

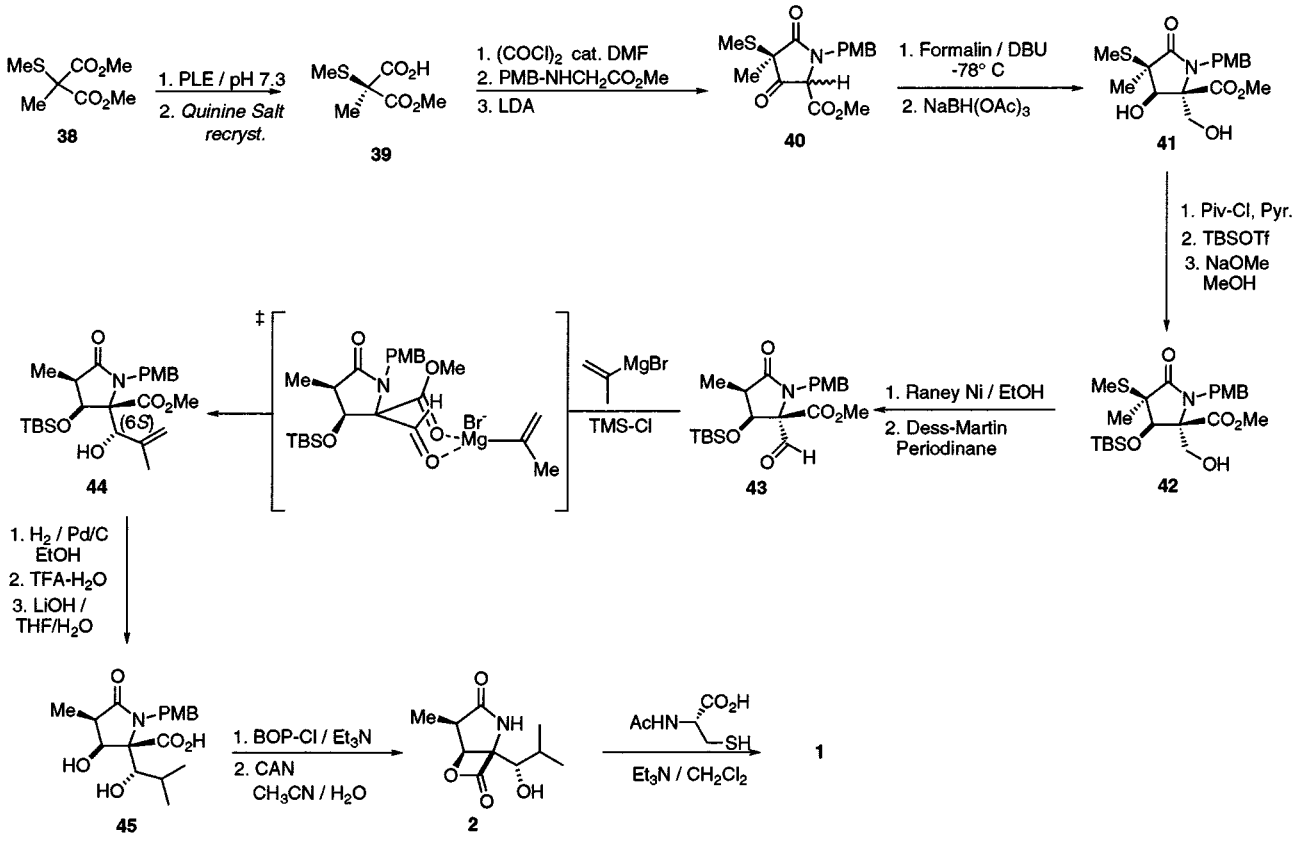
low levels of induction (*dr* ≈ 2:1). A later modification of this aldol bond construction under Mukaiyama-like conditions with a MgI_2 catalyst provided higher levels of induction, as will be seen in the following pages. Catalytic hydrogenolysis of **33** to remove the *N*-benzyl group, conversion of the resulting amino ester to the γ-lactam by heating in methanol, and desilylation of the primary TBS ether affords the γ-lactam **34**. Selective oxidation of the primary hydroxyl group of **34** to the acid using the Corey−Myers protocol,[29] followed by an acid-catalyzed methylene transfer to 1,3-propanedithiol gives the dihydroxyacid **26**. Dihydroxyacid **26** undergoes esterification by treatment with BOP-Cl, triethylamine (Et_3N), and *N*-acetyl-L-cysteine allyl ester to form the allyl ester of (+)-lactacystin (**35**). Deallylation of **35** under transfer hydrogenation conditions affords **1**.

As the evolution of the synthetic approaches to (+)-lactacystin emerged, a series of revised syntheses of **1** were reported. In the first modification of the Corey approach, the challenging *anti*-aldol step was revisited using a modification of the Mukaiyama aldol coupling of oxazolidine **32** and silyl-ketene acetal **36** (10:1 mixture of *E*/*Z* isomers) with MgI_2 as a catalyst (Scheme 8).[23] This aldol reaction proceeded with useful levels of stereoselectivity (*dr* = 9:1 *anti*/*syn*) and in good yield (77%) to afford the aldol product **37**.

The unique ability of MgI_2 to catalyze this aldol construction has been attributed to the ability of the small [IMg^+] species to form a chelate with the sterically congested aldehyde **32** (Scheme 8). The steric screening at the *re*-face of the formyl group due to the bulky isopropyl substituent and TBS ether forces nucleophilic attack at the *si*-face to give the *anti*-aldol product. The aldolate **37** was converted by an identical sequence as outlined for aldol product **33** (Scheme 7) into the dihydroxy acid **26**. Selective β-lactamization of **26** with BOP-Cl/Et_3N affords β-lactone **2**. Finally, the reaction of **2** with *N*-acetyl-L-cysteine produces **1** in high yields without the need to use a protected cysteine derivative or subsequent protecting group removal. This modification of the original Corey approach has been used successfully to produce multigram quantities of **1** and **2**.

The most recent synthesis from the Corey laboratories utilizes racemic starting materials whose enantiopurity was generated using enzymatic resolution techniques.[30] More specifically, the synthesis is initiated from the dimethylmethylmalonate derivative **38**, prepared from dimethylmethylmalonate in one step (Scheme 9).

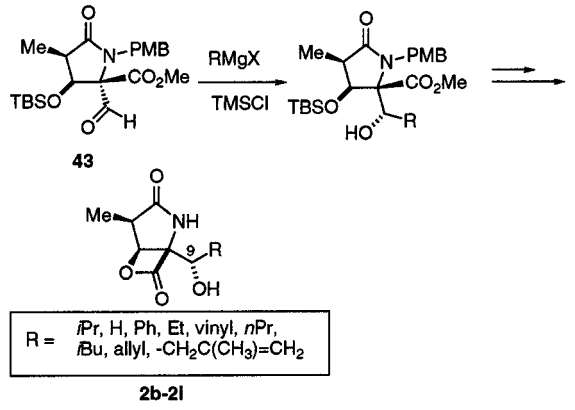
Enantioselective pig-liver esterase (PLE) catalyzed hydrolysis of **38** gives the enantioenriched acid. The crude acid is then purified by one recrystallization of its corresponding quinine salt from aqueous ethanol to give acid **39** in 95% enantiomeric excess (*ee*). Coupling of **39** as its acid chloride to the glycine ester component and subsequent Dieckmann cyclization gives the β-keto lactam **40** as a 1:1 mixture of diastereomers (with respect to the α-carbon of the β-keto ester moiety). Stereoselective α-hydroxymethylation of **40**



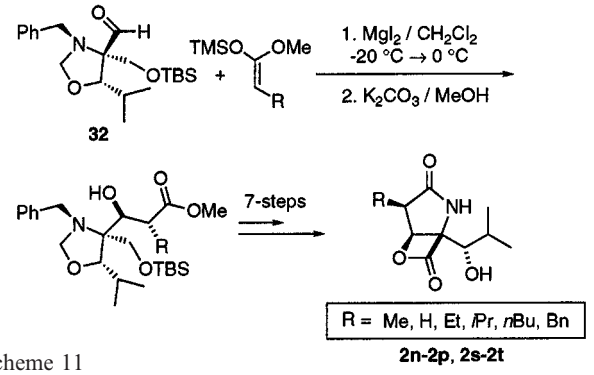
Scheme 9

(*dr* = 9:1) and substrate-directed reduction of the ester with $NaBH(OAc)_3$ affords **41** whose absolute stereochemistry was confirmed by an X-ray crystallographic analysis. In preparation for the construction of the C9-hydroxyl stereocenter, **41** was converted into the primary alcohol **42** in a three-step sequence: i) selective protection of the primary hydroxyl group of **41** as its pivolyl ester; ii) protection of the C6-hydroxyl as its TBS ether, and iii) deprotection of the pivoylate ester. At this point, all that remains is to unmask the C7-methyl stereocenters and construct the C9-hydroxyl stereocenter. The desulfurization of **42** with Raney nickel was surprisingly stereoselective (*dr* = 10:1) to afford aldehyde **43** after column chromatography and Dess−Martin oxidation. The reaction of **43** with 2-propenyl Grignard reagent in the presence of TMS-Cl (essential to prevent retro-aldol cleavage) provided the desired alcohol **44** stereospecifically. The TMS-Cl was found to be essential to the reaction as it traps the resulting alkoxide ion at a rate that is faster than retro-aldol cleavage, which **43** was found to be prone to in the absence of TMS-Cl. The stereoselectivity of the Grignard addition is consistent with a steric screening model by means of a bidentate chelation of the Mg^{II} complex with the carbonyl groups of the formyl and ester moieties (Scheme 8). Isomerically pure **44** is converted into the dihydroxy acid **45** by catalytic hydrogenation, desilylation of the C6-TBS ether, and saponification of the methyl ester. Selective β-lactonization of **45** with BOP-Cl and removal of the *p*-methoxybenzyl group with ceric ammonium nitrate (CAN) afforded the β-lactone **2** which was converted into **1** by the methods outlined in Scheme 8.

The Corey syntheses outlined in schemes 7−9 have been utilized to prepare a variety of analogs. The following section will highlight the synthesis of some of these analogs using the Corey synthetic methodologies and summarize the current state of knowledge regarding structure-activity relationships of (+)-lactacystin and these analogs. A more detailed table with the relative biological activity of these analogs is provided at the end of this review (see Table 1). Intermediate **43** (Scheme 9) provided the scaffold from which a variety of C9 substituents could be introduced to generate β-lactone analogs of the type shown in Scheme 10. By this methodology, unsaturated and saturated analogs were generated. The allyl and 2-methyl-1-propenyl analogs were prepared by allylation of **43** with the appropriate allyl bromide, manganese metal, and chromium chloride as a catalyst in the presence of TMS-Cl. The biological activity of these analogs was determined relative to **2** by measurement of the inactivation rate constants of purified bovine brain 20-proteasome. The methodology described by Corey has also been used to investigate structure-activity relationships for C7 analogs of **2**. The analogs were prepared using the Mukaiyama aldol methodology outlined in Scheme 11. The data for the analogs in Scheme 10 (see Table 1, entries 1−13) indicate that the biological activity (relative to **2**) is greatly diminished with C9-substituents which are slightly smaller than or larger than isopropyl, thus the C9-isopropyl



Scheme 10



Scheme 11

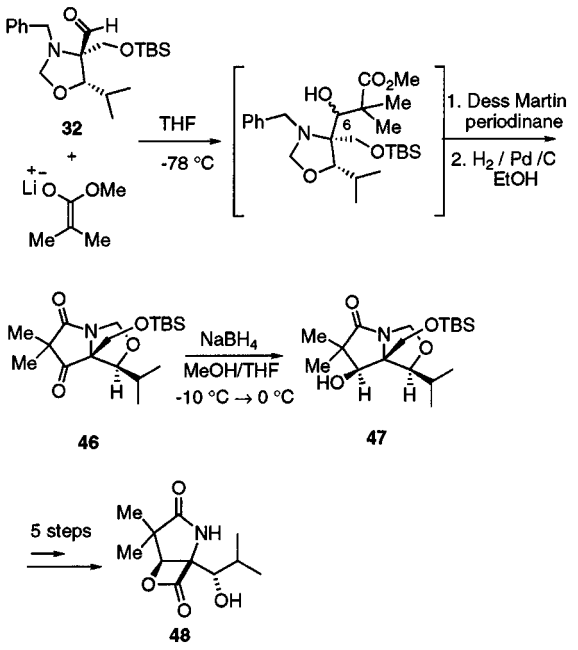
substituent in the natural product is optimal for proteasome inhibition. It appears the C9-hydroxyisobutyl moiety is the ideal structure for primary recognition of the target proteasome.

The data for the analogs in Scheme 11 (see Table 1) indicate that while replacement of the C7-methyl group of **2** by smaller groups (H) leads to reduced activity, replacement of the C7-methyl group with larger groups (entries 15−17, and 19) results in a doubling of the rate of proteasome inhibition relative to **2**.

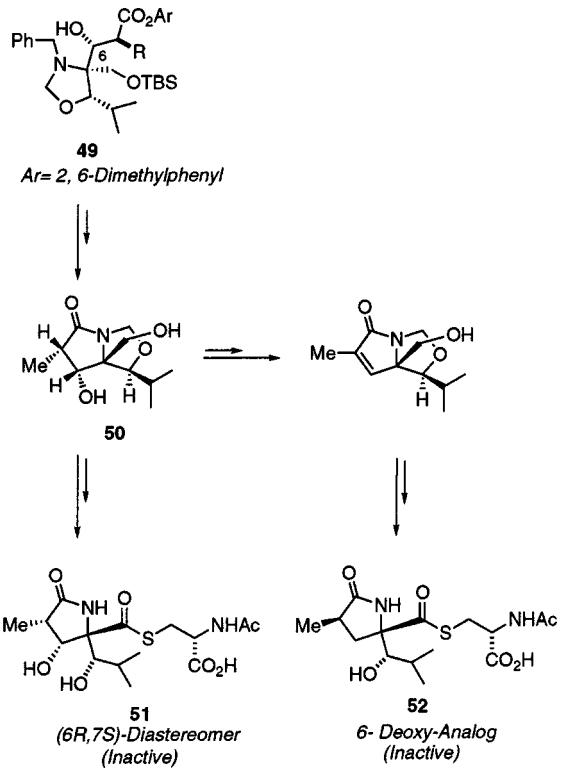
The preparation of a C7-gem-dimethyl analog of **2** was recently reported by Corey and co-workers as outlined in Scheme 12.[31] The key bond-construction utilizes similar methodology to that described in Scheme 7 and begins with the known aldehyde **32**.

Addition of **32** to the lithium enolate of methyl isobutyrate affords a diastereomeric mixture of β-hydroxy esters (*dr* = 1.2:1) which was directly oxidized under Dess−Martin conditions to give the C6-β-keto ester. Hydrogenation of the β-keto ester to liberate the amino group and simultaneous γ-lactam closure afforded the keto-γ-lactam **46**. Selective reduction of **46** with sodium borohydride ($NaBH_4$) in methanol/THF afforded the required β-hydroxy-γ-lactam **47** with 12:1 diastereoselectivity. Hydroxylactam **47** was converted into the β-lactone **48** by a reaction sequence paralleling that shown in Scheme 8. β-Lactone **48** displayed a lower activity than the parent lactone **2** (see Table 1, entry 22).

In order to test the initial hypothesis that β-lactone formation was crucial to the biological activity of (+)-lactacystin, several analogs were subsequently prepared which were incapable of cyclizing to form the β-lactone (Scheme 13). The minor aldol diastereomer **49** of the *anti*-aldol intermediate (**33**) was utilized for this purpose.



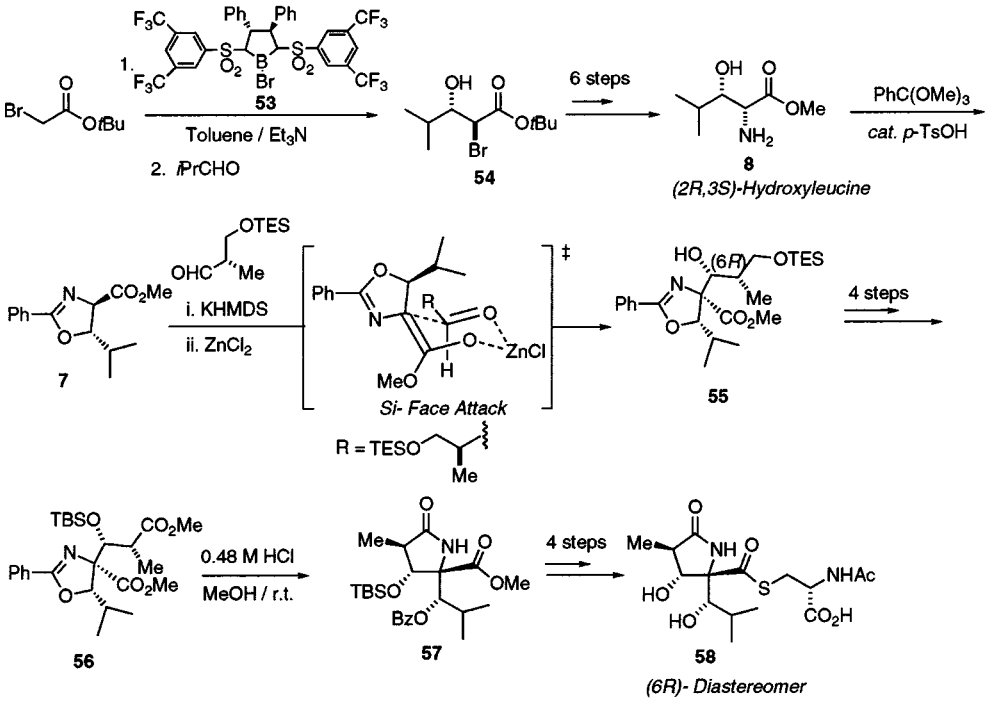
Scheme 12



Scheme 13

The aldolate **49** was converted via the lactam **50** to the (6*R*,7*S*)-diastereomer (**51**) by a reaction sequence analogous to that described in Scheme 7.[32] Lactam **50** was also utilized for the synthesis of the 6-deoxy analog **52** of (+)-

lactacystin.[32a] Both of these analogs proved to be biologically inactive, which suggested the possibility that β-lactone formation may be crucial to (+)-lactacystin′s bioactivity. In order to test this idea, the Corey group developed a synthesis of the (6*R*)-diastereomer of (+)-lactacystin which would be incapable of forming the β-lactone but possessed all of the other structural characteristics of the parent compound. The synthesis of this analog highlighted a new approach to the (+)-lactacystin core which utilized a (2*R*,3*S*)-hydroxyleucine synthon (**8**, Scheme 14).[33] The (2*R*,3*S*)-hydroxyleucine synthon was prepared by the enantioselective *anti*-aldol reaction of *tert*-butylbromoacetate with isobutyraldehyde in the presence of the Corey bromoborane catalyst (**53**)[34] followed by a six-step sequence to convert the derived bromohydrin **54** to the desired hydroxyleucine synthon **8**.



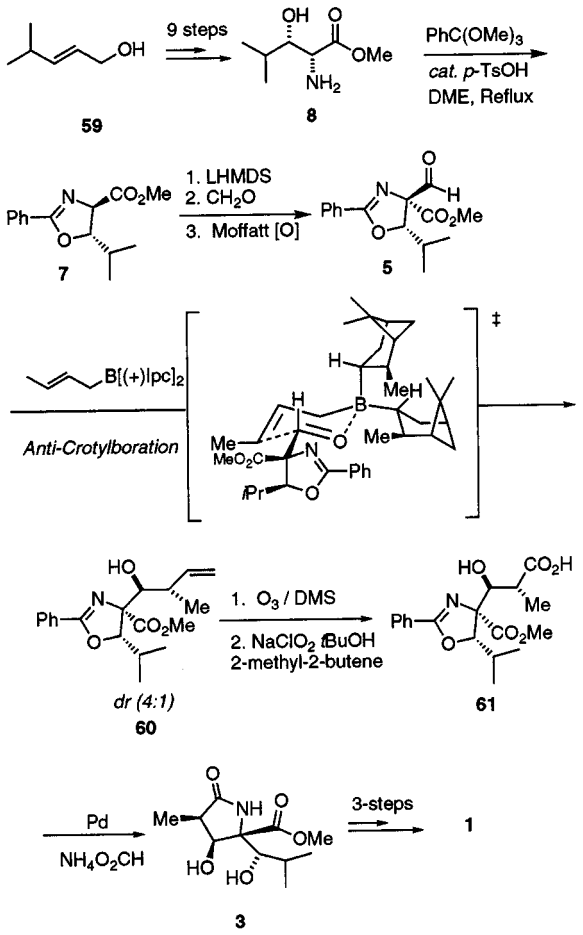
Scheme 14

Intermediate **8** was cyclized to oxazoline **7** using trimethylorthobenzoate in the presence of *p*-toluenesulfonic acid catalyst. The double stereodifferentiating aldol reaction of the zinc enolate of **7** with (*S*)-2-methyl-3-trimethylsilyloxypropionaldehyde afforded exclusively the *anti*-aldol product **55**. The observed (6*R*)-stereochemistry is consistent with the intervention of a chair-like transition state of the *E*(O)-enolate of the oxazoline with Felkin induction (Scheme 14). The oxidation state of the terminus of **55** was adjusted to afford oxazoline **56**, at which point the oxazoline moiety was unmasked by acidic hydrolysis to afford the γ-lactam **57** which was converted into the (6*R*)-diastereomer of (+)-lactacystin (**58**) in a straightforward four-step sequence. Diastereomer **58** was shown to be considerably less active than **1**, thereby underscoring the need for β-lactone formation to induce proteasome inactivation.

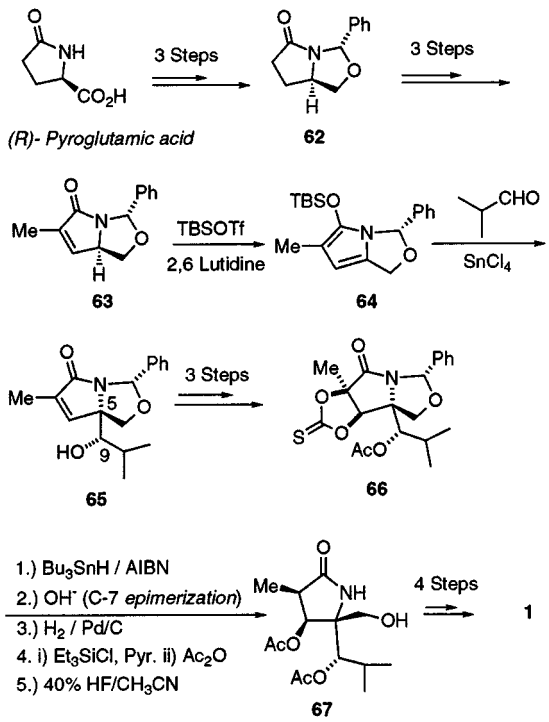
Omura−Smith Synthesis

The critical (2*R*,3*S*)-hydroxyleucine synthon has also been utilized by Omura and Smith in the total synthesis of **1**, as illustrated in Scheme 15.[35] In this work, the hydroxyleucine derivative **8** was prepared by a Sharpless asymmetric epoxidation of (*E*)-4-methyl-2-penten-1-ol (**59**) to give the chiral epoxide which was elaborated in a nine-step sequence to furnish amino ester **8**. The hydroxyleucine synthon was subsequently cyclized as in Scheme 4 to oxazoline **7**.

A key step in the Omura−Smith synthesis involves diastereoselective hydroxymethylation of oxazoline **7** where the observed chirality is controlled by the topology of the substrate. Oxidation of the primary hydroxyl group under Moffatt oxidation conditions affords the labile heterocyclic aldehyde **5**. The formation of the C6 and C7 stereocenters relies on a critical crotylboration between aldehyde **5** and Brown's (*E*)-crotylborane reagent.[36] This sequence affords the homoallylic alcohols as a 4:1 mixture of diastereomers favoring the desired product. A number of other crotylmetal reagents were surveyed, including the Hiyama (*E*)-crotylchromium,[37] Roush′s (*E*)-crotylpinacol borane,[38] and Roush′s (*E*)-crotyltartarate borane.[39] However, each of these reagents gave lower selectivities (*dr* = 2:1 → 3:1 *anti*/*syn*). The absolute stereochemistry can be rationalized in terms of the chair-like transition state shown in Scheme 15. The low stereoselectivity of the reaction is most likely due to the steric congestion associated with aldehyde **5** in the closed transition structure. The homoallylic alcohol **60** was converted into the labile acid **61** in a two-step sequence, involving oxidative cleavage of the double bond and subsequent oxidation of the resultant aldehyde. Unmasking of



Scheme 15



Scheme 16

the oxazoline under transfer hydrogenation conditions and spontaneous cyclization of the liberated amino alcohol furnishes the γ-lactam **3** which was converted into **1** by a sequence paralleling that of Scheme 7.

Baldwin−Uno Synthesis

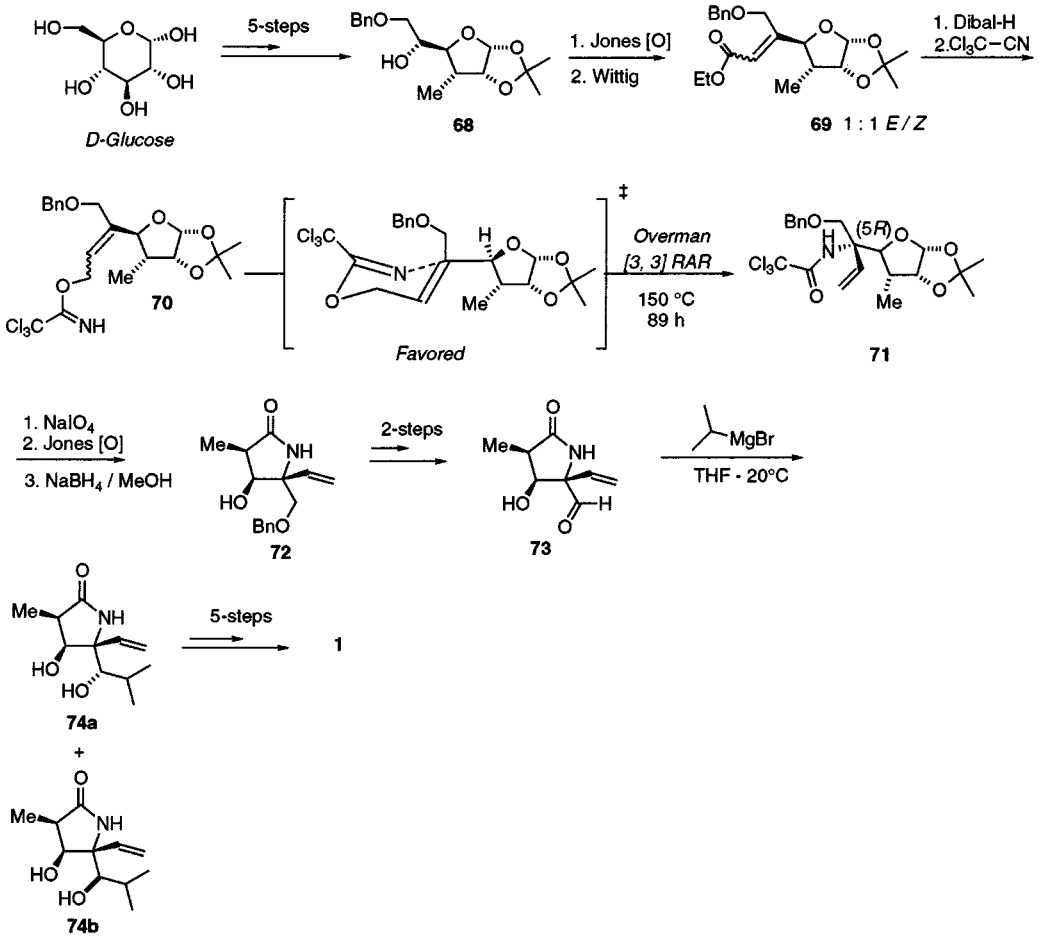
(+)-Lactacystin has also been synthesized by Baldwin and co-workers starting from (*R*)-pyroglutamic acid (Scheme 16).[40] The key feature of the Baldwin route is a Mukaiyama aldol reaction involving a silyloxy pyrrole and isobutyraldehdye to establish the C9 stereocenter. (*R*)-Pyroglutamic acid is converted into the bicyclic oxazolidine **62** in which the γ-lactam core is already in place and the additional chiral center serves as a diastereoselectivity controller for subsequent elaborations.

The bicyclic lactam **62** is converted into the α,β-unsaturated lactam **63** by a two-step sequence involving α-methylation and phenylselenation of the derived lactam enolate, followed by oxidative *syn*-elimination to afford the unsaturated lactam in 65% yield. Generation of the silyloxy pyrrole is accomplished by treatment with TBSOTf/2,6-lutidine to give intermediate **64**, which undergoes a vinylogous Mukaiyama aldol reaction with isobutyraldehdye to give aldol product **65**, thereby installing the C5 and C9 stereocenters with moderate levels of stereoselectivity (*dr* = 9:1). Installation of the C6 and C7 stereogenic centers requires a number of steps involving: i) dihydroxylation of **65**; ii) selective deoxygenation of the C7-hydroxyl group via the cyclic thiocarbamate **66**; iii) base-catalyzed epimerization of the C7-methyl group; iv) hydrogenolysis of the benzylidene *N,O*-acetal; and v) protecting group manipulations to afford the hydroxylactam **67**. Lactam **67** is converted into **1** by a sequence analogous to that of Scheme 7.

Chida Synthesis

Using a fundamentally different approach to the synthesis of (+)-lactacystin, Chida and co-workers utilize D-glucose as a scaffold to construct the γ-lactam core of **1** (Scheme 17).[41] Unfortunately, this approach is not very atom economical as only one of the five stereocenters of glucose is retained in the natural product. The synthesis does, however, feature as its key step an interesting Overman [3,3] sigmatropic rearrangement to install the quaternary C5 stereocenter with moderate levels of stereoselection.

The synthesis starts with furanose **68** which is oxidized at the C5 position and homologated under Wittig conditions to the α,β-unsaturated ester **69** (1:1 mixture of *E*/*Z* isomers). Reduction of the ester to the allylic alcohol is followed by in situ formation of the trichloroacetimidate **70**, which undergoes a thermal [3,3] Overman rearrangement to afford the aminofuranose **71** with 4.8:1 diastereoselectivity in favor of the correct C5-quaternary stereocenter. The observed (5*R*)-stereochemistry for the major product is consistent with a chair-like transition state which places the bulky furanose group in the pseudoequatorial position (Scheme 17). Periodate cleavage of the acetonide protecting group of **71** affords an *O*-formyl hemiaminal which is followed by oxidation under Jones conditions to furnish the



Scheme 17

O-formyl-γ-lactam. Finally, removal of the *O*-formyl group ($NaBH_4$/MeOH) gives the γ-lactam **72**. Construction of the C9-stereocenter involves Grignard addition of isopropylmagnesium bromide to the heterocyclic aldehyde **73**. This addition suffers from a lack of stereocontrol (**74a**/**74b** ≈ 1.2:1) and a low yield due to unwanted formyl reduction by the isopropylmagnesium bromide to give the primary alcohol side product. Completion of the synthesis of **1** involves oxidative cleavage of the terminal olefin at C5 and thioesterification with *N*-acetyl-L-cysteine using methods similar to those in Scheme 7.

Structure–Activity Relationships (SAR)

The early studies concerning SAR by Fenteany and coworkers demonstrated the importance of the requisite functionality and stereochemical relationships in the natural product.[3] Further detailed analysis has been pursued by the Corey laboratories,[30–34] and independently by the group at Millennium.[25] Both research groups showed that the SAR requirements were rather stringent. A pictorial

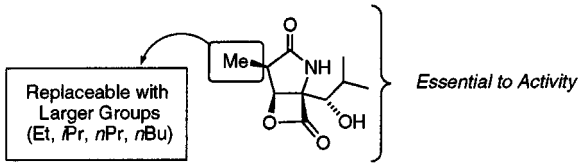
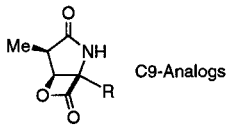


Figure 8. Summary of SAR studies

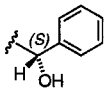
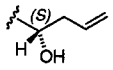
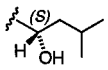
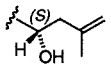
summary of these results is shown in Figure 8. There is an absolute requirement for the β-lactone ring, and the stereochemical fidelity as dictated by the natural product. In addition, *N*-methylation of the β-lactam abolishes activity. In general, most structural modifications to the natural product led to a dramatic loss of activity.

The one area of the molecule that supported chemical modification was the C7 alkyl group. Replacement of the methyl group at C7 with short aliphatic chains enhanced the potency of the lactone inhibitor. The best activities were recorded for ethyl, *n*-propyl, isopropyl, and *n*-butyl all possessing two to three times the potency of the corresponding (+)-lactacystin-β-lactone (Table 1, entries 15–17, and 19). The Millennium group has developed a route to the C7 *n*-propyl analog (PS-519, Table 1, entry 17) which has allowed for extensive in vivo biological testing. Table 1 below sum-

Table 1. Biological activity of (+)-lactacystin-β-lactone and its analogs



C9-Analogs

Entry	R	Compound #	Rel. $K_{obs.}/[I]^a$	Ref.
1.		**2**	1.0	3a
2.		**2b**	0.003	30a
3.		**2c**	Inactive	30a
4.		**2d**	0.095	30b
5.		**2e**	0.061	30a
6.		**2f**	0.063	30b
7.		**2g**	0.083	30a
8.		**2h**	0.006	30b
9.		**2i**	0.021	30a
10.		**2j**	Inactive	3b
11.		**2k**	0.021	3b
12.		**2l**	0.077	30b
13.		**2m**	0.032	30b

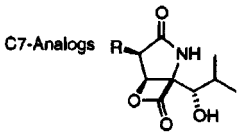
[a] Chymotrypsin-like activity with purified 20*S* proteasome reported relative to the parent lactone (**2**).

marizes the analogs prepared to date accompanied by their biological activity relative to the parent β-lactone **2**.

Concluding Remarks

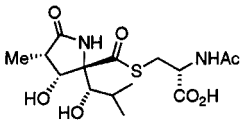
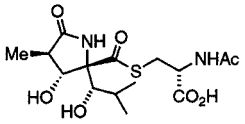
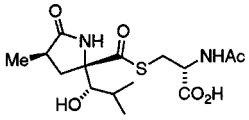
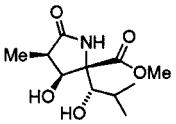
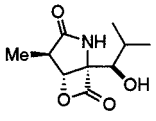
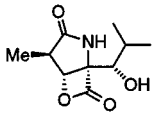
In this Microreview, we have demonstrated the important relationship between organic synthesis and the drug discovery process which is nicely illustrated in the case of PS-519

Table 1. (*Continued*)



Entry	R	Compound #	Rel. $K_{obs.}$	Ref.
13.	Me	**2**	1.0	3a
14.	H	**2n**	0.15	23
15.	Et	**2o**	2.18	23
16.	*i*Pr	**2p**	2.77	23
17.	*n*Pr	**2q**	2.33	25
18.	*i*Bu	**2r**	0.85	25
19.	*n*Bu	**2s**	2.38	23
20.	Ph-CH_2-	**2t**	0.73	23
21.	*epi*-Me	**2u**	0.41	23
22.	*gem*-dimethyl	**48**	0.75	31

Other Analogs

Entry	Analog	#	Rel. $K_{obs.}$	Ref.
23.	 *(6R,7S)-Diastereomer*	**51**	Inactive	32a
24.	 *(6R)-Diastereomer*	**58**	Inactive	33
25.	 *6- Deoxy-Analog*	**52**	Inactive	32a
26.		**3**	Inactive	35
27.		—	0.040	32b
28.		—	Inactive	32b

(Millennium, Inc.). This analog of (+)-lactacystin-β-lactone has been taken forward into clinical evaluation for the treatment of cerebral ischemia and stroke. It is the imagination and creativity of practicing medicinal chemists that allow for the production of this material in useful amounts for biological evaluation.

In the context of synthetic design, (+)-lactacystin is a deceptively simple natural product. Taken collectively, the synthetic approaches devised by the research groups working on this target have demonstrated considerable creativity and mechanistic insight en route to the total synthesis. The successful approaches presented represent an inventive application of several important strategies in the area of acyclic stereocontrol. These include the use of chiral metal enolate and chiral allylmetal-based bond construction methods. (+)-Lactacystin provides an excellent illustration of the ability of a small molecule to inhibit a biological cascade. Perhaps even more remarkable is (+)-lactacystin's capability to effect this inhibition with exquisite specificity for the 20*S* proteasome.

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