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THE UNIVERSITY OF ALBERTA

SERINE β-LACTONES IN SYNTHESES OF AMINO ACIDS

by

LEE D. ARNOLD

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled SERINE β-LACTONES IN SYNTHESES OF AMINO ACIDS

submitted by LEE D. ARNOLD

in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY

Supervisor

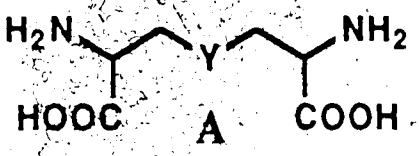
External Examiner

DATE April 29, 1987

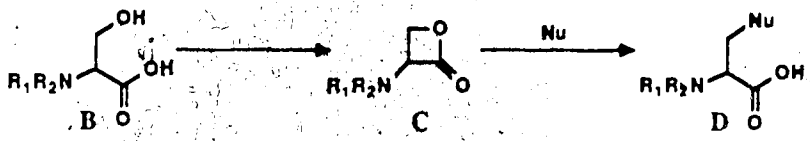
To Connie

ABSTRACT

The various stereoisomers of lanthionine (**A** (Y = S)) and its corresponding sulfoxides (Y = SO) and sulfones (Y = SO_2) have been prepared for studies with enzymes associated with the metabolism of diaminopimelic acid (Y = CH_2) in plants and microorganisms.



The syntheses of the lanthionine derivatives illustrate the problems and disadvantages often encountered in conventional syntheses of amino acids. An alternative route to amino acids via stable β-lactones of serine has been developed. Readily available optically pure N-acyl (eg., $PhCH_2C(O)$) or N-alkoxycarbonyl (eg., $PhCH_2OC(O)$) derivatives of serine (**B**) are cyclized under modified Mitsunobu conditions (Ph_3P, ROOC-N=N-COOR), in high yield. Treatment of the β-lactones (**C**) with a



(R1 =ROOC-, RCO-)
(R2 =H, Bn)
(Nu=X,O,N,S,P,R',Ar)

variety of halogen, oxygen, nitrogen, sulfur, or phosphorus nucleophiles provides optically pure N-protected β-substituted alanines (**D**) in excellent yields. Ring-openings by carbon nucleophiles, including Cu(I)-catalyzed Grignard (RMgCl) additions, can generate

N-protected aliphatic and aromatic amino acids (Nu = R', Ar in **D**) in good yield with complete (>99.4%) retention of optical purity, suitable for direct incorporation into peptides. Acid-mediated deprotection of N-(tert-butoxycarbonyl)-serine β-lactone (**C**, R_1 = ^tBuOOC, R_2 = H) affords 3-amino-2-oxetanone (R_1, R_2 = H) which may be isolated as the stable tosylate salt. These unprotected serine β-lactones (**C**) react chemoselectively with nucleophiles to directly provide free amino acids (**D**, R_1, R_2 = H). In many cases syntheses employing serine β-lactones are superior to previous methods with respect to optical purity, yields and simplicity. The alkyl azodicarboxylate reagent which is used in β-lactonization of the serine derivatives and in the preparation of many pharmaceutical and chemical products has been immobilized on a polystyrene matrix (i.e., Polymer~OOC-N=N-COOR). This allows regeneration of the reagent, simplifies isolation, and effectively reduces costs and dangers associated with the Mitsunobu reaction on large scale.

ACKNOWLEDGEMENTS

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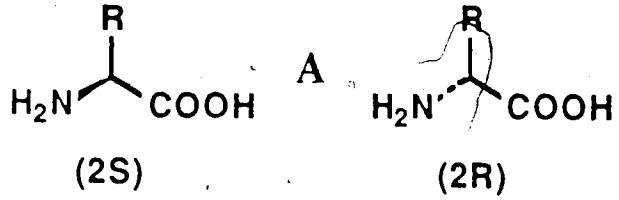
LIST OF ABBREVIATIONS

Ac	Acetyl (CH_3CO)
AcOH	Acetic acid
Bn	Benzyl ($PhCH_2$)
BOC	tert-Butoxycarbonyl
Bu	Butyl
cat.	Catalytic
CI	hemical ionization
DAP	,6-Diaminopimelic acid (2,6-diamino-heptanedioic acid)
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	N,N'-Dicyclohexylcarbodiimide
DEAD	Diethyl azodicarboxylate
DMAD	Dimethyl azodicarboxylate
DMAP	4-(Dimethylamino)pyridine
DMF	N,N-Dimethylformamide
EDTA	Ethylenediaminetetraacetic acid
EI	Electron impact
Enz	Enzyme
Et	Ethyl
LDA	Lithium diisopropylamide
Me	Methyl
MEA	β-Mercaptoethylamine
MPLC	Medium pressure liquid chromatography
MS	Mass spectroscopy
NBS	N-Bromosuccinimide

NEGFAB-MS	Negative ion fast atom bombardment MS
NMR	Nuclear magnetic resonance
nOe	Nuclear Overhauser effect
Ph	Phenyl
PLP	Pyridoxal phosphate
PMP	Pyridoxamine phosphate
POSFAB-MS	Positive ion fast atom bombardment MS
Pr	Propyl
pyr	Pyridine
R_f	Retardation factor
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMEDA	N,N,N',N'-Tetramethylethylenediamine
tosyl	p-Toluenesulfonyl
t_R	Retention time
Tr, Trityl	Triphenylmethyl
Z	N-(Benzyloxycarbonyl)

INTRODUCTION

An enormous number (>700) of α-amino acids (**A**) have been discovered in Nature[1] and many more have been produced synthetically.



(2S) (2R)

The 20 common (2S)-L-α-amino acids play a central role in the primary metabolism of all living organisms.[2,3] Peptide and protein products which are essential to all life processes can be assembled in an almost infinite array from this relatively small number of proteinogenic amino acids. A vast majority of the remaining naturally-occurring nonprotein amino acids are generated by various plants (mostly (2S)-**A**)[4] and lower organisms ((2S)-**A** and (2R)-**A**) as secondary metabolites,[5] products of detoxification of foreign compounds, or as defense mechanisms against predators or competitors.[1,4] Either free or as constituents in peptides or depsipeptides, the nonprotein amino acids are responsible for an incredible spectrum of biological activities.

Pharmacologically the most important of these activities are their action as antibiotic and antitumor agents and as antimetabolites and hormone analogs for the treatment of various diseases.[1,6] As a result, natural

nonprotein amino acids and their almost innumerable synthetic modifications function as important components in many drugs and pharmaceuticals.[1,7,8] Importantly, all the α-amino acids (**A**, R≠H) produced in Nature are enantiomerically pure, and often each enantiomer displays a specific biological activity.[9]

The natural α-amino acids represent an enormous pool of optically pure chiral units for organic chemists, who have recently begun to realize their potential as chiral synthons, reagents, catalysts, and auxiliaries in organic syntheses.[1a,10-12] In addition, with the emergence of peptide synthesis as a powerful tool in molecular biology there is an ever increasing demand for a wide range of N-protected amino acids with high optical purity.[13,14]

Of all the natural α-amino acids, only about 2-3% (i.e., the proteinogenic (2S)-L-isomers) occur abundantly in Nature. Most others are much rarer and often localized in a given species.[1,4,5,9a] Consequently, much recent work has focussed on both achiral,[15] and enantioselective syntheses of α-amino acids (**A**)[1,9,16] based on the use of chiral catalysts, reagents or auxiliaries. Asymmetric syntheses are still in an evolutionary state. As yet they are unable to match both the convenience and high optical purity of procedures employing derivatives of readily available proteinogenic amino acids for the synthesis of other new, rare or unusual amino acids.[11,17] In addition most asymmetric syntheses are not generally applicable to

production of many of the amino acids bearing a wide range of functionalities in the side-chain (R of **A**) which engender the most intriguing biochemical properties.

A primary objective of this research was the synthesis of both established and potential inhibitors of amino acid metabolism for use as drugs, antibiotics, herbicides, and mechanistic probes of the target enzymes. In amino acid metabolism a wide variety of chemical transformations are performed by pyridoxal phosphate (PLP) dependent enzymes.[2,18] Plants and microorganisms possess a number of unique PLP-dependent enzymes which are prime targets for expression of herbicidal or antibiotic action.[2,8,18,19]

Irreversible inactivation by covalent modification of the active site of a target enzyme has generally proven to be the most efficacious means of disrupting metabolism.[7,8,20,21] In contrast to active-site directed affinity labels in which a reactive functional group is already present in a substrate analog before reaching the target protein, mechanism-based suicide substrates are not indiscriminately reactive. Instead, suicide substrates incorporate a "masked" latent functionality which is revealed only in the microenvironment of the active site during an enzyme-catalysed transformation of the substrate analog. Capture of the "unmasked" reactive entity by an active-site residue of the enzyme or its cofactor thus constitutes a "suicidal" inactivation event.[8,20,21]

Suicide inactivation on a macroscopic level is characterized by several kinetic and chemical observables: the inactivator should exhibit binding equilibrium (K_D) followed by pseudo-first order time dependent inactivation (k_{inact}). This also implies protection by substrate; the covalent enzyme-inhibitor adduct should display 1:1 stoichiometry reflective of a specific modification; a "partition ratio" characteristic of the reactive intermediate should be measurable. This ratio is an estimate of the inactivation efficiency and represents the number of suicide substrate molecules processed per inactivation event. A partition ("kill") ratio of one is most desirable however values of 10^3 are not uncommon.[8,21]

Since suicide-substrates are mechanism-based inhibitors their design and rationalization requires some knowledge of the mode of action of the target enzyme. Underlying the activity of essentially all pyridoxal phosphate (PLP)-dependent enzymes is a simple, common mechanistic principle: the pyridoxal phosphate (PLP) cofactor acts as a temporary electron "sink" which transiently "stores" electrons of various carbanionic intermediates for later use in the formation of new bonds. This feature enables PLP to initiate events leading to: cleavage of any of the four bonds to the α-carbon (Category 1) (i.e., transamination, racemization, decarboxylation, retro-Aldol-type cleavage); electrophilic

or nucleophilic reactions at the β-carbon (Category 2) (i.e., decarboxylation, elimination, replacement); or elimination and addition reactions at the γ-carbon of α-amino acids (Category 3).[2,8,22]

Regardless of their specific role, all PLP-enzymic reactions have 3 common functional features[8,22] (Figure 1). First the amino group of the α-amino acid displaces the ε-amino group of an active site lysine residue to form a cationic imine. Since this imine (eg. C_1 or C_2) is conjugated with the pyridine ring, the PLP provides extensive charge delocalization and can exercise efficient electrophilic catalysis. Next, cleavage of one of the three C-C or C-H bonds to the α-carbon, facilitated by protonation of pyridine nitrogen, generates a stabilized α-carbanionic intermediate (eg. D_1 or D_2) which may experience a number of fates. The last step in all cases is hydrolysis or transaldimination of the product imine. The apoenzyme's role is in amino acid recognition, stereoelectronic control of the reaction rate and course primarily by fixing the PLP-Schiff's base (eg., C_1 or C_2) conformation,[2,9] and the determination of the stage at which the product imine is hydrolyzed.

Enzymes are subject to the same stereoelectronic considerations that apply to all heterolytic fragmentations.[22,23] Following the initial transaldimination the enzyme must orient the bond to be labilized perpendicular to the plane of the aldimine π-

system. In this conformation the cleavage of the orthogonal σ-bond to form an α-carbanionic intermediate is facilitated by maximum orbital overlap (eg., C→D, Figure 1).

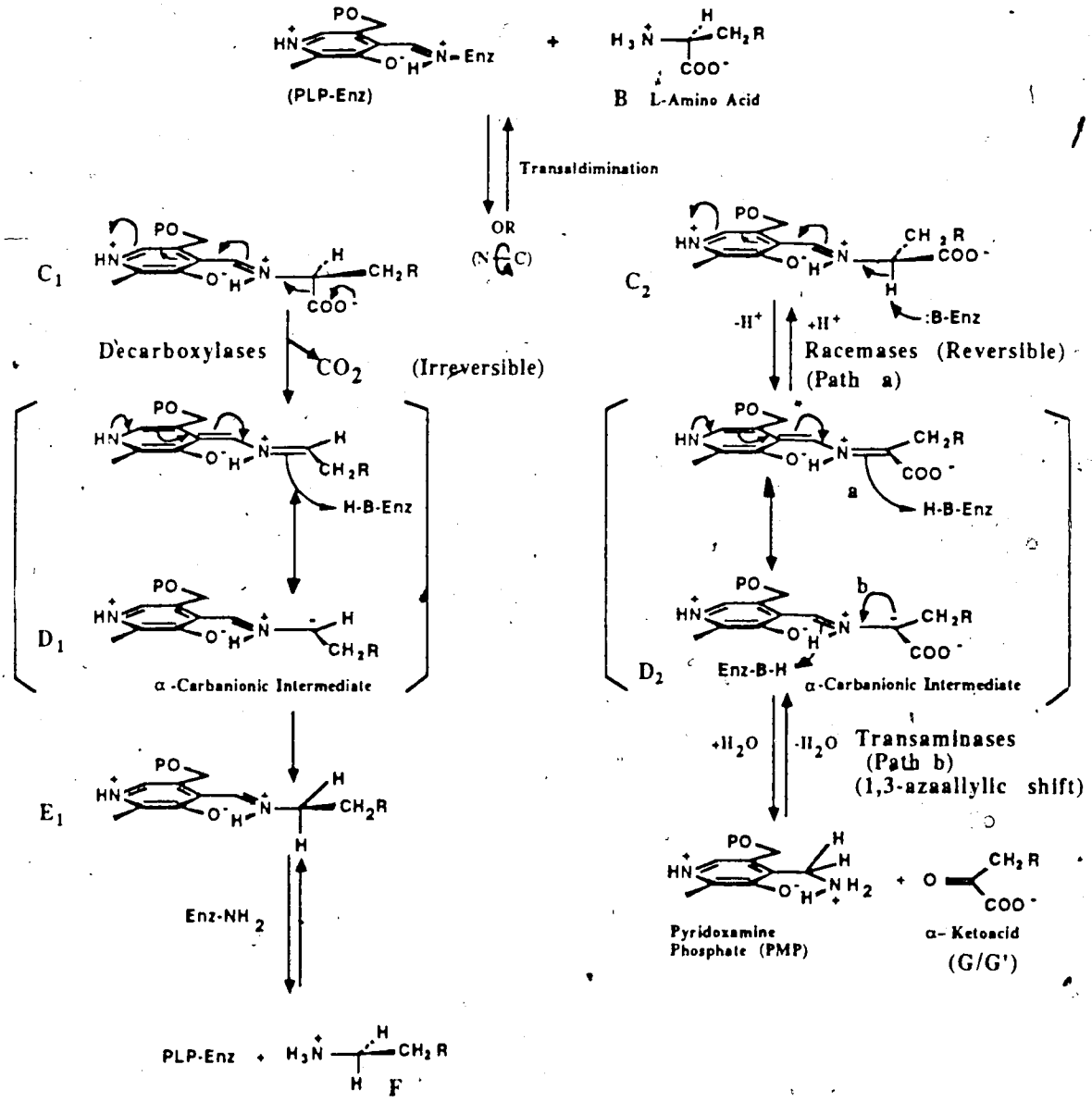


Figure 1. Mechanism of PLP-Dependent Enzymes Catalyzing Reactions at α-Carbon (Category 1)

Consider first reactions occurring at the α-carbon (Category 1). Pyridoxal phosphate (PLP) dependent decarboxylation of cationic imine C_1 (Figure 1) irreversibly generates the carbanionic intermediate D_1 which is stereospecifically reprotonated at the α-carbon on the re face (E_1). Hydrolysis of imine E_1 releases the amine product (**F**) (with overall retention of configuration for (2S), and inversion for (2R) substrates) and regenerates the PLP-enzyme.[8] Alternatively, reversible cleavage of the α-C-H bond of C_2 produces the α-carbanionic intermediate D_2 which is common to both epimerization and transamination. In epimerization reprotonation of the α-carbon on the opposite face by path a and hydrolysis generates the epimeric amino acid (epimer of **B**). In transamination, an overall 1,3-azaallylic suprafacial tautomerization of C_2 via D_2 (path b) followed by ketimine hydrolysis results in production of an α-keto acid (**G**) and pyridoxamine phosphate.[2,8,22] Completion of the transamination cycle with regeneration of the PLP-enzyme requires the analogous reverse reaction with an acceptor α-keto acid (**G'**) undergoing reductive amination. (We shall not be concerned with hydroxymethylase enzymes which effect cleavage of the remaining α-C-C bond in a retro-aldol fashion.)[2,8]

Various approaches have been proposed,[24] or investigated[7,8,21] for mechanism-based inactivation of PLP-enzymes of Category 1 (α-carbon reactions). The most

successful strategy involves rerouting the α-carba ic intermediate (D_1 or D_2) by elimination of a nucleofugal[8,20,21] leaving group from the β-carbon of the substrate analog. Whether the nucleofuge (X^-, Figure 2) takes the form of a pair of electrons in a β,γ-unsaturated substrate analog,[8,18,25] or a true leaving group resulting

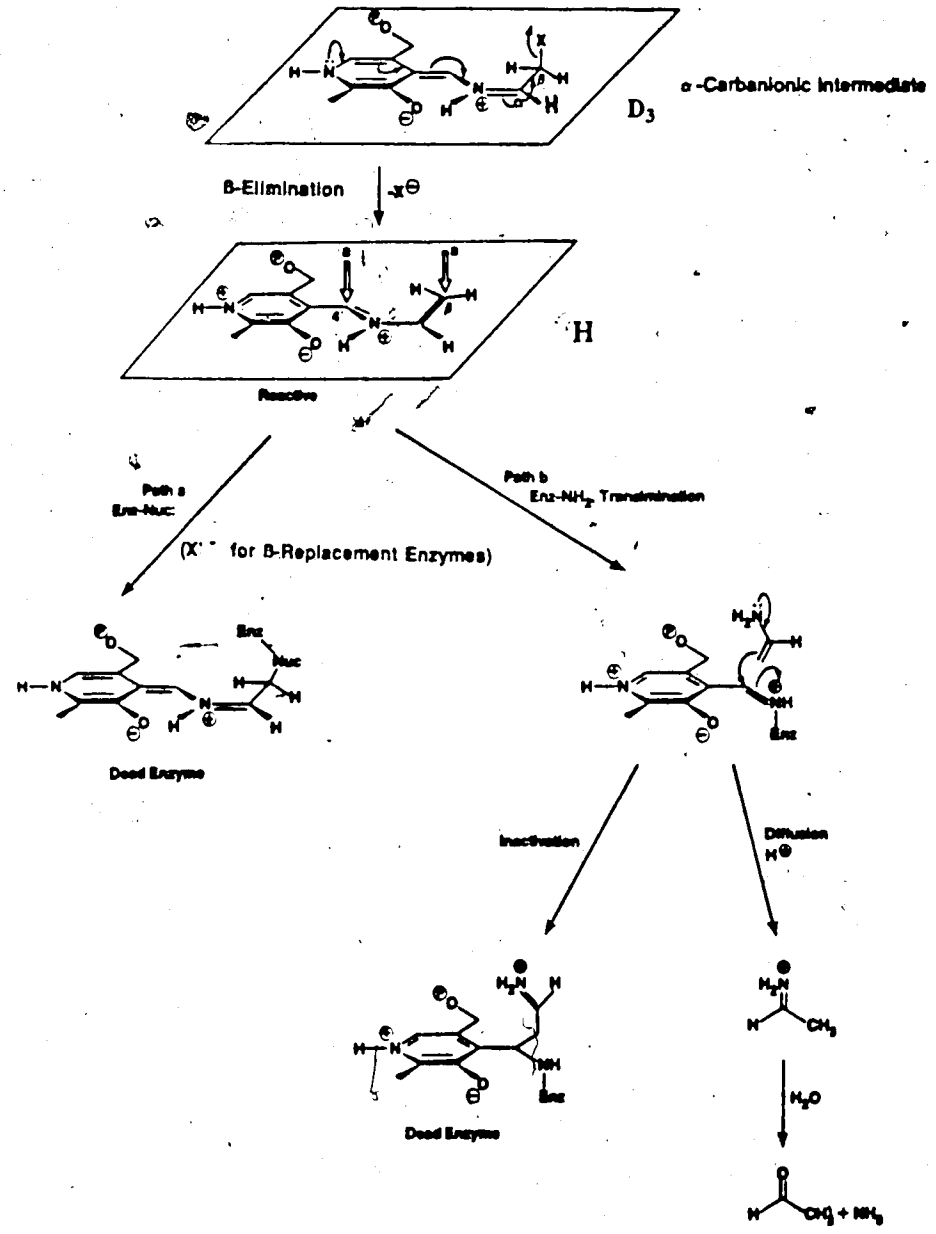
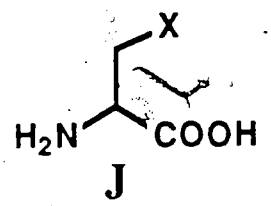


Figure 2. Potential Inhibition by ß-Elimination (Category 1) / Mechanism for ß-Replacement Enzymes (Category 2)

from heterolytic cleavage,[8,20,21] its elimination from the α-carbanionic intermediate D_3 generates an electrophilic imine (H) (Figure 2).

Originally it was believed that inactivation was generally the result of attack of an enzymic nucleophile (path a, Figure 2) at one of the two "a" sites of H.[8,21] Although this may be true for some enzymes,[26a] there is now substantial evidence indicating that the operative inactivation mechanism involves path b.[26] Presumably, transaldimination by an ε-amino sidechain of the enzyme liberates aminoacrylate. The aminoacrylate may passively diffuse out of the active site and harmlessly hydrolyze, or this enamine may attack the electrophilic internal Schiff's base to produce a covalently modified "dead" enzyme.

Interestingly, most of the PLP-enzymes of Category 2 which perform β-elimination/replacement reactions by expulsion of a β-leaving group produce the reactive intermediate H in their normal catalytic cycle. β-Replacement enzymes do not catalyze rapid hydrolysis of the enamino-PLP adduct (H), but rather await the attack of an external nucleophile (X'^-), essentially by path a (Figure 2), to eventually produce a new β-substituted alanine (X replaced by X' in J). Several protein (eg., cysteine, tryptophan)[2,8] and most nonprotein β-substituted alanines[1a,4,6,27-30a] are biosynthesized in this fashion.



J

A ß-Substituted Alanine

In β-elimination enzymes (eg., tryptophan, and cystathionine β-lyases) the enamino acid product is released (path b, Figure 2) and is hydrolyzed to ammonia and an α-keto acid without enzyme inactivation. In fact, many of the same compounds that act as potent suicide inhibitors for the PLP-enzymes of Category 1 (eg., β-chloroalanine (X = Cl in J), O-acetylserine (X = OAc), and serine-O-sulfate (X = SO_4^-)) are substrates for these β-elimination/replacement enzymes (Category 2).[2,6,26,31] This implies that these enzymes have evolved some "safety mechanism" which prevents inactivation by path a or b (Figure 2).

While expulsion of a β-leaving group generates an enimine with electrophilic character at the β-carbon (eg., H of Figure 2), loss of an electrofuge (i.e., E = H^+ or CO_2) from the β-position of the α-carbanionic intermediate (**D**) produces an enamine (**I**) with nucleophilic character at the β-carbon (Figure 3). This potential for reversed-polarity by loss of an electrofuge is exercised in the (Category 2) enzyme-catalyzed β-decarboxylation of aspartate (X = CO_2^- of J; E = CO_2^-, R = H in Figure 3). A subsequent reprotonation of the β- and α-carbons, and transimination eventually releases (2S)-L-alanine (X = H

in J' and PLP-enzyme (path a).[34c]

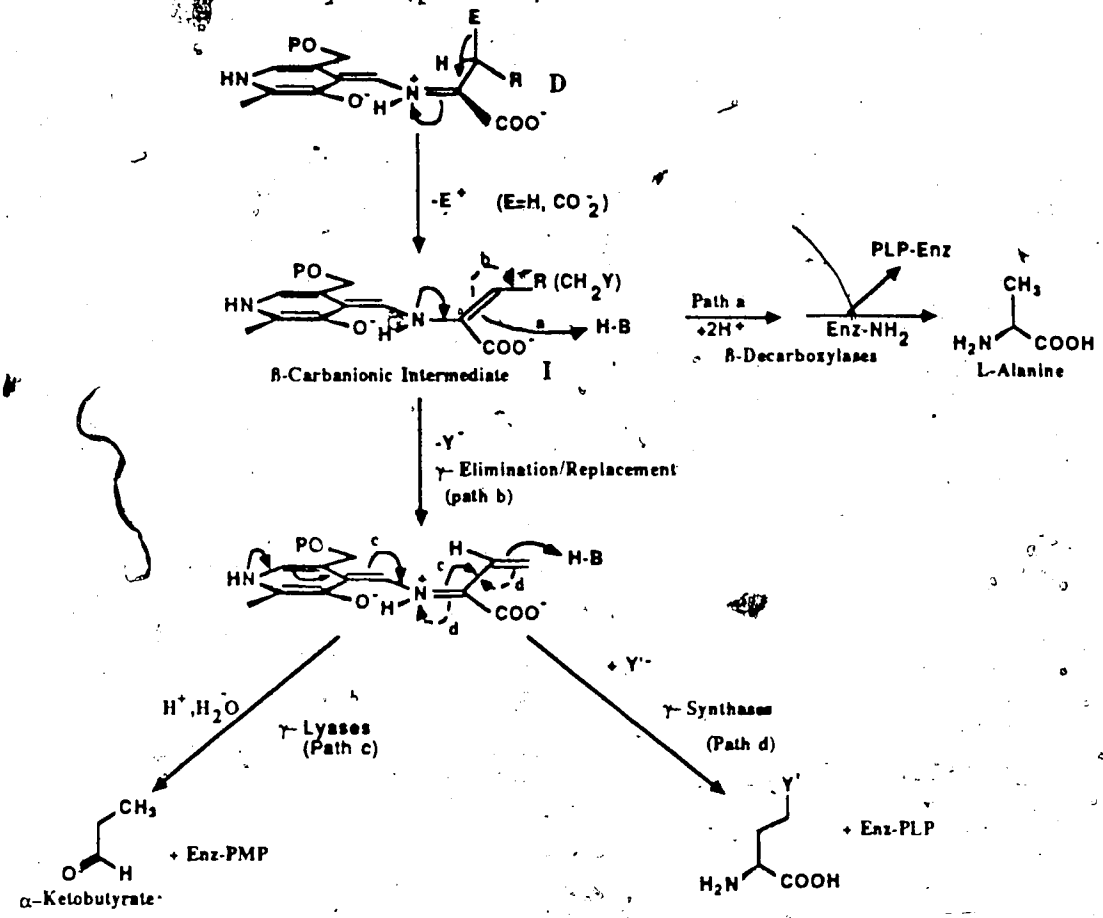


Figure 3. Mechanism of PLP-Enzymes Involving ß-Carbanionic (Enamine) Intermediates. (When R=CH_2Y, γ-elimination / replacement is possible).

In γ-elimination/replacement enzymes (path b, Figure 3) the removal of a proton (E = H) generates the β-carbanionic intermediate **I** which expels a γ-nucleofugal leaving group Y^-. As with reactions at the β-carbon, control of α-keto acid and amino acid production depends on the relative rate of protonation (at the γ-C, path c) versus attack by an external nucleophile (Y'^-) (path d),[8] respectively.

Suicide inhibition of the above enzymes in which β-carbanions are mechanistically involved (eg., cystathionine γ-lyase or γ-synthetase) is often achieved

through the use of propargylic substrate analogs (eg., propargyl glycine). Perhaps, as illustrated in Figure 4,[20,21] generation of the β-carbanion allows tautomerization to an extremely electrophilic conjugated allene which is highly irresistible even to enzymic nucleophiles of the normally cautious elimination/replacement enzymes.

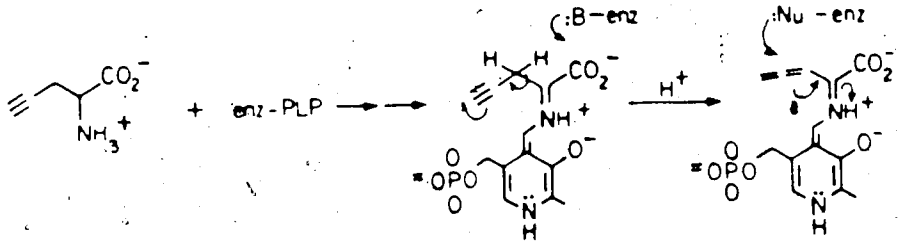


Figure 4. Inactivation by Acetylenic Substrate Analogs.

As one might expect from the substantial potential for error, it is not uncommon for these types of PLP-enzymes to mistakenly catalyze a reaction of another type,[22] especially on substrate analogs. This crossover is often an important consideration in rationalizing suicide inhibition.[8,18,20,21]

Most of the amino acid products synthesized in this thesis are β-substituted alanines having the general structure J. Essentially all of these can be classed as substrates, products, or established/potential inhibitors of pyridoxal phosphate enzymes, so we shall frequently return to the above mechanistic concepts.

In the rational design of suicide substrates as possible herbicides or antibiotics often differences in

plant/microbial and mammalian metabolism are exploited.[2,3,5,7] Ideally, in this manner, it may be possible to lethally disrupt the target organism's metabolism with minimal mammalian toxicity. Although PLP-dependent amino acid racemases (epimerases) exist in mammals,[20] they are essential in microorganisms for production of (2R)-D-amino acids (eg., D-Alanine, **J** (X = H)) for use in construction of cell walls.[2,4c] Hence, suicide inhibitors of D-alanine racemases with proven antibiotic properties[8,20,21,26c] were among our synthetic targets.

Another metabolic disparity between bacteria and mammals involves 2,6-diaminopimelic acid (DAP) (Figure 5).

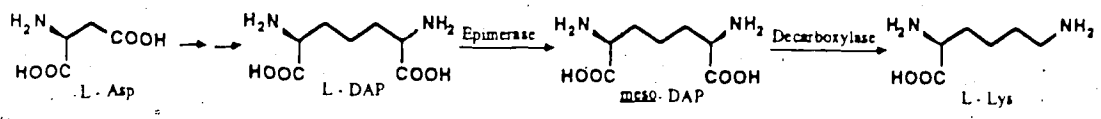
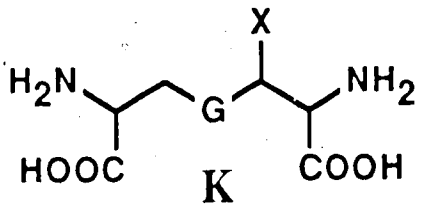


Figure 5. Biosynthesis of L-Lysine and Diaminopimelic Acid (DAP).

Various stereoisomers of 2,6-diaminopimelic acid (DAP) are essential constituents in the cell walls of nearly all bacteria.[25a,33] Furthermore, decarboxylation of the (2S,6R)-meso-isomer at the (6R)-position by a PLP-dependent meso-diaminopimelate decarboxylase (EC 4.1.1.20)[34,35] is the last step in the biosynthesis of L-lysine in bacteria[36] and green plants.[6] Lysine is an essential dietary amino acid for mammals since they lack this biosynthetic route.[2] L-Lysine is universally

required for protein biosynthesis and is itself involved in crosslinking cell walls of many gram-positive bacteria. DAP and small peptides containing it are rapidly excreted unaltered in urine.[37,38] As a result, DAP substrate analogs which disrupt diaminopimelate metabolism could be selectively lethal to bacteria by inhibiting both lysine production and cell wall biosynthesis.[2,39] Indeed, recent results with DAP analogs indicate this is a viable strategy for the design of antibacterial agents.[25,40]

Initial efforts of this thesis research were centered on the use of lanthionine, an unusual thia-analog of diaminopimelate,[41] in developing inhibitors of diaminopimelate DAP metabolism, with the PLP-dependent meso-DAP decarboxylase (Figure 5) as the primary target.



(G=CH$_2$, X=H) Diaminopimelate (DAP)
(G=S, X=H) Lanthionine

Lanthionine was first isolated from wool, feathers and hair[42a] as an artifact of alkaline hydrolysis.[43a] Later, lanthionine residues were found in the highly crosslinked antibiotic peptides nisin, subtilin, cinnamycin, and duramycin.[36] It has also been shown that lanthionine can be formed in a β-replacement reaction via amino acrylate by PLP-dependent cystathionine synthases

(as in Figure 2).[44]

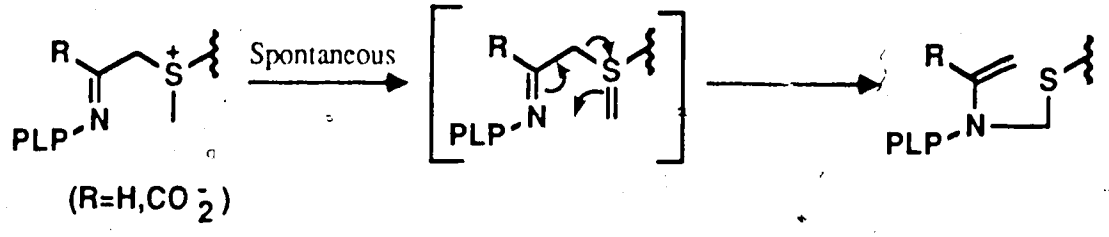
A comparison of the physical dimensions of lanthionine and diaminopimelate indicates that the increase in bond length (1.81 Å (C-S)/1.54 Å (C-C)) is partly offset by the smaller C-S-C bond angle (103.7° cf. 109.5° for C-C-C), so that the 2C-6C distance is only ~0.3 Å (<5%) longer in lanthionine. The van der Waal's radius of the sulfur is 1.70 Å compared with 2.00 Å for the sp^3-methylene group.[41] Although the electronegativity of S causes all COOH and NH_3^+ pKa's to decrease 0.5-0.8 units,[45a] relative to DAP, this decrease in acidity should have little effect at physiological pH. Not surprisingly then, lanthionine is incorporated into the cell walls of some bacteria in place of DAP,[46a,b] and actually acts as the essential diamino acid constituent in the peptidoglycan of some Fusobacterium species.[46c,47]

There has been one earlier report that a mixture of stereoisomers of lanthionine was turned over by meso-diaminopimelate decarboxylase at about 5% the rate of the normal substrate.[35c] It is known that β-elimination of cysteine thiolate (X = S^- in J) from lanthionine can be effected by pyridoxal phosphate (PLP) both nonenzymatically[48] and by β- and γ-cystathionases[49] (Category 2 and 3 enzymes) according to Figure 2. It seemed reasonable that these properties could allow a β-leaving group to be concealed in the methylene chain of DAP. Simple oxidation of the central sulfur of

lanthionine (G = S, X = H of **K**) to a sulfoxide (G = S(O)), or sulfone (G = SO_2) converts the potential thiolate (RS^-) leaving group to progressively better sulfenate (RSO^-), and sulfinate (RSO_2^-) nucleofuges.[50-52] Expulsion of one of these β-leaving groups during the catalytic cycle of meso-diaminopimelate decarboxylase would generate amino-acrylate in the active site, possibly leading to suicide inactivation as previously described for Category 1 PLP-enzymes (Figure 2). This strategy for increasing the leaving group ability to promote heterolytic fragmentation has been applied successfully in the development of penicillanic acid sulfones as suicide-inhibitors of β-lactamases.[53]

The presence of the central S-atom of lanthionine (G = S of **K**) is chemically advantagous for other reasons. S-Alkylation would generate a sulfonium salt (X = $\overset{+}{S}$-CH_3) which would be expected to undergo the desired E_1cB elimination reaction (to produce **H**, Figure 2) several orders of magnitude faster than the corresponding sulfone.[50-52,54] The S-methyl sulfonium salt of lanthionine would also have the potential for inactivation of the enzyme by a very facile spontaneous sulfonium ylid rearrangement of the α-carbanionic intermediate:[55]

Scheme 1



Because of their high reactivity however, sulfonium salts are rather unstable and could be guilty of undesirable nonselective alkylations.[51,52,54,56]

Furthermore, Pummerer-type rearrangements[57-59] on lanthionine derivatives (G = S, X = H of **K**) could introduce additional halide (**K**, G = S, X = halide) leaving groups at the β-position. Although α-halosulfides hydrolyze quite readily, the α-halo sulfoxides and sulfones (G = SO, SO_2, X = halide in **K**) should be resistant to undesirable nucleophilic attack,[50,60] but readily undergo the desired α,β-elimination which is requisite for suicide inactivation (as in Figure 2). Because its van der Waals radius (1.35 Å) and C-X bond length (1.4 Å) most closely resembles that of hydrogen (1.20 Å radius, 1.1 Å (C-H)), fluorine is the most desirable halide to introduce at the β-position for potential suicide substrates.[21,61,63]

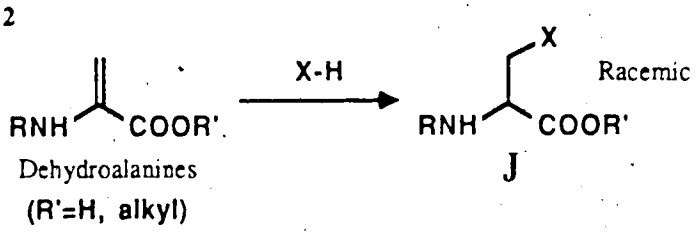
The interaction of all lanthionine analogs with L-diaminopimelate epimerase[64] and meso-diaminopimelate D-dehydrogenase (NADP-dependent)[65] enzymes is also of interest. The epimerase immediately precedes meso-DAP decarboxylase in the biosynthesis of lysine in most microorganisms and plants (Figure 5),[3,5,64] while the dehydrogenase represents a recently discovered shunt pathway which bypasses L-diaminopimelate[65] in certain bacteria. Even though these are not PLP-dependent enzymes, possible inhibition by lanthionine derivatives by

alternative mechanisms could still disrupt DAP metabolism.

The syntheses of lanthionine by established methods[42,66,67] are typical of the previous procedures for preparing β-substituted alanines (**J**) in general, and illustrate the associated problems, pitfalls and limitations.

Conjugate additions to N-protected aminoacrylates (dehydroalanines) usually proceed in good yield,[66,67,69,70] however, unlike their biological counterparts (β-replacement enzymes),[28] they suffer from an almost complete lack of stereocontrol (Scheme 2).

Scheme 2

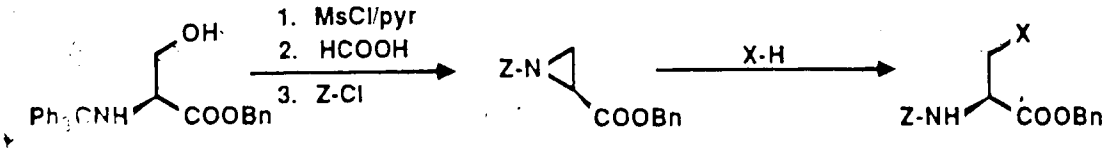


Nucleophilic displacement reactions on optically-pure O-tosyl-serine (X = TsO in **J**) or β-chloroalanine (X = Cl in **J**) are employed quite frequently but are often plagued by low yields and/or low optical purities resulting from elimination and subsequent conjugate addition (Scheme 2) side-reactions.[42,71,72] The classical general methods of amino acid synthesis (eg., Strecker, Bücherer-Bergs, etc.)[1a] are of course applicable but they also provide racemic products.

In Nature, the proteinogenic amino acid serine (X = OH in **J**) or its O-acetyl derivative (X = OAc in **J**) is the direct precursor of most β-substituted alanines.[1a,2,4,6]

In organic synthesis, since both enantiomers of serine are readily available[74] at relatively low expense they are also especially attractive chiral starting materials.[11] One earlier approach to the use of serine derivatives in the synthesis of optically-pure β-substituted alanines has involved their cyclization to an aziridine 2-carboxylic acid followed by nucleophilic ring-opening (Scheme 3).[11a,75]

Scheme 3



(Bn=CH_2Ph, Z=$C(O)OCH_2Ph$)

The synthesis of the aziridine requires several steps (<60% overall) since the trityl protecting group necessary for the desired cyclization must be replaced by an acyl moiety (eg., Z) for successful ring-openings. The reaction of the aziridine with most nucleophiles requires BF_3•etherate catalysis and proceeds well with simple thiols (RSH) and alcohols (ROH). However, yields of only 21-37% were achieved in the synthesis of lanthionine. With amine nucleophiles the predominant reaction is N-transacylation.

Difficulties in the satisfactory preparation of lanthionine derivatives by conventional routes lead to the conception of a new general route (Figure 6). According to this strategy, the cyclization of N-protected serines to the corresponding β-lactones would provide simultaneous

protection of the carboxyl group and activation of the hydroxyl as a leaving group. Nucleophilic ring-opening would introduce a side-chain substituent in the β-position while concomitantly deprotecting the carboxyl, so that products would be ready for use in peptide syntheses without further modification or could be readily N-deprotected to the free amino acid.

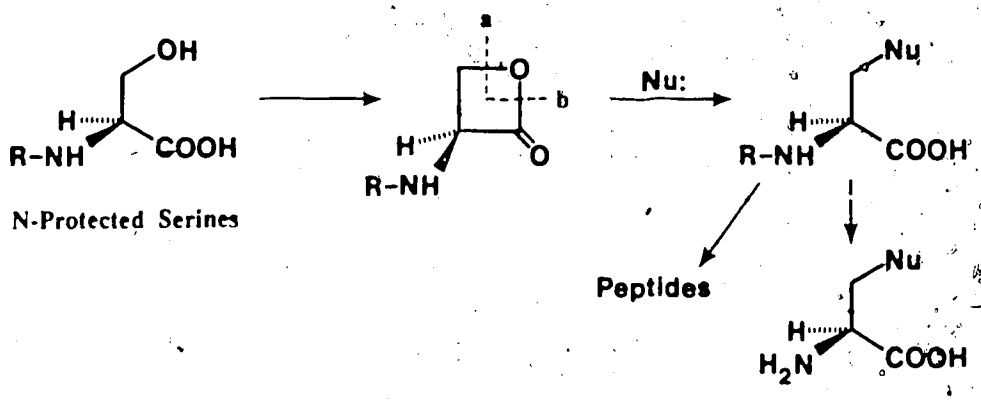


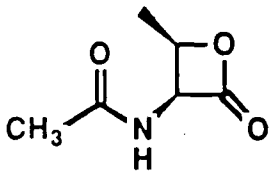
Figure 6. General Route to Amino Acids via Serine ß-Lactones.

β-Lactones are unique among the cyclic esters because the small-angle strain (~23 kcal mol^{-1})[1,9] promotes nucleophilic attack with alkyl-oxygen cleavage (**a**), in addition to the normal attack at the carbonyl with acyl oxygen cleavage (**b**). The chemistry of the parent heterocycle, β-propiolactone predicts that numerous heteroatom nucleophiles[76] and organocuprate reagents[77] could attack at the β-methylene group in S_N2 fashion (Figure 6), while "hard" nucleophiles such as alkoxides and organolithiums would likely be acylated by attack at the carbonyl.[76,78]

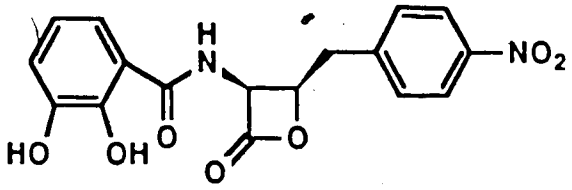
A survey of the literature revealed that the N-

protected α-amino β-lactones, N-acetyl-L-threonine β-lactone and obafluorin (below),[79] are among the few β-lactones which are produced naturally in microbes[80] and exhibit weak antibiotic activity.

The use of dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine (DMAP) in the cyclization of racemic



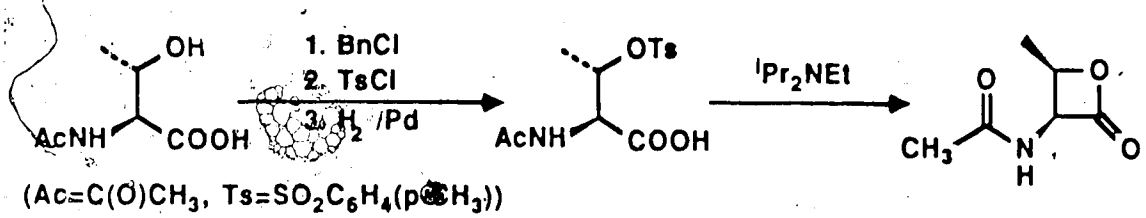
N-Acetyl-L-Threonine ß-Lactone



Obafluorin

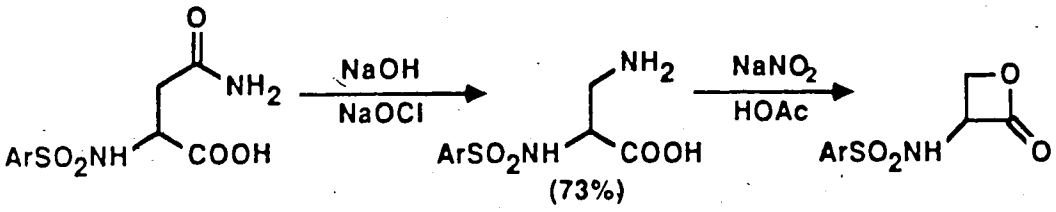
N-acetylthreonine provided the lactone in only 0.8% yield.[79b] Other attempts at lactonization of N-protected serine derivatives via carboxyl activation with carbodiimide reagents gave yields ranging from 1% or less (N-acyl) to 26% (N-trityl).[81] Cyclization of β-hydroxy acids using benzenesulfonyl chloride in pyridine[83] by a mixed anhydride intermediate is only successful for preparing tri- and tetrasubstituted β-lactones.

Alternative methods involving the generation of a leaving group at the β-position of the amino acid have been slightly more successful. The cyclization of N-acetyl-O-tosyl-DL-allo-threonine was achieved with the aid of a hindered base in 5.6% yield:[79b,81c]



(Ac=C(O)CH$_3$, Ts=SO$_2$C$_6$H$_4$(p-CH$_3$))

The Hofmann rearrangement and subsequent diazotization of N-(arenesulfonyl)-asparagines was previously the most rewarding approach, and provided the corresponding β-lactones in up to 45% overall yields.[84] However, this method appears to be restricted to the use of rather inconvenient N-arenesulfonyl protecting groups.[13]



None of the above syntheses were convenient, high yielding, or compatible with the N-alkoxycarbonyl protecting groups commonly employed in peptide synthesis. Thus, they were unattractive for use in the approach depicted in Figure 6. Of the methods available for cyclization of β-hydroxy acids to β-lactones, none is as direct or proceed under as mild conditions as the Mitsunobu reaction.[85,86] The Mitsunobu reaction (Ph_3P/ROOC-N=N-COOR) has recently proven to be of great synthetic utility in the formation of 4-membered β-lactam rings from hydroxamate (Y = NOR")[87,88b] and arylamide (Y = NAr) derivatives[89] of N-protected β-hydroxy α-amino acids (Figure 7). The analogous lactonization of β-hydroxy acids is somewhat more complicated. With most disubstituted (i.e., R_2=H, R_3 or R_4=H) acids, normal hydroxyl group activation usually results in primarily

decarboxylative dehydration to alkene (**L**). This olefin (**L**) formation ordinarily proceeds by an anti-elimination of CO_2 and $Ph_3P{=}O$ (however in the closely related Ph_3P-mediated deoxygenation of β-peroxylactones

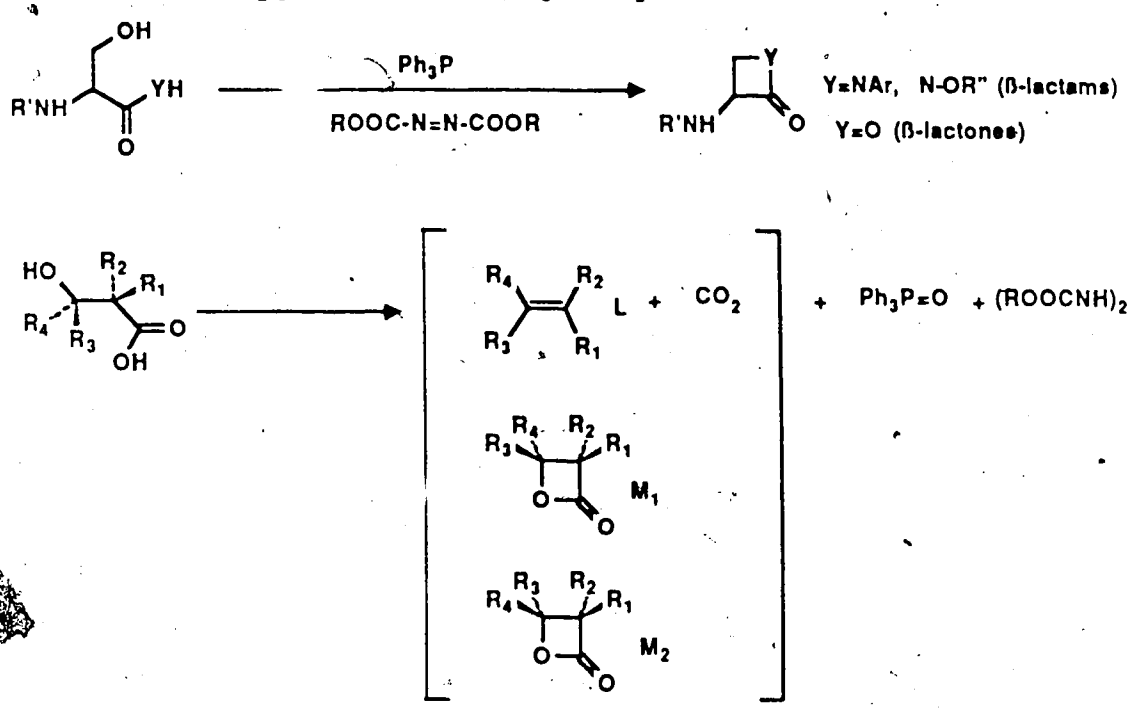


Figure 7. The Mitsunobu Reaction in the Formation of Four-Membered Rings.

when R_3 (or R_4) = Ph or vinyl a syn-elimination may occur).[86,90c] Yields of β-lactones (M_1) from anti-closure in disubstituted cases are typically 0-44% under normal conditions (THF, 25°C).[86] With hindered disubstituted, and tri- and tetrasubstituted acids abnormal carboxyl activation can occur to produce β-lactones (M_2) from syn-closure in high yield (up to 100%)[86,90] with a net retention of stereochemistry at the β-position.

Only one example of the lactonization of an α-monosubstituted β-hydroxy acid was found. N-Phenylacetyl-L-serine (R' = $PhCH_2C(O)$-, Y = O in Figure 7) was

reportedly lactonized in 1.4% yield using triphenylphosphine and diethyl azodicarboxylate in THF at 25°C.[79b] In spite of this discouraging result, and the possibilities of undesirable elimination,[86,90] or aziridine,[89a] oxazoline, or oxazolone[88] formation as the major pathway, the use of the Mitsunobu reaction in the lactonization of N-acyl and N-alkoxycarbonyl derivatives of serine was investigated.

Herein are reported successful direct syntheses of N-protected serine β-lactones (typically 70-80% yields) under modified Mitsunobu reaction conditions from serine derivatives commonly employed in peptide synthesis. Their utility as synthetic intermediates in the preparation of a wide variety of optically-pure amino acids by nucleophilic ring-opening (**a**) according to Figure 6 with both heteroatom and carbon nucleophiles was examined. This differs from the previous applications of N-protected (N-trityl, N-tosyl) serine β-lactones in which they were used primarily for synthesis of seryl esters, amides and peptides by attack of alkoxides[81a] or amines[81b,84] at the carbonyl resulting in acyl-oxygen cleavage (**b**).

The facile removal of the protecting group from N-(tert-butoxycarbonyl)serine β-lactones (R' = ^{t}BuOC(O)-, Y = O in Figure 7) enabled the study of the heretofore unknown 3-amino-2-oxetanone salts (R' = $^{+}H_2$, Y = O) as a direct synthetic analogy of the PLP-dependent β-replacement enzymes (Category 2).

Our advances in the preparation of serine β-lactones under modified Mitsunobu reaction conditions and realization of their synthetic utility prompted us to consider methods for large scale production of the β-lactones. The major problems associated with industrial-scale application of the Mitsunobu reaction have recently been elaborated by Miller[87a] as the expense of the azodicarboxylate reagent (ROOC-N=N-COOR), and the requisite chromatographic separation of product from triphenylphosphine oxide and dialkyl hydrazodicarboxylate (ROOC-NHNH-COOR) side-products. In addition, there is some danger of explosion on purification of the azodicarboxylate reagent by distillation.[91] Alternative methods of producing the same postulated oxyphosphonium intermediate for lactonization were considered,[86b,87] and dismissed because the facility with which β-lactones are ring-opened dictates the need for essentially neutral conditions and the absence of external nucleophiles.

Instead of avoiding the problems associated with Mitsunobu reactions on industrial scale, an attempt was made to overcome them. This would not only be important for production of the serine β-lactones, but could generally make the versatile condensation method expedient in the syntheses of many pharmaceuticals, and related compounds.

As illustrated in Figure 8, reactions employing triphenylphosphine and dialkyl azodicarboxylates are capable

of replacing hydroxyl groups with a large number of O, N, C, and halogen nucleophiles.[86,87] In contrast to many related condensation reactions, Mitsunobu-type conversions proceed under mild, essentially neutral conditions and exhibit stereospecificity, functional selectivity, and regioselectivity.[86,87] Because of these features it has many established applications in syntheses of macrolide antibiotics,[86] nucleosides (including azidothymidine (AZT))[86,92] and nucleotides,[86] amino acids,[93] amino sugars, steroids, natural products,[86] and various heterocycles[88] including monobactam antibiotics and precursors of other important β-lactams.[87,89]

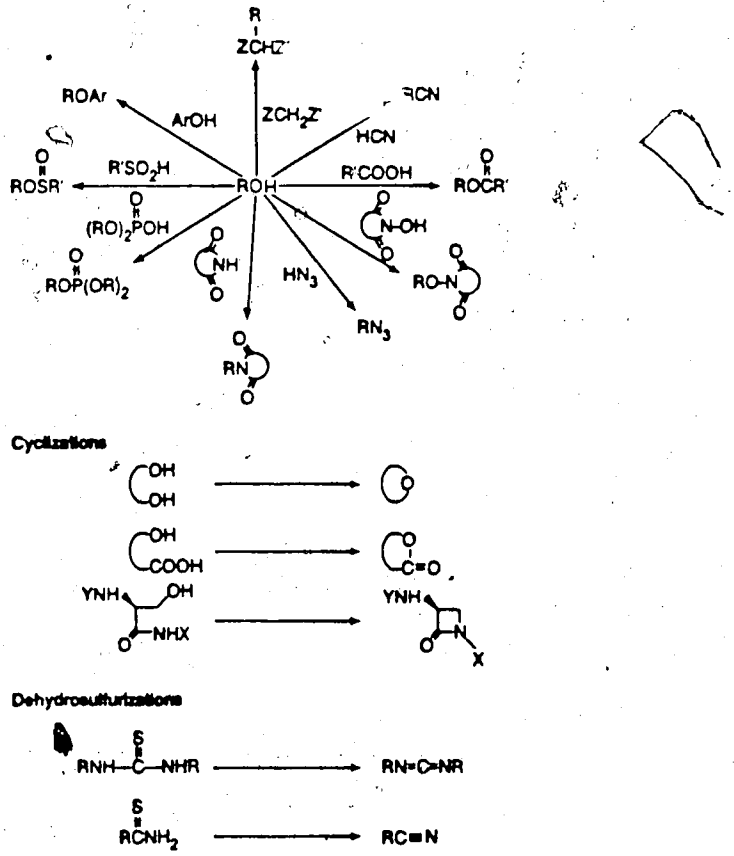


Figure 8. Applications of the Mitsunobu Reaction

Triphenylphosphine is an inexpensive reagent (i.e., ~$30/kg). Its progeny $Ph_3P=O$ may frequently be separated from Mitsunobu condensation products by selective crystallization. Therefore, a logical solution to the above practical problems involves the immobilization of the azodicarboxylate moiety on an insoluble polymeric support (i.e. Polymer~OOC-N=N-COOR). This could eliminate both the danger associated with distillation of ROOC-N=N-COOR, and in many cases the requirement for chromatographic purification of products. The high cost of the azodicarboxylate reagent would be greatly reduced since the "spent" resin in the hydrazodicarboxylate form (Polymer~OOC-NHNH-COOR) could be recovered by filtration. Regeneration of the polymer-supported reagent by reoxidation with one of a number of inexpensive oxidizing agents[91,94,95] would allow it to be used over and over. Similar immobilization of reagents[96] and catalysts[97] has previously proven to be very effective at simplifying purification and/or reducing costs by allowing regeneration. In addition, the advent of solid-phase peptide synthesis has provided much supporting technology for the preparation and use of reagents covalently bound to polymeric supports.[14]

RESULTS AND DISCUSSION

Resolution of Diaminopimelate Stereoisomers

A quantitative assessment of the degree of stereochemical preference of the enzymes acting on diaminopimelate (DAP) required the use of pure stereoisomers in biochemical studies. In addition, the preparation of optically pure L-(2*S*,6*S*)-isomer eventually allowed the utilization of a coupled spectrophotometric assay for DAP-epimerase (see Appendix 1). This necessitated the resolution of 2,6-diaminoheptanedioic acid (diaminopimelate; DAP) which is commercially available[98] as a statistical (2:1:1) mixture of *meso*-(2*S*,6*R*), L-(2*S*,6*S*)-, and D-(2*R*,6*R*)-stereoisomers (**1**) (Figure 9).

The separation of *meso* and racemic diaminopimelates was conveniently achieved by selective crystallization of the N^2,N^6-bis(benzyloxycarbonyl) derivatives (**2**) according to Wade *et al.*[99] In this procedure, DAP (**1**) is reacted with benzyl chloroformate under Schotten-Baumann conditions to produce **2** (88%), which after successive recrystallizations (typically thr) from ethyl acetate provides crystalline racemic bis(benzyloxycarbonyl) derivative **3d**. When this material is diastereotopically-pure, further recrystallizations do not alter the melting

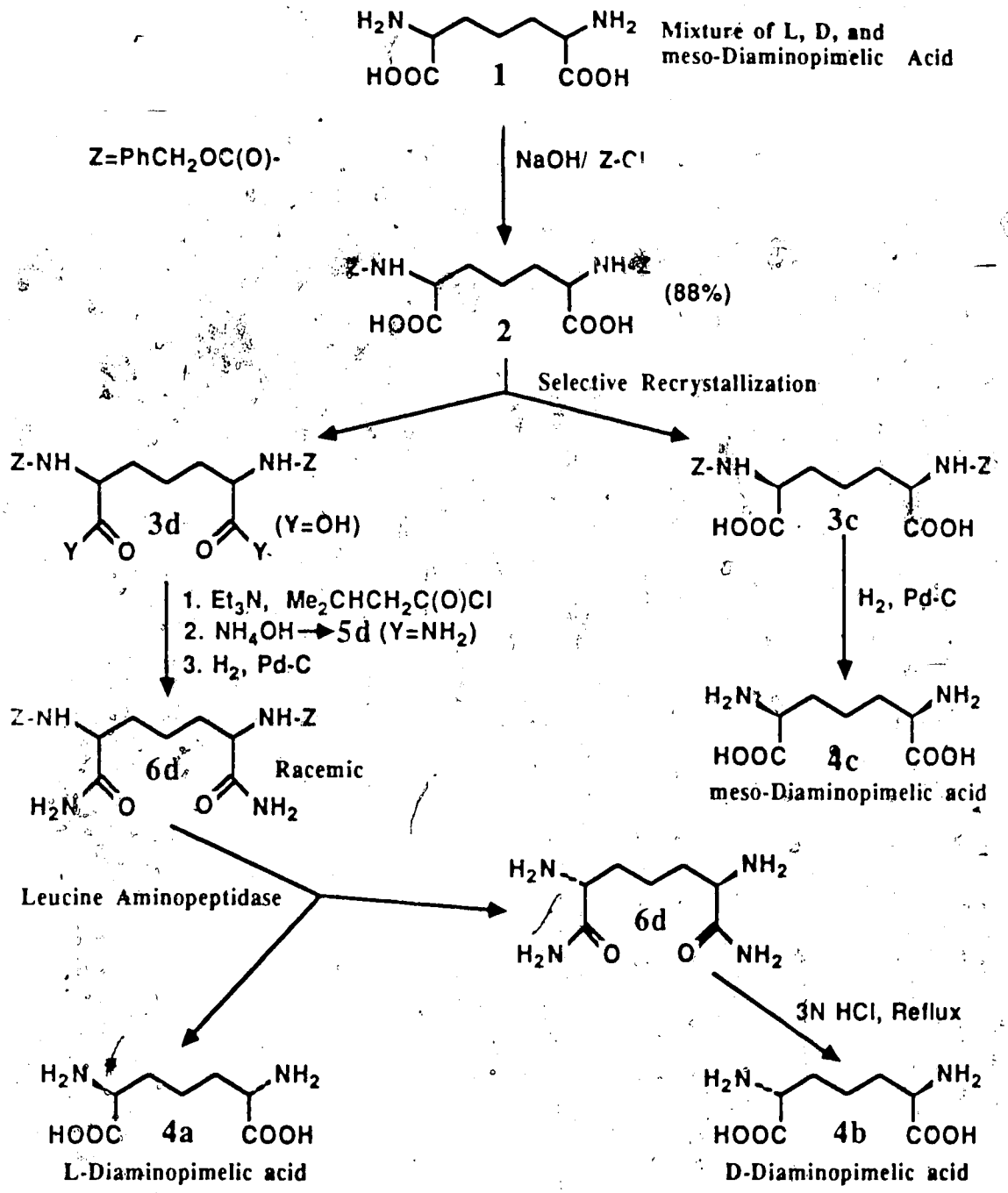


Figure 9. Resolution of Stereoisomers of Diaminopimelic Acid.

NOTE: All L, D, meso, and racemic compounds bear a, b, c, d, designators, respectively, throughout the thesis.

point (mp 164-165°C). The meso-diastereomer **3c** is subsequently obtained by concentration of ethyl acetate mother liquors and recrystallization from chloroform. The di-Z-meso compound **3c** was further purified by recrystallization from acetonitrile as suggested by Heijnoort and Bricas,[100] before hydrogenolytic deprotection to meso-(2S,6R)-diaminopimelic acid (**4c**) (58% from **2**). No diastereomeric (racemic) impurities could be detected by ^{13}C NMR in **4c** prepared by this method (>97% pure).

Both enzymic[99] and physical methods[100] have been employed in the literature for the resolution of L-(2S,6S) and D-(2R,6R) isomers of diaminopimelate as its diamide (**6d**). The physical method[100] utilizes selective recrystallization of the diastereomeric bis(dibenzoyl)-D-tartarate salts to provide partial (80%) resolution, followed by hydrolysis and numerous crystallizations of the bis(dicyclohexylamine) salt of the amino acid to provide optically pure material (**4a** and **4b**) (17-29% overall yields).[100] Instead, a modification of the published enzymic procedure[99,101] (employing leucine aminopeptidase in place of hog kidney amidase) was chosen. It provides a greater degree of confidence in the final optical purity, and higher yields, and may easily be performed on several grams of material with much less sample manipulation (Figure 9) than the physical method.

The racemic di-Z-derivative **3d** is converted to the

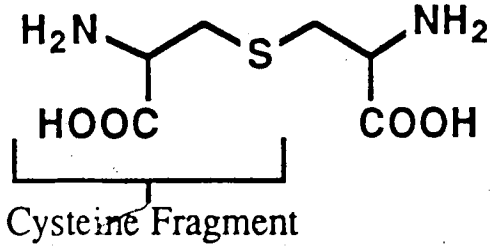
corresponding diamide **5d** (65%) by treatment of the mixed anhydride of **3d** and isovaleric acid with ammonium hydroxide.[99,101] Hydrogenolysis of **5d** quantitatively afforded the diamide of racemic DAP as its diacetate salt (**6d**) which was digested by leucine aminopeptidase at pH 8.0 to provide a mixture of L-DAP (**4a**) and D-diaminopimelate diamide (**6d**). These products were readily separated by cation exchange (Rexyn 102, Li^+ form), however the L-isomer **4a** which eluted with H_2O was found to be contaminated with much Li_2SO_4, which originated from the 2.9 M $(NH_4)_2SO_4$ solution in which the commercial enzyme was obtained. The steps required to remove Li_2SO_4 by precipitation as $BaSO_4$ reduced the final overall yield of **4a** to 52%, and should be avoided by removal of ammonium sulfate from the commercial enzyme preparation by dialysis or ultrafiltration before use.

D-(2R,6R)-Diaminopimelate diamide (**6b**) eluted from the ion-exchange resin chromatographically-pure and free of salts. Since reincubation of a portion of **6b** with leucine aminopeptidase caused no further appreciable hydrolysis, it was directly hydrolyzed in 3N HCl to liberate **4b**. Recrystallized L-(2S,6S)- (**4a**) and D-(2R,6R)-diaminopimelates (**4b**) possessed rotations matching reported values for pure isomers.[99-101] Subsequent incubations of **4a** and **4b** (10 mM) with meso-DAP D-dehydrogenase, which has a very strict specificity for meso-DAP (**4c**) (K_m = 1.1 mM, see Appendix 1), displayed

initial rates which were 0.0% and 0.2% respectively of that obtained with meso-DAP (**4c**), thus indicating very high diastereotopic purity.

Syntheses of Lanthionine and Its Derivatives

Lanthionine is also commercially available[98] as a mixture of all stereoisomers, however conditions for resolution have not been reported in the literature. Since lanthionine may be considered to be a β-substituted alanine (i.e., **J** (X = cysteine)), it appeared that synthesis of optically-pure material would be easier than resolution. The disadvantages and problems associated with the synthesis of stereochemically-pure lanthionines by established approaches are illustrative of those commonly encountered in the preparation of β-substituted alanines (**J**).



Lanthionine

Syntheses of totally racemic lanthionine[102] by classical methods offered no advantage and were not considered. The relatively high yield observed at minimal expense in the alkylation of cysteine (**9**) by acetamidoacrylate (**8**)[66] (analogous to biological syntheses) made that route attractive (Figure 10), especially in light of earlier reports that meso-(2S,6R)

and L-(2S,6S)-lanthionine diastereomers could be separated by selective crystallization.[67] In addition, it was envisioned that a selectively mono-N-acetyl-meso-lanthionine could later be useful in a stereospecific oxidation to provide a single sulfoxide diastereomer (Route a, Figure 10)[103] after hydrolysis.

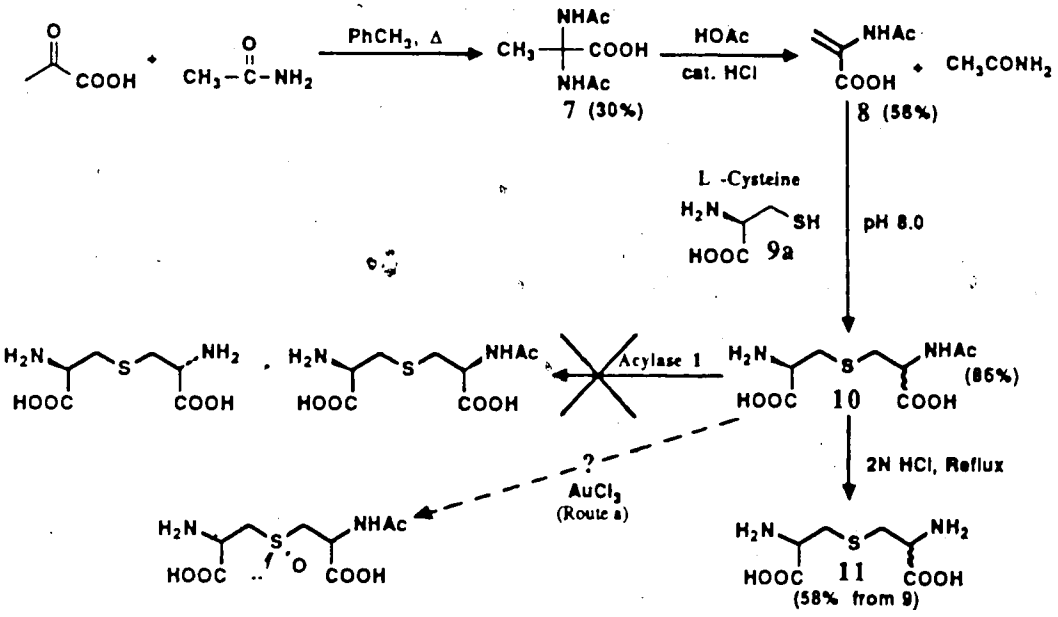
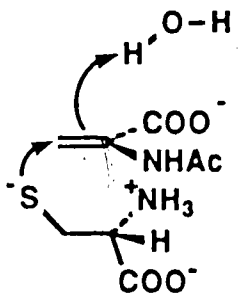


Figure 10. Dehydroalanine Route to Lanthionines.

Bis(2-acetamido)propionic acid (**7**) was prepared in low yield by condensation of inexpensive pyruvic acid and acetamide,[104,105] and converted to acetamidoacrylate (**8**) by heating in glacial acetic acid with a catalytic amount of HCl.[104,106] The conjugate addition of the proteinogenic amino acid, L-cysteine (**9a**) to 8 at pH 8.0, 95°C proceeded smoothly to provide an 86% yield of mono-N-acetyllanthionine as a mixture of meso-(2S,6R) and L-

(2S,6S) isomers (**10**) after purification by cation exchange chromatography.

Alternatively, the crude reaction mixture containing **10** could be directly hydrolyzed by refluxing 2N HCl, and (2S,6SR)-lanthionine (**11**) isolated by crystallization (58% from **9a**) near its isoelectric point (pI). A comparison of the optical rotation of **11** with that of pure L-lanthionine suggested 80% meso-(2S,6R), and 20% L-(2S,6S)-isomers. A possible rationalization for the diastereoselective predominance of the meso-isomer is the involvement of a transition state in which steric and electrostatic repulsions are minimized by an anti-addition to the re-face of 8:



Selective crystallization of the less soluble meso-isomer (0.22 mg/mL at pH 7, 25°C) in the presence of L-lanthionine (15 mg/mL)[67,102] proved to be very slow, tedious and inefficient. Instead, the possibility of enzymic resolution of meso-(2S,6R) and L-(2S,6S)-isomers by treatment of **10** with hog renal Acylase I, in a manner analogous to that used to resolve its disulfide homolog cystine (**12**),[107] was examined. Mono-N-acetyl-L-lanthionine proved to be extremely resistant to hydrolysis by Acylase I at pH 7.5, 37°C, with less than 3% conversion

in 24 h even in the presence of 25% by weight of enzyme (much denaturation of the enzyme did occur however). This approach was therefore abandoned in favor of direct chemical synthesis of optically-pure lanthionines.

Harpp and Gleason[71a] have reported a novel synthesis of optically-pure L-lanthionine from readily available L-cystine by selective desulfurization according to Figure 11. Some time later Olsen et al.[71b] reported a variation of this approach using cystine-S-sulfinates to prepare unsymmetrical lanthionines of undetermined optical purity.

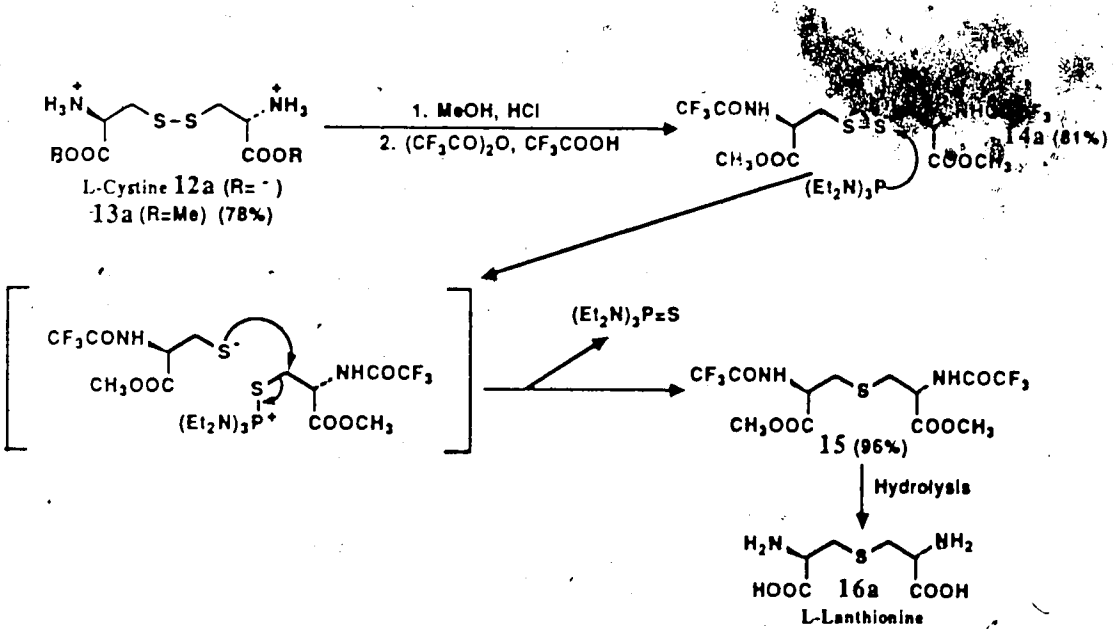
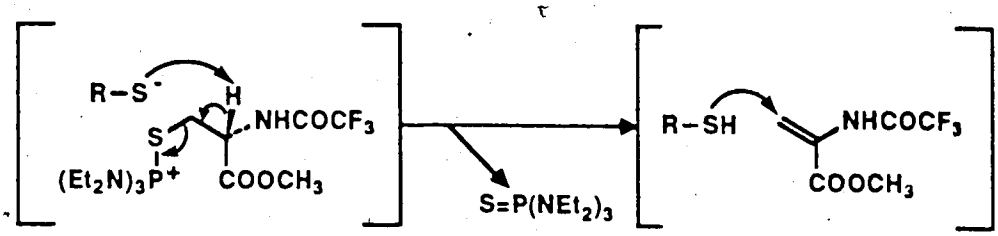


Figure 11. Selective Desulfurization Route to Lanthionine.

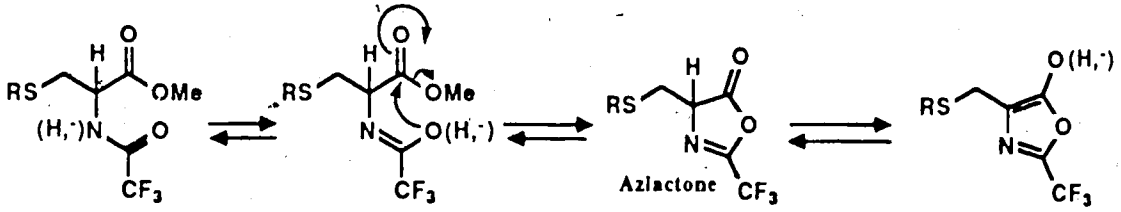
Esterification of L-cystine (**12a**) with methanol/HCl provided the dimethyl ester (**13a**), which was treated with

trifluoroacetic anhydride in TFA[71a] to yield **14a** (81%) with high optical purity (i.e., 97-100% based on $[\alpha]_D^{25}$). When this material was subjected to desulfurization with tris(diethylamino)phosphine under the conditions prescribed by Harpp and Gleason[71a] on a 5 to 50 mmol scale, excellent yields of **15** (96%) were obtained. Contrary to the literature report,[71a] the optical purity of the recrystallized product (**15**) was highly variable, with $[\alpha]_D^{25}$ values of -23°, -15.5° and -9.8° observed for reactions on 50, 10 and 5 mmol scale respectively (lit. $[\alpha]_D^{25}$ -21.6, -32.4° both reported in different locations of the same paper for the same compound, (c0.4, MeOH)). Furthermore, the authors reported[71a] that a brief alkaline hydrolysis (NaOH/aqueous dioxane, 5°C, 30 min) produced L-lanthionine with an $[\alpha]_D^{25}$ exceeding any previously reported by greater than 9%. In our hands, either alkaline hydrolysis as described by Harpp and Gleason,[71a] or acid hydrolysis (2.5N HCl reflux) of **15** ($[\alpha]_D^{25}$ -23°) yielded L-lanthionine (**16a**) with 21% and 30(±3)% optical purity, respectively. The presence of 60-65% meso-lanthionine and absence of salts in these preparations was confirmed by HPLC analysis.[108]

Racemization during the desulfurization of **14** by an elimination/addition side-reaction appears likely, especially considering the notoriety of esters and N-trifluoroacetyl groups[1a,13] in stabilizing an incipient α-carbanion and the proven facility of elimination of



similar thiolates.[13] Further losses in optical purity probably occur in acid or base hydrolysis of **15** via formation of the azlactone (oxazolinone) and subsequent favorable tautomerization which is frequently observed with N-trifluoroacetyl amino acid derivatives. Olsen et al.[71b] may have avoided these problems by the use of N-(alkoxycarbonyl) protecting groups,[13] but suffered considerably reduced yields.



Discouraged by the failure of the selective desulfurization route to provide optically-pure lanthionine, we turned to the method of Brown and du Vigneaud[42a] which was employed for the first syntheses of lanthionine stereoisomers. This procedure involves a nucleophilic attack on β-chloroalanines (**21a,b**) common in many syntheses of β-substituted alanines. For the production of L-(2S,6S)-lanthionine (**16a**) (Figure 12),[109] β-chloro-L-alanine (**20a**) was prepared in three steps from the common proteinogenic amino acid L-serine. This required esterification to **17a** (85-95%) reaction with

phosphorus pentachloride with acetyl chloride as a solvent[110] (79-81% of **18a**), and finally acid hydrolysis to **20a** (78-90%).

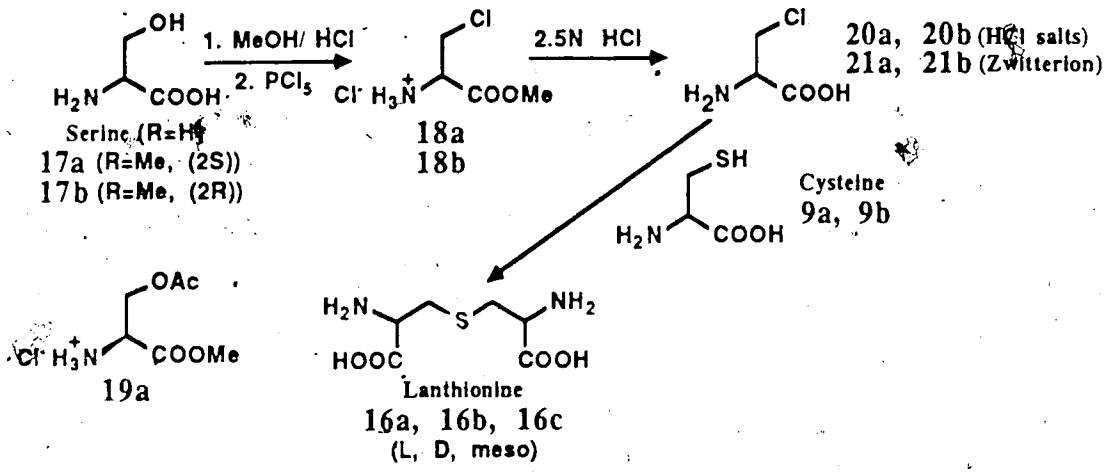


Figure 12. Synthesis of Lanthionine from ß-Chloroalanine.

The successful replacement of the hydroxyl of **17a** with Cl by reaction with PCl_5 required that L-serine methyl ester hydrochloride (**17a**) be totally freed of residual methanol by recrystallization, pulverization and drying in vacuo (over P_2O_5/KOH). Otherwise the O-acetyl-L-serine methyl ester·HCl (**19a**), a "dead-end" side-product (usually 3-5%), predominated in 50-73% yield. In order to minimize the amount of KOH required in the next step, and obtain a definitive melting point and rotation, **20a** was converted to the zwitterion of β-chloro-L-alanine (**21a**)[110] by recrystallization at its isoelectric point (pH 5.8).

The high optical-purity β-chloro-L-alanine (**21a**) was

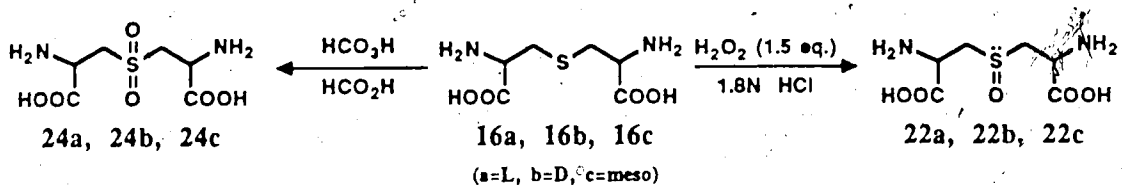
treated with L-cysteine (**9a**) under alkaline conditions to produce L-lanthionine (**16a**) which crystallized upon reduction of the pH to ~6. The yield of **16a** was increased from 56% to 81% (based on **21a**) by the use of 1.8 equivalents of cysteine rather than 1.3 equivalents originally employed by Brown and du Vigneaud.[42a] An analogous procedure utilizing relatively inexpensive D-serine[74] was enlisted to produce meso-(2S,6R)-lanthionine from β-chloro-D-alanine (**21b**) and L-cysteine (**9a**), while the synthesis of D-(2R,6R)-lanthionine required the use of **21b** and D-cysteine (**9b**).

HPLC analyses[108] on lanthionine stereoisomers produced according to Figure 12 indicated less than 3% diastereomeric impurity was present in all cases. Optical rotations of the L- (**16a**) and D-isomers (**16b**) suggested optical purities greater than 96 and 92%, respectively. Later enzymological studies with meso-DAP D-dehydrogenase suggested 1.0% and 2.7% meso-contaminant, respectively, in these materials (see Appendix 1). This procedure (Figure 12) was used successfully to prepare lanthionine isomers on large scale (>100 g)[109] without difficulty, but other workers[71a] have reported substantial losses in optical purity and low yields under the alkaline reaction conditions. Such problems are typical in the preparation of β-substituted alanines from β-chloroalanines,[72] and were eventually avoided through the use of the serine β-lactones.

It was found that the preparation of lanthionine sulfoxides and sulfones from lanthionines (**16**) could be conveniently effected by chemoselective S-oxidations under acidic conditions in which the amino and carboxyl functionalities were fully protonated (Scheme 4).

The sulfoxides (**22a, 22b, 22c**) were prepared by oxidation of the pure stereoisomers of lanthionine (**16a, 16b, 16c**) with hydrogen peroxide in aqueous acid. Under acidic conditions the oxidation essentially stops at the sulfoxide stage, and **22a** (L)/**22b** (D) may be isolated in 71% recrystallized yield. The meso-compound **22c** was obtained in 86% yield under identical conditions (probably due to its lower solubility) and is presumably a mixture of two optically-inactive diastereomers (i.e., (2S,4S,6R) and (2S,4R,6R)). Because of the instability of the sulfoxides in acid,[67] the reaction time should not be unnecessarily prolonged.

Scheme 4



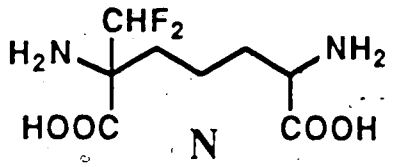
In the presence of 10 mol% molybdate ($MoO_4^=$) the oxidation with acidic H_2O_2 goes beyond the sulfoxide to the sulfone (**23**),[67] however over-oxidation to cysteic acid, low yields (30-38%), and product contamination with molybdate made this method unsatisfactory. A mixture of performic acid in formic acid, produced from H_2O_2 and

HCOOH, is known to achieve chemoselective oxidation of half-cystine and methionine residues of peptides and proteins under relatively mild conditions.[111] Simple treatment of the pure isomers of lanthionine (**16a, 16b, 16c**) with 3.5 oxidizing equivalents of the HCO_3H/HCOOH mixture (0°, 2 h), followed by removal of the solvent *in vacuo* and recrystallization, conveniently afforded the corresponding sulfones **24a, 24b, 24c** in 90-91% yields.[40,109]

The various lanthionine derivatives (**16**, **22** and **24**) were tested against diaminopimelate-associated enzymes by Dr. M. Palcic and Dr. L. Lam, and the results are tabulated in Appendix 1. Although none of these derivatives caused pseudo-first order inactivation of the enzymes (the first criterion for a suicide substrate), the results are still significant.

meso-Diaminopimelate decarboxylase from *B. sphaericus* and from wheat germ slowly acted on only *meso*-lanthionine in accord with previous results,[35c] and its conversion to thialysine (i.e., the thia-analog of lysine) was later confirmed by synthesis of authentic material. This enzyme usually displays extremely strict specificity for *meso*-DAP, with its L- and D-isomers acting neither as substrates nor competitive inhibitors.[34] This "tight-fit" of enzyme to substrate accounts for the nonacceptance of the α,α-difluoromethyl analog of DAP (**N**) (prepared by Dr. J. Kelland) at the active site, and its resultant failure

as a suicide substrate for the decarboxylase, in spite of ample precedent with related decarboxylases.[8,21,61,63]



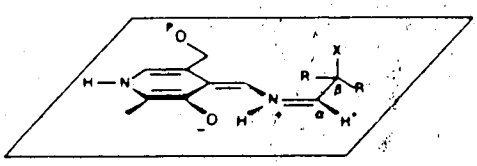
Similar exclusions of saturated and unsaturated analogs of DAP bearing substituents in α- and γ-positions by meso-DAP decarboxylase have been observed by other researchers.[25b] In violation of this enzyme's normally high stereospecificity,[34] all of the stereoisomeric sulfoxides were reasonably good competitive inhibitors of meso-DAP decarboxylase with L-(2S,6S) (K_i ~0.9 mM)) > meso > D-(2R,6R) in order of effectiveness. The unexpectedly greater effectiveness of the L-(2S,6S)-lanthionine sulfoxide (**22a**) relative to the meso (**22c**) may suggest that only one of the diastereomeric meso-sulfoxides (i.e., either (2S,4S,6R) or (2S,4R,6R)) is capable of binding at the active site.[103]

meso-Diaminopimelate D-dehydrogenase (NADP-dependent) from B. sphaericus utilized meso-lanthionine (**16c**) as a relatively poor substrate (K_m 5.8 mM, V_{max} ~1% that of meso-DAP) and largely ignored all other isomers and derivatives. In contrast, meso-lanthionine (**16c**) displayed effective mixed inhibition (K_i = 0.18 mM, K_i' = 0.66 mM) of DAP epimerase (not a PLP-dependent enzyme), while the L-isomer (**16a**) acted as a competitive inhibitor (K_i = 0.43 mM). A comparison of binding affinities for

lanthionine derivatives (see Appendix 1) with the decarboxylase and epimerase indicates that meso- and L-lanthionines are ~40-50 fold better inhibitors of the epimerase than the decarboxylase. Inversely, the corresponding sulfoxides, especially the L-isomer, are about ten-fold better inhibitors of the decarboxylase than the epimerase. This implies that the specificity of lanthionine derivatives for either of these two enzymes may be controllable by simple manipulation of the oxidation state of the sulfur.

Since no suicide substrate for any of these enzymes has yet been found, the lanthionine backbone provides a most promising skeleton on which to append reactive functionalities which may lead to mechanism-based inactivation. Since it was demonstrated that all meso and L-isomers of the lanthionine derivatives bind to some extent (Appendix 1) the lack of suicide inhibition of the PLP dependent decarboxylase by these compounds may be due to any or all of several factors. The first is that the enzyme must exercise part of its function before the latent reactive group is revealed. In this case, generation of the α-carbanionic intermediate D_3 (below) must occur. However actual turnover has only been verified for meso-lanthionine (**16c**). The second

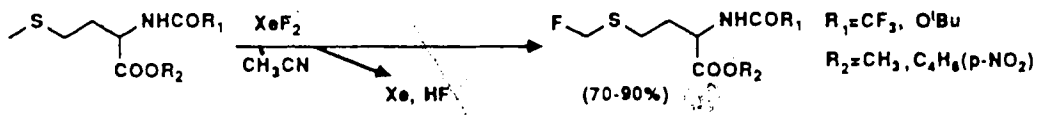
D_3 of Figure 2



possibility is that intermediate D_3 forms, but perhaps due to lack of protonation of the sulfur-containing fragment, the leaving group-ability of the β-nucleofuge (X) may have been insufficient to effectively reroute D_3 from its normal course (recall Figure 1). A third consideration is that in order to exert its stereochemical preference for meso-DAP, the decarboxylase must bind the substrate at both chiral centers. The binding of the distal groups may "misalign" the bond between the β-carbon and the sulfur so that it occupies the nonorthogonal R or R' sites which are stereoelectronically disfavorable for elimination. Finally, the enzyme may be immune to inactivation according to Figure 2 as many β-elimination (Category 2) enzymes appear to be, likely due to a lack of an appropriately placed nucleophilic group.

If an unfavorable conformation is responsible for failure of elimination (i.e., from D_3 above) the replacement of one or both β-hydrogens with a halogen (e.g., fluorine) might afford a suicide inactivator for the decarboxylase.[61,62] The introduction of a halogen in the β-position of the lanthionines should be facilitated by the potential for Pummerer-type rearrangements.[57-59,112,113] The most successful methods of introducing fluorine on a carbon adjacent to sulfur involve the reaction of (diethylamino)sulfur trifluoride with a sulfoxide,[58] or direct fluorination of sulfides with xenon difluoride.[59,112,113] Both of these reactions

are believed to proceed through the same sulfonium cation intermediate (i.e., $RCH=\overset{+}{S}R'F^-$).[58,59,113] It was hoped that the lower temperatures[113b] and more polar acetonitrile solvent commonly used for the XeF_2 reactions[59,112,113] would promote substitution and reduce HF-elimination.[60] Janzen et al.[113a] had recently reported high yields in the oxidative fluorination at the 6-methylthio position of protected methionine derivatives by XeF_2 under mild conditions, and the integrity of the CFH_2S-moiety in

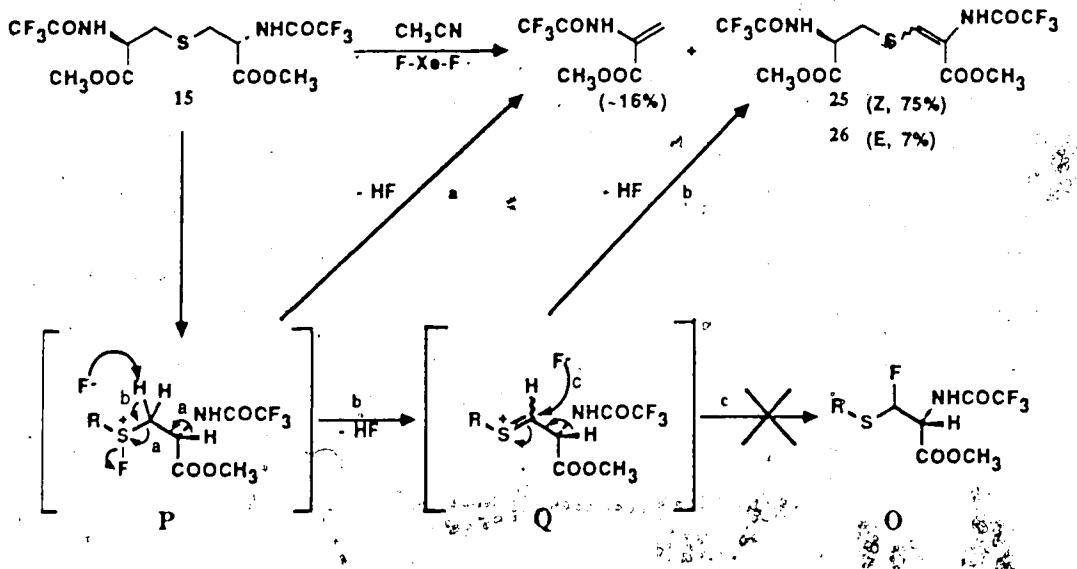


aqueous alkaline media. Hence, these conditions appeared ideal for generation of the α-fluorothioether of the previously prepared N,N'-bis(trifluoroacetyl)-L-lanthionine methyl ester (**15**), which could later be deprotected by treatment under mild alkaline conditions.[71]

Injection of **15** in acetonitrile into a solution of XeF_2 at -23°C followed by slow warming to 20°C resulted in the evolution of greater than 2 mole equivalents of gas (Xe + >1 eq. HF). Quenching of the reaction with hexamethyldisilazane (HMDS) and removal of solvent was followed by NMR analysis. ^{19}F and ^1H NMR indicated that none of the desired fluorinated product (**O**, Scheme 5) was present, but only the olefinic materials **25**, **26** and methyl [N-trifluoroacetylamino]acrylate (~16% by ^1H NMR). The Z-

and E-bis(TFA)-L-dehydrolanthionine methyl esters (**25** and **26**) were subsequently isolated by chromatography in 75% and 7% yields, respectively.

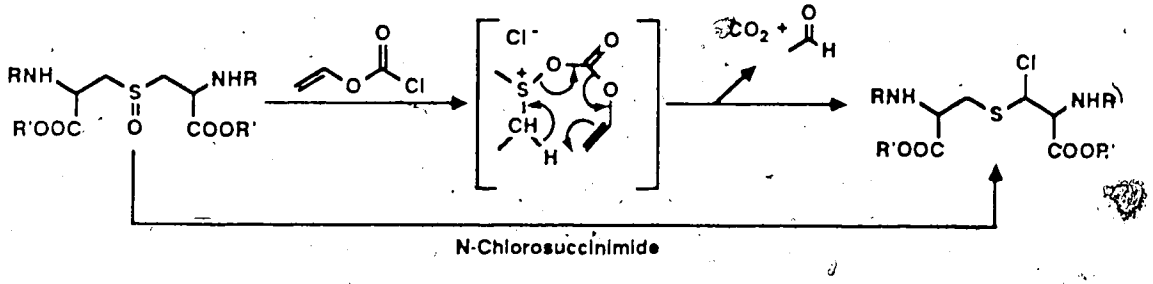
Scheme 5



Although it was not observed in the methylthio fluorination of methionine (above), α,β-olefin formation from isopropyl sulfides[113b] or γ-ketosulfides bearing acidic hydrogens[112,113c] in the β-position is not uncommon. In retrospect, this behavior is not surprising in the case of **15** which has demonstrated the lability of its 2-C-H before in desulfurization and deprotection. Since the desired fluorinated product was totally absent, and greater than the theoretical amount of gas was evolved before quenching with HMDS, olefin formation probably does not result from a simple loss of HF from the α-fluorothioether (**O**), but rather as depicted by Scheme 5. The undesirable basic character of the "naked" fluoride anion predominates over its nucleophilic behavior and

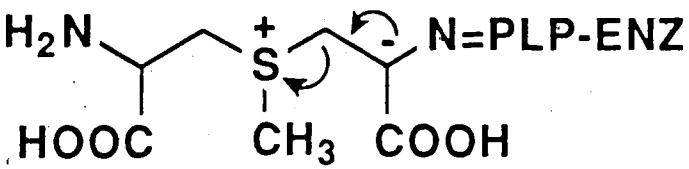
either causes elimination of RSF from P (path a) with formation of an aminoacrylate or loss of a second mole of HF from Q (path b) to produce **25/26** as the observed products, in lieu of the desired O (path c).

These results discouraged further attempts at introducing fluorine by Pummerer-type rearrangements. However, since fluoride is unique among the halides in possessing considerable basic character,[114,115] it may be possible to produce β-chlorolanthionines by reaction of the corresponding sulfoxide with N-chlorosuccinimide (NCS)[57a] or vinyl chloroformate[57b] under essentially neutral conditions without the complications of elimination.

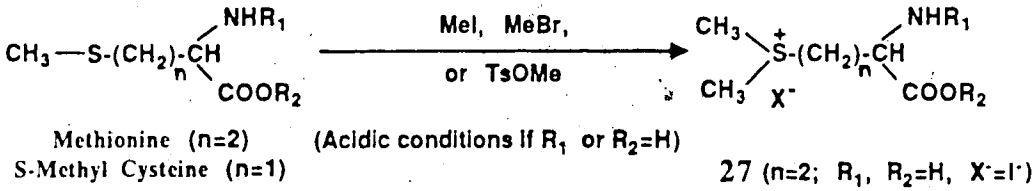


If the leaving group ability of the β-nucleofuge (X) was insufficient to reroute the α-carbanionic intermediate (D_3 above), then S-methylation of the sulfur of lanthionine should greatly increase the potential for heterolytic fragmentation[23] of D_3 in the active site (see below).[50-52,54] In addition, there is a chance of the aforementioned (Scheme 1) spontaneous sulfonium ylid rearrangement occurring with D_3.

Since it was known that methionine (either free[56] or



in proteins[116]) and N-protected derivatives of S-methyl cysteine[116b] could be directly and chemoselectively alkylated at low pH (<3.5), it seemed logical to extend these reactions to the S-alkylation of lanthionine.

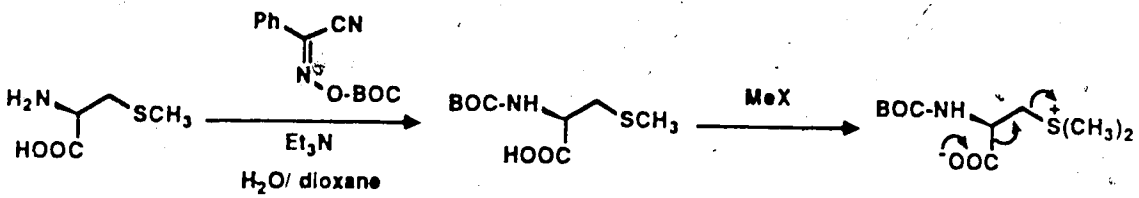


All attempts at S-methylating unprotected lanthionine in acidic media (pH 3[116a] → HCO_2H/AcOH[56a] (1:1)) with methyl iodide (4-15 eq.) were unsuccessful. Even when the reaction was stirred two weeks with repeated additions of CH_3I, ^1H NMR indicated that only solvolysis of methyl iodide had occurred. Similarly no S-methylation could be detected with the more soluble mono-N-acetyllanthionine (**10**) at pH 3.5 with 15 equivalents of MeI after two weeks. As a check of this procedure, L-methionine was subjected to identical conditions with only 2 equivalents of CH_3I in D_2O and the reaction was monitored by ^1H NMR.

Within 4 h at 25°C S-methylation had proceeded 45%. After 23 h the reaction was 87% complete and the S-methylmethionine sulfonium salt (as the iodide, **27**) was isolated in 64% recrystallized yield. A similar ^1H NMR experiment with the lower homolog S-methyl L-cysteine required more reactive dimethyl sulfate (3 eq.) and 13 days to reach 60% completion. These results suggest that increasing β-branching greatly slows the reaction, presumably for steric reasons. In further attempts, treatment of lanthionine in neat CF_3COOH or CF_3COOH/H_2O with excess methyl iodide and silver trifluoroacetate resulted in a small amount of S-methylation (~20%), but was plagued by much decomposition, and rapid solvolysis of CH_3I. Lanthionine remained unaltered after 9 weeks in the presence of methyl p-toluenesulfonate (4.5 eq.)/p-toluenesulfonic acid (2 eq.) in CF_3COOH. Finally, treatment of the ditosylate salt of lanthionine in CF_3COOH with trimethyloxonium fluoborate[117] resulted in the formation of only CF_3COOCH_3 and TsOMe.

S-Methyl L-cysteine was treated with 2-(tert-butoxycarbonyloximino)-2-phenylacetonitrile ("BOC-ON")[118] to provide N-(tert-butoxycarbonyl)-S-methyl-L-cysteine (**29**) which was used to investigate nonprotic conditions for S-alkylation of lanthionine derivatives. Studies with **29** indicated that S-methylation only occurred at an appreciable rate in polar solvents such as CH_3CN and DMF (DMF > CH_3CN), and that attempted isolation by

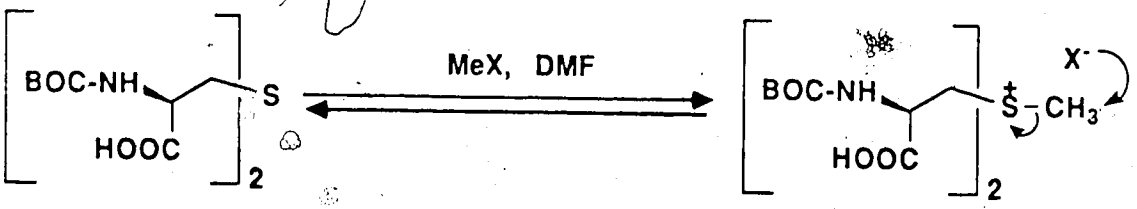
crystallization of the zwitterion led to rapid decarboxylative elimination of dimethyl sulfide.



BOC=$(CH_3)_3COC(O)$—

29

With this knowledge, N,N'-bis(tert-butoxycarbonyl)-lanthionine (mixture of isomers) (**28**) was prepared by reaction of lanthionine with di-tert-butyl pyrocarbonate, and its reaction with methyl iodide (8.5 eq.) in DMF was studied by ^1H NMR. S-Methylation proceeded to 45% completion in less than 22 h and then stopped. Neither prolonging the reaction time nor further additions of CH_3I altered the extent of reaction, suggesting an equilibrium had been reached ($\mathbf{X}^- = I^-$):



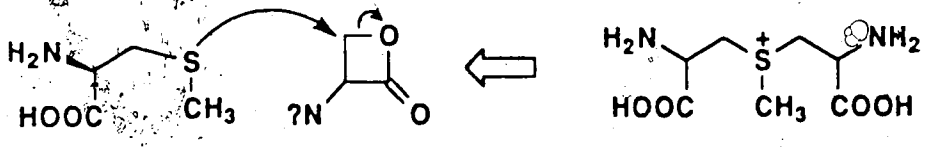
28 **30** ($X^- = BF_4^-$)

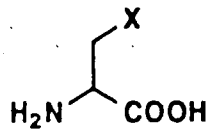
The analogous use of dimethylsulfate (6 eq.) as the alkylating agent, in which the counterion ($\mathbf{X}^-$) is the less nucleophilic $CH_3OSO_3^-$, required 12 days for >80% reaction and several other products were also produced. Instead, equilibrium in the methyl iodide reaction was shifted to the right by addition of $AgBF_4$, which precipitated the nucleophilic I^- counterion and replaced it with BF_4^-. This reduced the reaction time to 18 h and allowed

isolation of the unstable methylsulfonium tetrafluoborate salt **30** as a yellow solid containing 1.5 mole equivalents of DMF. Frustratingly, all attempts at CF_3COOH deprotection of this material and its isolation as various acid salts (eg. p-TsOH), provided impure, hygroscopic, material which rapidly decomposed (>90% in 16 h as solid; >90% in H_2O in 30 min) to predominantly lanthionine and S-methyl cysteine (i.e., rapid cleavage of one of the C-S bonds). β-Chloroalanine (**21**) was also detected among the decomposition products of the hydrochloride salt (TLC and POSFAB-MS evidence).

The problems encountered in the syntheses and attempted S-methylation of lanthionine and its derivatives led to the consideration of alternative approaches to assembling these molecules. One possibility which could avoid in part the steric effects of β-branching of the sulfide would be the formation of one of the other C-S bonds by alkylation of S-methyl cysteine. For this

Scheme 6





J

alkylation, a serine β-lactone or its equivalent seemed ideal (Scheme 6). The parent heterocycle, β-propiolactone (2-oxetanone), was known to react with simple sulfides

(R'R"S) in polar aprotic solvents to provide the corresponding sulfonium salts ($R'R"\overset{+}{S}CH_2CH_2C\overset{-}{O}O$) in good yield.[78] Furthermore, β-propiolactone had been employed under aqueous conditions to chemoselectively S-alkylate methionine residues of proteins at pH <3.5.[119] Of course, alkylations of various other nucleophiles by the serine β-lactones also had the potential to provide numerous interesting β-substituted alanines (**J**),[1,4,6] for which satisfactory general synthetic routes to several did not previously exist.

Synthesis of N-Protected Serine β-Lactones

Ideally, the synthesis of the serine β-lactones should be convenient and proceed in high yield from the relatively inexpensive N-protected serines commonly employed in peptide syntheses. This requirement would allow synthesis of N-protected β-substituted alanines which could be directly incorporated into peptides or readily deprotected to free amino acids by established routes (recall Figure 6). It would also take advantage of the established commercial sources of very high optical purity (>99.8%)[120,121] N-protected serines currently provided for peptide synthesis. None of the previous literature preparations[79,81,83,84] were able to meet these criteria to provide an attractive general synthetic route to β-substituted alanines.

Initial investigations were carried out with serines

which were mono-N-protected with either benzyloxycarbonyl (Z) (**35**) or phenylacetyl (**38**) moieties. These represent the most common carbamate and acyl protecting groups employed in peptide synthesis that may be removed hydrogenolytically (H_2/Pd-C), or by dissolving metal reduction.[1a,13] The methodology was later extended to N-(tert-butoxycarbonyl)-protected (BOC) derivatives (**41**) which can be conveniently deprotected under mild acidic conditions (eg., CF_3COOH).[1a,13] When necessary, these N-protected derivatives were generated from serine under Schotten-Baumann conditions (i.e., **35b**, **38**, **41b**). For the preparation of BOC derivatives the procedures using di-tert-butyl pyrocarbonate as described for **41b** and 51 were most convenient and provided the highest yields.

In all cases these N-alkoxycarbonyl (Z) (**35**), BOC (**41**) or N-acyl (**38**) serine derivatives reacted with triphenylphosphine (Ph_3P) and a dialkyl azodicarboxylate (ROOC-N=N-COOR, R = Et, Me (**34**))[86] to produce only two products, along with the normal Ph_3P=O and dialkyl hydrazodicarboxylate byproducts (Figure 13). The β-lactone and enamine products could be isolated with >90% recovery by flash chromatography on silica. Cyclization of N-phenylacetyl-L-serine (**38**) employed diethylazodicarboxylate (R = Et) (DEAD) typical of most Mitsunobu reactions. Use of the dimethyl analog (R = Me) (DMAD) was preferred in lactonizations of Z- (**35a**, **35b**) and BOC-serines (**41a**, **41b**) since it facilitated the

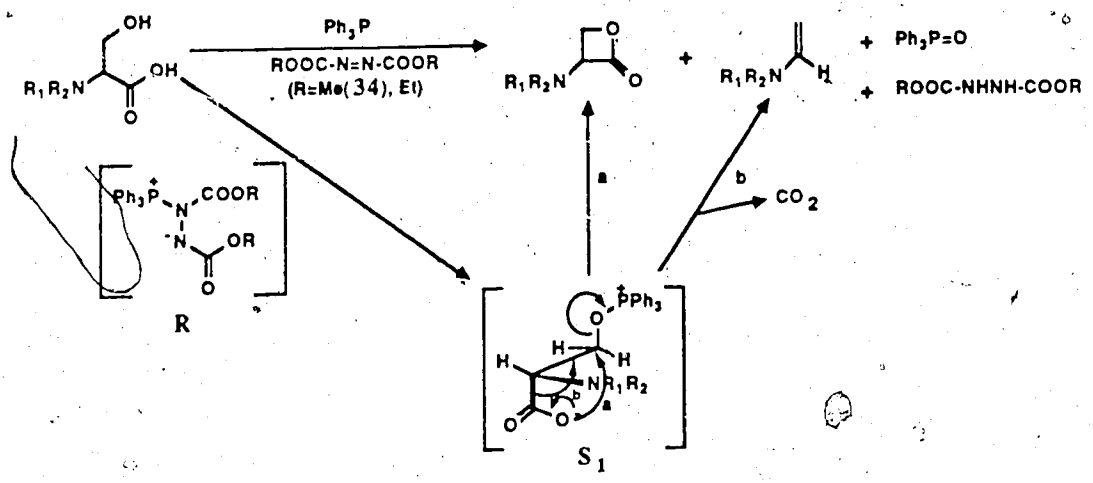


Figure 13. Triphenylphosphine / Dialkyl Azodicarboxylate Mediated Lactonization of N-Protected Serines

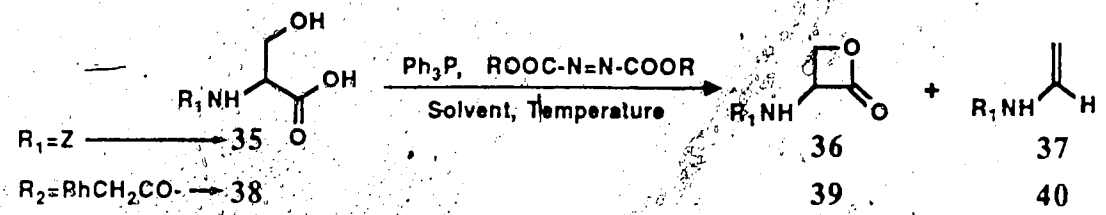
chromatographic separation of the desired β-lactones (**36a, 36b** and **42a, 42b**, respectively) from the hydrazodicarboxylate byproduct (R = Me). In contrast to analogous β-lactam syntheses which utilize the less acidic amide or hydroxamate N-H in place of COOH (Figure 7) no problems with production of aziridines[89] or oxazolines[87,88] were observed.

The production of enamine (**37** (R_1 = Z, R_2 = H), **40** (R_1 = $PhCH_2CO$, R_2 = H)) and β-lactone products from a common intermediate may be rationalized according to Figure 13. Attack of the serine hydroxyl at the phosphorus atom of the phosphonium-adduct (**R**), formed from triphenylphosphine and the azodicarboxylate,[85,86] produces an alkoxyphosphonium species (**S_1**).[122] Intramolecular

nucleophilic displacement of triphenylphosphine oxide by the carboxylate anion (anti-closure) generates the desired β-lactones according to **path a.** However, since an excellent leaving group ($Ph_3P=O$) has been generated in the β-position to a carboxylate, the common intermediate (S_1) may undergo a net decarboxylative dehydration[90] by a Grob-type fragmentation[23] (**path b**) to produce the enamine.

In order to maximize the yield of β-lactone, a significant departure from the usual Mitsunobu reaction conditions (Entry 1a, 1b, Table 1) was required. The results of these investigations for Z-(**36**) and N-phenylacetylserine β-lactones (**39**) are presented in Table 1. Between -20°C and 25°C in THF yields of β-lactone were low (29-45%) and essentially independent of the order of addition of reagents (Entries 1a→3a, 1b). Decreasing the temperature of addition of the final component to between -50°C and -78°C considerably reduced the extent of decarboxylative dehydration, and increased lactonization. Further improvements in the yield of β-lactone were obtained by preformation of a slurry of the Ph_3P/ROOCN=NCOOR adduct (**R**)[60] followed by addition of the serine derivative at low temperature, and by the use of more polar CH_3CN cosolvent. The inclusion of at least 9% THF was required to suppress the freezing point of the solvent and increase solubility of reagents. Increasing the "ionic strength"[60] by the presence of 1.1 equivalent of tetra-n-butylammonium tetrafluoroborate (0.12 M) in THF

Table 1. Lactonizations of Mono-N-Protected Serines



Entry	Method	Solvent	Temp. (°C)	Yields β-Lactone (%)	Yields Enamine[b] (%)
For Z-Serine:					
1a	A1 or A2	THF	25	35-40 (**36**)	(50) (**37**)
2a	B	THF	0	40[a]	n.d.
3a	C	THF	-20 (1h)→25	36-45[a]	(44)
4a	A2	THF	-50 (0.5h)→25	57[a]	38 (35)
5a	C	THF	-78 (0.5h)→25	60-64[a]	(32)
6a	C	CH_3CN/THF (8:2)	-50 (0.5h)→25	62[a]	n.d.
7a	C	CH_3CN/THF (10:1)	-50 (0.5h)→25	76-81[a]	13-17 (15)
For N-Phenyl-L-acetylserine:					
1b	A1	THF	25	29[c] (**39**)	71[c] (70) (**40**)
2b	C	THF	-50 (0.5h)→25	68[c]	32[c] (30)
3b	D	THF	-50 (0.5h)→25	64[c]	36[c] (40)
4b	C	CH_3CN/THF (9:1)	-50 (0.5h)→25	76[a,c]	24[c] (25)

[a] Isolated by chromatography on silica.

[b] Values in parentheses represent the percentage of elimination (±5%) estimated from ratio of 1840/1650 cm^{-1} bands in IR.

[c] HPLC yield (±3%) (see Experimental).

Methods:

A - DEAD (1) or DMAD (2) added to **35**/**38** and Ph_3P;

B - Ph_3P added to **35**/**38** and DMAD;

C - **35**/**38** added to DMAD/Ph_3P-adduct;

D - As for **C** but 1.1 eq. n-$Bu_4N^+BF_4^-$ present.

had essentially no effect (Entry 3b). All reactions were complete within 2 h.

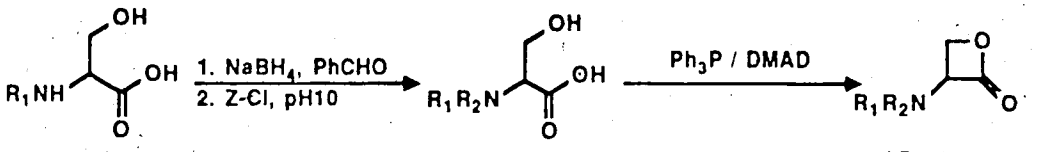
In the syntheses and subsequent reactions of serine β-lactones, infrared (IR) spectroscopy served admirably for both establishing the presence, and estimating the extent of β-lactone formation/consumption. Observation of the ~1840 cm^{-1} carbonyl stretching band in the reaction mixture (0.1 mm cells) provided a sensitive and fairly accurate measure of the β-lactone concentration (2-10 μmol/mL, ±5%).

The optimal isolated yields of 76-81% for β-lactones **36** and **39** compare favorably with the 1.4% yield of N-phenylacetylserine β-lactone previously reported under normal Mitsunobu conditions.[79b] Extension of the optimized conditions to BOC-serine (**41**) (R^1 = $OC(O)C(CH_3)_3$, R^2 = H) provided the corresponding β-lactone **42** in 68-72% yield.

It must be mentioned that in a single report König and Geiger claimed to produce Z-L-serine β-lactone (**36a**) in 91% yield by carboxyl activation with dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HBT).[125] However, the authors provided only elemental analysis as evidence, and the melting point of their material (177-179°C) is 44°C greater than our lactone **36a**. In our hands the synthesis with DCC/HBT could not be repeated, nor could we detect the presence of β-lactone by IR at any point during the reaction.

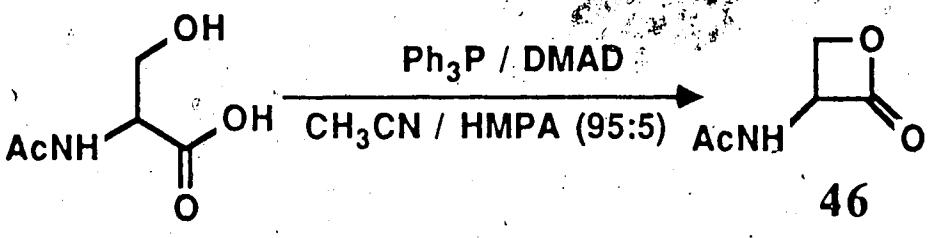
For later investigations of reactions with organometallics the N,N-diprotected serine derivative (R_1 = CH_2Ph(Bn), R_2 = Z) was prepared.[124] N-Benzylserines (**43a** or **43b**) were produced by reductive amination of benzaldehyde with the aid of $NaBH_4$, and reacted with benzyl chloroformate at pH 10 to provide N-benzyl-N-(benzyloxycarbonyl)serines (**44a** and **44b**). In the lactonization of **44** to **45** (**a** or b) temperature effects outweighed those of solvent polarity and the best yields

Scheme 7



(70-71%) were obtained using THF at -78°C (Method C, Table 1). The choice of N-benzyl (Bn) and N-benzyloxycarbonyl (Z) as protecting groups conveniently allows deprotection of ring-opened products in a single step (eg., by H_2/Pd-C or Na/NH_3).

The preformation of the Ph_3P/DMAD-adduct (**R**) at -42°C in acetonitrile, followed by addition of anhydrous N-acetyl-DL-serine in CH_3CN/HMPA and warming to 25°C provided hygroscopic **46** in 51% yield (60% based on



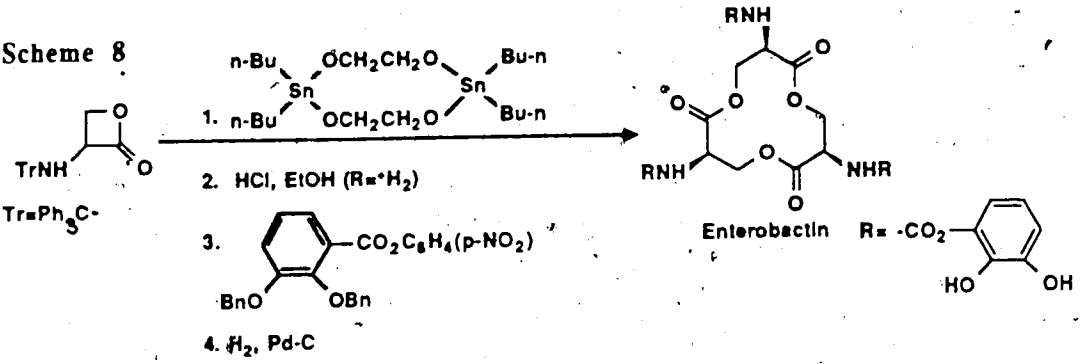
46

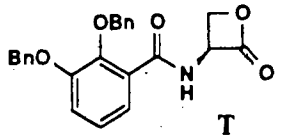
recovered N-Ac-serine). The chromatographic purification of **46** was hampered by the similar mobility of $Ph_3P=O$ on silica. This problem could be avoided by substitution of $(n\text{-}Bu)_3P$ for triphenylphosphine only at the expense of yields (33%) and reaction rate (8 h for completion).

With the exception of **46** above, all of these N-acyl (**39**) and N-alkoxycarbonyl (**36a, 36b, 42a, 42b, 45a, 45b**) β-lactones are stable crystalline solids which are not appreciably hygroscopic. They may be handled in air without any special precautions, or stored dry at -20°C for over one year without measurable decomposition. Solutions of the β-lactones in pure organic solvents are stable for days, and in aqueous mixtures (pH 2-5) hydrolysis occurs only slowly.

In their synthesis of the siderophore enterobactin Shanzer and Libman[81a] reported a novel one-step cyclooligomerization of N-trityl-L-serine β-lactone using an organotin template. The authors also reported that all attempts to prepare N-acylated serine β-lactones (including **T**) with diisopropylcarbodiimide/dimethylaminopyridine (DMAP) failed. However, the apparent general applicability of the above Ph_3P/dialkyl azodicarboxylate-mediated cyclization to N-acyl serines could enable direct preparation of N-[2,3-bis(benzyloxy)benzoyl]-L-serine β-lactone (**T**). The cyclization of **T** to the triester with the above stannoxane could then provide the protected enterobactin in a single step.[81a]

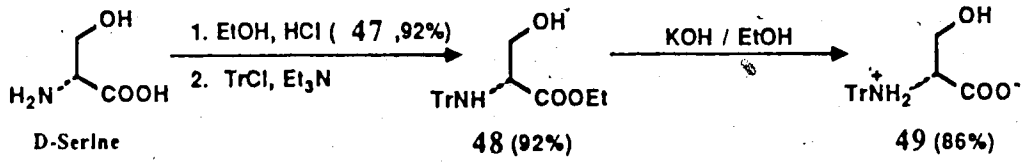
Scheme 8





T

N-Trityl-serine had been prepared (15-26%) by carboxyl-activation on two occasions in the literature.[81] We investigated the possible lactonization with the modified Mitsunobu conditions. D-Serine was esterified with ethanolic-HCl, and then reacted with triphenylmethyl chloride in the presence of triethylamine to provide **48**.[126] Alkaline hydrolysis of **48**[127] yielded N-trityl-D-serine (**49**). Although the material was anhydrous,



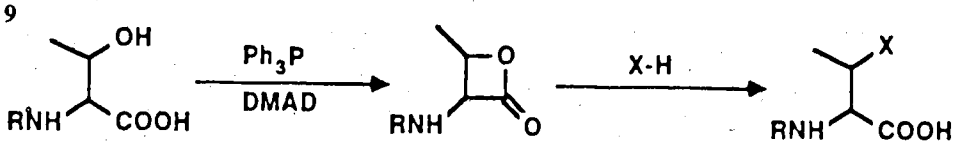
attempts to produce the β-lactone using Ph_3P/DMAD were unsuccessful, and no β-lactone was detected at any point in the reaction. Since none of the three products was isolated, it is uncertain whether aziridine[89a] formation occurred. IR and ^1H NMR of N-trityl-D-serine (**49**) in the

solid state and in solution in THF indicate that it exists primarily in the zwitterionic form, which is perhaps incompatible with Ph_3P/DMAD-mediated lactonization.

N-Acetyl-L-threonine β-lactone has been isolated from the fermentation broth of Bacillus and exhibits weak antibacterial activity.[79b] Ring-openings of threonine β-lactones (Scheme 9) analogous to those proposed for the serine β-lactones could potentially provide access to β-methylamino acids, many of which occur naturally.[1a] Thus, attempted lactonization of N-protected threonines was a logical extension of our methodology.

L-Threonine and L-allo-threonine were converted to their respective N-(tert-butoxycarbonyl) (BOC) derivatives **50** and **51** using either "BOC-ON" or the more convenient di-

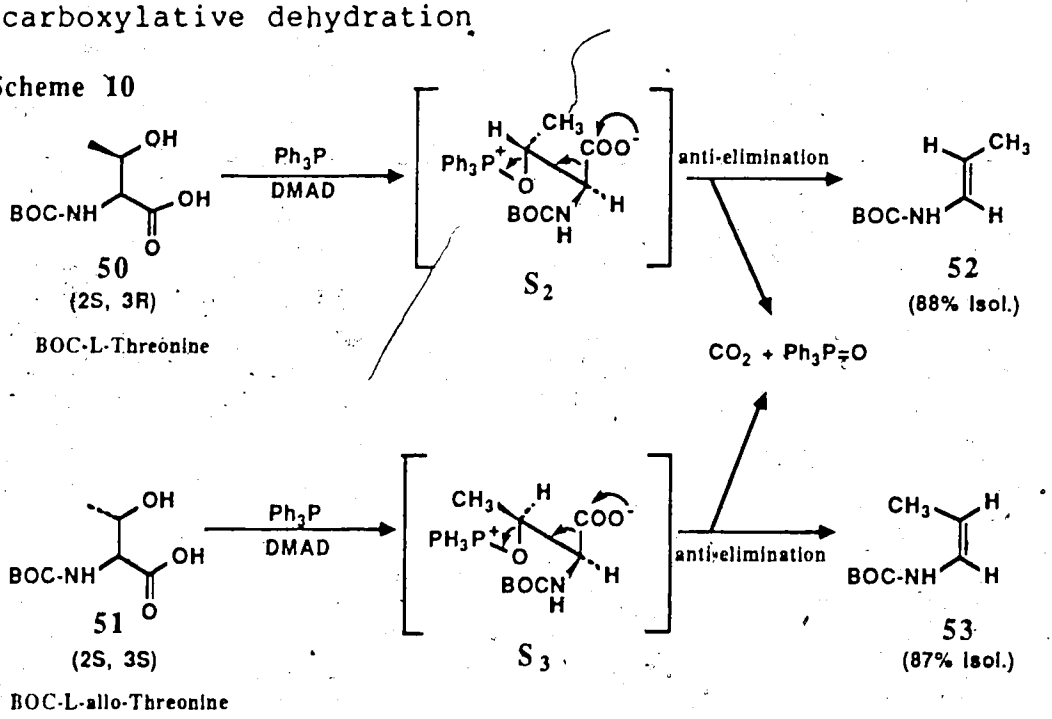
Scheme 9



tert-butyl pyrocarbonate. When BOC-L-threonine (**50**) was subjected to the preformed adduct of Ph_3P/DMAD (**R**) in THF at -78°C a single product, the trans-enamine **52**, was observed and isolated in 88% yield by chromatography. Similarly, BOC-L-allo-threonine reacted under identical

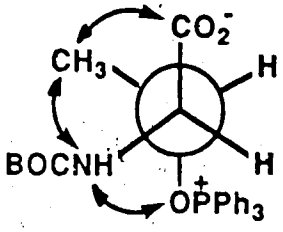
conditions to give exclusively the more labile cis-enamine **53**. As depicted in Scheme 10, the stereospecific generation of **52** and **53** from L-threonine and L-allo-threonine derivatives, respectively, implies that the decarboxylative dehydration

Scheme 10

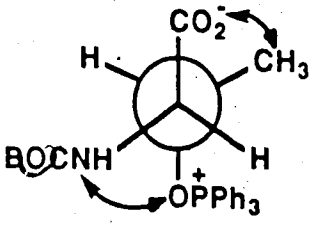


proceeds by exclusive anti-elimination with no observable products of syn-elimination.[86,90b] This also provides compelling evidence that the lactonization and competing elimination with the serine derivatives both occur by the proposed intermediate S_1 (Figure 13) in the anti-conformation, with no partitioning toward alkene via syn-elimination.

The single less serious gauche interaction in the intermediate S_3 generated from (2S,3S)-**51** appears to be of no significant consequence, since both lactonization and elimination must proceed via S_2 or S_3. In classic



S_2 from **50**



S_3 from **51**

studies of substitution versus elimination it was demonstrated that introduction of substituents at the displacement center greatly retards nucleophilic attack, while either causing little effect or increasing the rate of elimination.[60,128] Consistently, the 3-4 kcal mol^{-1} difference in activation energy ($\Delta\Delta G^{\ddagger}$) required to cause elimination to predominate (>99%) over intramolecular substitution in progressing from the serine to threonine derivatives is largely accountable by the difference in substitution rates on primary versus secondary centers (eg., $\Delta\Delta G^{\ddagger}$ (for Et vs. i-Pr) ~2-2.5 kcal mol^{-1}).[128] These results unfortunately suggest that the production of α-amino β-lactones by Ph_3P/DMAD-mediated cyclizations are probably limited to serine derivatives.

Reactions of N-Protected Serine β-Lactones with Heteroatom Nucleophiles

As illustrated in Figure 14, the serine β-lactones are ambident electrophiles susceptible to nucleophilic attack at the β-methylene (Path A) or carbonyl carbons (Path B). Attack at the carbonyl (Path B, nucleophilic acylation) is a characteristic shared by β-propiolactones

with their higher homologs, however reactivity is enhanced considerably due solely to ring strain[78] in the 4-membered heterocycle. It is this "acyl halide"-like reactivity of serine β-lactones which was persued primarily by previous investigators in attempts to introduce serine residues into peptides without the need for the usual hydroxyl protection.[81b,84]

The enormous potential for attack at the β-position (Path A) with alkyl-oxygen cleavage of β-propiolactone by many nucleophiles has long been realized,[78] however this pathway has been largely unexploited (and in fact undesirable) in the earlier studies with serine β-lactones.[81b,84] Although usually attributed to ring strain (~23 kcal mol^{-1}),[129] the nucleophilic ring-opening of β-lactones by path A is probably also facilitated by the "transition-state"-like configuration imposed by the geometric constraints of the β-lactone as illustrated below (i.e., the 'p'-character (θ ~93°)[130] in the O_1-C_4-C_3

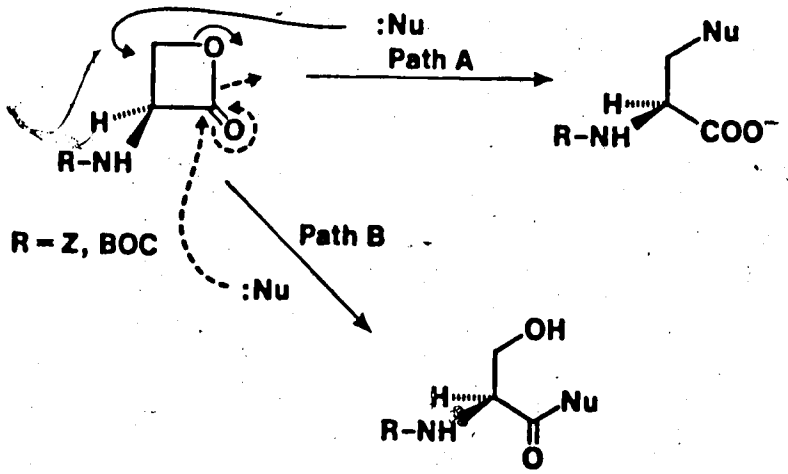
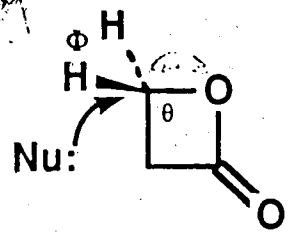


Figure 14. Possible Modes of ß-Lactone Ring-Openings.

bonding would be expected to cause considerable 'sp^2'-like bonding in the $H\text{-}C_4\text{-}N$, hence an "early" transition state. These two arguments are however conceptually inseparable according to the Hammond postulate).



$\Phi \sim 115^{\circ}$

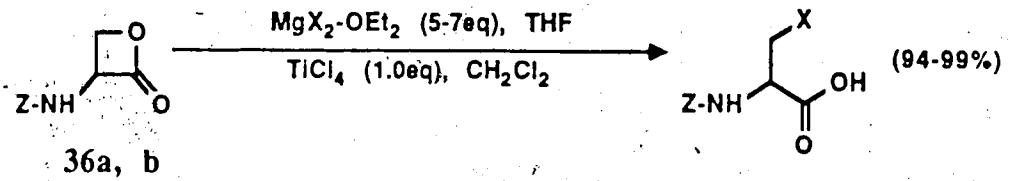
$\theta = 93 (\pm 1.5)^{\circ}$

As this thesis shall illustrate, Path A is operative in numerous instances with serine β-lactones causing them to behave as chiral dehydroalanine or alanine β-cation equivalents. In this manner they may be considered synthetic analogs of the biological conversion of serine (or O-acetylserine) to β-substituted alanines by PLP-dependent β-replacement enzymes.[26,131]

Since they are employed most widely in peptide synthesis, N-alkoxycarbonyl protecting groups were employed with the serine β-lactones used to study nucleophilic additions. The N-(benzyloxycarbonyl) (Z) moiety was usually employed because it is compatible with the widest range of conditions, however in almost all cases identical conditions should be applicable to BOC derivatives. The results of these investigations are summarized in Table 2 at the end of this section.

Halide Nucleophiles

Z-Serine β-lactones (L-(**36a**) or D-(**36b**)) reacted almost instantly with magnesium dibromide etherate to produce the corresponding Z-β-bromoalanine (**55a**, **55b**) in 99% isolated yield. The analogous reaction of **36b** with magnesium dichloride etherate required somewhat longer (6.5 h) but provided optically-pure Z-β-chloro-D-alanine (**56b**) in 94% yield. The reaction with magnesium bromide etherate is in direct contrast to reports that β-lactones incapable of ring expansion normally decarboxylate under these conditions.[129a]



X

55a Br (2S)-L
55b Br (2R)-D
56a Cl (2S)-L
56b Cl (2R)-D

In these halide ring-openings, Lewis-acid (eg., Mg^{++}) complexation of the lactone carbonyl appears to catalyze and possibly direct the nucleophilic attack. Further evidence for this comes from examination of the IR spectra of solutions of Z-serine β-lactones (**36**) in the presence of $TiCl_4$. In solvents such as THF or acetonitrile which may act as Lewis donors, $TiCl_4$ reacts very slowly (~3 days) and little complexation of the lactone carbonyl is initially apparent. In dichloromethane however $TiCl_4$ immediately broadens and shifts the carbonyl stretching

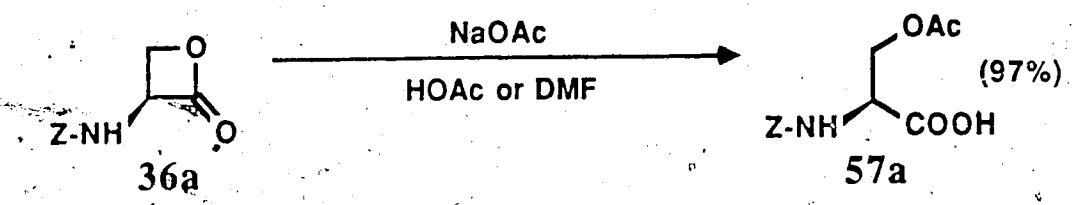
band of **36a** (1835 → 1790 cm^{-1}) indicative of complexation, and after 30 min a near quantitative yield of Z-L-β-chloro-L-alanine (**56b**) was isolated.

As in many of the reactions with N-protected serine β-lactones, isolation of these N-protected amino acids simply involved acidification and extraction into organic solvent with no required chromatographic purification.

The N-protected β-haloalanines (**55**, **56**)[72] produced above, and the corresponding deprotected amino acids[110] have been frequently employed as synthetic intermediates, usually in the preparation of other β-substituted alanines (eg., lanthionine).[132] In addition, because they possess a leaving group in the β-position, the β-haloalanines are well established as suicide substrates for several Category 1 PLP-dependent enzymes (according to Figure 2) including bacterial amino acid (eg., alanine) racemases,[26] various transaminases (aminotransferases),[20] and L-aspartate β-decarboxylase (Category 2).[8]

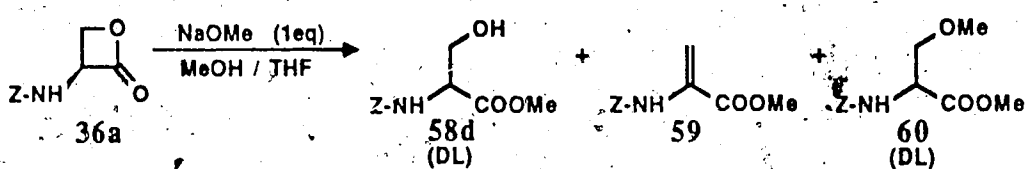
Oxygen Nucleophiles

The syntheses of the β-haloalanine derivatives from serine β-lactones are the first of many examples of β-substituted alanines which are not accessible by current methods of asymmetric synthesis of amino acids.[9,16] Another instance is the synthesis of N-Z-O-acetyl-L-serine (**57a**) from **36a** in 97% yield by reaction with excess sodium acetate in acetic acid or DMF. O-Acetyl-D-ser[illegible]o



a suicide inhibitor of bacterial amino acid racemases (Category 1, Figure 2)[26] while its L-antipode functions as an immediate precursor of β-substituted alanines with the aid of β-replacement enzymes (Category 2) in higher plants.[1a,4,28]

The reaction of Z-L-serine β-lactones (**36a**) with more basic alkoxides was an exception to the general observation of optically-pure products from heteroatom nucleophiles. Treatment of **36a** with sodium methoxide (1 equiv.) in methanol/THF for 25 min at 25°C provided only racemic **58d** and **59**, as products of attack at the carbonyl (Path A, Figure 14). Z-Dehydroalanine methyl ester **59** arises by dehydration of the initial racemized product **58d**, however the reaction is not preparatively useful for generating **59**. Prolonging the exposure to methoxide in methanol only slightly increases the yield of **59** and results in the production of methyl ether **60** by conjugate addition of MeOH to **59**. A similar dehydration/addition



25°C (25min)	88%	12%	--
25°C (20min), 0°C (50min)	67%	20%	13%

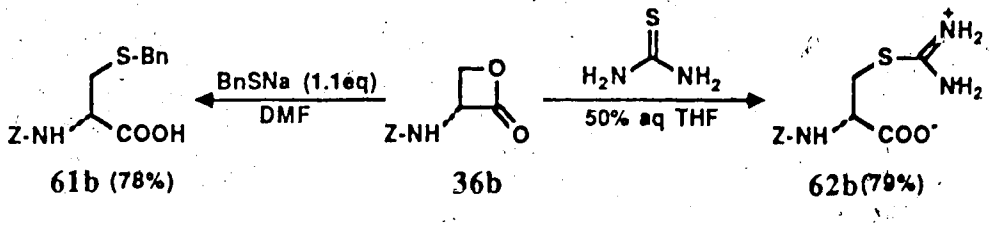
sequence has been observed in the methoxide opening of β-propiolactone.[76d]

Sulfur Nucleophiles

Z-D-Serine β-lactone (**36b**) reacted with sodium benzylthiolate (1.1 eq.) in DMF to provide optically-pure N-Z-S-benzyl-D-cysteine (**61b**) (78%) which was ready for direct incorporation into antibiotic peptides such as malformin.[1c] This illustrates how the β-lactone methodology may be economically used to convert relatively inexpensive D-serine (<$100/mole) into much more expensive D-amino acids (eg., D-cysteine, ~$2900/mole).[133] A similar reaction with the thiolate of N-tritylcysteine may be useful in generating lanthionines which are differentially protected at the N^2 and N^6-positions.

A similar ring-opening with thiourea in 50% aqueous THF provided Z-β-(isothiureido)alanines **62b** and **62d** (from L-(**36a**) and DL-(**36d**))[109] which are analogs of the plant amino acid albizzine,[1a,134] and may be hydrolyzed with dilute alkali to provide the corresponding cysteines.[60]

In principle these reactions demonstrate that many of the numerous sulfur-containing amino acids found in Nature (eg., in radishes, garlic, onions, chives, etc.)[1a,4]



61b (78%) **36b** **62b(78%)**

areaccessible by reaction of the appropriate S-nucleophile with the β-lactone. In addition, the selenium containing analogs[1a,4,6] which have been used as radiopharmaceuticals (^{75}Se)[135] should be easily prepared by this strategy.

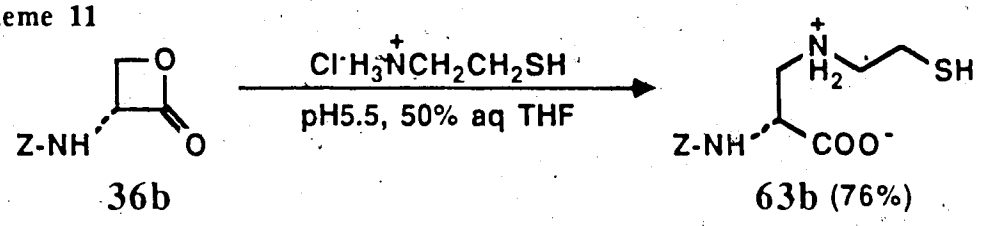
Although the N-protected serine β-lactones reacted quite eagerly with most sulfur nucleophiles, the BOC-(**42a**) and Z-L-serine β-lactones (**36a**) reacted sluggishly with dimethyl sulfide to produce the corresponding S-dimethylcysteine sulfonium derivative. In THF virtually no reaction occurred until 1 equivalent of the Lewis acid titanium(IV) isopropoxide was added, and then the reaction required 48 h. The rate of reaction of **36a** with Me_2S in d_7-DMF (^1H NMR study) was increased so that in 4 days all the lactone was consumed, however on concentration the product $Me_2\overset{+}{S}CH_2CH(NHZ)COO^-$ fragmented to benzyl vinylcarbamate (**37**) with the evolution of CO_2 and Me_2S. Disappointingly, BOC-S-methyl-L-cysteine (**29**) in DMF failed to react (>3 weeks) with the N-protected serine β-lactones (**36** or **42**) to provide an N-protected lanthionine S-methyl sulfonium salt. Similar attempts at reaction of S-methyl-L-cysteine with BOC-L-serine β-lactone (**42a**) at pH 3 in H_2O/CH_3CN resulted only in lactone hydrolysis.

Nitrogen Nucleophiles

In an attempt to produce Z-thialysine (i.e., $H_3\overset{+}{N}CH_2CH_2SCH_2CH(NHZ)COOH$), which is the N-protected form of the amino acid resulting from decarboxylation of

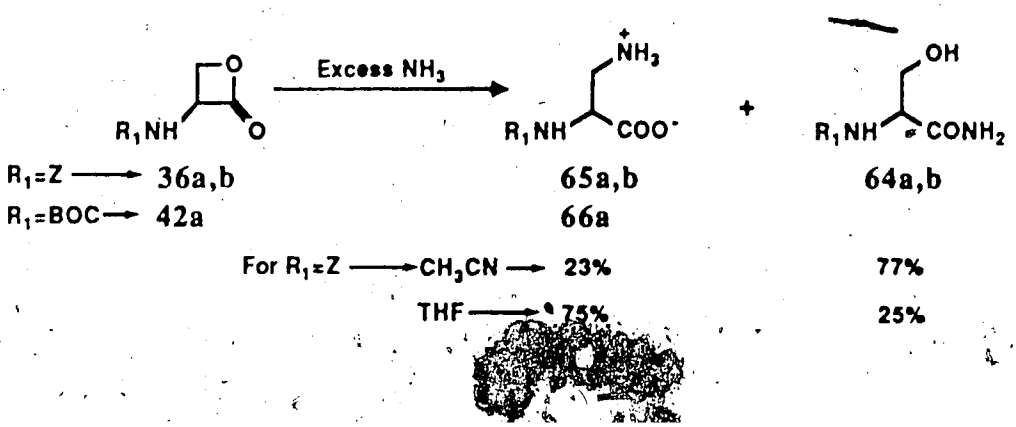
lanthionine by meso-DAP decarboxylase, Z-D-serine β-lactone (**36b**) was treated with mercaptoethylamine (MEA) (2 eq.) in 50% aqueous acetonitrile or THF at an apparent pH of 5.5 (±0.5) (Scheme 11). Surprisingly, this provided aminothiol **63b** as a result of amino rather than thiol attack of MEA

Scheme 11



in 76% recrystallized yield. This is in contrast to reactions of mercaptoethylamine (MEA) with β-propiolactone at pH 5.5 in H_2O.[136] Since this problem was later avoided in the reaction of MEA with 3-amino-2-oxetanone salts in H_2O at pH 5.5, it appears that the predominant amino attack to produce **63b** is due to suppression of ionization of MEA by the presence of organic solvent (i.e., $H_2\overset{+}{N}CH_2CH_2SH$ attacks rather than $H_3\overset{+}{N}CH_2CH_2S^-$).

The Z-(**36a, 36b**) and BOC-serine β-lactones (**42a**) reacted quantitatively with ammonia to provide mixtures of the corresponding $\underline{N}^2$-protected serine amide (**64a, 64b** from **36a,b**) and α,β-diaminopropionic acid derivatives (**65a, 65b**

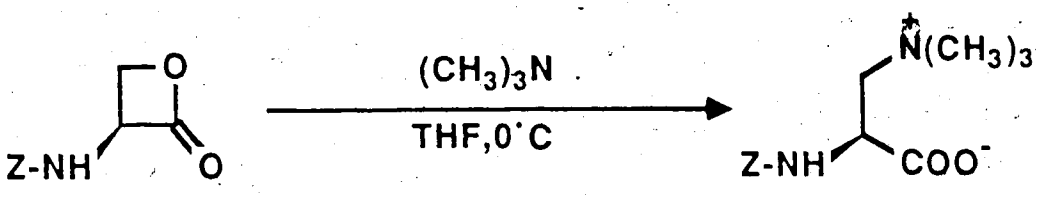


from **36a,b**, and **66a** from **42a**). Both products were optically-pure based on optical rotation, and were easily separated by extraction. Interestingly the choice of solvent determined which of these was the major product. In less polar THF the amine predominated 3:1 over amide, whereas in acetonitrile this ratio was reversed.

As one might expect, α,β-diaminopropionic acid is produced biosynthetically from serine by β-replacement PLP-enzymes[30a] (Category 2). In addition to its presence in many monobactam antibiotics, α,β-diaminopropionic acid is an important constituent in numerous antitumor and antibiotic peptides such as bleomycins, malonomycin, tuberactinomycins, galantins, and edeines.[30,137] For the synthesis of these compounds optically-pure α,β-diaminopropionic acid derivatives in which $\underline{N}^2$ and $\underline{N}^3$ are differentially protected (eg., **65a**, **65b**, **66a**) are essential starting materials. Simple routes to mono-N-protected α,β-diaminopropionic acids are lacking since selective N-acylation is not possible,[72a] thus much effort has recently been devoted to alternative syntheses.[70,72,93a,138,139] The reaction of serine β-lactones with NH_3 as described above represents perhaps the simplest route to these molecules.

In contrast to NH_3, trimethylamine caused ring-opening of **36a** with exclusive alkyl-oxygen cleavage to quantitatively provide the quaternary ammonium salt **67a**.[140] The parent amino acid derived from **67a** is the

betaine analog of the natural neurotoxic β-N-methyl-L-α,β-diaminopropionic acid.[6]



36a 67a

The β-lactone **36b** also reacts with relatively poor N-nucleophiles such as pyrazole in acetonitrile at 50°C to provide Z-β-(pyrazol-1-yl)-D-alanine (**68b**) in 71% recrystallized yield. This is the first reported synthesis of an optically-pure β-(pyrazol-1-yl)alanine,[141] an amino acid which has been isolated from watermelon seeds[6,142] and employed as a histidine analog. More importantly, its synthesis illustrates how the serine β-lactones may provide convenient access to a number of interesting heterocyclic β-substituted alanines (Figure 15) such as mimosine, willardiine, isowillardiine, quisqualic acid,[93b] and stizolobic acid which occur in

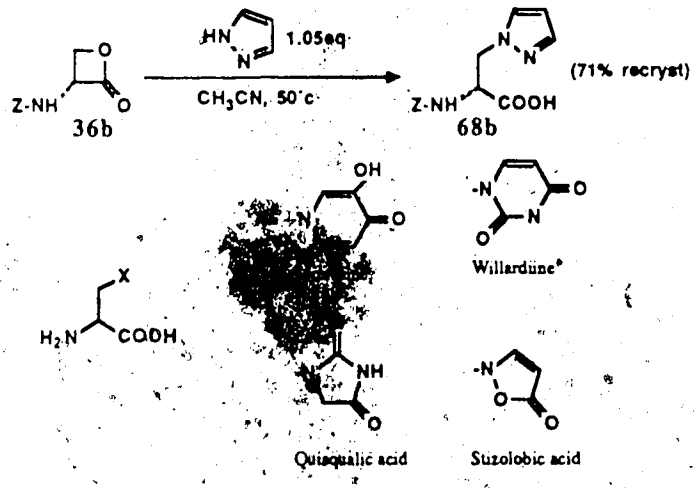
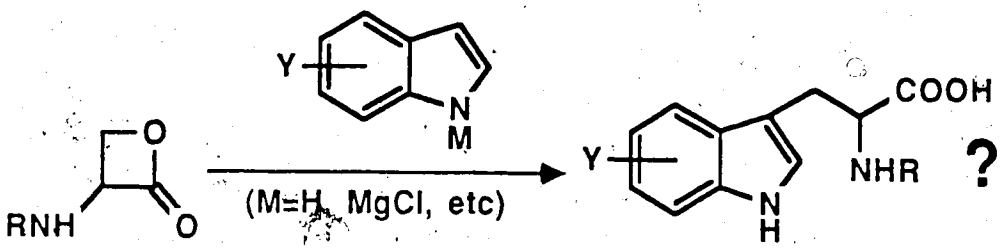


Figure 15. Natural Heterocyclic ß-Substituted Alanines

higher plants as products of β-replacement of O-acetylserine by PLP-enzymes (Category 2).[1,6,28,131,143]

The proteinogenic amino acid tryptophan is also produced from serine and indole by a β-replacement reaction catalyzed by a PLP-enzyme.[2,8] Since β-propiolactone reacts with indole (120°C, 6 h, 40-50%) at the 3-position,[78] it is likely that the analogous

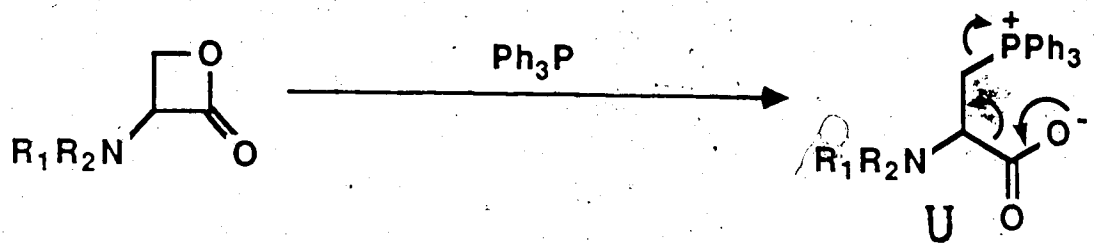


"β-replacement" reaction of the serine β-lactones with indole derivatives could provide many of the bioactive indole amino acids.[1a]

Phosphorus Nucleophiles

Triphenylphosphine is usually considered an excellent nucleophile,[60,128,144] and initially in the optimization of the lactonization conditions there was concern that Ph_3P could react with the product β-lactones and decrease yields through formation of **U** according to Scheme 12. Since Corey *et al.* had demonstrated the synthetic utility of $Ph_3P^{+}-^{-}CHCH_2COO^{-}$ in Wittig reactions for preparing β,γ-unsaturated acids under special conditions,[145] we later considered the use the ylid of **U** for similar preparations of β,γ-unsaturated amino acids, which often act as suicide substrates for Category 1 PLP-enzymes (Figure 2).

Scheme 12



Surprisingly, when β-lactone **42a** (R_1 = BOC, R_2 = H) was stirred with 5 equivalents of Ph_3P in THF for 7 weeks at 25°C only ~65% of the β-lactone had been consumed. Additional heating to 70°C (5 h) resulted in only ~6% further reaction. Attempted isolation of **U** from the resulting mixture by reverse-phase MPLC provided only unreacted β-lactone **42a** (29%), and Ph_3P, and some Ph_3P=O (from air oxidation). None of the desired **U** was isolable possibly due to decarboxylative elimination of Ph_3P similar to that observed by Corey under normal Wittig conditions.[145] Any further attempt to produce **U** should utilize a more polar solvent to speed the reaction and also incorporate stabilization of the product by protonation, etc.

Importantly, these results establish that negligible decomposition by attack of Ph_3P occurs in the formation of the serine β-lactones. The increase in lactonization yield achieved by preformation of the Ph_3P/DMAD-adduct (**R**) (~10%) is probably attributable to the promotion of intra versus intermolecular condensation achieved by maintaining a low concentration of N-protected serine in the reaction

mixture.

Although the nucleophilic character of phosphites is generally considered lower than that of the corresponding phosphines,[128,146b] BOC-L-serine β-lactone (**42a**) did react when heated to 50-70°C in an excess of trimethylphosphite (Figure 16). Analytically pure phosphonate product **69a** resulting from a novel internal Michaelis-Arbuzov rearrangement[144,146,147] was obtained in 98% yield by simply removing excess $(MeO)_3P$ in vacuo, and filtering ether insoluble impurities. ^1H and ^{31}P NMR, as well as IR and MS-fragmentation confirmed the structure as **69a**, and not a pentacoordinate phosphorane intermediate (**V**) similar to those sometimes observed in Arbuzov rearrangements.

The product **69a** is a protected β-phosphonoalanine. Numerous racemic syntheses of protected β-phosphonoalanines have appeared in the literature,[148] along with a single report of an optically-active (~50% e.e.)

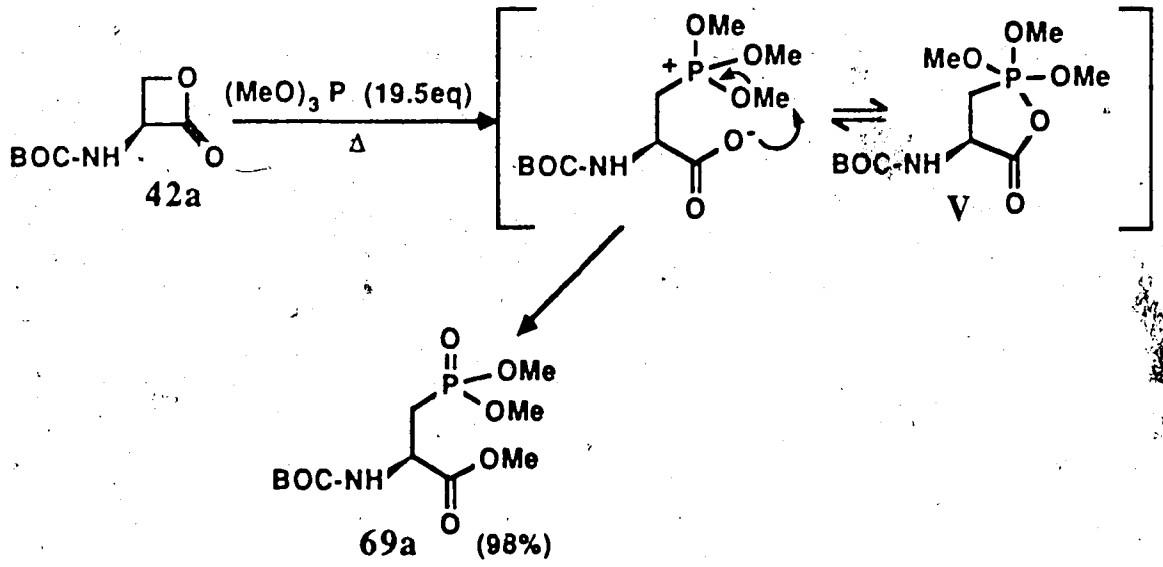
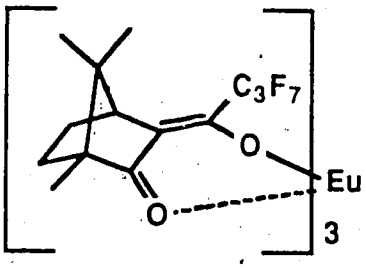


Figure16. ß-Phosphonoalanine Synthesis by Internal Michaelis-Arbuzov Reaction.

derivative prepared by an asymmetric Strecker-type synthesis.[149] Although **69a** was optically-active, $(MeO)_3P$ does have some appreciable basic character,[60,128] and thus partial racemization of the methyl ester product[13] under the reaction conditions was a concern.

In order to obtain an estimate of the optical purity of **69a**, its interaction in $CDCl_3$ (0.1 mmol/mL) with successive 0.1 equivalent additions of the chiral NMR-shift reagent, tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]europium(III) derivative (hfc-Eu(III))[150] was investigated. Due to probable complexation of P=O by



hfc-Eu(III) ^1H NMR
Chiral Shift Reagent

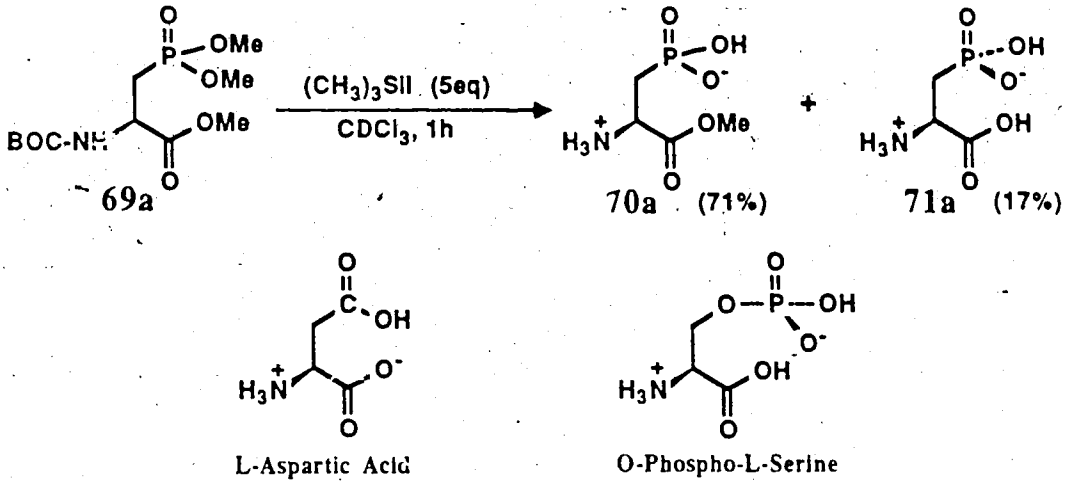
this Lewis acid, the ^{31}P NMR (^1H decoupled) immediatel broadened ($W_{1/2}$ ~450 Hz with 0.1 eq. hfc-Eu(III)) and was of no help. In the ^1H NMR spectrum, the adjacent $\underline{CH_2}P$ peak originally centered at δ2.38 ppm (^{31}P decoupled) was split into two broad singlets δ2.59 (1.84H) and δ2.88 (0.16H) by the addition of 0.1 equivalent hfc-Eu(III) suggestive of a 92/8 ratio of (2S)/(2R)-enantiomers. Unfortunately other resonances (i.e., PO$\underline{Me}$, CH) were not split enough to allow accurate integration, and further additions of shift reagent caused extensive broadening and overlap of peaks disallowing additional estimates. This

sort of behavior, along with a complicated dependence of the magnitude of induced differential shift on reagent/substrate concentration, is common for these chiral shift reagents.[150]

Although not indisputable evidence, these NMR measurements suggest approximately 84% enantiomeric excess in **69a** and indicate that racemization did occur to some extent under the reaction conditions. This procedure still represents the most direct synthesis of optically active phosphonoalanine to-date. The alternative use of the phosphite salt $(MeO)_2PONa$, which also undergoes Arbuzov rearrangements,[146,148c] could allow preparation of the Na^+-carboxylate salt $(MeO)_2P(O)CH_2CH(NHBOC)COONa$ which would be less prone to epimerization than methyl ester **69a**.

Since the free β-phosphono-L-alanine (**70a**) was desired for enzymic studies it had to be deprotected. Most previous literature deprotections of β-phosphono-alanines involved vigorous hydrolyses under acidic[148,151] or basic[148a] conditions in low yields (50-69%). These harsh conditions were conveniently avoided by the use of trimethylsilyl iodide (TMSI) for deprotection.[152,153] This reagent (5 eq. in $CDCl_3$) effected almost immediate dealkylation of the phosphonate moiety[152] and removal of the BOC-group,[153] but cleavage of the -COOMe required considerably longer.[153a] After 1 h at 25°C the desired β-phosphono-L-alanine (**71a**) and its corresponding methyl

ester (**70a**) were isolated in 17% and 71% yield respectively.



L-Aspartic Acid

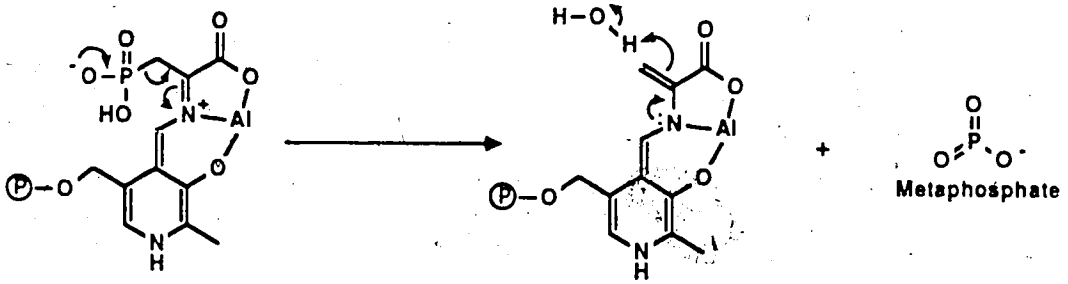
O-Phospho-L-Serine

β-Phosphono-L-alanine (**71a**) is an analog of both O-phospho-L-serine and the proteinogenic L-aspartic acid which are both important primary metabolites.[1a,2] It has been isolated in small amounts from sea anemone and ciliate microorganisms, in which it appears to arise from phosphoenolpyruvate (PEP) $H_2C{=}C(PO_4H_2)COOH$ by a unique rearrangement to 3-phosphonopyruvic acid ($(HO)_2P(O)CH_2C(O)COOH$) followed by transamination (Category 1, PLP-enzyme).[154,155]

As an analog of O-phospho-L-serine,[156] β-phosphono-L-alanine is a competitive inhibitor of serine phosphatase (K_i = 0.2 mM),[237] because its C-P bond is incapable of being hydrolyzed by ordinary phosphate cleavage enzymes.[154a] β-Phosphono-L-alanine is nontoxic to mammalian cells[144,154a] and readily transported into bacterial and mammalian cells using the system established for aspartate,[154] however there have been few studies of

its therapeutic potential.

In general the phosphonate moiety has been shown to be a readily accepted substitute for substrate carboxylates by many enzymes;[1,157] often such compounds are potent competitive inhibitors. The "de-phosphonylation" of β-phosphonoalanines by pyridoxal phosphate and metal ions (eg., Al^{+3}, Cu^{+2}, Ga^{+3}) in solution,[158] exactly analogous to that of the β-decarboxylation of aspartic acid (Category 2, PLP-enzyme, Figure 3), has been observed. As yet no enzyme has been found which executes this C-P bond cleavage ("dephosphonylation"):



For these reasons, the interaction of β-phosphonoalanine with various aspartate enzymes (aminotransferase,[82] α-[68] and β-decarboxylases[34c]) is currently being studied in collaboration with Dr. M. Palcic. Initial results with aspartate aminotransferase indicate it is a potent competitive inhibitor (Appendix 1). If indeed metaphosphate[23] is released by one of the enzymes as proposed for the nonenzymic dephosphonylation above,[158] it could phosphorylate a nucleophile at the

active site causing inactivation. The aspect of active site phosphorylation is similar to the action of nerve gases and organophosphorus insecticides,[2,8,21b] but β-phosphonoalanine would be acting as a suicide substrate rather than an "affinity label".

Whereas aspartate aminotransferase (Category 1) and β-decarboxylase (Category 2) are PLP-dependent enzymes, the aspartate α-decarboxylase instead utilizes a pyruvoyl residue (Enz-NH-C(O)C(O)CH_3) as a 2-electron "sink" to effect α-decarboxylation in a manner analogous to PLP-catalysis.[2,68] As yet there are no known suicide inhibitors for the α-decarboxylase, however since it is the major metabolic pathway for producing β-alanine required for Coenzyme A [2] biosynthesis in microorganisms,[68] inhibitors could be potent antibiotics.

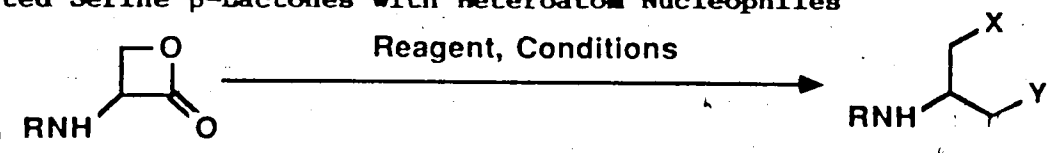
Because $MeP(OEt)_2$ reacts in Arbuzov reactions even more readily than its phosphite counterpart, the patented fungicide,[159] $Me(HO)P(O)CH_2CH(NH_2)COOH$ (nontoxic to mammals), should be readily accessible via the route we have established for β-phosphonoalanine (Figure 16). Similarly, amidophosphites (eg., $(RO)_xP(NR'_2)_{3-x}$) could be used to produce the corresponding phosphonamides.[146] Since phosphonamides generally mimic the tetrahedral carbinolamine intermediate in the hydrolysis of their carboxamide analogs, they frequently act as slow binding inhibitors[160] of the corresponding hydrolase enzyme (eg., asparaginase[2]).

Table 2 summarizes the reactions of heteroatom nucleophiles with N-protected serine β-lactones. It amply illustrates how ring-opening with alkyl-oxygen cleavage occurs in most instances to produce the optically-pure β-substituted alanine derivatives, analogous to the action of PLP-dependent β-replacement enzymes. These reactions can provide a convenient means of incorporation of isotopically-labelled heteroatoms (eg., S, P,[154a,c] N, O,[247] X) for use in biological or mechanistic studies. Only the "hardest bases" such as alkoxides and ammonia give substantial amounts of products resulting from attack at the "harder acid" carbonyl.[60,128]

Carbon Nucleophiles

An obvious extension of the serine β-lactone methodology is the formation of carbon-carbon bonds through reactions with C-nucleophiles to produce amino acids with homologated sidechains. With the exception of CN^- additions, reactions of the N-protected serine β-lactones with carbon nucleophiles represent a departure from their behavior as mimics of the action of β-replacement enzymes. Although these reactions proved more challenging to refine than the additions of heteroatom nucleophiles, they rewarded us with access to most major classes of amino acids.

Table 2. Reactions of N-Protected Serine β-Lactones with Heteroatom Nucleophiles



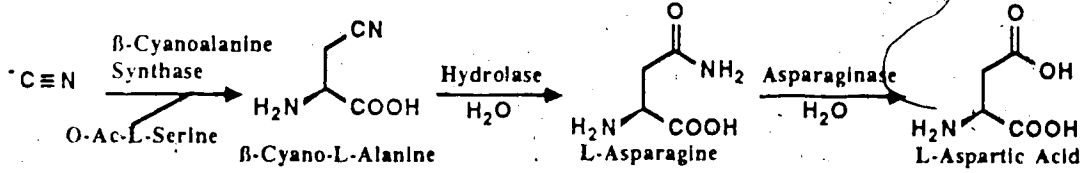
Product	Nucleophilic Reagent	Conditions[a] (°C)	Enantiomer[b]	Product Structure R	X	Y	Yield[c]
55a	$MgBr_2 \cdot Et_2O$ (7 eq.)	Et_2O, 5 min	L(2S)	Z	-Br	-OH	99 (67)
55b			D(2R)	Z	-Br	-OH	99
56a	$TiCl_4$ (1.0 eq.)	CH_2Cl_2, 30 min	L	Z	-Cl	-OH	99
56b	$MgCl_2 \cdot Et_2O$ (5 eq.)	Et_2O, 6.5 h	D	Z	-Cl	-OH	94 (69)
57a	NaOAc (13 eq.)	HOAc, 45°, 7 h	L	Z	-OAc	-OH	97
58d	NaOMe (1 eq.)[d]	MeOH/THF, 25° (25 min)	DL	Z	-OH	-OMe	88
58d		25°(20 min), 0°(50 min)	DL	Z	-OH	-OMe	67
60			DL	Z	-OMe	-OMe	13
61b	$PhCH_2SNa$ (1.1 eq.)	DMF, 30 min	D	Z	$-SCH_2Ph$	-OH	78 (65)
62b	$(H_2N)_2C=S$ (1.5 eq.)	50% aq. THF, 2 h	D	Z	$-SC(NH_2)NH_2^+$	$-O^-$	(79)[109]
62d			DL	Z	$-SC(NH_2)NH_2^+$	$-O^-$	(56)
63b	$HSCH_2CH_2NH_3^+Cl^-$ (2 eq.)	pH 5.5, 50% aq. CH_3CN or THF, 20 min	D	Z	$-\overset{+}{N}H_2CH_2CH_2SH$	$-O^-$	(76)
64a	NH_3(g) (excess)	CH_3CN, 0°, 20 min	L	Z	-OH	$-NH_2$	77
65a			L	Z	$-NH_3^+$	$-O^-$	23
64b	NH_3(g) (excess)	THF, 0°, 3 h	D	Z	-OH	$-NH_2$	25
65b			D	Z	$-NH_3^+$	$-O^-$	75
66a	NH_3(g) (excess)	THF, 0°, 3 h	L	BOC	$-NH_3^+$	$-O^-$	79
67a	Me_3N (6 eq.)	THF, 0°, 2 h	L	Z	$-\overset{+}{N}Me_3$	$-O^-$	100 (91)
68b	Pyrazole (1.05 eq.)	CH_3CN, 50°, 12 h	D	Z	-(pyrazol-1-yl)	-OH	(71)
69b	$(MeO)_3P$ (19.5 eq.)	50° (3 days), 70° (2 days)	L[e]	BOC	$-[P(O)(OMe)_2]$	-OMe	98

[a] Unless otherwise noted reactions were done at 25°C.
[b] Optically-pure (based on rotation) and corresponds to starting lactone unless noted.
[c] Isolated chromatographically-pure, recrystallized yields in parentheses.
[d] Racemized products. The balance of material was Z-dehydroalanine methyl ester (59).
[e] Approximately 84% e.e.

1-Carbon Nucleophiles

Cyanide is produced as a byproduct of ethylene biosynthesis in plants.[161] In plants,[1a,161] herbivorous insect larvae[162] and cyanide producing bacteria,[163] CN^- is rapidly detoxified by incorporation into β-cyano-L-alanine with the aid of a β-replacement PLP-enzyme (Category 2).[165] Although some insect larvae utilize β-cyanoalanine in their defense secretions,[162] and some legumes accumulate it (possibly) as a deterent to herbivores,[164] in most cases it is rapidly degraded enzymically to L-asparagine and aspartic acid.[161,165]

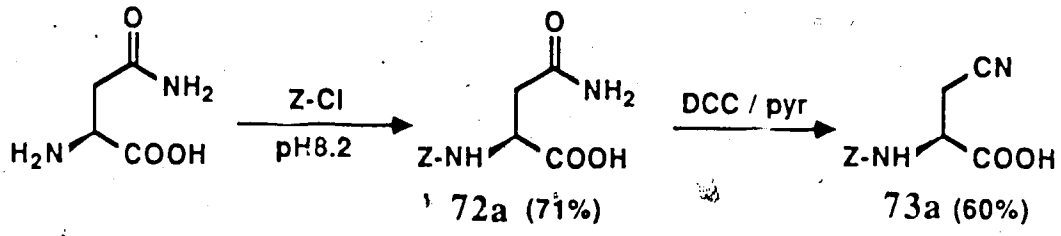
Synthesis of N-protected β-cyanoalanine by CN^- addition to serine β-lactones was attractive for a number of reasons. It is an example o a case where attack on



β-haloalanines fails. Previous syntheses by conjugate addition of isotopically labelled CN^- to acetamidoacrylate (8), followed by resolution, allowed the preparation of 4-C-isotopically-labelled asparagine and aspartic acid for biological studies.[166] Recently optically-pure β-cyanoalanine derivatives have been employed as chiral educts in alkaloid syntheses, etc.[167] Our interest in β-cyanoalanine was primarily due to its biological activity as a neurotoxin[164] and inhibitor of many enzymes. It is a

competitive inhibitor of β- and γ-cystathionases, aspartate β-decarboxylase (Categories 2 and 3), and glutaminase.[164] More importantly it acts as a suicide inhibitor of PLP-dependent alanine[168a] and D-amino acid transaminases (Category 1),[168b] possibly via a ketenimine-PLP adduct exactly analogous to allenic intermediate proposed for propargylglycine inactivations (recall Figure 4).[8,168]

The nucleophilic ring-opening of Z-L-serine β-lactone (**36a**) by cyanide anion was not trivial, and to facilitate the identification and purification of products, authentic Z-β-cyano-L-alanine was prepared.[167a,169,170] N-(Benzyloxycarbonyl)-L-asparagine (**72a**) was prepared from L-asparagine at pH 8.2 (±0.2), and dehydrated to **73a** using dicyclohexylcarbodiimide in pyridine according to the method of Ressler and Ratzkin.[169]

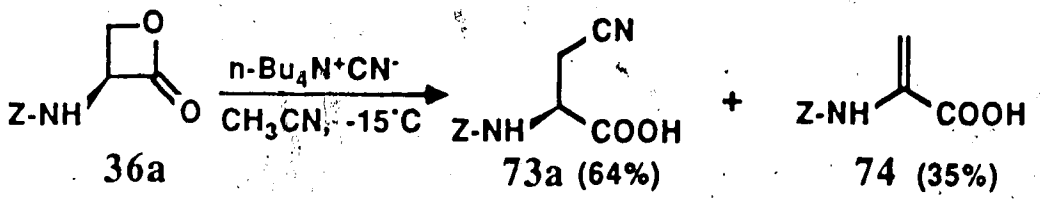


72a (71%) **73a** (60%)

The reactions of Z-serine β lactones (**36**) with cyanide anion were plagued by the nucleophile's action as a base. Use of KCN in methanol with **36a** gave exclusively Z-serine methyl ester (**58**) from attack of methoxide at the carbonyl (Path B, Figure 14). The use of KCN in DMF, CH_3CN, or DMSO, or with 18-crown-6 in aprotic solvents (THF, DMF)[171] gave complex mixtures from which **73a** was

difficult to purify. Although trimethylsilyl halides and azides (X = Cl, Br, N_3) have been used with β-propiolactone and a catalytic amount of pyridine to provide $XCH_2CH_2COOSi(CH_3)_3$ (44-75%)[172] which could be readily hydrolyzed, the analogous reaction of $(CH_3)_3SiCN$ and **36a** (with cat. DMAP) was very sluggish, and on warming to 45°C provided a mess.

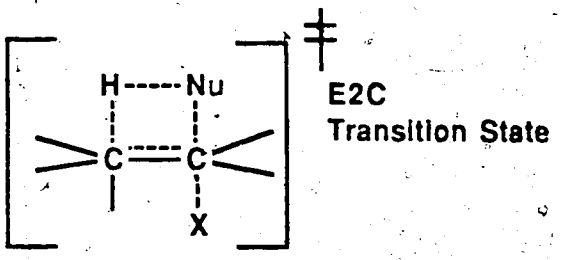
In the reactions of β-lactone **36a** with KCN in aprotic solvents much decomposition of the β-lactone by a "forbidden" elimination to Z-dehydroalanine (**74**) was apparent. With cyanide (as well as fluoride) anion it has been demonstrated that the less the anion is encumbered by a counterion in an ion-pair the more substitution is favored at the expense of elimination.[173] Consistent with this observation, utilization of highly dissociated tetra-n-butylammonium cyanide[241] in acetonitrile at -15°C was able to provide optically-pure Z-β-cyano-L-alanine **73a** in 64% yield, with the balance of material (**74**) resulting from elimination. Importantly none of the Z-β-cyanoalanine was generated from CN^- addition to **74** which would exist as the conjugate anion in the reaction mixture.



36a **73a (64%)** **74 (35%)**

Although it was not mentioned before, similar problems were encountered in attempts at producing the antibiotic enzyme inhibitor, β-fluoro-D-alanine[21b,6Ic,174] (X = F in J), from reactions of **36b** with fluoride anion from various sources (eg., KF/18-C-6; n-$Bu_4N^+F^-$ in THF; TAS-F in CH_3CN).[175] In all instances with F^-, the anion behaved as a base,[114,115,176] and **74** or its decomposition products predominated. Never was any of the desired fluorinated product detectable in the reaction mixture by ^{19}F NMR. Acidic fluoride sources (eg., $(HF)_x$-pyridine or HF/CH_2Cl_2) reacted slowly with the N-protected β-lactones to give only serines on aqueous workup, probably by hydrolysis of the acyl fluoride from F^- ("hard-base")[60,128] attack at the carbonyl.

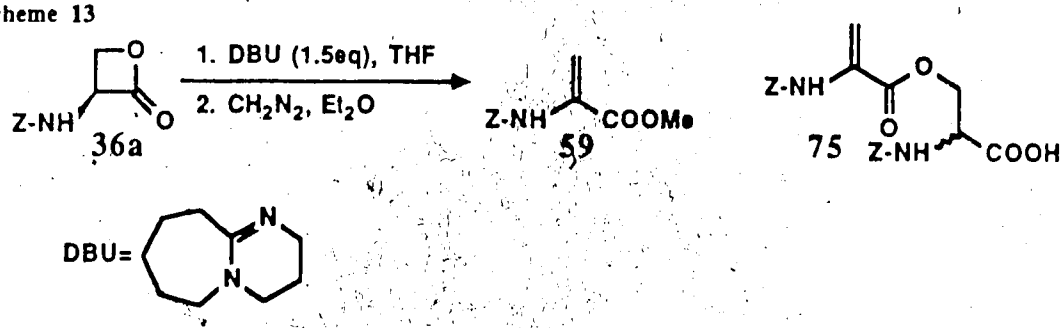
The eliminations to N-protected dehydroalanines (eg., **74**) caused by F^- and CN^- nucleophiles behaving as bases while amines and thiolates do not, has important mechanistic implications. In attempt to rationalize the phenomenon in which eliminations of good leaving groups proceed at rates dependent on C-nucleophilicity and H-bond accepting abilities with little or no correlation with H-basicity, Parker *et al.*[177] introduced the E2C mechanism. In the E2C-transition state the base interacts both with the carbon and the adjacent proton as a kind of $S_N2/E2$ hybrid. While the actual mechanism is controversial,[60] it appears that the observable phenomenon is operative and recurrent with the N-protected serine β-lactones.



Indeed, the internal elimination of Z-L-serine β-lactone (**36a**) caused by 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) occurs quantitatively, but considerably slower than with CN^- and F^- (Scheme 13) The products isolated depend on the conditions of workup. If the reaction is quenched by addition to 0.1N HCl, alkylation by diazomethane and chromatography yields only 18% of **59** and 68% of the acid-catalyzed dimerization product **75**. Alternatively, if the pH is maintained at 3-5 during aqueous workup, **59** may be isolated in 87% yield.

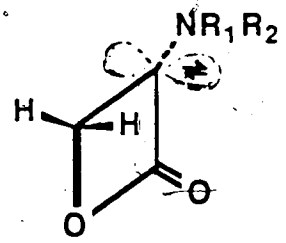
Another interesting mechanistic feature of the eliminations of the N-protected serine β-lactones is that they are stereoelectronically "forbidden". Since the

Scheme 13



orbital of the incipient carbanion is strictly fixed orthogonal to the leaving group[60,178] by the planar geometry of the β-lactone, elimination is greatly

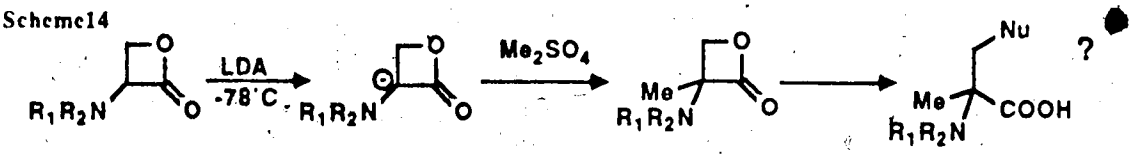
disfavored (see below). Because of this orthogonality



Mulzer et al. have successfully generated the α-carbanions of alkyl-substituted β-lactones with LDA and alkylated them at low temperatures.[178] At temperatures above -30°C these α-carbanions eliminate rapidly by an ElcB mechanism (i.e., 2 step),[178] which is quite unlikely when F^-, CN^- or even DBU are the bases.

In view of Mulzer's achievements at alkylation of the carbanions generated from β-lactones, it may be possible to alkylate carbanions produced from the di-N-protected serine β-lactones at low temperatures to produce the corresponding α-methyl serine β-lactones (Scheme 14). These α-methyl β-lactones would be incapable of elimination and could provide access to numerous α-methyl amino acids which have many important biological activities.[9a,17] It is even possible that the presence of the electronegative/π-donor amino group on the α-carbon could raise the barrier of inversion of the β-lactone carbanion sufficiently to favor pyramidal geometry with a

Scheme14



(eg. 45 (R_1=Bn, R_2=Z))

low inversion rate. This could allow a "self-reproduction of chirality"[17] through alkylation of a chiral α-carbanion and provide optically-active α-methyl amino acids. To date this remains unexplored and π-electron acceptor-stabilized carbanions of 3-membered rings are the only known pyramidal cases.[178]

Although unreactive with diazomethane in ether or dichloromethane (even on addition of $BF_3 \cdot OEt_2$), in the presence of DMF Z-L-serine β-lactone (**36a**) undergoes a unique ring-expansion reaction (Figure 17). When excess diazomethane and CH_2Cl_2/DMF solvents were removed in vacuo at 25°C, chromatography yielded 60% of ketene acetal **77**, 9% of Z-homoserine (**78**) and 30% of Z-L-serine methyl ester (**58a**). If the excess of CH_2N_2 from reaction in Et_2O/DMF was quenched by addition of an excess of CF_3COOH followed by removal of the solvents in vacuo, benzyl carbamate (**76**) and **58a** were isolated in 74% and 25% yield, respectively. Even if the diazomethane solution is dried over KOH and redistilled, Z-L-serine methyl ester (**58**) is produced from hydrolysis and esterification of **36a**. The generation of the remaining products **77**, **78a** and **76** may be rationalized according to Figure 17.

Ring-expansion of β-lactone **36a** probably produces Z-L-homoserine lactone which may hydrolyze to **78** or undergo enolization and methylation to **77** with loss of chirality by further reaction with diazomethane.[179] Treatment of purified **77** in CH_2Cl_2 with trifluoroacetic acid (TFA)

produced benzyl carbamate (**76**) as the only UV active product, thus suggesting the origin of **76** (74%) in the reaction quenched with TFA. Whereas reactions of

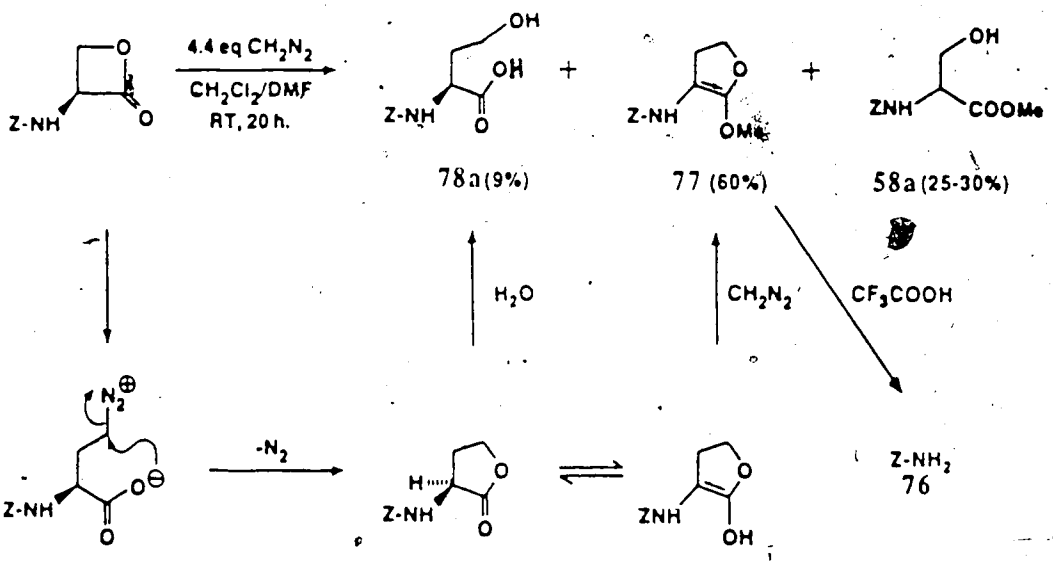


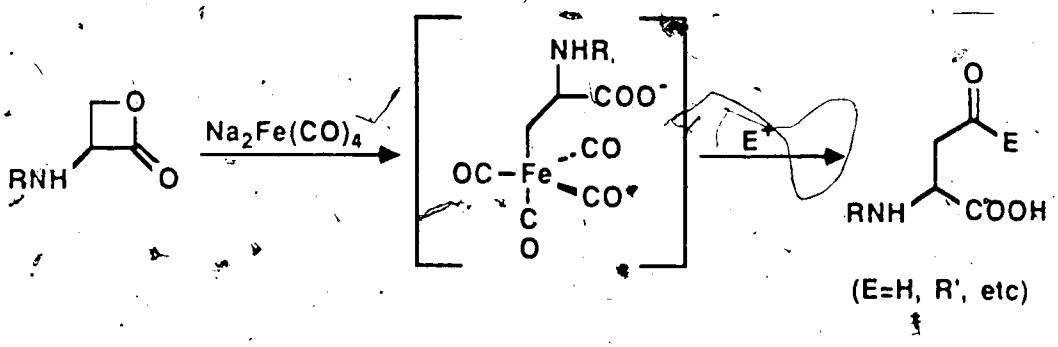
Figure 17. Ring Expansion of Z-L-Serine ß-Lactone by Diazomethane.

CH_2N_2 with cyclic and heterocyclic ketones are well known, this appears to be the first report of a ring-expansion of a β-lactone by diazomethane.

Unfortunately, the synthetic utility of this unique ring-expansion may be limited. Attempts to stop the reaction after addition of 1 equivalent of CH_2N_2 in DMF (60% of β-lactone consumed) still resulted in a mixture of products. Consumption of the β-lactone by reaction with phenyldiazomethane in DMF required 24 equivalents of $PhCHN_2$ due to rapid polymerization of the diazoalkane in that solvent and precluded isolation of products. Ethyl diazoacetate (2.5 eq.) required several days for reaction in DMF and produced >12 products all in less than 15%

yield.

The successful ring-expansion of β-lactone **36a** with diazomethane does however provide hope that simple "carbenoid"-type ($^{\pm}CH_2$) additions may succeed. For example, homoserine lactones may be accessible by a Simmons-Smith reaction (CH_2I_2/Zn-Cu couple). Furthermore, the use of the chemoselective Collman's reagent, $Na_2Fe(CO)_4 \cdot 1.5$ dioxane,[62] with the N-protected serine β-lactones could be quite effective at producing N-protected aspartate semialdehyde (E = H) (an important synthetic intermediate[180,181]) or various other 4-oxo amino acids.[1b] This would involve a reaction directly analogous

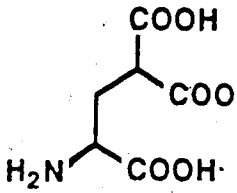


to that of the hydroacylation of Michael acceptors by Collman's reagent.[62]

Malonate Additions

γ-Carboxyglutamic acid (Gla) residues are produced in select proteins by a post-synthetic vitamin-K dependent carboxylation of glutamate side-chains.[1a,182] The γ-carboxyglutamate residues are responsible for the calcium (Ca^{++})-binding activity of numerous blood clotting factors

(eg., prothrombin) and bone proteins such as osteocalcin.[1a,182]



γ-Carboxy-L-Glutamic Acid

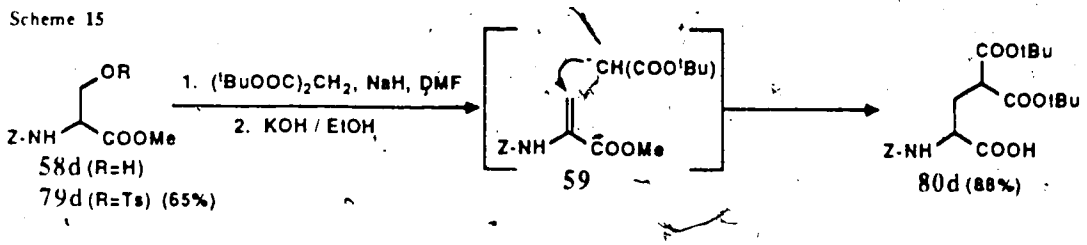
Since the discovery of this elusive amino acid (i.e., it readily decarboxylates) in 1974, many syntheses have appeared in the literature.[73,183-186] For use in peptide syntheses differential protection of the γ-carboxyl and amino functionalities are essential. Previous syntheses typically involved alkylation of malonate diesters by β-haloalanine or O-toluenesulfonyl serine derivatives and resulted in racemic product due to elimination and subsequent conjugate addition (see Scheme 15 below).[183a,c,186] N-Protected dehydroalanine esters have also been employed directly[185] (rather than generated *in situ*) to provide racemic products, which are inevitably resolved by tedious recrystallizations as diastereomeric salts.[183] Despite reports of an asymmetric Strecker synthesis[184a] (10% yield), and biomimetic "carboxylations" of glutamate derivatives,[73,184b] optically-pure differentially-protected γ-carboxyglutamic acid derivatives for peptide synthesis remain an extremely expensive commodity ($400/g for **80a** from Bachem).

Alkylation of malonate diesters by N-protected serine

β-lactones possessed the potential to produce differentially protected derivatives of γ-carboxyglutamic acid (Scheme 16). Importantly, if elimination were to occur subsequent Michael-addition of the malonate anion to the aminoacrylate salt was unlikely. Thus the desired product should be optically-pure.

Because of the ambident nature of both the malonate anion and the β-lactone, complicated product mixtures could be envisioned, so authentic N-Z-γ,γ-di-tert-butyl-DL-γ-carboxyglutamic acid (**80d**) was prepared.[183a] Z-DL-Serine was quantitatively converted to its methyl ester (**58d**) with diazomethane and treated with p-toluenesulfonyl chloride in pyridine[187a] to produce **79d** in 65% recrystallized yield. This O-tosyl-DL-serine derivative (**79d**) was treated with di-tert-butyl sodiomalonate in DMF. This operation generates Z-dehydroalanine methyl ester (**59**) in situ[183a] which subsequently adds the malonate anion in Michael-fashion. The crude product

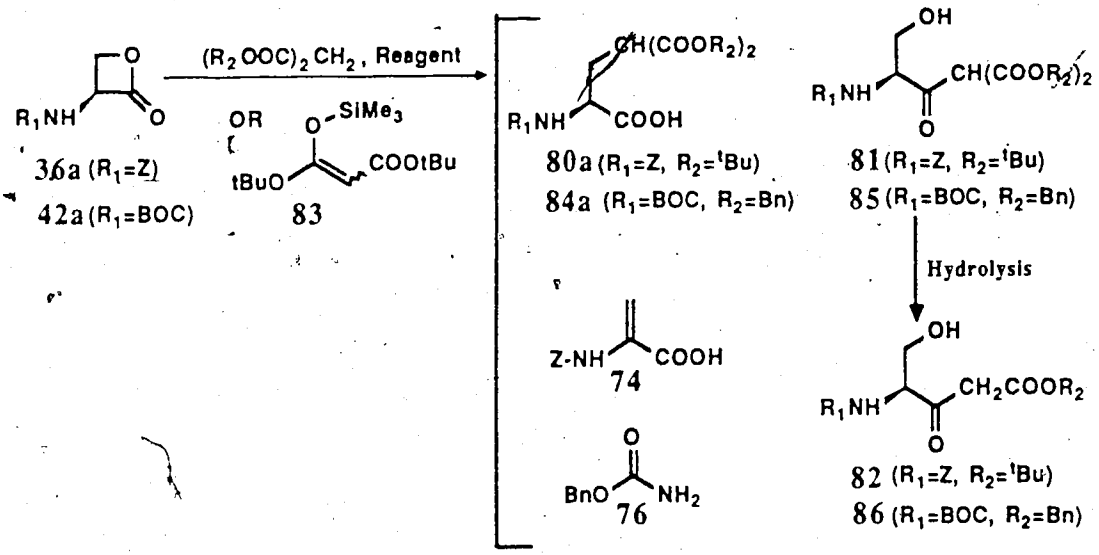
Scheme 15



methyl ester was directly saponified to provide authentic **80d** in 88% yield from **79d**. Since citric acid is soluble in EtOAc or CH_2Cl_2 used in extractions, the use of aqueous citric acid in workup as described in several literature

preparations[183] was problematic. Instead, recoveries in this and later cases were maximized and product decomposition minimized by aqueous workup while maintaining pH 3-5 during quenching, and adjusting to pH 2.5 for extraction.

Scheme 16 to accompany Table 3



The results of some of the attempts to optimize the yield of the γ-carboxyglutamic acid derivatives (**80a** or **84a**) are presented in Table 3. Typical experimentals have been reported for anionic (Entry 5) and Lewis acidic (Entry 13) conditions. Although the desired products (**80a** or **84a**) were obtained optically pure, the maximum observed yields were only 7% and 36% respectively.

In most instances the major products were those of acylation of the malonate anion by the β-lactones (i.e.,

Table 3. Malonate Additions to N-Protected Serine β-Lactones[a]

Entry	R^1	R^2 (eq.)	Reagent (eq.)	Conditions (°C)	Isolated Product 74	76	80/84	81/85	82/86
1	Z	^{t}Bu (1.5)	$(Me_3Si)_2NLi$ (1.3)	THF,[b] -45(1h)→-23(1.5 h)	36	17	3	43	
2				THF,[b] 0° (75 min)	42		7	49	
3		^{t}Bu (2.0)	^{t}BuLi/TMEDA (1.5)	THF,[b] -78° (21 h)	20		7	31[c]	
4	Z	^{t}Bu (2.2)	NaH (1.5)	DMF,[b] 0° (1 h)			<5	>90[c]	
5	BOC	Bn (2.0)	NaH (1.5)	DMF,[b] 25° (24 h)			nd	62	6
6	Z	^{t}Bu (1.5)	KH (1.3)	DMF,[b] 0° (1[illegible] min)	12	33	<2	35	16
7	BOC	Bn (1.5)	KH (1.3)	DMF,[d] 0° [illegible]			36	64[c]	
8	Z	^{t}Bu (1.5)	KO^tBu (1.4)	DMF,[b] 0° (1 h)				85	
9	Z	^{t}Bu (2.0)	^{i}PrMgCl (1.5)	THF,[b] 0° (3.5 h)				20[e]	
10	Z	^{t}Bu (2.0)	K_2CO_3(1), $Ni(acac)_2$ (.1)	THF[b] (6 days)[188]				>95[c]	
11	Z	^{t}Bu (2.0)	DBU (1.5)	ØH/THF (4:1)(1.5h)	>90				
12	Z	**83** (2.0)	$TiCl_4$[f] (2.0)	CH_2Cl_2, -15° (3 h)			>5	18	20[g]
13	Z	**83** (2.0)	$TiCl_4$ (0.7)/$Ti(O^iPr)_4$ (2.5)	CH_2Cl_2, -15°(3h)→ 20°(1h)				87	

[a] See Scheme 16 for product structures.

[b] β-lactone added to malonate anion.

[c] Sum of **81/85** and **82/86** was constant (±5%) however the actual ratio varied between experiments.

[d] Malonate anion added to β-lactone.

[e] 60% of Z-β-chloro-L-alanine (**56**) produced.

[f] Added to mixture of β-lactone and **83**.

[g] 24% of Z-β-chloroalanine (**56**) produced.

81/85 and **82/86**) and as a result no real trends with respect to counterion can be deduced. The formation of these acyl adducts i[illegible]ally caused much confusion since their behavior by [illegible] and [illegible] (in several literature systems) is almost indistinguishable from that of the desired product. In addition **81/85** decompose rapidly on silica or in H_2O at pH <2 or >8 to a number of products including the corresponding serine derivative (**35** or **41**) and the β-dicarbonyl compound **82/86**. The characterization and separation of the products with minimal decomposition required mild workup conditions followed by reverse-phase chromatography (RP-8 MPLC). Under these conditions very high yields of the acyl adducts **81/85** could be isolated, and surprisingly were found to be optically-active.

Disappointingly, the only times acylation did not predominate were when elimination did. Elimination was especially pronounced with the Li^+ counterion (Entries 1-3). Hydrolysis of Z-dehydroalanine (**74**) resulted in the formation of benzylcarbamate (**76**), so that their sum represents the extent of elimination. Although DBU and F^- have been used as bases for selective C-monoalkylations of active methylene compounds,[114,115d] their use in this case gave only elimination products. These results at least indicate that no conjugate addition of malonate to Z-dehydroalanine (**74**) occurs.

With the MgCl enolate of di-tert-butyl malonate, the reaction with malonate was so slow that nucleophilic

attack by chloride competed to produce 60% of Z-β-chloro-L-alanine. Formation of Z-β-bromo-L-alanine (**55a**) in situ by reaction with $MgBr_2 \cdot OEt_2$, followed by addition of the K^+-enolate provided only unreacted **55a** and Z-dehydroalanine (**74**).

The trimethylsilyl ketene acetal of di-tert-butyl malonate was prepared by reaction of the Na^+-enolate with trimethylsilyl chloride and triethylamine,[189] since the usual conditions of Danishefsky (i.e., Et_3N, Me_3SiCl, cat. $ZnCl_2$) were unsuccessful.[190] It was hoped that the use of **83** under Lewis acid conditions might alter the regiochemistry of the ring-opening by directing the attack through complexation of the β-lactone carbonyl as was previously observed with $TiCl_4$. The use of $TiCl_4$[191] (Entry 12) with **83** provided β-chloro-L-alanine (**56**) as the major product. This was effectively suppressed by "buffering" the reactivity of $TiCl_4$ with $Ti(O^iPr)_4$[181] to afford the acyl adduct **81** in 87% yield. The alternate use of tris(diethylamino)sulfonium difluorotrimethyl-siliconate, $[Et_2N]_3S^+Me_3SiF_2^-$, (0.1 or 1.0 equivalents) to produce the "naked" enolate[175] from **83** was also unsuccessful at providing appreciable amounts of the desired γ-carboxyglutamic acid derivative **80a**.

There was a significant difference between both the rates of reaction and optimal conditions for formation of **80**/**84** from the pairs of protecting groups (compare Entries 4 and 5, 6 and 7). Since reactions of dibenzylmalonate

with Z-L-serine β-lactone (**36**) were as fast as those of di-tert-butyl malonate under identical conditions, it appears that it is the N-BOC moiety which greatly reduces the rate of reaction and may even direct the attack.

In the course of the investigations with malonate additions, several sets of conditions were found to provide the acyl adducts **81/85** in very high yield (eg., Entries 5, 8, 13, Table 3). Comparison of the structure of these acyl adducts and their more stable decomposition products **82/86** with that of the unusual amino acid statin[20] indicates obvious structural similarities (Figure 18). It is believed that the extremely strong binding of pepstatin to pepsin ($K_i \sim 10^{-11}$ M) and other peptidases[192] is due to the fact that statin component resembles the tetrahedral intermediate related to the transition state for peptide hydrolysis.[20] Similarly aldehyde and ketone analogs of peptide substrates are demonstrated potent competitive inhibitors of cysteine and serine proteases, as well as carboxypeptidases.[193] In some instances, hydration or addition of an enzymic nucleophile to the carbonyl as illustrated in Figure 18 has been postulated to account for the potent inhibition.[193]

Based on this knowledge, it is quite likely that the products of malonate acylation by the serine β-lactones (**81/85** or **82/86**) could be useful as, or in "transition-state analog" inhibitors of peptidases. Because of their highly electrophilic character they would be expected to

readily hydrate or add an enzymic nucleophile. Simple reduction of this β-carbonyl would produce a secondary alcohol analog of statin which would be less susceptible to hydrolysis.

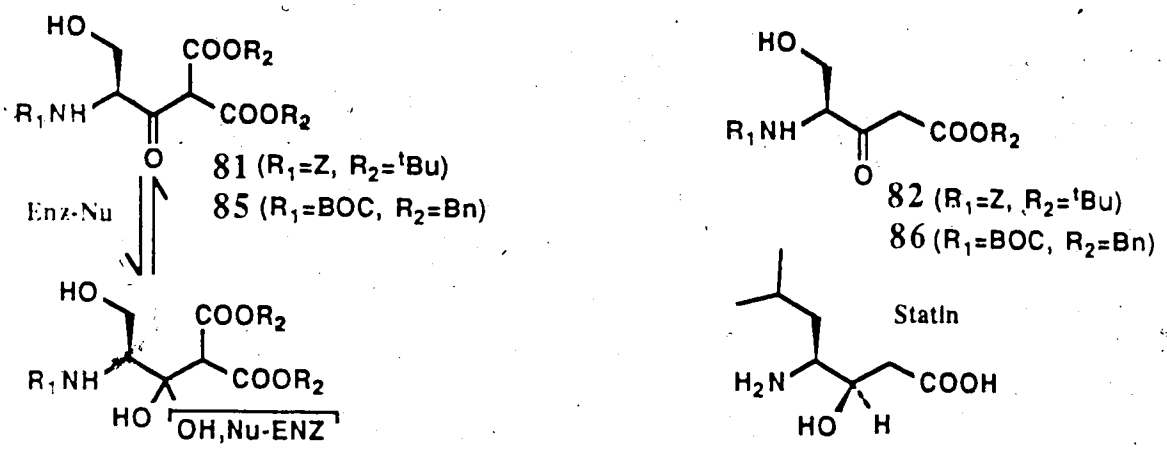


Figure 18. Comparison of Acylated Malonate Products with Statin.

Organo-Copper Nucleophiles

Reactions of copper-containing organolithium and organomagnesium reagents with N-protected serine β-lactones could produce a wide variety of amino acids with homologated aliphatic and aromatic side chains. Previous work on β-propiolactone[76] indicated that most Grignard and organolithium reagents attack the carbonyl of the lactone with acyl-oxygen cleavage to generate the corresponding ketone or tertiary alcohol products. While some organocadmium compounds reacted to produce β-substituted

carboxylic acids, the method was not generally applicable.[76b] More recently Normant et al.[77] established that the desired regiospecific ring-openings of β-propiolactone could be accomplished with either stoichiometric (ie. R_2CuLi or R_2CuMgX) or catalytically-generated (10 mol % Cu(I) salt/RMgX) organocuprate reagents in excellent yield (R = n-Bu, i-Pr, t-Am, Ph). Such approaches to three carbon homologation have proven successful in the synthesis of numerous natural products,[77,194,195] although they have not previously been applied to optically-active 3-substituted 2-oxetanones.

We have examined the ring-opening reactions of optically-pure N-protected serine β-lactones by organometallic reagents with respect to regiospecificity and stereochemical integrity. Conditions under which these serine β-lactones react with aliphatic and aromatic carbanions, with essentially no loss in optical purity, to produce N-protected amino acids suitable for direct incorporation into peptides (Scheme 17) have been determined.

With mono-N-protected Z-serine β-lactones (i.e., **36**), an organometallic reagent may abstract the relatively acidic NH proton to form an amidate anion (see below, Scheme 20, **X**) which could open the lactone or repel attack by another equivalent of organometallic species. To assess the influence of the NH on the outcome of reactions of serine β-lactones with organometallics, the diprotected

stereochemical purity encountered in the addition of organometallics to the β-lactones, a measure of the enantiomeric excess (e.e.) of both the starting lactones **36** and **45** and the addition products is required. The assay developed to measure the optical purity of the serine β-lactones **36** and **45** utilizes regiospecific ring-opening by the potassium salt **87** of (S)-2-methoxy-2-(trifluoromethyl)phenylacetate[196] (MTPA) in DMF (analogous to the previously discussed opening by acetate) to produce diastereomers from enantiomers (Figure 19). Acidification, extraction, and esterification with diazomethane produces mixtures of **88a** and **88b**, or **89a** and **89b**, along with side product **92**. Elimination to N-protected-dehydroalanine is minimized (ie. < 0.6% of product) by performing the reaction with $K^{\oplus}MTPA^{\ominus}$ (**87**) in DMF at 0-5 °C. Diastereomers **88a,b** or **89a,b** in the product mixture were directly separated and quantitated using HPLC. Complementary ^{19}F and ^{1}H NMR results were obtained after separation of the MTPA derivatives **88** or **89** from methyl MTPA (**92**) by chromatography.

The accuracy and validity of the HPLC and ^{19}F NMR analyses on **88** or **89** were determined by subjecting known mixtures of the enantiomers of **36** or **45** to the analysis. In the case of the mono-N-protected β-lactones **36a,b**, derivatization and analyses of a standard mixture containing 65.22% **36a**[197] and 34.78% **36b**[198] gave standard **90** which provided ratios of 65/35 by ^{1}H NMR (δ 3.66 and

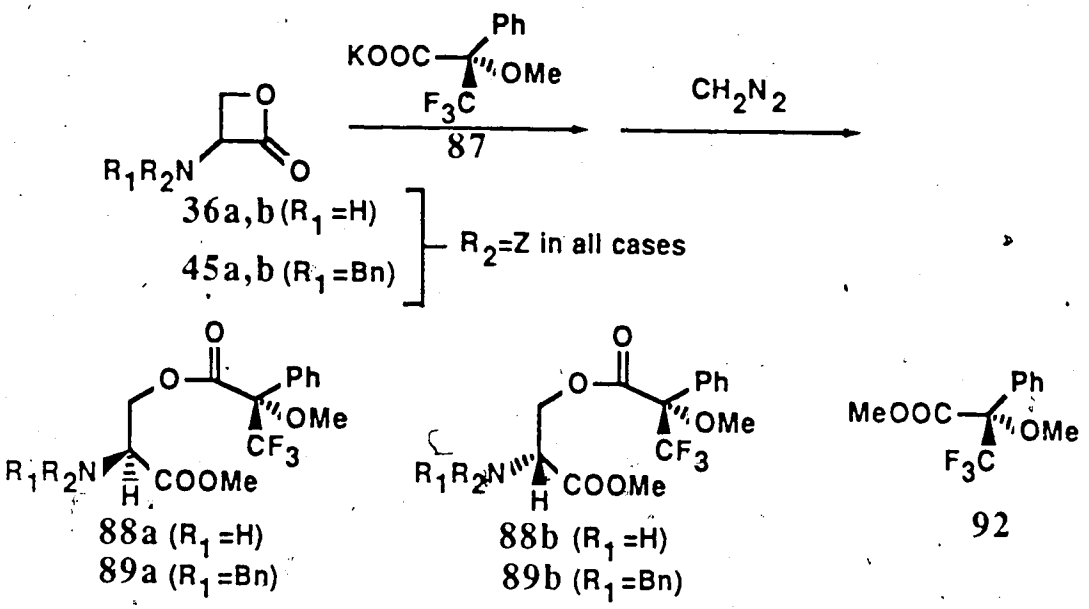


Figure 19. Derivatives for Determination of Optical Purity of Serine ß-Lactones.

3.73 ppm, respectively, for $COOCH_3$'s), and ^{19}F NMR (δ -76.26 and -76.23 ppm, respectively, for CF_3's),[199] and 64.8/35.2 (±0.11) by HPLC. The values reported for the optical purity of **36a** and **36b** were obtained by HPLC and, when possible, confirmed by NMR.

For the di-N-protected β-lactones **45a** and **45b** a reference standard containing 67.12% **45a** (S)[200] and 32.88% **45b**[201] was derivatized to provide **91** which was analyzed to yield ratios of 2/1 by ^1H NMR (δ 3.46 and 3.43, respectively, for $COOCH_3$'s), 67/33 by ^{19}F NMR (δ -72.14 and -71.96 , -72.04, respectively, for CF_3's),[202] and 67.4/32.6 (± 0.30) by HPLC. Although HPLC and ^{19}F NMR results complemented each other, the excellent resolution and accuracy of ^{19}F NMR[203] with the di-N-protected derivatives made it the method of choice. In all cases the measured optical purity of the serine β-lactones **36** or

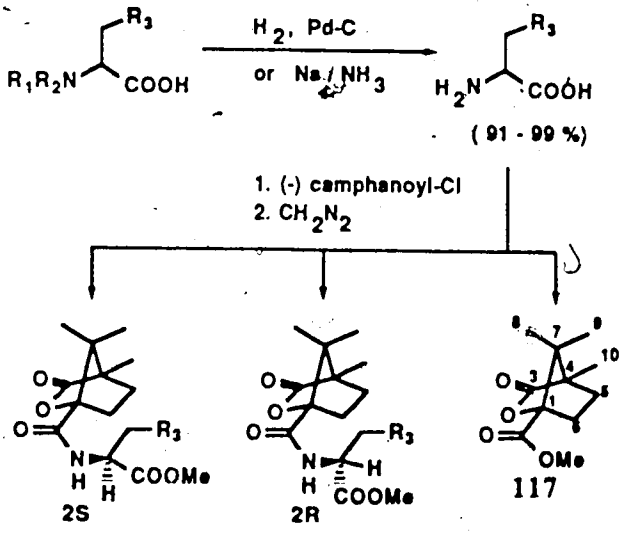
45 exactly matched that of the starting materials (i.e., **35**, **43**, **44** or free serine), thereby indicating no detectable loss in optical purity in lactonization.[121] This is consistent with our previous observations of optically-pure ring-opened products based on [α] values (Table 2).

Whereas β-substituted alanines (Table 2) are not generally amenable to analysis of optical-purity by GC as diastereomeric derivatives due to facile eliminations,[204] the more stable amino acids bearing aliphatic or aromatic sidechains are. Recently Dr. L.A. Trimble and Mr. J.G. Drover of our research group demonstrated that several such amino acids could be analyzed for optical purity by GC as their N-(1S,4R)-camphanoyl methyl esters.[9i,124] This method was found to be generally applicable to all amino acids produced from the organometallic additions. In addition, these analyses were facilitated by the commercial availability of the optically-pure amino acids[98] in all but one case.

To assess the optical purity of the amino acid derivatives resulting from organometallic additions to the serine β-lactones (Scheme 17, Table 4), the corresponding free amino acids were liberated from mono- and di-N-protected products by hydrogenolysis (or Na/NH_3 reduction for **105a**, **106a** to avoid reduction of the sidechain C=C), and then analyzed as their N-(1S,4R)-camphanoyl methyl esters (Figure 20, Table 5). In all instances

chromatographically-pure but unrecrystallized N-protected products were deprotected, and the free amino acids obtained were derivatized directly without recrystallization in order to avoid possible enrichment of one enantiomer. In two cases (**96b** and **107a**), the complete analysis was carried out on both recrystallized and unrecrystallized materials with identical results.

It was found that derivatization of as little as 1 mg of amino acid is conveniently effected in 80-95% yield using (-)-camphanoyl chloride (2 equiv.) in 1 M sodium carbonate/bicarbonate buffer (pH 10, 20 mole eq.) and toluene (0.2 volumes) (Figure 20). These mild conditions eliminate the need to monitor and adjust the pH during the reaction.[205] Following esterification of the intermediate acids with diazomethane, a mixture of diastereomers and methyl camphanoate (**117**) is produced (Figure 20). ^{1}H NMR and gas chromatographic (GC) analyses may be carried out



See Table 5 for Results and Compound numbers.

Figure 20. Derivatives for Determination of Optical Purity of Amino Acid Products.

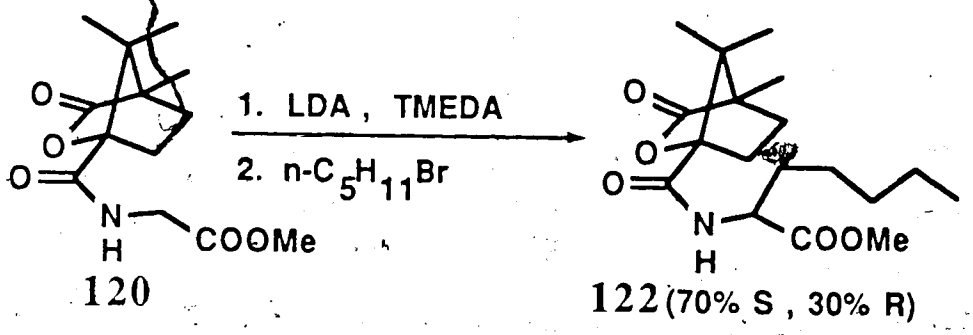
directly on this mixture or after removal of **117** by sublimation or chromatography.

Although excellent resolution of 8'-CH_3 peaks of the (2S)- and (2R)-isomers of all of the examined N-camphanoyl-amino acid methyl esters (Table 5) in ^1H NMR[206,207] easily allows accurate estimation of the diastereomeric ratio down to approximately 2 (±1)% cross-contamination, the results of GC analysis are reported because of their greater sensitivity and accuracy. In all cases standard mixtures of (2R)- and (2S)-isomers N-camphanoyl-amino acid methyl esters (**119, 122, 124, 126, 128, 130**) were used to develop GC conditions and estimate accuracy. Invariably the (2R)-isomer emerged ahead of the (2S)-isomer, and sufficient resolution to establish limits of detection at 0.2→0.5 % of diastereomeric impurity was easily obtained. With the exception of the 2-aminoheptanoate reference standard **122**, all GC standards (**119, 124, 126, 128, 130**) were generated by derivatization of known mixtures of commercially available optically-pure amino acids.[98] Since 2-aminoheptanoic acid was not commercially available, a standard mixture of (S)- and (R)-isomers **122** was produced by diastereoselective alkylation of the corresponding glycine derivative according to Scheme 18.[216] The ^1H NMR spectrum of **122** suggested an S/R-ratio of 70/30 in agreement with the result of 69.8/30.2 (± 0.1) by GC analysis.

When sufficient amounts of the various amino acids

were deprotected to allow accurate measurement of optical rotation, the ratios agreed with those obtained by GC and ^{1}H-NMR analysis within experimental error (see Table 5). Values for the % decrease in enantiomeric excess (e.e.) reported in Table 4 are obtained by subtraction of the optical purity of the products (Figure 20, Table 5) from that of the serine β-lactone starting materials (Figure 19).

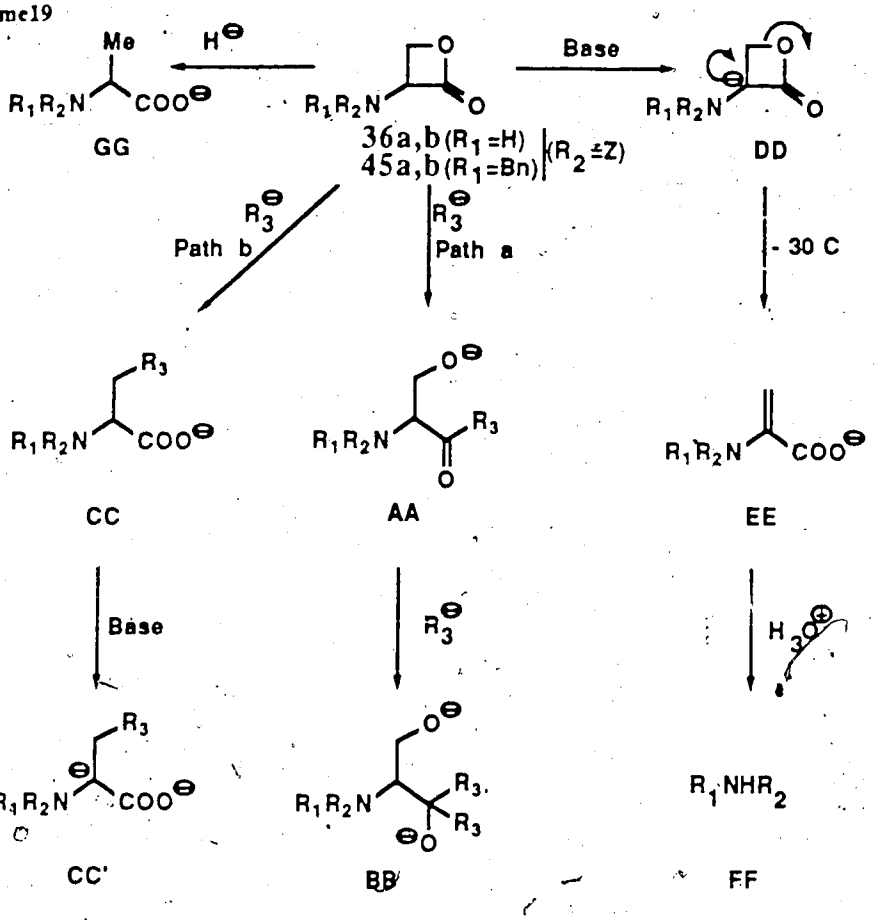
Scheme 18



General Features of Reactions of Serine β-Lactones with Organometallic Reagents.

Organometallic reagents may attack serine β-lactones at two sites (Scheme 19). Undesirable attack at the carbonyl carbon (Path a) produces the corresponding ketone (**AA**, eg., **95**, **98**) which may add a second equivalent of organometallic species to generate a tertiary alcohol (**BB**, eg., **108a**). To produce the desired N-protected amino acids, the serine β-lactones must behave like "chiral enone equivalents" with "1,4-attack" of the carbanion at the β-methylene group (Path b) and concomitant ring-opening to liberate the carboxylate functionality (**CC**).

Scheme19



Organometallic substitutions on N-protected O-tosyl or ω-halogeno derivatives of serine or homoserine methyl esters give products which are susceptible to racemization under the reaction conditions or in the subsequent hydrolysis.[11c] In contrast, the N-protected 2-aminocarboxylate products (**CC**) derived from β-lactone cleavage should be rather resistant to racemization since it requires a proximal dianion (**CC'**, Scheme 19). Interestingly, previous work[76,77,194,195] with β-propiolactones indicated that organocuprate reagents which add in 1,4-fashion to α,β-unsaturated carbonyl systems also add to the methylene group of β-lactones. The same

organometallic reagents are also useful in alkylations by primary alkyl halides and tosylates.[11c] To gain further insight into the behavior of β-lactones with organometallic reagents, the reactions of some of the more contemporary reagents with the serine β-lactones (**36** and **45**) were examined. Recently, BF_3-etherate has been reported to promote addition of alkyllithiums to oxetanes and oxiranes.[217] Under similar conditions the attack of RLi/BF_3-OEt_2 on **45a** is <u>not</u> directed toward the β-methylene carbon, but instead the only products are ketones **AA** and alcohols **BB** resulting from reaction at the carbonyl (Path a, Scheme 19).[124] Organocerium reagents $RCeX_2$,[218] which display enhanced oxaphilicity and reduced basicity relative to their RLi and RMgX counterparts, similarly add in 1,2-fashion to the serine β-lactones (Path a, Scheme 19), in direct analogy to their behavior with enones. For example, reaction of $MeCeCl_2$ (1 equiv.) with **45a** yields only the ketoalcohol **95** (11%), diol (19%), and unreacted β-lactone (57%).[124] Lower order cyanocuprates RCu(CN)Li, in which $CN^{\ominus}$ economically functions as the residual ligand, have been reported to possess reactivity comparable to R_2CuLi, but with higher thermal stability.[219,220] Disappointingly, PhCu(CN)Li (7 equiv.) reacts with **36a** to provide only a 4% yield of Z-phenylalanine (**107a**). In contrast, higher-order cyanocuprates $R_2Cu(CN)Li_2$[220,221] add to the mono- and di-<u>N</u>-protected β-lactones to give the desired amino acids

(Table 4), and are discussed below. Please note that 6 of the entries in Table 4 involving cuprate additions to **45** have been exerpted from Mr. J. Drover's thesis[124] for clarity in the discussion.

Organolithium-Derived Cuprate Reagents.

Lipshutz and coworkers have illustrated the advantages and utility of higher-order cyanocuprates $R_2Cu(CN)Li_2$ in reactions with primary and secondary alkyl halides and tosylates, and in conjugate additions to α,β-unsaturated carbonyl systems.[220,221] Earlier work by Normant et al.[77] had also established that R_2CuLi reagents add to β-propiolactones in the desired manner. The results in Table 4 show that both types of reagents add to N-protected serine β-lactones in the required fashion. Similar yields were obtained with both reagents, but the cyanocuprates $R_2Cu(CN)Li_2$ may be preferred due to their higher thermal stability.

Yields of $R_2Cu(CN)Li_2$ additions are usually higher with diprotected β-lactones **45a,b** than with monoprotected β-lactones **36a,b** (eg., compare Table 4 Entries 1/4, 5/6, but 16/18). In the case of vinyllic transfer from $(CH_2{=}CH)_2Cu(CN)Li_2$ to **36a** none of the desired allylglycine derivative was detected, while a 56% yield was secured with **45a** (Entry 14). In order to obtain comparable yields with mono-N-protected serine β-lactones, an excess of cuprate reagent was required (typically 5 equivalents were

Table 4. Results of Organo-Copper Additions to N-Protected Serine β-Lactones

Entry	Starting β-Lactone[a]	R_2	Reagent (eq.)	Conditions[b] (°C)	R_3	Product (Yield %)	% Decrease in e.e.[c]
1	36a	H	CuCN (5), MeLi (8)	-23° (2 h), 0° (15 min)	Me	93a (47)	1.7 (±.4)%
2	45a	Bn	$CuBr \cdot SMe_2$ (3.5), MeLi (6.7)	-45° (2.5 h)	Me	(70)	2.4 (±.4)%[d]
3	45a	Bn	CuCN (1.8), MeLi (3)	-78° (40 min), -46° (3 h)	Me	(72)[i]	17.5 (±.6)%
4	45b	Bn	CuCN (1.8), MeLi (3.0)	-78° (1 h), -45° (0.5 h)	Me	94b (92)[e]	1.0 (±.7)%
5	36b	H	CuCN (5.2), n-BuLi (10)	-23° (2 h)	n-Bu	96b (62)[i]	0[f,m]
6	45a	Bn	CuCN (2.1), n-BuLi (3.5)	-78° (40 min), -46° (1 h)	n-Bu	97a (76)[i]	11.7 (±.9)%
7	36a	H	$CuBr \cdot SMe_2$ (0.19), i-PrMgCl (6)	-23° (1.5 h)[h]	i-Pr	99a (44)[i]	<0.5%
8	45a	Bn	$CuBr \cdot SMe_2$ (0.21), i-PrMgCl (5.2)	-23° (2 h)[h]	i-Pr	100a (83)[i]	0[f]
9	45a	Bn	CuCN (2.3), sec-BuLi (4.5)	-78° (20 min), -45° (1.2 h), -18° (1 h)	sec-Bu	(76)[j]	n.d.[g]
10	36a	H	CuCN(3.3), MeLi(3.1), t-BuLi(3.1)	-23° (1 h)	t-Bu	101a (48)	0[f]
11	45a	Bn	CuCN (1.9), t-BuLi (3.4)	-78° (1 h), -46° (1 h), -15 (.5 h)	t-Bu	(38)[j,k]	5.6 (±.6)%[d]
12	45a	Bn	$CuBr \cdot SMe_2$ (4.4), t-BuLi (7.8)	-46° (7 h), -10° (1 h)	t-Bu	102a (51)[j,k]	0[f]
13	36a	H	$CuBr \cdot SMe_2$ (0.25), $CH_2CHMgCl$ (5)	-23° (2 h)[h]	CH_2CH-	105a (47)	0[f]
14	45a	Bn	CuCN (1.8), CH_2CHLi (3.0)	-78° (1 h), -45° (3 h), 0° (.5 h)	CH_2CH-	106a (56)	27.2 (±.8)%
15	36a	H	$CuBr \cdot SMe_2$ (0.3), PhMgCl (6.0)	-23° (2 h)[h]	Ph	107a (55)[l]	0[f]
16	36b	H	CuCN (5.13), PhLi (10)	-15° (2 h)	Ph	107b (46)	67.4 (±.4)%
17	45a	Bn	$CuBr \cdot SMe_2$ (2.5), PhMgBr (4.9)	-12° (4 h)[h]	Ph	109a (60)	3.3 (±.8)%
18	45a	Bn	CuCN (1.8), PhLi (3.1)	-78° (1 h) → -15° (over 3 h)	Ph	(25)	4.7 (±.6)%
19	45a	Bn	$CuBr \cdot SMe_2$ (3.0), PhLi (6)	-35° (4 h)	Ph	(36)	14.2 (±.8)%[d]

(Continued)

Table 4 Footnotes:

[a]Unless noted optical purities of β-lactones **36a**. **36b**, **45a**, **45b** were 99.5%, 97.0 (±0.2)%, 98.7 (±0.3)% and 96.9 (±0.3)%, respectively.

[b]THF solvent unless indicated.

[c]Determined by comparison with enantiomeric excess (e.e.) of starting β-lactone (see a).

[d]By comparison of $[\alpha]_D^{25}$.

[e]Based on 22% recovered β-lactone; 72% isolated yield of **94b**.

[f]Within experimental error (±0.3%).

[g]Mixture of at least two diasteromers.

[h]THF/Me_2S (20:1) solvent.

[i]Ketone product isolated: 5% (Entry 3 (**95**), 6 (**98**)), 14% (Entry 5), 8% ketone (Entry 7), 16% (Entry 8).

[j]Z-NH-Bn (**104**) isolated: 4% (Entry 9), 19% (Entry 11), 18% (Entry 12).

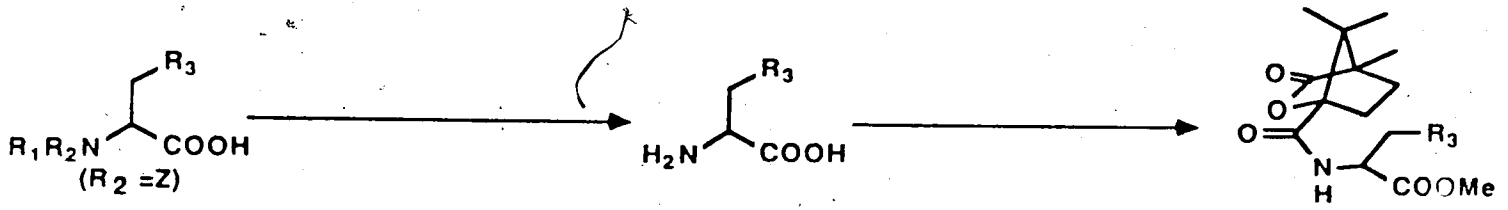
[k]N-Z-N-Bn-L-Alanine (**103a**) isolated: 14% (Entry 11), 23% (Entry 12).

[l]Tertiary alcohol sideproduct (**108a**) isolated in 43% yield.

[m]The S-isomer produced under analogous conditions also exhibited no detectable decrease in optical purity. Identical yield using DME solvent at -23°C.

Note: For entries 12 and 17 the cuprate addition was performed by J.C.G. Drover,[124] with deprotection and derivatization by LDA. Entries 2, 3, 9, 11, 18, and 19 are exerpts from J.C.G. Drover, M.Sc. Thesis,[124] with β-lactone optical purities determined by LDA.

Table 5. Results of Deprotection and Derivatization of Organocuprate Addition Products



Addition Product[a]		Amino Acid		N-Camphanoyl Methyl Ester[c]	
Compd. (Table 4 Entry)	R_3	Compd. (% Yield)	$[\alpha]_D$[b] (lit.)	Compd.	% 2S-isomer (GC)[d]
93a (1)	Me	**111a** (97)	n.d.	**118a**	98.91 (±.07)[208]
94b (4)	Me	**111b** (97)	-40.5° (+42.0)[209]	**118b**	2.07 (±.21)[201]
96b (5)	n-Bu	**112b** (96)	-32.3° (+33.0)[209]	**121b**	1.24 (±.16)[198]
97a (6)	n-Bu	**112a** (94)	+28.5°	**121a**	94.49 (±.30)[200]
99a (7)	i-Pr	**113a** (97)	+22.5° (+22.49)[210]	**123a**	>99.5[208]
100a (8)	i-Pr	**113a** (99)	+22.5°	**123a**	>99.5[200]
101a (10)	t-Bu	**114a** (99)	+16.0° (+14.7,[211b] +16.3[212])	**125a**	99.64 (±.20)[208]
102a (12)	t-Bu	**114a** (99)	+15.6°	**125a**	99.62 (±.06)[200]
105a (13)	H_2C=CH	**115a** (93)	n.d.	**127a**	99.20 (±.10)[32]
106a (14)	H_2C=CH	**115a** (95)	n.d.	**127a**	85.74 (±.22)[200]
107a (15)	Ph	**116a** (91)	-35.0 (-34.5,[213] -35.1[214])	**129a**	>99.6[206]
107b (16)	Ph	**116b** (99)	n.d.	**129b**	35.2 (±.2)[198]
109a (17)	Ph	**116a** (99)	-30.5	**129a**	94.25 (±.11)[215]

[a] J.G. Drover's results have not been included.

[b] Measured when >10 mg produced. See Experimental for concentration and solvent. Opposite literature values correspond to L-isomers.

[c] GC standards were **119** (Me), **122** (n-Bu), **124** (^{i}Pr), **126** (^{t}Bu), **128** (H_2C=CH), **130** (Ph).

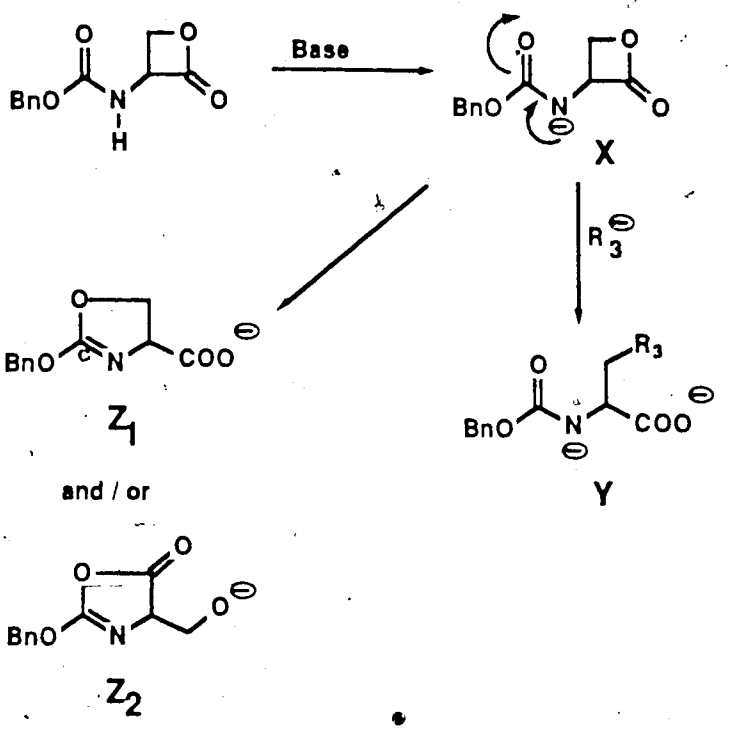
[d] GC results are average of at least 3 runs. The balance is 2R-isomer.

employed). This is due in part to consumption of an equivalent of reagent in removing the "acidic" NH proton from **36** to form **X**, or from the addition product to form **Y** (Scheme 20). In some cases a 20-25% excess of CuCN relative to RLi was also required to suppress attack at the carbonyl (Path a, Scheme 19). For example, when exactly 2:1 MeLi/CuCN was employed with **36a**, 28% ketone (**AA**), 37% tertiary alcohol (**BB**), and 18% of the desired acid (**CC**) were obtained (cf. Entry 1, Table 4). Lipshutz et al.[220,221] have observed the equilibrium between $R_2Cu(CN)Li_2$ and a mixture of RCu(CN)Li and RLi. They found that the percentage of free RLi increases with temperature. Presumably, this equilibrium accounts for the increase in Path a (Scheme 19) products encountered at the higher reaction temperature (-23°C) used with the monoprotected lactones, and the corresponding reduction in these undesired products on addition of excess CuCN. A reduction in the equilibrium concentration of RLi on switching from THF to DME might also be expected,[220,221] however such a solvent substitution for Entry 5 (Table 4) had no effect on product yields. Even under optimal conditions with $R_2Cu(CN)Li_2$, between 5 and 15% of ketone products (eg., **95**, **98**; **AA** of Scheme 19) were usually observed.

With the mono-N-protected β-lactones **36a** and **36b** additional temperature-dependent side reactions require that the addition of β-lactone to $R_2Cu(CN)Li_2$ be done at

-23 to -15 °C for optimum yield. At -78 °C no observable reaction occurs in 1.5 hours. Upon warming to -46 °C the β-lactones are slowly consumed, but considerable amounts

Scheme 20



(18-35%) of optically pure Z-serine are generated on aqueous workup using conditions which do not hydrolyze the β-lactones. At temperatures greater than -15 °C the yield of desired products is lowered by increasing production of Z-dehydroalanine (**74**) (Scheme 19, EE). The formation of Z-serine at low temperatures suggests intramolecular rearrangement to an oxazoline (Z_1), or oxazolone (Z_2) (Scheme 20) which would readily hydrolyze to Z-serine in the acidic workup.[88] This reaction predominates only at low temperatures where intermolecular nucleophilic addition of "R_3^-" to the anion **X** is most significantly retarded by Coulombic repulsion. Consistent with this argument are the observations that the diprotected β-

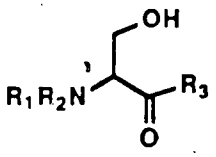
lactones (**45a,b**) generally react much more rapidly with organometallics than **36**, and that solution IR on the reaction mixtures of the Z-serine β-lactones (**36**) indicate the absence of the NH proton (broad, ~1615 cm^{-1} for C=O). As expected, no corresponding serine derivative **44a** or **44b** is produced in reactions of N-diprotected β-lactones **45a** or **45b**.

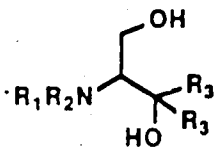
Although $R_2Cu(CN)Li_2$ and R_2CuLi additions to the di-N-protected β-lactones **45a** and **45b** appear superior with respect to yield and amount of organometallic reagent required, they often suffer from major losses in optical purity (Table 4). In contrast, with the exception of Entry 16, additions of $R_2Cu(CN)Li_2$ reagents to the monoprotected serine β-lactones **36a** and **36b** proceed with little or no decrease in enantiomeric excess (eg., Entries 1,5,10; Table 4). Comparison of Entries 3 and 4 which differ only in reaction times suggests that racemization of the di-N-protected products may occur on prolonged exposure to the organometallic reagent at -46°C. Despite the fact that $R_2Cu(CN)Li_2$ additions to the mono-N-protected lactones were done at higher temperatures (e.g., -23 °C), little or no racemization is observed, presumably because deprotonation of species **X** or **Y** (Scheme 20) which already possess an anionic nitrogen is disfavored. Racemization could in principle also occur by formation of the α-carbanions **DD** (Scheme 19), which are known to undergo rapid "forbidden" elimination to **EE** at

temperatures above -30 °C.[178] Although reaction of **45a** and **45b** with hindered sec- or tert-butyl reagents produced some benzyl N-benzylcarbamate (**104**) (**FF**, Scheme 19) after hydrolytic workup due to this elimination (Entries 9 (4%), 11 (19%), 12 (18%)), nucleophilic addition of "R_3^-" to the anion **DD** or its "elimination" product **EE** seems unlikely and probably does not account for loss of stereochemical purity.

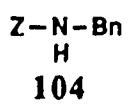
Lipshutz and coworkers have noted that relative to other $R_2Cu(CN)Li_2$; $Ph_2Cu(CN)Li_2$ exhibits low reactivity, poor yields, and lack of regiospecificity with enones.[221] Additions of $Ph_2Cu(CN)Li_2$ to the diprotected β-lactone produced only a low yield of the desired product (25%, Entry 18) as did Ph_2CuLi reagent (36%, Entry 19, Table 4). A moderate yield of Z-phenylalanine (**107b**) was

Side-Products of Organometallic Additions

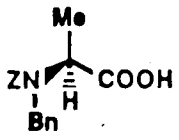




95 (R_1 = Bn, R_2 = Z, R_3 = Me) **108a** (R_1 = H, R_2 = Z, R_3 = Ph)
98 (R_1 = Bn, R_2 = Z, R_3 = n-Bu)



104



103a

obtained with the monoprotected lactone **36b** (46%, Entry 16), however substantial losses (5-67%) in optical purity were apparent in all three cases.

In the reactions of (tert-Bu)$_2$Cu(CN)Li$_2$ (Entry 11) and (tert-Bu)$_2$CuLi (Entry 12) with β-lactone **45a**, yields of the desired neopentylglycine derivative **102a** were reduced considerably (ie., 14-23%) due to the formation of N-Z-N-Bn-alanine (**103a**). Since **103a** is optically active, the alanine derivative probably arises from hydride transfer to the β-lactone (**GG**, Scheme 19) from the organometallic compound, or from "CuH" type reagents which are generated in the thermal decomposition of labile cuprates such as (tert-Bu)$_2$CuM.[220,222,223] Sato et al. previously found pivalic acid was the major product of the Cu(I)-catalyzed ring-opening of α,α-dimethyl-β-propiolactone by tert-BuMgCl.[195c] The hydride transfer reaction was effectively abolished in tert-butyl addition to the mono-N-protected β-lactone **36a** through use of the sterically-less hindered mixed cuprate, tert-Bu(Me)Cu(CN)Li$_2$, to provide Z-L-neopentylglycine **101a** in 48% yield (Entry 10, Table 4). In accord with the findings of Lipshutz et al.[220,221] with enones, this reagent exclusively transfers its tert-butyl ligand, and no product of methyl transfer (i.e., **93a**) was detected.

Initially problems were encountered with Cu^{++} contamination of the products since they chelate this cation. Removal of cupric ion from products with Chelex resin (BioRad) was successful but also resulted in significant product losses.[124] To avoid this, reactions were quenched by addition to cold degassed 0.5 N HCl,

which precipitates most of the copper as cuprous(I) chloride. The use of ether rather than ethyl acetate in extractions and washing of the extracts with aqueous EDTA (pH 3.0) and saturated brine efficiently removes any residual copper from the organic phases. Purification by reverse-phase chromatography (RP-8 MPLC) was generally most effective at resolving all of the products of the reactions.

Grignard-Derived Organocuprates.

Most of the disadvantages associated with organolithium derived cuprate reagents, such as losses in optical purity, yield decreases due to elimination, Cu(0)-contamination, and requisite large excesses of organometallic reagent, can be avoided by the use of organomagnesium-derived reagents.[223] Utilization of the stoichiometric cuprate $Ph_2CuMgBr$ derived from PhMgBr and $CuBr\text{-}SMe_2$[224] (Entry 17, Table 4) with the di-*N*-protected β-lactone **45a** resulted in a considerable increase in both the yield (60%) and optical purity relative to the PhLi-derived cuprates (Entries 18 (25%) and 19 (36%)).

Whereas organolithiums RLi are generally more reactive with enones than their respective cuprate adducts R_2CuLi, Grignard reagents RMgX are considerably less reactive than the corresponding cuprate R_2CuMgX.[223] This difference in reactivities has been exploited for Cu(I)-catalyzed 1,4-additions of Grignard reagents to enones,[223]

and to β-propiolactones.[77,194c]

Enlistment of only a catalytic amount of CuBr-SMe_2[224] in the reactions (Entries 7,8;13,15, Table 4) simplifies workup, eliminates problems with Cu(II)-contamination, and reduces the amount of organometallic reagent required by at least 50%. Furthermore, Grignard reagents RMgCl are less expensive, more stable, and easier to generate and handle than their organolithium counterparts. The use of Grignard reagents derived from alkyl chlorides rather than bromides is advantageous because $MgBr_2$-etherate reacts much more rapidly with β-lactones **36a** and **36b** than the corresponding dichloride (Table 2).

The unoptimized yields of desired N-protected amino acid products (44-83%) are superior in all instances to those obtained with $R_2Cu(CN)Li_2$ and R_2CuLi_2. For example, a 47% yield of Z-L-allylglycine **105a** was secured (Entry 13, Table 4) with catalytic CuBr/$CH_2CHMgCl$, whereas none of this desired material was detected with $(CH_2CH)_2Cu(CN)Li_2$. As before, yields obtained with mono-N-protected β-lactones **36a** and **36b** are somewhat lower than with **45a** and **45b** (e.g., 44% versus 83% for i-PrMgCl, Entries 7,8). Further refinement of mole ratios should increase yields, and reduce ketone (Entries 7,8) and optically active tertiary alcohol (43% in Entry 15) side products resulting from organometallic additions at the carbonyl (Path a, Scheme 19). Unlike reactions involving organolithiums, the copper-catalyzed RMgCl additions were

conveniently carried out at -23 °C with no observable formation of elimination products.

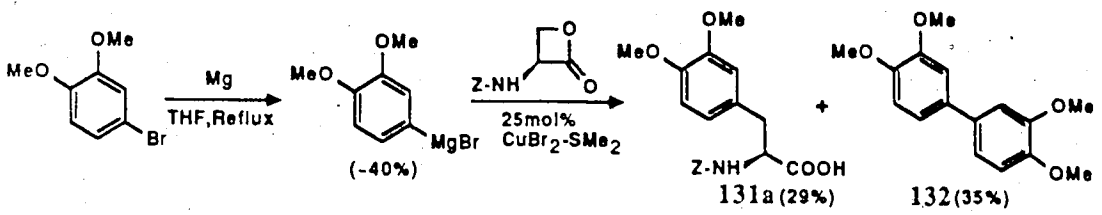
Most importantly, in all cases in which Cu(I)-catalytic RMgCl additions were employed (Entries 7,8,13,15, Table 4), greater than 99.4% retention of optical purity was observed. The phenyl addition results (Entry 15) dramatically contrast the large decrease in optical purity measured with $Ph_2Cu(CN)Li_2$ (Entry 16). In virtually all respects, copper-catalyzed organomagnesium chloride additions to both mono- and di-N-protected serine β-lactones (**36**, **45**) are superior to alternative stoichiometric cuprate additions (R_2CuLi, $R_2Cu(CN)Li_2$ or R_2CuMgX) for production of N-protected amino acids.

These investigations have established conditions for the additions of organometallic reagents to both mono- and di-N-protected serine β-lactones (**36** and **45**) to afford N-protected amino acids in fair to excellent yields with 99-100% retention of optical purity. The use of Cu(I)-catalyzed Grignard (RMgCl) additions avoids low yields, loss of optical purity, and cupric ion contamination which are often encountered with stoichiometric cuprates (R_2CuLi, $R_2Cu(CN)Li_2$, R_2CuMgX). Our procedure conveniently produces derivatives which are suitable for direct incorporation into peptides (ie., in terms of optical purity and protecting groups), or can be deprotected in a single step (91-99% yield) to the free amino acids (as in Figure 6). The general synthetic

utility of this methodology in providing access to most major classes of amino acids bearing aliphatic or aromatic side chains has been demonstrated by the addition of methyl, primary (n-Bu),[225] secondary (i-Pr, sec-Bu), tertiary (tert-Bu), vinyllic (H_2C=CH), and aromatic (Ph) carbanion reagents to both the D- and L-isomers of the readily accessible N-protected serine β-lactones.

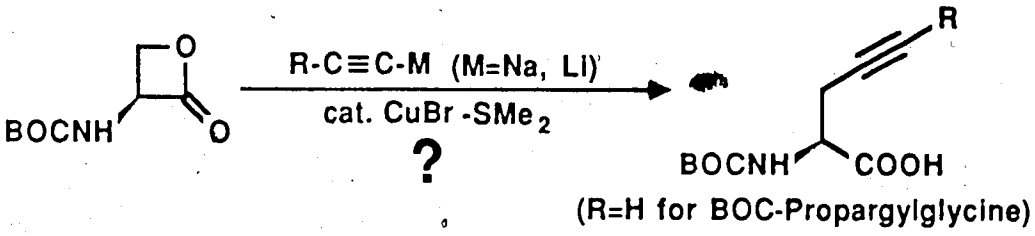
Among these products are D-leucine (R_3 = i-Pr), (2R)-2-aminobutanoic acid and D-phenylalanine (R_3 = Ph) which are constituents in numerous microbial peptides with antibiotic/antitumor activities and impart resistance to peptidases.[1,214] C-Allylglycine (R_3 = $CH=CH_2$ is a naturally-occurring enzyme inhibitor, neurotoxic amino acid, and useful chiral synthon.[180,226] Neopentylglycine (R_3 = tert-Bu) is a highly lipophilic amino acid with unique space-filling and steric properties which make it useful in synthetic analogs of bioactive peptides.[211]

L-Dihydroxyphenylalanine (Levo-DOPA) is not only a plant nonprotein amino acid,[6] and a precursor to cuticle crosslinking agents in insects,[1a] but also a highly successful drug in the treatment of Parkinson's disease. Because of this numerous patents and literature reports have appeared on its synthesis.[227] Using the Grignard-reagent produced from 4-bromoveratrole, optically-pure Z-3,4-dimethoxy-DOPA (**131a**) was conveniently prepared in a single step from Z-L-serine β-lactone (**36a**).

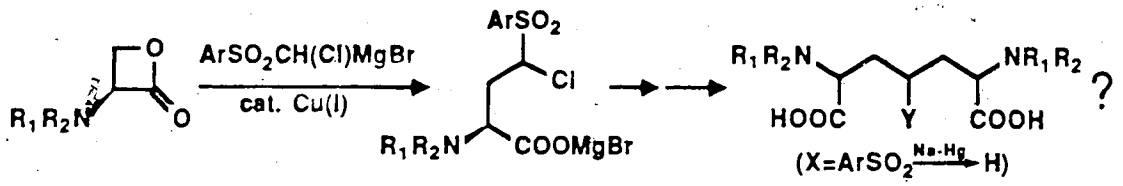


The deprotection of **131a** to L-DOPA can be achieved by treatment with BBr_3.[228] The low yield is most probably a result of problems encountered in preparing the bromoveratrole-Grignard reagent and its extensive dimerization to **132.** However, this single unoptimized attempt does further illustrate the potential of these cuprate additions in preparing bioactive amino acids.

A similar extension of this methodology to include copper-catalyzed ring-openings by acetylide anions[60,229] could potentially enable the simple preparation many of the acetylenic/allenic amino acid analogs which act as suicide substrates for Categories 2 and 3 PLP-enzymes analogous to propargylglycine (Figure 4).[8,20,21b]



Through the use of stable α-haloorganometallics such as $ArSO_2CH(Cl)MgBr$[230] it may even be possible to synthesize various stereoisomers of diaminopimelic acids by dialkylations with N-protected β-lactones:



These are but a few possible applications of reactions of the N-protected β-lactones with C-nucleophiles which remain untapped.

3-Amino-2-oxetanone Salts

Although deprotection of the N-protected derivatives generated by nucleophilic ring-openings of BOC-(**42**), Z-(**36**) or N,N-Z-Bn-serine β-lactones (**45**) will produce many free amino acids in near quantitative yield, some side-chain functionalities (eg., $-N_3$) cannot withstand typical deprotection methods.

The easily accessible N-(tert-butoxycarbonyl)serine β-lactones (**42a,b**) may be readily deprotected by treatment with trifluoroacetic acid (TFA) to produce 3-amino-2-oxetanone salts (**140, 141**) in near quantitative yield (Scheme 21 in Table 6). The unprotected serine β-lactone may be obtained as its trifluoroacetate salt (**140**) simply by removal of TFA and tert-butyl trifluoroacetate (b.p.$_{60\ mm}$ ~30°C)[231] in vacuo at 25°C. This material (**140**) is usually obtained as a syrup which is best used immediately in subsequent reactions since traces of salts and residual TFA can cause decomposition. More conveniently, 3-amino-2-oxetanone may be isolated, characterized, recrystallized

(from DMF/Et_2O) and stored dry as a stable solid salt of p-toluenesulfonic acid (**141**).

In contrast to their β-lactam counterparts (3-aminoazetidinones) which may be N-acylated under aqueous or nonaqueous conditions,[87c] the unprotected serine β-lactones eagerly add even the poorest of nucleophiles (eg., CF_3COO^-, TsO^-)[232] to produce the corresponding stereochemically-pure (based on [α]) free amino acids in high yield (Table 6). This direct synthetic mimicry of PLP-β-replacement enzymes (Category 2) has proven successful in the preparation of several β-substituted alanines which were previously inaccessible (Entries 1, 2, 7, 12 in Table 6), and significantly expands the utility of the serine β-lactones.

In many respects the 3-amino-2-oxetanones are very similar to their N-protected counterparts, however there are significant differences. Reactions may be carried out in THF (with **140**) or the usual polar aprotic solvents, acetonitrile and dimethylformamide. In addition, TFA may be used as the reaction solvent with generation of 3-amino-2-oxetanone in situ from **42a** (eg., Entries 1, 2). Despite the fact that the 3-amino-2-oxetanone salts hydrolyze quite rapidly in water ($t_{1/2}$ ~2.5 h in unbuffered H_2O; $t_{1/2}$ = 10.6 (±0.5) min in pH 6.78, 50 mM potassium phosphate as determined by quantitative solution IR), with adequate nucleophiles (eg., thiols) extremely high yields and chemoselectivity can be attained through

control of pH (Entries 9-11). The use of the high polarity TFA and H_2O solvents may account in part for the considerably enhanced reactivity of these β-lactones.

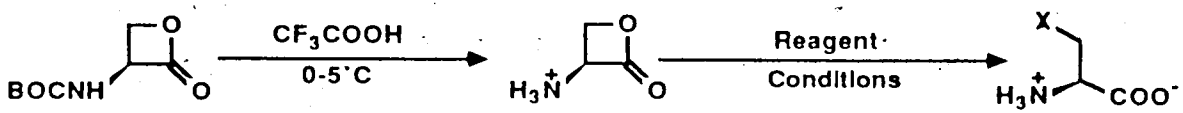
In the absence of impurities, the TFA salt of 3-amino-2-oxetanone (**140**) is stable for over one week in CF_3COOH, however if impurities are present O-trifluoroacetyl-L-serine (**142**) rapidly forms (Entry 1, Table 6). For this reason **140** is usually generated and used immediately. Because it is difficult to liberate **140** of all residual CF_3COOH and CF_3COO^tBu it is usually employed under aqueous conditions with controlled pH (Entries 4, 9, 10, 11) or with an excess of nucleophile (Entry 3, 5, 8).

Isolation of 3-amino-2-oxetanone as its tosylate salt (**141**) offers distinct advantages. It may be prepared and stored (dry, 4°C) analytically pure in multigram quantities and handled without problem in air. β-Lactone **141** is free of residual CF_3COOH and CF_3COO^tBu which can consume nucleophilic reagents, and thus is particularly well-suited for reactions in nonprotic solvents with a minimal amount of nucleophile or for reactions involving acid sensitive reagents/products (eg., Entry 7).

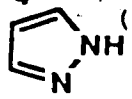
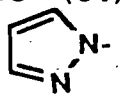
Reaction in TFA and subsequent isolation of the product as the tosylate salt by precipitation from ether was desirable for products bearing electrophilic or nucleofugal β-substituents (eg., Entries 1, 2, 12) in order to prevent decarboxylative elimination or

Table 6. Reactions of Nucleophiles with (S)-3-Amino-2-oxetanone Salts

Scheme 21



140 (CF_3COO^- salt)
141 (TsO^- salt)

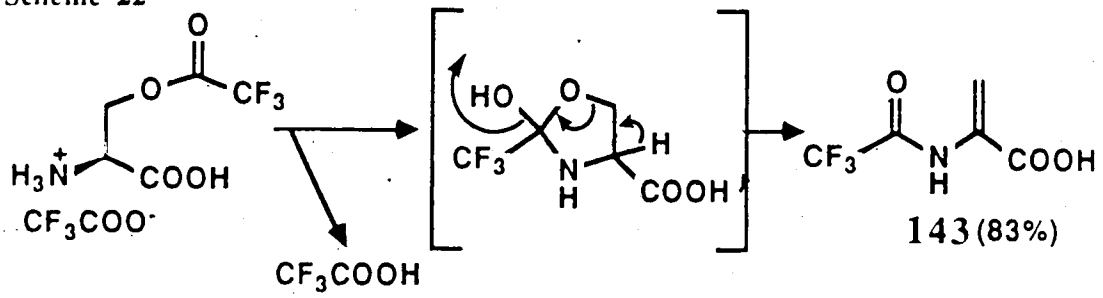
Entry	Method	Reagent (eq.)	Conditions	X (Yield)	Compd.
1	A	CF_3COO^-/AG1 (1.3)	CF_3COOH, 16 h	CF_3COO- (87)[a]	**142**
2	A	TsOH (2), CF_3COO^-/AG1 (1.8)	CF_3COOH, 7 days	TsO- (75)[a,b]	**144**
3	B	K_2PO_4 (3)	18-crown-6(1 eq), DMF, 72h	H_2PO_4- (87)[c]	**145**[233]
4	A/B	conc. HCl (24-5)	30 min	Cl- (92)[d]	**13a→14a**[233]
5	B	n-$Bu_4N^+CN^-$ (1.5)	DMF, -10°C(.5h)→25°C(.5h)	N≡C- (84)[c]	**145**
6	C	 (5)	DMF, 2.5 h	 (77)[c]	**147**
7	C	NaN_3 (3)	DMF, 1 h	N_3- (96)[c]	**148**
8	B	LiSH (3)	CH_3CN/THF, 1 h	HS- (88)[c]	**3a**[233]
9	B	$H_2NCH_2CH_2SH \cdot HCl$ (2)	pH 5.5, H_2O, 35 min	$H_2NCH_2CH_2S$- (85)[c,d]	**149**[233]
10	B	L-cysteine (3)	pH 5.5, H_2O, 40 min	(S)-$^-OOC(H_3N^+)CHCH_2S$- (93)[c]	**16a**[233]
11	B	$Na_2S_2O_3$ (2)	pH 5.0, H_2O 1 h	$Na^{+-}O_3SS$- (83)[c]	**150**
12	C	Me_2S (4)	TsOH (1.5 eq), CF_3COOH, 15 min	Me_2S^+- (88)[a]	**151**

Methods: **A.** **140** generated *in situ*. **B** **140** produced and used immediately after removal of TFA. **C.** **141** (TsO^- salt) employed.

Footnotes: For method A/B yield based on **42a**. Isolation by: [a]Precipitation as tosylate salt. [b] ^1H NMR yield. [c]Ion exchange chromatography. [d] Recrystallization as HCl salt.

decomposition. For example, when **140** was treated with anhydrous AG1 resin in the CF_3COO^- form in TFA, O-trifluoroacetyl-L-serine was produced in high yield. Attempted isolation of this product by removal of excess TFA *in vacuo* resulted in O→N-acyl transfer and dehydration to produce a sublimate of N-(trifluoroacetyl)dehydroalanine (**143**) in 83% yield (Scheme 22).[234] The presence of an equivalent of nonvolatile p-TsOH during the

Scheme 22



isolation allowed O-trifluoroacetyl-L-serine to be secured in 88% yield as its tosylate salt (**142**). When treated with nucleophiles (eg., N_3^-) **142** produces exclusively serine, and any serine observed in nonaqueous reactions of β-lactone **140** probably arises in this manner from O-TFA-serine impurities.

A prolonged exposure of 3-amino-2-oxetanones to the poorly nucleophilic p-tosylate anion[232] in TFA eventually generates O-tosyl-L-serine which was isolated in 75% purity (25% serine impurity) as its tosylate salt **144** (Entry 2).

O-Phospho-L-serine (**145**) is a metabolic precursor of serine in plants,[1a] a suicide inactivator of glutamate α-

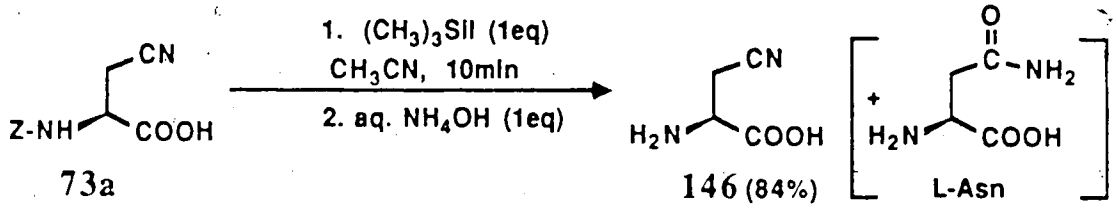
decarboxylase[26a] (Category 1, PLP-enzyme), and important constituent in phosphorylated secretory and other proteins.[1a,235-237] It is usually synthesized by a roundabout protection/deprotection strategy,[235] however it may be produced simply and directly in 87% yield by attack of phosphate anion on 3-amino-2-oxetanone in DMF (Entry 3).[233] Use of concentrated H_3PO_4 on **140** produced only serine on aqueous workup.

Based on the results with these relatively non-nucleophilic oxygen nucleophiles, it is probably safe to predict that the analogous reactions of sulfate and acetate anions with 3-amino-2-oxetanone could directly produce serine O-sulfate (X = SO_4^-)[238] and O-acetylserine. These compounds are suicide substrates for several α-decarboxylase, aminotransferase and racemase PLP-enzymes (Category 1).[26a-d]

β-Chloroalanine (**13a**) may be produced either from **140** or directly from BOC-L-serine β-lactones (**42a**) in 92% recrystallized yield by treatment with concentrated HCl (Entry 4) (cf. previously described conventional 3-step synthesis[110] from serine). This material (**13a**) was used in the previous synthesis of optically active lanthionines (**16**), and also acts as a suicide substrate for aspartate β-decarboxylase,[8,21b] alanine aminotransferase[239] and bacterial amino acid racemases.[26,240] In collaboration with Dr. Monica Palcic we have found **13a** and **142** to also be suicide inhibitors of aspartate α-decarboxylase (see

Appendix 1).

The origins and activities of β-cyano-L-alanine as a neurotoxin and enzyme inhibitor[4,161-164,166,167] were previously mentioned in the synthesis of its N-(benzyloxycarbonyl) (Z) derivative (**73a**). As in syntheses of Z-β-cyano-L-alanine (**73a**), the nucleophilic additions by cyanide anion were initially problematic, but in this case elimination was not observed. To facilitate investigations, authentic β-cyano-L-alanine (**146**) was produced from **73a** by deprotection with trimethylsilyl iodide[153c] (TMSI) in CH_3CN. This also proved to be



somewhat tricky since use of an alternative solvent, >1 equivalent of TMSI, prolonged reaction, or simple H_2O quench resulted in 18-40% hydrolysis to L-asparagine (L-Asn). The deprotection conditions eventually developed with TMSI (84% recrystallized) are however a considerable improvement over those in the literature using $Na/NH_{3(l)}$ (50% yield[169]). Hydrolyses of β-cyano-L-alanine on AG50 (H^+ form) resin and in recrystallizations from hot H_2O were also encountered. β-Cyanoalanine could not be produced by reactions of β-chloroalanine with KCN in H_2O.

Reaction of β-lactone **140** with aqueous KCN at pH 5 provided a 60/40 mixture of serine and β-cyanoalanine

(**146**). Treatment of 3-amino-2-oxetanones (**140/141**) with NaCN in DMF yielded a ~2:1 mixture of the desired nitr (R-CN) (**146**) and the corresponding isonitrile ($R-^{+}N{\equiv}C^{-}$) (IR: 2250, 2160 cm^{-1}; ^{13}C NMR (D_2O) δ119.8, 160.5 ppm for nitrile and isonitrile, respectively). Use of the more highly dissociated n-$Bu_4N^{+}CN^{-}$ [241] in DMF[60,128] followed by desalting under neutral conditions on ion-retardation resin and recrystallization (H_2O/dioxane, 25°C) avoided these problems and provided β-cyano-L-alanine (**146**) in 84% yield. β-Cyano-L-alanine (**146**) is now under scrutiny as an inhibitor of the various aspartate enzymes by Dr. M. Palcic.

Pyrazole reacted with 3-amino-2-oxetanone [illegible] to provide free β-(pyrazol-1-yl)-L-alanine **147**[72,142] in good yield (77%). This suggests that the related unprotected heterocyclic β-substituted alanines (eg. quisqualic acid, willardiine, mimosine, etc. of Figure 15)[4,28,93b,131,143] could be produced in this manner.

β-Azidoalanine (**148**) is a mutagenic metabolite recently isolated from Salmonella grown in the presence of azide.[242] It is an example of induced production of a β-substituted alanine by a microorganism via PLP-β-replacement enzymes (from O-acetylserine) in attempt to detoxify an external nucleophile (N_3^{-}).[242] The reaction of NaN_3 with β-lactone **141** in DMF to afford β-azido-L-alanine in 96% yield represents the first chemical synthesis of this labile compound.[243] It also illustrates

how the 3-amino-2-oxetanone tosylate salt serves admirably in the preparation of materials which could not withstand deprotection by acid (eg., TFA) or hydrogenolysis. Because nothing is known about the biological properties of β-azido-L-alanine (**148**) aside from its mutagenicity to Salmonella,[242] its interaction with various asparate-associated enzymes is currently being investigated in collaboration with Dr. M. Palcic.

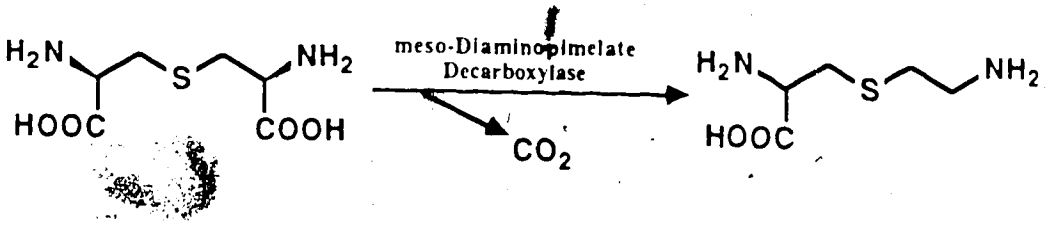
All of the sulfur nucleophiles in Table 6 reacted rapidly with the unprotected serine β-lactones (**140**, **141**). Reaction with lithium hydrosulfide produced L-cysteine in 88% recrystallized yield,[233] thereby demonstrating the potential to generate expensive unprotected D-amino acids (eg., D-cysteine) from inexpensive D-serine. Although this reaction (Entry 8, Table 6) was carried out in CH_3CN/THF,[233] the results with other thiolate nucleophiles (Entries 9-11) suggest that the same result could be achieved in H_2O at pH 5.

Both L-cysteine and mercaptoethylamine (MEA) reacted rapidly and chemoselectively with 3-amino-2-oxetanone **140** at pH 5.5 to produce the diaminopimelate (DAP) analog, lanthionine (**16a**) and thialysine (**149**), respectively. Interestingly, this is in direct contrast to the previously observed N-alkylation of MEA by the N-protected β-lactones in mixed aqueous/organic solvents. Since thialysine would be generated if lanthionine were decarboxylated (Scheme 23), this material (**149**) was used

to establish that indeed meso-lanthionine (**16c**) was actively converted to thialysine by meso-DAP decarboxylase. The stereochemistry of this CO_2/H replacement is currently under investigation in collaboration with Dr. M. Palcic.[34]

The synthesis of L-lanthionine (**16a**) from the unprotected L-serine β-lactone (Entry 10, Table 6) in 93%

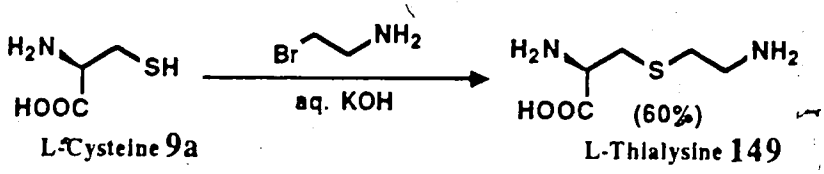
Scheme 23



recrystallized yield is the most efficient yet reported. Notably, the product **16a** is produced without the need for the strongly alkaline conditions previously required with β-chloroalanines (i.e., **16a** from **14a**), and provides L-lanthionine of higher optical purity than we have ever encountered.

L-Thialysine (**149**) was also produced according to Cavallini et al.[244a] by alkylation of L-cysteine with 2-bromoethylamine at high pH. Isolation and purification of **149** produced by this method was considerably more difficult due to the presence of much salt, and yields were substantially lower (60% yield).

L-Thialysines (**149**) prepared by either method possessed indistinguishable physical properties.



L-Cysteine 9a L-Thialysine 149

Thialysine is produced naturally in mushrooms from serine and mercaptoethylamine by a β-replacement enzyme (analogous to preparation from 3-amino-2-oxetanones[41,244b]) and acts as a lysine antimetabolite in biological systems.[245]

In view of the extremely high chemoselectivity of alkylation of the above poly-functional thiols by 3-amino-2-oxetanone **140** at pH 5.5, this methodology should prove useful not only for the preparation of the numerous natural β-thia-substituted amino acids,[1a] but also in the chemical modification of peptides and proteins.[119] Considerable manipulation has previously been required to produce differentially N-protected lanthionines[71b] necessary for synthesis of the antibiotic peptides in which it is a constituent.[105] Use of the unprotected serine β-lactones could allow simple synthesis of mono-N-protected lanthionines, or the post-synthetic conversion of cysteinyl residues of a peptide to lanthionine residues by chemoselective S-alkylation at pH 5.5 in the presence of all other side-chain functionalities.

The Bunte salt, S-sulfo-L-cysteine (**150**, Entry 11, Table 6; X = $-SSO_3^-$) is the immediate metabolic precursor of L-cysteine produced from serine and thiosulfate (i.e.,

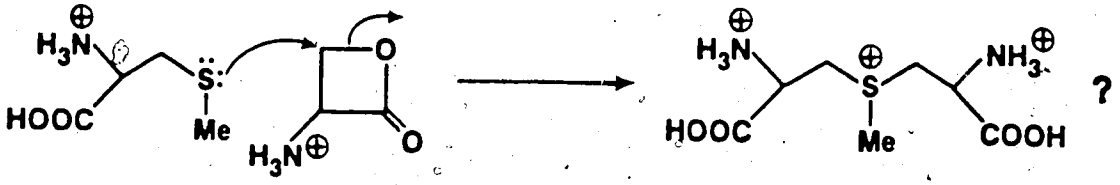
β-replacement) in Aspergillus and other organisms.[52,246a] It also functions as the natural direct donor of sulfur in the biosynthesis of the antibiotic cephalosporin C from Cephalosporium acremonium.[246b] This material is readily prepared (83% yield) from 3-amino-2-oxetanone **140** by ring-opening with sodium thiosulfate in H_2O at pH 5.0.

In contrast to the rather sluggish reactions of N-protected serines with Me_2S in DMF, the unprotected serine β-lactones (**140** or **141**) reacted almost instantly and quantitatively (by ^1H NMR) with dimethyl sulfide in CF_3COOH to produce the dimethylsulfonium salt of cysteine which was isolated in 88% recrystallized yield as the stable bis(tosylate) salt (**151**, Entry 12, Table 6). The enormous increase in reaction rate relative to that in DMF is likely due in part to the increased solvent polarity and Lewis acid-catalysis of the ring-opening in this Type-2[128] nucleophilic attack (i.e., charged products generated).

The success of the reaction with dimethyl sulfide provided much hope that the synthesis of the initial target, S-methyl lanthionine sulfonium salt might finally be realized (Scheme 24). In the reaction of S-methyl-L-cysteine with 3-amino-2-oxetanone (**140**) in trifluoroacetic acid no problem was expected or indeed encountered with regard to attack on the lactone by amino or carboxyl moieties. However when monitored by ^1H NMP it was obvious

that no detectable S-alkylation was occurring either! After 7 days at 25°C, O-trifluoroacetyl-

Scheme 24



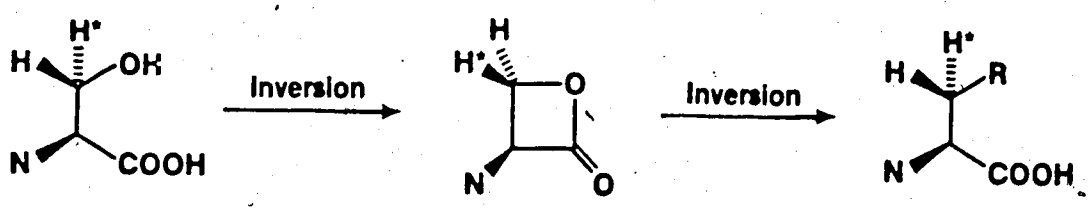
L-serine (**142**), resulting from ring-opening of the lactone by the solvent, was the sole product. Use of the tosylate salt of S-methyl-L-cysteine in hope of reducing attack by the TFA solvent resulted only in the production of O-tosylserine (**144**) instead! Employment of DMF as the solvent with tosylate salts again resulted in the eventual (3 weeks) production of O-tosylserine (**144**) as the only product. It appears that because of the steric hindrance due to the presence of the amino and carboxyl moieties in S-methyl-L-cysteine, S-alkylation is greatly retarded so that even a poor nucleophile like tosylate successfully competes.

A number of syntheses of serine stereospecifically-labelled with deuterium or tritium at the C-3 position have recently been reported.[247] Recently Mr. S.E. Ramer of our laboratory utilized such labelled serines to verify that the Mitsunobu-type lactonization of Z-L-serine does in fact proceed by hydroxyl-activation with resultant inversion of C-3 as expected and depicted in Figure 13.[247] He further proved that as anticipated nucleophilic

ring-opening by acetate anion again inverts the stereochemistry at C-3. Based on these results a wide variety of β-substituted alanines stereospecifically labelled at the C-3 position with isotopic hydrogen may be generated from the corresponding serine via the β-lactones with a net retention of C-3 stereochemistry (Scheme 25). Since attack of organocopper reagents on secondary substrates bearing oxygen leaving groups (eg., tosylate or mesylate) has been demonstrated to proceed with inversion,[220,221,223] C-3 stereospecifically-labelled amino acids bearing aliphatic and aromatic side-chains should be accessible by this route. These labelled compounds are often valuable probes in studies of the stereochemical course of enzyme mechanisms and biological pathways.[8,22]

The serine β-lactones which are readily accessible from optically-pure inexpensive serine derivat ves have

Scheme 25



proven to be stable, convenient, versatile synthetic intermediates. In many instances the β-lactone approach has been demonstrated to be superior to previous methods in terms of optical purity, yield, economy, or ease of

preparation of known amino acids. Clearly the N-protected and free 3-amino-2-oxetanone salts have enormous potential appications in organic syntheses, biochemistry and the pharmaceutical industry for producing both discovered and as yet unconceived amino acids.

Polymer-Supported Alkyl Azodicarboxylates in Mitsunobu Reactions

Because of the established potential of the serine β-lactones as versatile synthetic intermediates, convenient large scale methods for their preparation were investigated. On an industrial scale, the prohibitive cost and hazards of the dialkyl azodicarboxylate reagent, and required chromatographic purification of the product from $Ph_3P=O$ and ROOC-NHNH-COOR sideproducts, disfavor the Mitsunobu reaction.[87a] Immobilization of the alkyl azodicarboxylate moiety on a polymeric support would render it macroscopically insoluble yet reactive in a "quasidissolved state".[14] The "spent" immobilized reagent could be physically removed after the lactonization reaction, regenerated by reoxidation with a number of inexpensive oxidizing agents,[91] and used over and over in this fashion. This would effectively avoid the dangers associated with distillation of the azodicarboxylates and considerably reduce costs. Furthermore, purification of the serine β-lactones would be simplified by the absence of dialkyl hydrazodicarboxylate in the product mixture

(i.e., filtrate).

The polymeric support matrix employed must be inert to the reaction conditions used in immobilization, oxidation and utilization of the alkyl azodicarboxylate functionality. Ideally it should be initially free of nitrogen in order to allow measurement of loading by elemental N analysis. Finally, it should possess mechanical stability to physical degradation but swell considerably on solvation by organic solvents to facilitate reactions. These considerations for a suitable polymer are very similar to those of Merrifield solid-phase peptide synthesis,[13,14] and consequently 1% crosslinked polystyrene resin was found to be most suitable.[14,96,97] This choice also enabled us to take advantage of commercial sources of derivatized polystyrene resins and the well-established chemistry developed for solid-phase peptide synthesis (SPPS).[14,248]

To examine the viability of this approach, commercially available hydroxymethyl polystyrene resin (**152**) (1 meq/g, ~10 mol% load, 1% crosslink) swollen in dichloromethane was converted to the corresponding chloroformate by reaction with phosgene and pyridine (Figure 21). Excess reagents were removed by filtration and the "activated" resin was treated with triethylamine and methyl hydrazinocarboxylate to produce the methyl hydrazodicarboxylate derivatized resin **153**. Incorporation of this functionality was evident from the very strong

carbonyl band in the IR (Fluorolube® mull, 1790-1680 cm^{-1}), and analysis was consistent with the derivatization of 88% of the available hydroxymethyl moieties (i.e., 0.75 meq/g, 8.75 mol% of units).

Oxidation of dialkyl hydrazodicarboxylates (ROOC-NHNH-COOR) to the corresponding azo compounds (ROOC-N=N-COOR) can be accomplished with many inexpensive oxidizing agents such as Cl_2, N_2O_4, or tert-butyl hypochlorite,[91,95] which are compatible with the resin. On small scale N-bromosuccinimide/pyridine is most rapid and convenient. Initially, dichloromethane was used for this oxidation, but later results suggest acetonitrile is preferable since the succinimide side product is soluble

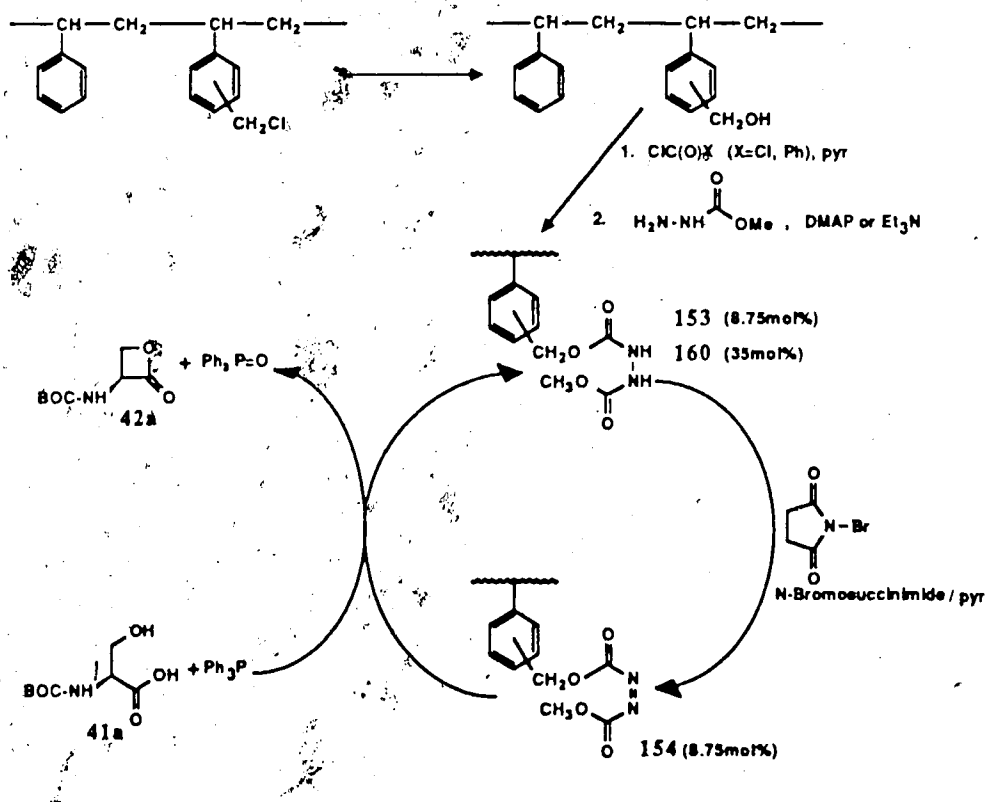


Figure 21. Preparation and Use of Polymer-Supported Methyl Azodicarboxylate

in CH_3CN, thus potentially allowing the utilization of continuous flow methods. On oxidation the snowy-white hydrazodicarboxylate resin turns bright orange, and IR on the resulting azo resin (**154**) indicates >94% conversion of hydrazo units by comparison of relative intensities of N-H bands at 3360 cm^{-1}. Semiquantitative infrared spectroscopy on **154** resin also suggested 8 (±3)% of underivatized hydroxymethyl units.

This immobilized alkyl azodicarboxylate reagent **154** served admirably in Mitsunobu condensations. Estimation of the concentration of accessible (i.e., synthetically usable) alkyl azodicarboxylate functionalities on the resin was conveniently achieved by reaction with a known excess triphenylphosphine, alcohol, and acidic component followed by chromatographic recovery and determination of unreacted Ph_3P and/or $Ph_3P{=}O$ product. Analysis of resin **154** in this manner indicated 0.61 (±0.03) meq/g of usable azodicarboxylate units corresponding to 86% of the 0.74 meq/g possible. This activity of the resin showed no dimunition (±0.03 meq/g) over 5 redox cycles of the resin (Figure 21).

Synthesis of benzyl benzoate (**155**) from benzyl alcohol and benzoic acid using exactly 1 equivalent of Ph_3P and 1.3 equivalents of resin **154** proceeded in 65% yield. This compares favorably with 80-85% yields reported for this reaction in the literature,[86] with the losses being due primarily to moisture. In later

lactonization reactions ~0.5 equivalent excess of Ph_3P and resin **154** were typically employed to minimize losses due to moisture.

For lactonization of BOC-L-serine (**41a**), Ph_3P was added to a mixture of the β-hydroxy acid (**41a**) and resin **154** at -45°C in THF and allowed to warm to 0°C. After 3.5 h the reaction was complete according to solution IR on the supernatant. The filtrate and washings of the resin containing mostly Ph_3P, Ph_3P=O and β-lactone yielded 56% BOC-L-serine β-lactone (**42a**) by flash chromatography. This yield rivals that of 60-65% obtained in the preparation of **42a** by the analogous homogeneous reaction in THF. As an alternative, the β-lactone (**42a**) could be isolated in 51% yield (91% recovery) by precipitation of >90% of the Ph_3P=O from ether followed by crystallization of β-lactone from chloroform/CCl_4/hexane. Such selective recrystallizations were not possible in the presence of dialkyl hydrazodicarboxylate and illustrate how use of the resin **154** can eliminate the need for chromatographic purification of products from Mitsunobu reactions.

THF was preferred to CH_3CN as the solvent employed with the resin due to greater solubility of reagents and increased swelling (2-2.5X)[14] of the resin, thereby facilitating faster reactions in the former solvent. Preformation of the Ph_3P/azo-resin (**154**) adduct did not significantly increase yields, perhaps due to more significant losses to moisture. Other workers have noted

considerable enhancements of the intra- versus intermolecular reactions and increased "lifetimes" of labile intermediates for reagents immobilized on polymeric supports, which are attributable to reduced diffusion rates in the polymer matrix.[249] For this reason, preformation of the N-phosphonium adduct probably achieves no further gains in promoting lactonization versus intermolecular condensation.

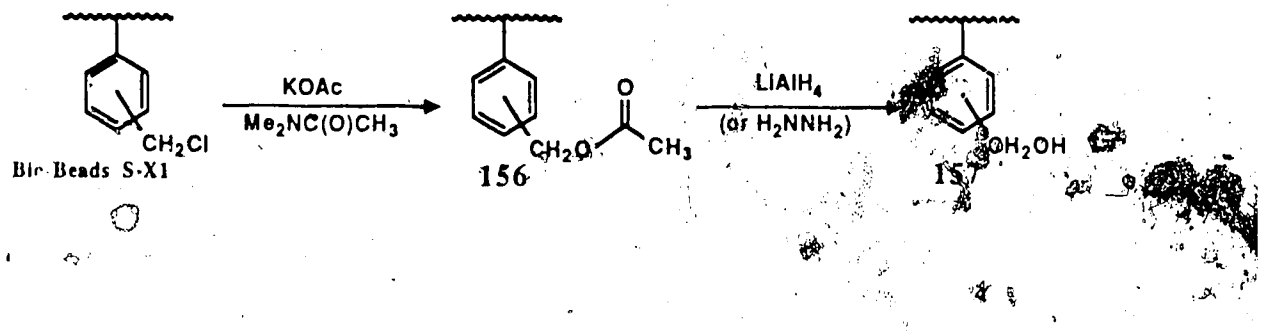
All resin reactions and manipulations including drying in vacuo could be conveniently carried out in a stirred, jacketed reaction vessel equipped with a sintered glass filter at the base (Figure 23 in Experimental), thereby avoiding transfers of the resin. The polymer supported alkyl azodicarboxylate (**154**) is also amenable to column/continuous flow and related production methods currently utilized in solid-phase peptide syntheses.[14,250]

The cost of recycling recovered hydrazodicarboxylate resin **153** by oxidation with N-bromosuccinimide/pyridine to **154** is less than 1/10 that of purchasing dialkyl azodicarboxylates (eg., DMAD or DEAD) and a saving of approximately $140/cycle/mole is easily realized through the use of the resin. Further savings are procured by elimination of the need for chromatography, and the expense of production of serine β-lactones (eg., **42a**) is reduced 3-4 fold relative to the homogeneous solution procedure.

Heavier loading of the polystyrene resin with

reactive dialkyl azodicarboxylate units would economize on the volume of solvent required for reactions and could reduce losses due to residual moisture in the solvents and resin. Since the cost of the polystyrene resin starting material is essentially independent of the degree of loading, an increase in the number of meq/g would substantially reduce the initial production costs of the resin. With resin **154** it is possible to produce ~70 g of β-lactone **42**/kg resin/cycle, however an increase to 35 mol% loading of the resin could allow production of ~300-350 g β-lactone **42**/kg resin/cycle.

Because heavily-loaded hydroxymethyl polystyrene resin is not commercially available, it was prepared from Merrifield chloromethyl peptide resin (BioBeads S-X1, 3.90 meq/g, 50 mol% loading, 1% crosslink) according to Wang.[300] This involved conversion to the acetoxymethyl form (**156**) by heating in dimethylacetamide with potassium



acetate to react >97% of the CH_2Cl moieties. Reductive deacylation was next effected by treatment with lithium aluminum hydride. In view of the extensive washing required to free the resulting hydroxymethyl resin (**157**)

of aluminates, the alternate hydrazinolysis procedure appears more attractive especially on large scale.[300] IR on hydroxymethyl resin **157** indicates >99% removal of acetyl groups and elemental chloride analysis confirms <3% residual chloromethyl functionalities. Conversion of the chloromethyl to hydroxymethyl resin via the acetoxymethyl form avoids the crosslinking by Williamson-type benzyl ether formation possible in a direct hydrolysis.

The heavy-loaded hydroxymethyl polystyrene resin (50 mol% hydroxymethyl units) was subjected to phosgene/pyridine followed by methyl carbazate ($H_2NNHCOOMe$)/Et_3N analogous to the light-loaded case (Figure 21) but with extended reaction times to provide **158**. Analyses indicated ~40% unreacted chloroformate residues (Cl anal.) and ~40-50% hydrazodicarboxylate units (N anal.). The balance appeared to be rather unreactive carbonate crosslinked units. In order to reduce the possibility of crosslinking by formation of unreactive carbonate (Polymer~OC(O)O~Polymer) residues and avoid the presence of residual reactive chloroformate groups, an alternative phenylcarbonate activation was attempted.

On small scale, heavy-loaded hydroxymethyl resin **157** was suspended in CH_2Cl_2 and treated with phenyl chloroformate/pyridine. After 16 h no detectable CH_2OH functionalities remained according to IR, and these were replaced by a strong carbonyl band at 1760 cm^{-1} for the phenyl carbonate (X = Ph in Figure 21). Oxygen analyses

were consistent with near quantitative conversion to (phenyloxycarbonyl)oxymethyl polystyrene resin (**159**) (50 mol% loading, 2.8 meq/g).

This phenyl carbonate activated resin (**159**) reacted only slowly with methyl carbazate and Et_3N (pK_b = 2.99) in DMF at 25°C, however over threefold enhancement in rate was obtained by substitution of the hypernucleophilic acylation catalyst 4-dimethylaminopyridine (DMAP) (pK_b = 4.35)[251] in triethylamine. After 5 days 70 (±2)% incorporation of methyl carbazate (N anal.) corresponding to 1.91 meq/g hydrazodicarboxylate units (35 mol%) in **160** was achieved. Semiquantitative IR, elemental analyses and solid-state ^{13}C NMR (see below, Figure 22) on **160** indicated that the unreacted functionalities remained very conveniently "capped" as relatively unreactive phenyl carbonates (15 mol%) and no free hydroxymethyl moieties were detectable. Higher incorporation was not attempted since unreacted functionalities are probably relatively inaccessible. However if desired, reaction at higher temperatures or activation as the more reactive p-nitrophenylcarbonate form of the resin (from p-nitrophenylchloroformate + **157**) could probably achieve this. With yields and conditions similar to those with the light loaded resin (0.61 meq/g usable azodicarboxylate units), this 35 mol% loaded resin has the potential to produce 30-35% of its mass in BOC-L-serine β-lactone (**42a**) per cycle, easily allowing complete recovery of production costs in 3

cycles or less. This methyl hydrazodicarboxylate derivatized resin (**160**) has been oxidized with 75% conversion by 1.5 equivalents of NBS/pyr in acetonitrile. The resulting oxidized resin (1.43 meq/g azo-units) produced a 34% yield of β-lactone **42a** when employed under conditions analogous to those used with **154**. Although optimization of conditions is still required this indicates that the heavy-loaded resin is indeed useful in Mitsunobu reactions.

The difficulties and shortcomings with analytical methods for quantitatively determining the exact state of functionalization of the polymer support which are inherent in solid-phase peptide syntheses (SPPS) were also encountered in this work. Mass recovery of resin is only at best a rough estimate of the extent of reaction, especially with the resin's tendency to stick to untreated glassware. Elemental analyses were often successful when redoubled combustion was employed. The H, N, O, and X values were usually consistent with other estimates, but carbon determinations were frequently variable and low by ~1%. Nitrogen determinations on azodicarboxylate resins (**154**) were often low, probably because of thermal decomposition and loss of N_2 in the analysis. With the light loaded resin (0.61 meq/g), the ±0.4% accuracy of the analyses represents only ±20% accuracy in determining loading! IR spectroscopy is commonly used in SPPS to qualitatively assess the success of a reaction on the

resin. FT-IR of the resins as a Fluorolube® mull reliably provided a semiquantitative measure (±5%) of the extent of generation or loss of OH, NH, and C=O functionalities by comparison of band intensities with those of relatively constant (±5%) C=C and aromatic C-H bands. The previously described "back-titration" with an excess of Ph_3P is, however, the only reliable way to obtain an estimate of the number of usable alkyl azodicarboxylate units available, and is simple to perform.

As expected, conventional ^1H NMR spectra of suspensions of the resin in $CDCl_3$ were completely useless and were totally lacking any fine structure. To the best of our knowledge there has been one previous report of solid state ^{13}C NMR on underivatized polystyrene in the literature.[252,253] With the kind assistance of Nancy Cyr of Alberta Research Council we were able to obtain the solid state ^{13}C NMR spectra on the 50 mol% loaded resins (**156, 157, 159, 160**) shown in Figure 22. Although the considerable presence of spinning sidebands (SSB) associated with the magic angle spinning method reduce sensitivity and obscure the high field region somewhat,[254] the important structural features of the derivatized resin are clearly visible. Notably, the chemical shifts of the carbons are within a few ppm of those expected in normal solution phase ^{13}C NMR.[27] The **A**, **B** and C peaks of spectrum 1 (Figure 22) represent overlapping resonances of methine and methylene carbons of the polystyrene backbone,

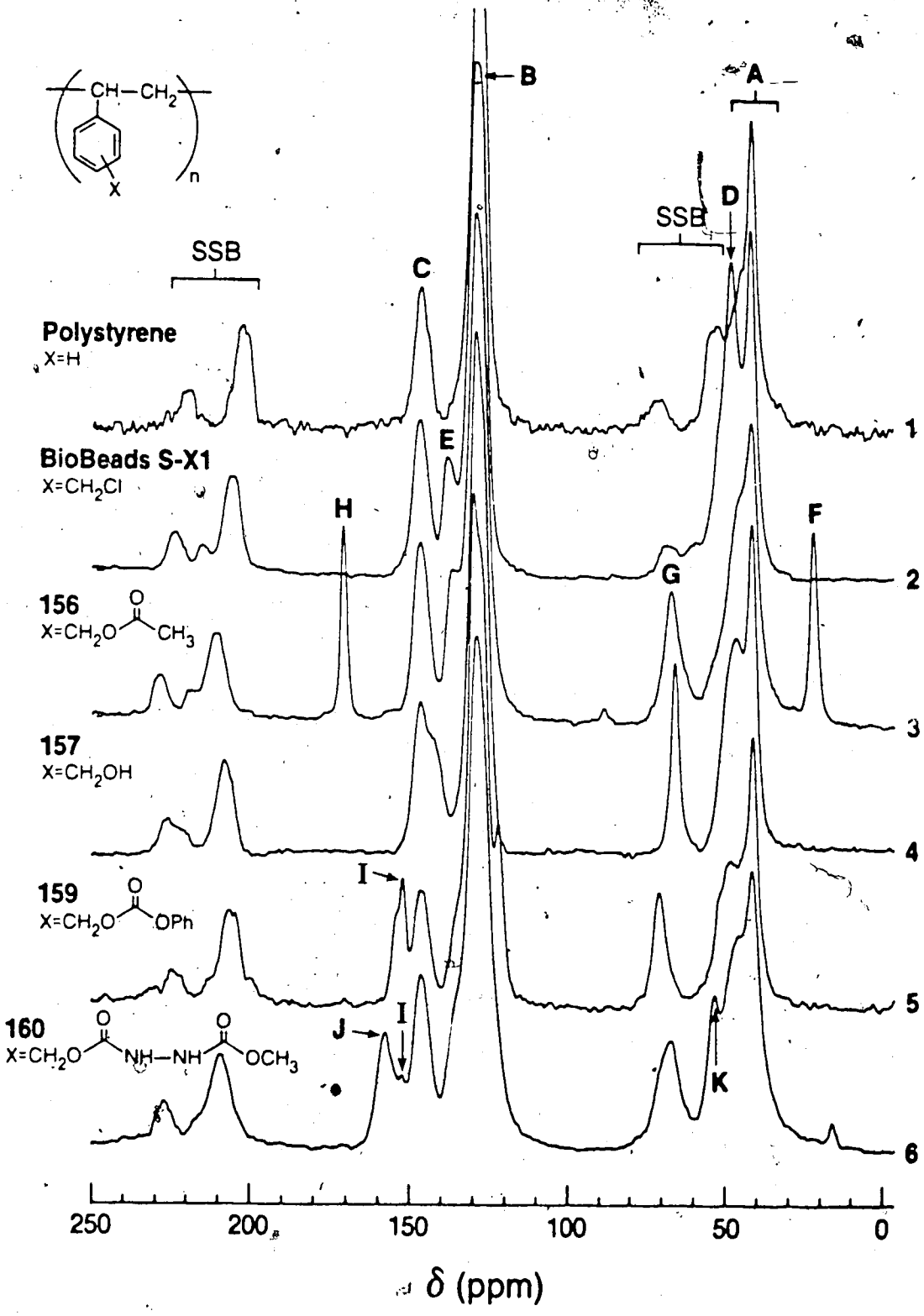


Figure 22. Solid State ^{13}C NMR on Derivatized Polystyrene Resins.

and tertiary aromatic, and C-1 quaternary carbons of polystyrene, respectively. Spectrum 2 of the 50% chloromethylated polystyrene resin (BioBeads S-X1) possesses peak **D** for the CH_2Cl and **E** for the quaternary C-4 aromatic carbon to which the chloromethyl group is attached. The presence of the acetyl methyl is obvious at 22 ppm (**F**), in the acetoxymethyl form of the resin **156** as is the acetyl carbonyl at 171 ppm (**H**). The linking CH_2O is shifted to 67 ppm (**G**) from its previous position **D** in the chloromethyl resin. Hydrolysis of the acetoxymethyl resin **156** produced hydroxymethyl resin **157**, for which the ^{13}C NMR spectrum **4** indicates the complete absence of the acetyl methyl and carbonyl (**F** and **H**). The resonance of the quaternary aromatic carbon to which these functional groups are attached (**E**) is also sensitive to these transformations but is not always well resolved. The ^{13}C NMR of (phenoxycarbonyl)oxymethyl polystyrene resin **159** (spectrum **5**) produced from **157** illustrates the presence of the carbonate carbonyl (Peak **I**) at 153 ppm and an unresolved new aromatic peak at 123 ppm, due to the introduction of the phenoxy ring. In spectrum **6** for the 35 mol% methyl hydrazodicarboxylate resin **160**, peaks **K** and **J** represent the newly introduced methyl and carbamate carbons respectively. The residual peak **I** of reduced intensity is consistent with the presence of ~15 mol% of residual phenylcarbonate units. These six spectra quite satisfyingly reflect the success of all the important

functional group manipulations on the resin and provide useful insight as to the state of the resin which is not readily available from elemental or IR analyses. In the future with the constant improvements in resolution, sensitivity, and integration which are being achieved in solid-state NMR,[252,254] it is likely this technique will provide a very useful quantitative method of examining the functionalization of polymeric supports.

In summary, lanthionine derivatives have been prepared and appear to represent attractive skeletons on which to append further functionalities in developing suicide inhibitors for DAP enzymes. The synthetic utility of serine β-lactones has been firmly established through additions of typical heteroatom and carbon nucleophiles, and a method has been devised to enable their economical large scale production through immobilization of the alkyl azodicarboxylate reagent on a polystyrene support. This polymer supported reagent is generally applicable to all Mitsunobu reactions and eliminates the major disadvantages associated with these condensations. It should therefore facilitate industrial production of numerous chemical and pharmaceutical products (Figure 8)[86-89,92-93] (in addition to the formation of serine β-lactones) which are accessible through utilization of this versatile condensation reaction.

EXPERIMENTAL

General

All reactions requiring anhydrous conditions or involving air-sensitive reagents (eg., thiols) were done under slight positive pressure of dry Ar. Dry reactions were performed using oven-dried glassware (>6 h at 140°C) which was cooled under Argon. All organic layers from extractions were dried over Na_2SO_4. The term "in vacuo" refers to the removal of solvent on a rotary evaporator followed by evacuation (<0.010 torr) to constant sample weight. All solid products were dried in vacuo over P_2O_5 and KOH pellets. Dry solvents were prepared under an Ar atmosphere according to Perrin et al.:[256] benzene, toluene, and tetrahydrofuran (THF) were distilled from Na or K/benzophenone; acetonitrile (CH_3CN), diisopropylamine, and pyridine (pyr) were distilled from calcium hydride; methanol and ethanol were distilled from Mg/catalytic I_2; dimethylformamide (DMF) was stirred with BaO (16 h), decanted and distilled at reduced pressure; dichloromethane was distilled from P_2O_5; trifluoroacetic acid (TFA) was dried over P_2O_5, and distilled. Ethyl ether and hexamethylphosphoramide (HMPA) were obtained and used as anhydrous reagents. Any solvent used for chromatography was distilled. Water was Milli-Q quality which when

necessary was degassed by boiling, further degassing at reduced pressure while hot, and cooling under Ar. Aqueous HCl was prepared free of metal ions from Milli-Q quality H_2O and glass distilled constant-boiling (~110°C) 5.7 N HCl.

All reagents employed were ACS grade or finer. Methyl iodide was percolated through silica and distilled under Ar at 43°C onto Cu wire for storage. Commercial diethyl azodicarboxylate, dibenzyl malonate, and di-tert-butyl malonate were distilled at reduced pressure before use (60°C/0.65 mm, 156°C/0.1 mm, and 90°C/8.0 mm Hg, respectively). Xenon difluoride was purchased from SCM Specialty Chemicals. Cuprous cyanide was obtained from Fisher Chemicals and was dried and stored in vacuo in an Abderhalden pistol at 64°C over P_2O_5. Copper(I) bromide·dimethylsulfide complex ($CuBr \cdot SMe_2$) was prepared according to Theis and Townsend[257] and was recrystallized and stored in a dessicator in darkness. All commercial organometallic reagents were obtained from Aldrich Chemical Co., except for vinyllithium from Organometallics Inc., and vinyl and isopropylmagnesium chlorides from Ventron. Organometallic solutions were titrated immediately before use against either 1,3-diphenyl-2-propanone tosylhydrazone (RLi reagents),[258a] or menthol/phenanthroline (RLi or RMgX reagents).[258b] Potassium tert-butoxide was sublimed at 220°C/1 mm immediately before use. Anhydrous p-toluenesulfonic acid (p-TsOH) was prepared from the monohydrate by dissolving

it in hot benzene with the aid of EtOAc, azeotropically removing H_2O by boiling to 50% volume, and cooling to 0°C; crystalline anhydrous p-TsOH (mp 94-95°C) was filtered, dried in vacuo and stored in a dessicator.

Hydroxymethyl polystyrene resin (1 meq/g, 1% crosslink) was obtained from Bachem Inc. and chloromethylated polystyrene resin (3.90 meq/g, 1% crosslink, Bio-Beads S-X1) was from BioRad Laboratories. Cation exchange resins were BioRad AG50W-X8 (H^+ form, 50-100 mesh) and Fisher Rexyn-102 (H^+ form, 100-200 mesh). Anion exchange resin was BioRad AG1 X8 (Cl^- form, 50-100 mesh), and ion retardation resin was BioRad AG11 A8.

Whenever possible the progress of reactions was monitored by thin-layer chromatography (TLC) using one or more of the following for visualization: UV absorption by fluorescence quenching; I_2-staining; (1:1) methanol/H_2SO_4 spray with charring; dodecaphosphomolybdic acid spray for reducing compounds; bromocresol green spray for acids; ninhydrin or fluorescamine (Sigma) sprays for amino acids and amines; nitroprusside spray for thiols. All spray reagents were prepared and used as described by Krebs et al.[259] For TLC of amino acids and their derivatives on silica, four solvent systems were commonly employed: System A = pH 5.80, 50 mM potassium phosphate buffer/ethanol (30/70); System B - n-BuOH/HOAc/H_2O (4:1:1); System C - MeOH/pyridine/11.6 M HCl/H_2O (80/10/2.5/17.5); System D - CH_3CN/ethylene glycol/pH

7.15, 0.1 M NH_4OAc (70/15/15). For monitoring reactions in DMF or HOAc, the solvent was removed from the plate *in vacuo* before developing. Unless otherwise noted the specified R_f values are on silica plates.

Reactions involving N-protected serine β-lactones were monitored by TLC using bromocresol green spray (0.04% in EtOH, made blue by NaOH)[259] followed by heating of the plate, for detection of the β-lactone as a yellow spot on a blue background. Consumption or generation of the N-protected serine β-lactones could be followed quantitatively in the infrared region by observation of the β-lactone carbonyl stretching band at ~1840 cm^{-1}. Use of 0.1 mm path length solution IR cells allowed estimation of between 2.0 to 110 μmole/mL β-lactone with ±5% accuracy.

Commercial thin-layer chromatography (TLC) plates were silica, Merck 60F-254, or reverse-phase, Merck RP-8F_{254}S. Silica gel for column chromatography was Merck type 60, 70-230 mesh. Flash chromatography was executed according to Still *et al.*[260] using Merck type 60, 230-420 mesh silica gel. Normal phase medium pressure liquid chromatography (MPLC) employed a column of Merck Kieselgel 60H (~50 g, 2.5 × 30 cm). Reverse-phase MPLC was performed on two Merck Lobar Lichroprep RP-8 columns (Size A and B) in series. All solvent mixtures are listed as volume ratios (v/v), and all column chromatography was performed using solvents which were previously degassed *in*

vacuo.

High pressure liquid chromatography (HPLC) was performed on either a Hewlett Packard 1082B or 1090 instrument equipped with a variable wavelength UV detector. Silica gel HPLC columns were either Whatman Partisil M9 (1.0 × 25 cm, semi-preparative), or Beckman 5 μm Ultrasphere-Si (analytical, 0.5 × 15 cm) columns. Analyses of amino acids were carried out on an aminopropyl reverse phase column (4.6 × 200 mm, packed with 5 μm Li-Chrosorb-NH_2 available from Hewlett Packard) using a binary system of extensively degassed 0.010 *M* KH_2PO_4, pH 4.3 (*A*), and CH_3CN/H_2O (500:70) (*B*) with detection at 200 nm essentially as described by Schuster[108] (gradients: 0 min (95% *B*), 5 min (95% *B*), 20 min (70% *B*), 27 min (50% *B*), 30 min (0% *B* for column washing), 50 min (0% *B*), 55 min (95% *B*), 85 min (95% *B* for reequilibration)).

Gas chromatography (GC) was executed on a Hewlett Packard 5890A instrument fitted with either an Alltech FSOT RSL-300 polyphenylmethylsiloxane column (0.53 mm × 10 m) or a J&W Scientific fused-silica Megabore (FSOT, DB-17+) phenylmethylpolysiloxane column (0.53 × 15 m). Injector and detector temperatures were constant at 250°C and flame-ionization detection (FID) was used in all determinations. The He carrier gas pressure specified in the text was measured at the indicated initial temperature. All GC results reported are the average of at least 3 runs.

All literature compounds had ^{1}H NMR, MS and IR spectra consistent with the assigned structures. Melting points were determined on a Thomas Hoover or Buchi oil-immersion apparatus using open capillary tubes and are uncorrected. Optical rotations were measured on a Perkin Elmer 241 polarimeter with a microcell (10.00 cm, 0.9 mL) at ambient temperature (25 ± 2°C). Optical rotations on compounds having no previously reported rotation were determined at two concentrations (50-75% dilution) to ensure the correct magnitude of the reported value. Infrared spectra of pure materials were measured on a Nicolet 7199 FT-IR spectrometer. The C-H stretching bands are not necessarily reported. The kinetics of hydrolysis of 3-amino-2-oxetanone salts (**140**, **141**) were followed by FT-IR using 0.1 mm IR-Trans cells (Kodak, polycrystalline ZnS). Other reactions involving N-protected serine β-lactones were monitored semi-quantitatively on a Perkin Elmer 297 IR spectrometer using 0.141 mm KBr solution cells. Mass spectra (MS) were recorded on Kratos AEI MS-50 (high res., electron impact ionization (EI)-MS), MS-12 (low res. EI-MS and chemical ionization (CI)-MS), and MS-9 (fast-atom bombardment (FAB) with Ar) instruments with an ionizing voltage of 70 eV.

Nuclear magnetic resonance (NMR) spectra were measured on Bruker WP-80 (CW), WH-200, AM-300, WM-360, or WH-400 instruments in the specified solvent with either tetramethylsilane (TMS) or deuterated sodium 3-(trimethyl-

silyl)-1-propanesulfonate (TSP) in D_2O as internal standards in ^1H NMR. Deuterated solvent peaks were used for reference in ^{13}C NMR.[27] ^{19}F NMR spectra were recorded at 376.5 MHz using ^1H-broadband decoupling, and $CDCl_3$ solvent with $CFCl_3$ as an internal standard at 298 (±0.3)K. ^{31}P NMR was performed at 161.96 MHz at 298 (±0.3)K using an external H_3PO_4 standard. Solid state ^{13}C NMR spectra on polystyrene resins were aquired at 50.3 MHz on a Bruker CXP-200 NMR spectrometer using "magic" angle spinning and cross polarization techniques. Samples were packed in a sapphire rotor equipped with Kel F® end caps and spun at 4 kHz. The contact time (or CP time) was 2.0 ms, with a 10 s recycle delay. Typically 500-2000 scans were accumulated.

2,6-Bis[N-(benzyloxycarbonyl)amino]heptanedioic acids (2 and 3d (racemic)).

The procedure was that of Wade et al.[99] A solution of 2,6-diaminoheptanedioic acid (**1**) (Sigma, statistical mixture of LL, DD and meso-isomers) (19.02 g, 100 mmol) in 2N NaOH (250 mL) was cooled to 0-5°C and stirred vigorously while benzyl chloroformate (39 mL, 270 mmol) was added dropwise over 30 min. Vigorous stirring was continued for 3 h at 25°C and the mixture was extracted with EtOAc (2 × 200 mL). The aqueous layer was cooled on ice/H_2O and acidified to pH 1.5 with 5N HCl. The mixture was extracted with ethyl acetate (3 × 200 mL), and the

organic layers were pooled, dried over Na_2SO_4 and concentrated *in vacuo* to leave 40.3 g (88%) of **2** as a foam (mixture of LL, DD, and *meso*-isomers). This material was >95% pure by ^1H NMR: (80 MHz, $CDCl_3$) δ10.3 (br s, 2H, COOH), 7.30 (s, 10H, Ph), 5.80 (br s, 2H, NH), 5.09 (s, 4H, $PhCH_2O$), 4.35 (m, 2H, CH), 2.1-1.1 (m, 6H, $(CH_2)_3$).

A portion of **2** (25.0 g) was recrystallized thrice from boiling EtOAc (cooling to 0°C) to provide white crystals of **3d** (racemic) which were suction filtered, washed with a little chilled (-20°C) ethyl acetate and dried *in vacuo* (8.02 g, 64%). All of the ethyl acetate filtrates and washings were pooled and saved for recovery of the *meso*-isomer (**3c**). The melting point of 164-165°C for **3d** was unaltered by further recrystallization (lit. mp 164-165°C[99], 165.5°C[100]). **For 3d**: IR (CH_2Cl_2 cast) 3500-2300 (mult, br, m), 1717 (vs), 1529 (s), 1454 (w), 1341 (m), 1290 (m), 1190 (m), 1050 (m), 696 (m) cm^{-1}; ^1H NMR (80 MHz, d_6-acetone) δ~8.75 (brs, 2H, COOH), 7.34 (s, 5H, Ph), 6.45 (d, 2H, ~9 Hz, NH), 5.08 (s, 4H, $PhCH_2O$), 4.28 (m, 2H, CH), 2.0-1.3 (br m, 6H, $(CH_2)_3$); EI-MS, 306.1212 ((M-$PhCH_2OCO$, OH), 306.1216 calcd. for $C_{15}H_{18}N_2O_5$); POSFAB-MS (glycerol) 459 (MH^+).

(2*R*,6*S*)-2,6-Bis[*N*-(benzyloxycarbonyl)amino]heptanedioic acid (3c).

According to the procedure of Wade *et al.*[99] the pooled ethyl acetate mother liquors secured from **3d** above

were evaporated in vacuo to a gummy residue which was recrystallized from hot $CHCl_3$ (cooling to 0°C, 2 weeks). The white solid obtained (9.38 g, mp 118-122°C; lit. mp 123-125°C[99], was further purified by recrystallization from minimal hot acetonitrile (cooling to 4°C, 1 week) as suggested by Heijenoort and Bricas[100] to yield **3c** (meso) (8.13 g, 55%): mp 123-125°C (lit. mp 123-125°C[99]; 123.5-124.5°C[100]); IR, ^1H NMR (80 MHz), and MS characteristics were essentially as described for **3d** above.

(2R,6S)-2,6-diaminoheptanedioic acid (4c).

A solution of **3c** (3.00 g, 6.54 mmol) in acetic acid/H_2O (30 mL/10 mL) was stirred with 5% Pd on carbon (150 mg) for 12 h under an atmosphere of H_2. The catalyst was removed by suction filtration (5 μ frit) and washed with HOAc/H_2O (2:1, 3 × 2 mL). The filtrate was evaporated to dryness in vacuo (35°C), and the residue was redissolved in H_2O and evaporation repeated. The crystalline solid was dissolved in a minimum of hot H_2O, and the hot solution was filtered. The pH was adjusted to 6.0 with 1N LiOH, and **4c** crystallized by addition of hot EtOH and slow cooling to 0°C. The fluffy white solid was filtered and washed with MeOH and Et_2O and dried over P_2O_5 in vacuo to yield 0.72 g (58%) of **4c** (meso-isomer): $[\alpha]_D^{25}$ 0.00° (c 2.0, 1N HCl); IR (KBr disk) 3300-2400 (m, br), 2100 (w, br), 1631 (s), 1599 (vs), 1510 (m), 1503 (m), 1413 (m), 1397 (m), 1363 (m), 1317 (s), 534 (m) cm^{-1}; ^1H

NMR (300 MHz, D_2O + DCl) δ4.14 (t, 2H, 6.3 Hz, CH), 2.16-1.90 (m, 4H, $CHCH_2$), 1.80-1.50 (m, 2H, CH_2); ^{13}C NMR (75.5 MHz, D_2O + DCl) 174.04, 54.89, 31.63, 22.75 (no detectable diastereomer); POSFAB-MS (glycerol/HCl) 191 (MH^+), 283 ($M(gly)H^+$), 381 (M_2H^+).

Racemic 2,6-Bis[N-(benzyloxycarbonyl)amino]heptanedioic acid diamide (5d).

Following the method of Wade et al.,[99] di-Z-DL-diaminopimelic acid (**3d**) (7.50 g, 16.3 mmol) and triethylamine (4.55 mL, 32.5 mmol) were dissolved in 1,4-dioxane (100 mL) and cooled on ice/H_2O. To this solution was added isovaleryl chloride (4.00 mL, 32.7 mmol) dropwise with rapid stirring while maintaining the temperature below 10°C. The mixture was stirred 1 h at 10°C and concentrated aqueous ammonia (4.3 mL, 60 mmol) was added dropwise causing precipitation of the diamide. The mixture was allowed to stand 4 h at 4°C, a little 1N NH_4OH was added, the slurry was filtered, and the solid was washed with 1N NH_4OH and H_2O. This material was dried on the funnel and recrystallized from hot DMF by addition of H_2O and cooling to 4°C to yield 4.80 g (65%) of **5d** (dried in vacuo over P_2O_5): mp 227.0-227.5°C (lit. mp 223-224°C[99]); IR (KBr disk) 3333 (s), 3314 (s), 3203 (m), 3100-2750 (m, mult), 1683 (s), 1658 (vs), 1547 (s), 1456 (m), 1434 (m), 1424 (m), 1313 (s), 1292 (m), 1256 (vs), 1051 (m), 695 (s) cm^{-1}; ^1H NMR (80 MHz, D_6-DMSO) δ7.32 (s,

10H, Ph), 7.16 (d, 2H, 9 Hz, NH); 6.92 (br s, 4H, $C(O)NH_2$), 5.02 (s, 4H, $PhCH_2O$), 3.90 (m, 2H, CH), 1.90-1.10 (br m, 6H, $(CH_2)_3$); Anal. Calc. for $C_{23}H_{28}N_4O_6$: C, 60.52; H, 6.18; N, 12.27. Found: C, 60.72; H, 6.15; N, 12.26; CI-MS (NH_3) 474 ($M+NH_4^+$), 457 (MH^+).

Racemic 2,6-diaminoheptanedioic acid diamide, diacetate salt (6d).[99]

Di-Z-DL-diaminopimelic acid diamide (**5d**) (4.44 g, 9.73 mmol) was suspended in HOAc (85 mL) and stirred with 5% Pd on carbon (150 mg) under an atmosphere of H_2 for 12 h. The catalyst was removed by filtration and the filtrate concentrated *in vacuo* at 40°C. The syrupy residue was dissolved in H_2O (15 mL) and evaporated (2×), and dried *in vacuo* over P_2O_5 to produce **6d** (dihydrate) as a glassy colorless hygroscopic solid (3.35 g, quantitative): IR (KBr disk) 3500-2000 (vs; br), 1692 (vs), 1620 (m), 1560 (vs), 1406 (s) cm^{-1}; ^1H NMR (80 MHz, D_2O) δ4.06 (~t, 2H, CH), 1.95 (s, 6H, CH_3COO^-), 2.25-1.78 (m, 4H, $CHCH_2$), 1.77-1.25 (m, 2H, CH_2); POSFAB-MS (glycerol) 189 (MH^+). This material was used directly in the enzymic resolution below.

Enzymic Resolution of (2S,6S)- and (2R,6R)-diaminoheptanedioic acids (4a and 4b).

The procedure employed was a modification of Wade *et al.*[99] A portion of the racemic hydrogenolysis product

(**6d**) (1.86 g, 5.40 mmol) was incubated for 72 h at 25°C with leucine aminopeptidase (3000 units, Sigma, from Hog Kidney, 7 mL of 200 units/mg in 2.9 M $(NH_4)_2SO_4$) in 0.01 M manganous acetate at pH 8.0. The enzyme was removed by ultrafiltration using an Amicon apparatus (PM-10 membrane) and the filtrate applied to a column of Fisher Rexyn 102 (methacrylate resin, Li^+ form, 2.5 × 24 cm, ~90 mL at 3.6 meq/mL). The resin was eluted successively with H_2O (350 mL), 0.5% HOAc (300 mL), and 1.0% HOAc (1 L), and 25 mL fractions were collected. L-Diaminopimelic acid eluted in the H_2O wash, and a small amount of monoamide emerged early in elution with 1% HOAc followed by the D-diaminopimelic acid diamide (**6b**). The early H_2O fractions containing **4a** were pooled and evaporated in vacuo and recrystallized (2×) from H_2O by addition of excess EtOH to yield 1.92 g of white solid (100% theory = 513 mg). The excess material was found to be primarily Li_2SO_4 generated fro the $(NH_4)_2SO_4$ in the commercial enzyme preparation. The sulfate was removed by precipitation as $BaSO_4$ by addition of a solution of $BaCl_2 \cdot 2H_2O$ (2.56 g, 10.5 mmol) in H_2O (20 mL) to a solution of the recovered material in H_2O (20 mL) at pH 2.0. The suspension was stirred 30 min, filtered and the precipitate washed well with H_2O (pH 2). The combined filtrate and washings were concentrated in vacuo at 35°C and the residue was dissolved in a minimum of hot H_2O, and the pH adjusted to 6.0 with dilute NH_4OH. Addition of methanol (100 mL) and cooling to 4°C

precipitated a white gelatinous solid which was recrystallized twice more from H_2O/MeOH to yield **4a** (267 mg, 52%): $[\alpha]_D^{25}$ +45.1 (±0.1)° (c 1.37, 1N HCl) (lit. $[\alpha]_D^{26}$ +45.0 (c 1-5, 1N HCl)[99,101]); IR (KBr disk) 3420 (m, br), 3300-2400 (br, s), 2100 (m), 1590 (vs, br), 1540 (s), 1410 (s), 1345 (m), 1325 (m) cm^{-1}; ^1H NMR (400 MHz, D_2O + DCl) δ4.11 (m, 2H, CH), 2.00 (m, 4H, $CHCH_2$), 1.58 (m, 2H, CH_2); Anal. Calc. for $C_7H_{14}N_2O_4$: C, 44.20; H, 7.42; N, 14.53. Found: C, 43.80; H, 7.32; N, 14.53 (after drying 1 week *in vacuo* at 64°C); POSFAB-MS (glycerol/formic acid) 191 (MH^+), 381 (M_2H^+), 571 (M_3H^+). Incubation of **4a** (10 mM) with *meso*-DAP D-dehydrogenase (see Appendix 1) displayed no measurable activity.

Late 1% HOAc column fractions containing D-diaminopimelic acid diamide (**6b**) were pooled and evaporated *in vacuo* to provide 914.0 mg (98% of diacetate dihydrate) of solid. As a check of stereochemical purity 12.0 mg (1.3%) of this material was reincubated with leucine aminopeptidase for 24 h at 37°C. Since no free diaminopimelate and only a faint trace of monoamide was generated in this digestion, the product was judged to be of high optical purity. The diamide **6b** (900 mg, 2.61 mmol) was directly hydrolyzed by refluxing 3N HCl (150 mL) for 6 h under an atmosphere of Ar. The solvent was removed *in vacuo* at 45°C, the crystalline residue was redissolved in H_2O (50 mL), and the solvent was again removed *in vacuo*. The solid was dissolved in minimal hot

H_2O, filtered, and the pH raised to 6.5 with 3N NH_4OH. Addition of MeOH (100 mL total vol.) and cooling precipitated **4b** which was recrystallized twice more from H_2O/MeOH (final yield 350 mg, 69% from **6d**): $[\alpha]_D^{25}$ -45.7 (±0.2)° (c 1.05, 1N HCl) (lit. $[\alpha]_D^{26}$ -45.5° (c 1; 1N HCl)[100,101]; IR, ^1H NMR, and POSFAB-MS characteristics were identical to **4a** (L-isomer); Anal. Calc. for $C_7H_{14}N_2O_4$: C, 44.20; H, 7.42; N, 14.73. Found: C, 43.89; H, 7.17; N, 14.58 (after drying in vacuo at 64°C for 7 days). Incubation of **4b** (10 mM) with meso-DAP D-dehydrogenase (see Appendix 1) displayed an initial reaction rate 0.16% of that obtained with meso-DAP (**4c**) (K_m = 1.1 mM).

Bis(2-acetamido)propanoic acid (7).[104,105]

According to the procedure of Arnstein and Clubb,[104] pyruvic acid (347 g, 3.94 mol) and acetamide (465 g, 7.87 mol) were suspended in toluene (1.2 L) and refluxed with azeotropic removal of H_2O for 19 h. The mixture was cooled (4°C) and the toluene decanted from the crystalline brown solid. This material was suspended and washed with warm ethanol to provide 221.1 g (30%) of **7** as a white solid: mp 190-192°C (dec) (lit. mp 197°C (dec)[104]); IR (KBr disk) 3410 (s), 3230 (m), 1735 (s), 1689 (vs), 1600 (s), 1545 (s), 1521 (s), 1142 (s) cm^{-1}; ^1H NMR (80 MHz, D_2O) δ1.76 (s, 3H, $CH_3C(NHC(O)CH_3)_2COOH$), 1.93 (s, 6H, $CH_3C(O)NH$); EI-MS (low res.) 143 ($M-CO_2H$), 101

(M-H_2C=C=O), 59 (M-2(H_2C=C=O)), 43 (CH_3CO).

2-(Acetamido)propenoic acid (8).[104,106]

Using the method of Arnstein and Clubb,[104] a solution of **7** (15.0 g, 80.0 mmol) in glacial acetic acid (100 mL) containing 1 drop of conc. HCl was boiled gently for 20 min. The hot solution was filtered through glass wool and on cooling to 10°C α-acetamidoacrylic acid (**8**) crystallized. This solid was filtered, washed with H_2O and dried in vacuo over P_2O_5 to yield 6.10 g (59%) of **8**: mp 189-192°C (dec) (lit. mp 205°C,[214] 185-186°C[261]); IR (KBr disk) 3335 (vs), 1710 (s), 1635 (vs), 1610 (s), 1535 (vs), 1372 (m), 1300 (s), 1270 (s), 1190 (s), 901 (s), 735 (m), 572 (m) cm^{-1}; ^1H NMR (80 MHz, d_6-DMSO) δ9.03 (br s, 1H, COOH), 6.21 (s, 1H, CHH), 5.63 (s, 1H, CHH), 2.02 (s, 3H, CH_3); EI-MS: 129.0423 (129.0426 calcd. for $C_5H_7NO_3$).

(2S,6SR)-Mono-N^6-acetyllanthionine (10) and (2S,6SR)-lanthionine (11).

The procedure of Schöberl[66] was employed. To L-cysteine (**9a**) (hydrochloride salt, monohydrate; 1.60 g, 9.11 mmol) and 2-acetamidoacrylic acid (**8**) (1.78 g, 13.8 mmol) in degassed H_2O (10 mL) under Ar was added 1N NaOH (28 mL, degassed) dropwise over 10 min with stirring (final pH 8.0). The solution was heated under Ar to 95°C for 25 min, and evaporated in vacuo at 40°C to a slightly yellow foam of crude (2S,6SR)-mono-N^6-acetyllanthionine

(**10**) (6.0 g). One half of this crude material (3.0 g) was purified by ion exchange chromatography on a column of AG50 X8 (H^+ form, 3.5 x 20 cm, 220 mL). The resin was washed with H_2O (800 mL) and **10** eluted with 0.5N NH_4OH (1.3 L, 100 mL fractions collected). Fractions containing **10** (R_f 0.45, System C) were pooled and lyophilized to yield 1.05 g (86%) of (2S,6SR)-mono-N^6-acetyllanthionine (**10**) as its hygroscopic ammonium salt: IR (KBr disk) 3420 (br s), 3100-2300 (br, s), 2100 (w), 1640 (vs), 1625 (vs), 1600 (s), 1595 (s), 1525 (s), 1392 (m), 538 (m) cm^{-1}; ^1H NMR (80 MHz, D_2O) δ4.41 (~dd, 1H, 6, 7 Hz, $\underline{CH}NH_3^+$), 3.89 (~dd, 1H, 6, 7 Hz, $\underline{CH}NHAc$), 3.15-2.93 (overlapping m's, 4H, $\underline{CH_2}S\underline{CH_2}$), 2.03 (s, 3H, $\underline{CH_3}$); POSFAB-MS (glycerol) 252 (MH^+). Treatment of **10** (264 mg, 0.98 mmol) with Acylase I Sigma Grade II from Hog Kidney, 25-75 mg, 46-138 x 10^3 units) in H_2O (8 to 20 mL) at pH 7.5 at 37°C for 24 h resulted in <3% hydrolysis of **10** (by ^1H NMR on dialysate).

The remaining half of crude **10** was refluxed in 2.5N HCl (25 mL) for 4.5 h under an atmosphere of Ar and the solvent removed *in vacuo* at 40°C. The residue was dissolved in H_2O and the solvent again evaporated. The recovered solid was dissolved in hot H_2O (7 mL) and a saturated solution of NaOAc (2.8 mL) was added. The white crystals produced after 16 h at 4°C were filtered, washed with ethanol, and dried *in vacuo* to yield 0.60 g (58% yield from **9a**) of (2S,6SR)-lanthionine (**11**): $[\alpha]_D^{25}$ +1.2 (±0.2)° (C 1.0, 2.4N NaOH) (lit. $[\alpha]_D^{25}$ +6°,[42a] +8.4°[42c] (c

1.0, 1N NaOH), +8.6° (c1.4-5.0, 2.4N NaOH)[42d,102] for the pure L-isomer); IR (KBr disk) 3200-2000 (br s, mult), 2100 (w), 1625 (s), 1590 (s), 1505 (m), 1525 (w), 1433 (m), 1403 (m), 1395 (m), 1350 (m), 1340 (m), 555 (m) cm^{-1}; ^1H NMR (80 MHz, D_2O + DCl) δ4.50 (~t, 2H, ~5.5 Hz, CH), 3.38 (~d, 4H, 5.5 Hz, CH_2S); POSFAB-MS (glycerol/HCl) 209 (MH^+).

L-Cystine dimethyl ester dihydrochloride (13).

L-Cystine (30.0 g, 125 mmol, Sigma) was suspended in anhydrous methanol (650 mL) and dry HCl(g) was rapidly passed into the stirred mixture without external cooling. When saturation with HCl(g) without external cooling was achieved, the mixture was cooled to -10°C and addition of HCl was continued until ~225 g total had been added. The vessel was sealed and allowed to stand 16 h at 4°C. The unreacted cystine dihydrochloride precipitate (~6.5 g) was removed by filtration and washed with cold methanol. The filtrate and washings were concentrated in vacuo at 30°C to a thick slurry (~150 mL), anhydrous ether was added (400 mL) and the mixture cooled to -20°C. The white solid was filtered, washed with dry ether (~300 mL), and dried in vacuo over KOH and P_2O_5 to provide 33.3 g (78%) of **13**: mp 182-3°C (dec) (lit. mp 173°C[262a]); $[\alpha]_D^{25}$ -45.5° (c 4.0, MeOH) (lit. $[\alpha]_D^{20}$ -38.4° (c 4, MeOH)[262b]); IR (KBr disk) 3200-2400 (br s), 1745 (vs br), 1580 (m), 1505 (s), 1432 (m), 1330 (m), 1260 (s), 1200 (m), 1150 (w)

cm^{-1}; ^1H NMR (80 MHz, D_2O) δ4.70 (m, 2H, CH), 3.94 (s, 6H, $COOCH_3$), 3.48 (~d, 4H, ~6 Hz, CH_2S); POSFAB-MS (glycerol) 269 (MH^+).

N,N'-Bis(trifluoroacetyl)-L-cystine dimethyl ester (14).

According to the procedure of Harpp and Gleason,[71] a suspension of **13** (9.00 g, 26.4 mmol) in trifluoroacetic acid (30 mL) was cooled to -5°C and trifluoroacetic anhydride (20 mL) was added dropwise over 15 min. The solution was stirred 1 h at -5°C and 1 h at 25°C. The mixture was poured over ice/H_2O (400 mL), stirred 10 min and filtered. The crystalline product was washed well with H_2O and dried in vacuo over KOH and P_2O_5 to yield 9.85 g (81%) of **14**: mp 152-153°C (lit. mp 152-154°C[71]); $[\alpha]_D^{25}$ -188° (c 2.5, MeOH) (lit. $[\alpha]_D^{25}$ -183°,[71] -194°[263] (c 2.5, MeOH)); IR (KBr disk) 3260 (s), 1747 (s), 1700 (vs), 1560 (s), 1175 (s), 463 (w) cm^{-1}; ^1H NMR (80 MHz, d_6-DMSO) δ9.93 (d, 2H, 8 Hz, NH), 4.65 (m, 2H, CH), 3.65 (s, 6H, $COOCH_3$), 3.20 (m, 4H, CH_2S); EI-MS: 460.0202 (M^+, 460.0197 calcd. for $C_{12}H_{14}N_2O_6S_2F_6$).

N,N'-Bis(trifluoroacetyl)-L-lanthionine dimethyl ester (15).

The method of Harpp and Gleason[71] was utilized. Tris(diethylamino)phosphine (12.2 mL, 55.0 mmol) was added dropwise to a stirred suspension of pulverized **14** (22.8 g, 49.5 mmol) in dry benzene (250 mL). The resulting mixture

was stirred 7 to 10 min during which time **14** dissolved and the product reprecipitated as a gel. Hexane (500 mL) was added and the granular precipitate was filtered, washed well with hexane, and dried <u>in vacuo</u> to yield 20.2 g (96%) of **15**, mp 106-108°C. This material was twice recrystallized from MeOH/H_2O (81% overall yield) to provide **15** with mp 112-113.5°C (lit. mp 103-109°C, 117-118°C (3 récryst.)[71]) and $[\alpha]_D^{25}$ -23 (±0.3)° (c 0.4, MeOH) (lit. $[\alpha]_D^{25}$ -21.6°, -32.4° (c 0.4, MeOH) both reported in the same paper by Harpp and Gleason[71]). The experiment was also repeated on a 10 mmol and a 5 mmol scale and the optical rotation of the analytically pure products (mp 112-113 (±0.5)°C) after two recrystallizations from MeOH/H_2O was $[\alpha]_D^{25}$ -15.5 (±0.3)° and -9.8 (±0.3)° (c 0.4, MeOH), respectively. For **15**: IR (acetone cast) 3295 (m, br), 1758 (s), 1750 (s), 1697 (s), 1558 (m), 1179 (vs) cm^{-1}; ^1H NMR (300 MHz, d_6-acetone with 1% D_2O) δ4.18-4.73 (m, 2H, C<u>H</u>), 3.75 (s, 6H, COOC<u>H_3</u>), 3.30-3.22 (m, 2H, SC<u>H</u>H), 3.11-3.02 (m, 2H, SCH<u>H</u>); Anal. Calc. for $C_{12}H_{14}N_2O_6SF_6$: C, 33.57; H, 3.30; N, 6.54. Found: C, 33.76; H, 3.31; N, 6.63; EI-MS: 428.0478 (M^+, 428.0477 calcd.); CI-MS (NH_3) 446 ($M+NH_4^+$).

Alkaline Hydrolysis of 15 to L-lanthionine (16a).

The method employed was that of Harpp and Gleason.[71] A solution of **15** (2.58 g, 6.00 mmol; $[\alpha]_D$ -23° (c 0.4, MeOH)) in dioxane (30 mL) was cooled to 5°C and

chilled 1N NaOH (54 mL) was added dropwise over 10 min with stirring. After 30 min at 5°C the mixture was acidified with cold 2N HCl (24 mL) and the pH adjusted to 5.5 to 6.0. The solvent volume was reduced *in vacuo* to 12 mL and the mixture cooled to 4°C for 16 h. The precipitate of lanthionine was filtered, washed with cold H_2O (5 mL) and EtOH, and recrystallized by dissolution in H_2O (6 mL) by addition of conc. NH_4OH, cooling to 5°C and neutralization (pH 6) with formic acid. Upon standing at 4°C (2 days), 0.75 g (60%) of **16a** was recovered by filtration, washed with EtOH and dried *in vacuo*: $[\alpha]_D^{25}$ +1.8° (c 1.4, 2.4N NaOH) (lit. $[\alpha]_D^{25}$ +9.4° reported by Harpp and Gleason,[71] +8.6° (c 1.4-5.0, 2.4N NaOH)[42d,102]) (also cf. later syntheses of **16a**); IR, NMR and MS behavior were identical to **11**. HPLC analysis[108] of this product indicates it is composed of 60 (±0.8)% *meso* (2S,6R) (t_R = 24.3 min) and 40 (±0.8)% L/D((2S,6S) or (2R,6R))-lanthionines (t_R = 23.5 min) and is free of salts.

Acidic Hydrolysis of 15 to L-lanthionine (16a).

N,N'-Bis(trifluoroacetyl)lanthionine dimethyl ester (**15**) (1.50 g, 3.50 mmol; $[\alpha]_D^{25}$ -23° (c 0.4, MeOH)) was suspended in 2.4N HCl and refluxed 18 h under Ar. The solvent was removed *in vacuo* at 35°C and the residue dissolved in H_2O (50 mL) and evaporation repeated (twice). The white solid was recrystallized from H_2O with the aid of NH_4OH and formic acid as described for **16a**

above. The L-lanthionine **16a** obtained possessed IR, NMR and MS properties identical to **11** and **16a** above, and $[\alpha]_D^{25}$ +2.6 (±0.3)° (c 1.4, 2.4N NaOH). HPLC analysis[108] of this product indicates 65 (±1.0)% meso (2S,6R) (t_R = 24.3 min) and 35 (±1.0)% L/D((2S,6S) or (2R,6R))-lanthionine (t_R = 23.5 min) which is free of salts.

L- and D-Serine methyl ester hydrochloride (17a and 17b).

Typically, dry HCl(g) was rapidly passed into a suspension of L- or D-serine (Sigma) (35.0 g, 333 mmol) in dry MeOH (1 L). After all of the serine dissolved the solution was cooled to 20°C and addition of HCl(g) was continued until 300-350 g total had been added. The flask was equipped with a drying tube and allowed to stand 16 h at RT. The solvent was removed in vacuo at 40°C, the residue was dissolved in dry methanol and evaporation repeated several times. The crystalline product was dried in vacuo over P_2O_5 and KOH pellets to afford a quantitative yield (51.7 g) of **17(a or b)**, which was recrystallized from dry MeOH/Et_2O in 85-95% overall yield.

For 17a: mp 151-154°C (dec) (1st recryst), 162-164°C (2nd recryst) (lit. mp 163-165°C,[261] 167°C[102]); $[\alpha]_D^{25}$ +3.4 (±0.1)° (c 4.0, MeOH) (lit. $[\alpha]_D^{21}$ +3.4°[261]); IR (KBr disk) 3350 (s br), 3330-2450 (vs, br), 1945 (w), 1745 (vs), 1595 (s), 1515 (vs), 1255 (vs), 1095 (s), 1040 (vs) cm^{-1}; ^1H NMR (80 MHz, D_2O) δ4.22 (m, 1H, CH), 3.98 (m, CH_2), 3.81 (s, 3H, CH_3); POSFAB-MS (glycerol) 120 (MH^+), 239 (M_2H^+).

For 17b: mp 156-157° (dec); $[\alpha]_D^{25}$ -3.6 (±0.1)° (c 4.0, MeOH) (cf. **17a** above); IR, NMR and MS properties were identical to **17a**

L- and D-β-Chloroalanine methyl ester hydrochloride (18a and 18b) and O-acetyl-L-serine methyl ester hydrochloride (19).

The procedure used was that of Fischer and Raske.[110] To freshly distilled acetyl chloride (500 mL) with rapid stirring at 0°C was added serine methyl ester hydrochloride (**17a** or **17b**) (51.7 g, 0.333 mol; pulverized and dried *in vacuo* over P_2O_5) followed by pulverized PCl_5 (78.9 g, 0.379 mol) added in 5 portions over 15 min. The mixture was allowed to slowly warm to 25°C and stirred 2 h. The suspension was cooled to -10°C, and suction filtered. The solid was washed with acetyl chloride (50 mL) and pet. ether (1 L) and dried *in vacuo* to yield 50.0 g (86%) of crude L- or D-β-chloroalanine methyl ester hydrochloride (mp 143-144°C (dec)), typically containing 3-5 mol% of O-acetylserine methyl ester hydrochloride (**19**) by ^1H NMR. Recrystallization from methanol/ether yielded pure **18a** (L) or **18b** (D) (45.6-46.8 g, 79-81%). **For 18a:** mp 156-157°C (dec) (lit.[102] mp 157°C); IR (KBr disk) 3300-2200 (s, br, mult), 2020 (w), 1750 (s), 1619 (w), 1518 (m), 1446 (m), 1335 (m), 1249 (s), 1205 (m), 1070 (m) cm^{-1}; ^1H NMR (80 MHz, d_6-DMSO) δ9.14 (br s, 3H, $\underline{NH_3}^+$), 4.65 (m, 1H, $\underline{CH}$), 4.18 (m, 2H, $\underline{CH_2}Cl$), 3.75 (s, 3H,

$COOCH_3$); POSFAB-MS (glycerol) 139 (MH^+), 141 ($(M+2)H^+$, 33% of MH^+).

For 18b: mp 148-150°C (dec); IR, NMR and MS behavior was identical to **18a.**

If the serine methyl ester hydrochloride (**17**) was not freed of residual MeOH by recrystallization, pulverization and drying *in vacuo* before use, **19** became the major product (50-73%): mp 154-156°C; $[\alpha]_D^{25}$ +9.10 (±0.08)° (c 3.0, MeOH); IR (KBr disk) 3700-2400 (s, vbr), 2050 (m), 1749 (vs, br), 1580 (m), 1517 (m), 1432 (m), 1378 (m), 1257 (s), 1233 (s), 1045 (s), 775 (m) cm^{-1}; ^1H NMR (80 MHz, d_6-DMSO) δ9.03 (br s, 3H, NH_3^+), 4.5-4.30 (m, 3H, CH-CH_2O), 3.73 (s, 3H, $COOCH_3$), 2.00 (s, 3H, CH_3COO); EI-MS: 162.0766 (MH^+, 162.0766 calcd. for $C_6H_{12}NO_4$), 102.0551 ($M-CH_3COOH$); POSFAB-MS (glycerol) 162 (MH^+).

L- and D-β-Chloroalanine hydrochloride (20a and 20b) and free base (21a and 21b).

β-Chloroalanine methyl ester hydrochloride (**18a** or **18b**) (30.42 g, 175 mmol) was stirred 75 min in refluxing 2.5N HCl (300 mL) under an atmosphere of Ar. The solvent was removed *in vacuo* at 40°C. The residue was dissolved in H_2O (100 mL) and solvent again evaporated and dried *in vacuo* over P_2O_5. The dried solid was recrystallized from anhydrous methanol/ether to afford 21.8-25.2 g (78-90%) of **20a** or **20b.** This material is reported to have an "$[\alpha]_D$ close to zero in H_2O, and no distinct melting point."[102]

For **20a** or **20b**: IR (KBr disk) 3450-2200 (s, vbr, mult), 1970 (w), 1740 (s), 1596 (m), 1498 (m), 1415 (m), 1346 (m), 1230 (m), 1196 (m), 1067 (m), 894 (m), 850 (m), 792 (s), 680 (s) cm^{-1}; ^1H NMR (80 MHz, D_2O) δ4.60-4.45 (m, 1H, CH), 4.25-4.07 (m, 2H, CH_2Cl); POSFAB-MS (glycerol) 124 (MH^+)), 126 ($(M+2)H^+$, 33% of MH^+); R_f 0.76 (System A). The free amino acids were prepared by dissolving either **20a** or **20b** (eg., 20.25 g, 127 mmol) in a minimal amount of H_2O, raising the pH to 5.8 with 2N LiOH, diluting with EtOH (1.5 volumes) and cooling to -20°C (16 h). The crystalline solid was filtered, washed with EtOH, and dried in vacuo over P_2O_5 to yield 13.3-14.1 g (85-90%) of **21a** or **21b**.

For 21a: mp 164-165°C (dec) (lit.[110] mp 160°C); $[\alpha]_D^{25}$ -17.0 (±0.2)° (c 2.0, H_2O) (lit. $[\alpha]_D^{20}$ -15.5° (c 1.0, H_2O),[102] -15° (c 9.9, H_2O)[42a]); IR (KBr disk) 3420-2250 (br s, mult), 2080 (w), 1625 (s), 1605 (s), 1535 (m), 1435 (m), 1399 (s), 1345 (m), 1295 (m), 1190 (m), 1054 (m), 1015 (m), 642 (s) cm^{-1}; ^1H NMR (80 MHz, D_2O) δ4.60 (m, 1H, CH), 4.28-4.06 (m, 2H, CH_2Cl); EI-MS: 123.0088 (123.0087 calcd. for $C_3H_6NO_2Cl$); POSFAB-MS (glycerol) 124 (MH^+), 126 ($(M+2)H^+$, 33% of MH^+); CI-MS (NH_3) 124 (MH^+), 141 ($M+NH_4^+$).

For 21b: mp 160-161°C (dec); $[\alpha]_D^{25}$ +17.0 (±0.2)° (c 2.0, H_2O) (cf. **21a** above) (lit. $[\alpha]_D^{20}$ +15.0° (c 1, H_2O)[102]); IR, ^1H NMR, and MS characteristics were as described for **21a** above.

L-Lanthionine (16a) from β-chloro-L-alanine (21a).[40]

A modification of the method of Brown and du Vigneaud[42a] was employed. Potassium hydroxide (77.4 g, 1.38 mol) was dissolved in degassed H_2O (110 mL) and L-cysteine hydrochloride monohydrate (Sigma, 62.25 g, 0.354 mol) was added under Ar atmosphere. The stirred solution was heated to 60°C and β-chloro-L-alanine (**21a**) (24.3 g, 0.197 mol) was added in small portions over 1 h. The mixture was removed from the heat, degassed H_2O (45 mL) was added and stirring was continued 3 h under Ar. H_2O (150 mL) was added, the pH was lowered to 5.5-6.0 with 5.7N HCl while cooling on ice/H_2O, and the mixture was stored 16 h at 4°C. The crystalline L-lanthionine (33.2-36.5, 81-89%) was filtered under suction, washed with EtOH, and dried briefly on the funnel. This material was directly recrystallized from H_2O (175 mL) by addition of conc. ammonia, cooling on ice/H_2O, and neutralization (pH 5.5-6.0) with formic acid. Upon standing 16 h at 4°C a 70-74% yield (28.7-30.4 g) of crystalline L-lanthionine (**16a**) was recovered by filtration, washing with a little cold H_2O and EtOH, and drying in vacuo over P_2O_5: mp 279-281°C (dec) (lit.[214] mp 293-295°C (dec)); $[\alpha]_D^{25}$ +6.8 (±0.2)° (c 1.4, 2.4N NaOH), +8.1 (±0.1)° (c 5.0, 2.4N NaOH), (lit. $[\alpha]_D^{22}$ +6 (±1)° (c 1, 1N NaOH),[42a] +8.4° (c 1.0, 1N NaOH,[42c] or c 1.4, 2.4N NaOH[42d]), +8.6° (c 5.0, 2.4N NaOH)[42a,102]; IR (KBr disk) 3200-2400 (s, br, mult),

2075 (m), 1610 (vs, br), 1595 (vs, br), 1518 (s), 1415 (m), 1390 (s), 1348 (s), 534 (m) cm^{-1}; ^1H NMR (400 MHz, D_2O + DCl) δ4.45 (dd, 2H, 4.4, 7.4 Hz, CH), 3.38 (dd, 2H, 4.4, 15.0 Hz, CHHS), 3.26 (dd, 2H, 7.4, 15.0 Hz, CHHS); Anal. Calc. for $C_6H_{12}N_2O_4S$: C, 34.60; H, 5.81; N, 13.45; S, 15.40. Found: C, 34.46; H, 5.81; N, 13.35; S, 15.40; POSFAB-MS (glycerol/HCl) 209 (MH^+), 417 (M_2H^+); R_f 0.33 (System A). HPLC analysis[108] of **16a** prepared by this method was unable to detect the presence of the meso-isomer (**16c**) (i.e., <3%) in the sample (t_R = 23.5 (±0.1) min (L) and 24.3 min (meso)); R_f 0.40 (System C). Incubation of **16a** with meso-DAP dehydrogenase suggested 1.0% meso-contaminatant.

D-Lanthionine (16b) from β-chloro-D-alanine (21b).[40]

This material was prepared in 70-71% recrystallized yield as described for the L-isomer (**16a** above) using β-chloro-D-alanine (**21b**) and D-cysteine (generated from $Na/NH_3(l)$ reduction of D-cystine (Sigma)[102]) and KOH (17.9 g, 0.32 mol): mp 277-278°C (dec) (lit. mp 293-295°C (dec)[214]); $[\alpha]_D^{25}$ -6.1° (c 1.0, 2.4N NaOH), -7.9 (±0.1)° (c 5.0, 2.4N NaOH) (lit $[\alpha]_D^{22}$ -8.0 (c 5.0, 2.4N NaOH),[102] and cf. **21a** above); IR, ^1H NMR and MS were identical to **21a** above; Anal. Calc. for $C_6H_{12}N_2O_4S$: C, 34.60; H, 5.81; N, 13.45; S, 15.40. Found: C, 34.33; H, 5.79; N, 13.39; S, 15.23. HPLC analysis[108] of **16b** prepared by this method indicated no detectable (i.e., <3%) meso-isomer (**16c**) in

the sample (t_R = 23.5 (±0.1) min for D, 24.3 min for meso); R_f 0.[redacted]ystem C). Incubation of **16b** with meso-DAP dehydrogenase suggests 2.7% meso-contaminant (**16c**).

meso-Lanthionine (16c) from β-chloro-D-alanine (21b).[40]

This material was prepared in 81% recrystallized yield from **21b** and L-cysteine hydrochloride monohydrate according to the procedure described for the L-isomer (**16a**) above: mp 277-278°C (dec) (lit. mp 304°C (dec, 207°C softens)[214]); $[\alpha]_D^{25}$ 0.00° (c 5.0, 2.4N NaOH); IR (KBr disk) 3400-2360 (s, br, mult), 2290 (w), 1626 (s), 1575 (s), 1488 (m), 1405 (m), 1337 (m), 1290 (m), 1145 (m), 558 (m) cm^{-1}; ^1H NMR (400 MHz, D_2O + DCl) δ4.26 (dd, 2H, 4.4, 6.4 Hz, CH), 3.31 (dd, 2H, 4.4, 14.5 Hz, CHHS), 3.23 (dd, 2H, 6.4, 14.5 Hz, CHHS); Anal. Calc. for $C_6H_{12}N_2O_4S$: C, 34.60; H, 5.81; N, 13.45; S, 15.40. Found: C, 34.54; H, 5.99; N, 13.42; S, 15.36; POSFAB-MS (glycerol/HCl) 209 (MH^+). HPLC analysis[108] of **16c** indicated <3.0% of the D/L isomer (t_R = 23.5 (±0.1) min for D/L, 24.3 min for meso); R_f ~0.4 (System C).

L-Lanthionine sulfoxide (22a).[40]

A modification of the procedure of Zahn and Osterloh[67] was used. L-Lanthionine (**16a** from **21a**) (2.0 g, 9.61 mmol) was suspended in H_2O (10 mL) and distilled 5.7N HCl (5.0 mL) was added. To the stirred solution was added 30% H_2O_2 (1.5 mL, 14.4 mmol) dropwise and stirring was

continued 24 h. The mixture was cooled on ice/H_2O and the pH was adjusted to 7.0 with conc. ammonia. After standing at 4°C several days, the white crystalline solid was filtered, ...ed with cold H_2O and EtOH, and recrystallized from H_2O in the manner described above for **16a**. L-Lanthionine sulfoxide (**22a**) was obtained as a monohydrate in 70-71% yield (1.63-1.65 g): mp 220-221°C (dec); $[\alpha]_D^{25}$ +61.4 (±1.2)° (c 1.0, 1N HCl); IR (KBr disk) 3100-2300 (br, s), 1950 (w), 1640 (s), 1620 (m), 1500 (s), 1390 (m), 1070 (m), 1010 (s), 545 (s) cm^{-1}; ^1H NMR (400 MHz, D_2O + DCl) δ4.54 (dd, 2H, 5.5, 6.5 Hz, CH), 3.64 (dd, 2H, 6.5, 14.5 Hz, CHHS(O)), 3.55 (dd, 2H, 5.5, 14.5 Hz, CHHS(O)); Anal. Calc. for $C_6H_{12}N_2O_5S \cdot H_2O$: C, 29.75; H, 5.82; N, 11.56; S, 13.23. Found: C, 29 ; H, 5.84; N, 11.35; S, 13.27; POSFAB-MS (glycerol/HCl) 225 (MH^+), 449 (M_2H^+); R_f 0.15 (System C).

D-Lanthionine sulfoxide (22b).[40]

This compound was prepared in 70% recrystallized yield as the monohydrate from D-lanthionine (**16b** from **21b**) exactly as outlined for the L-isomer (**22a**) above: mp 220-221°C (dec); $[\alpha]_D^{25}$ -59.8 (±1.0)° (c 1.0, 1N HCl) (cf. **22a** above); IR, ^1H NMR, MS, and chromatographic properties were identical to the L-isomer **22a**. Anal. Calc. for $C_6H_{12}N_2O_5S \cdot H_2O$: C, 29.75; H, 5.82; N, 11.56; S, 13.23. Found: C, 29.68; H, 5.91; N, 11.51; S, 13.30; R_f 0.15 (System C).

meso-Lanthionine sulfoxide (22c).[40]

This material was produced in anhydrous form in 86% recrystallized yield from meso-lanthionine (**16c** from **21b**) as described for **22a** above. Presumably the product is a mixture of two optically inactive diastereomers (i.e., mixed chirality at S): mp >300°C (lit. mp 260-270°C (dec)[67]); $[\alpha]_D^{25}$ 0.00° (c 1.0, 1N HCl); IR (KBr disk) 3300-2200 (br, s), 2090 (w), 1637 (s), 1588 (s), 1507 (w), 1436 (m), 1408 (m), 1335 (m), 1325 (m), 1029 (s), 533 (m) cm^{-1}; ^1H NMR (200 MHz, D_2O + DCl) δ4.71 (m, 2H, CH), 3.88-3.54 (m, 4H, $CH_2S(O)$); Anal. Calc. for $C_6H_{12}N_2O_5S$: C, 32.13; H, 5.39; N, 12.49; S, 14.30. Found C, 32.07; H, 5.41; N, 12.69; S, 14.55; POSFAB-MS (glycerol/HCl) 225 (MH^+); R_f ~0.15 (System C).

Lanthionine Sulfone (mixture of all stereoisomers) (23).

This material was prepared according to the method of Zahn and Osterloh.[67] Lanthionine (Sigma, mixture of DD, LL, and meso) (1.00 g, 4.80 mmol) stirred in H_2O (10 mL) and 20% $HClO_4$ (8 mL) was treated with 10% aqueous $(NH_4)_2MoO_4$ (1 mL) and dropwise with 30% H_2O_2 (2 mL, 19.2 mmol). The mixture was stirred 24 h at 25°C, cooled on ice/H_2O, and neutralized (pH 6) with isobutylamine. The red-brown mixture was allowed to stand 3 days at 4°C and the precipitated solid was filtered, washed with a little cold H_2O and EtOH and dried in vacuo to provide a slightly

yellow solid (0.49 g, 43%). TLC (System C) indicated ninhydrin-positive impurities (eg., cysteic acid, etc.) in the lanthionine sulfone (R_f ~0.25). These were removed by stirring successively with acetone (15 mL) and H_2O (10 mL at 4°C) and recrystallizing the solid (recovered by filtration) from H_2O as described for lanthionine (**16a**) above to yield 0.35 g (30%) of **23** (cf. 87% recrystallized yield for **24** below): mp 187-189°C (dec); IR (KBr disk) 3430 (m, br), 3300-2200 (s, br, mult), 2080 (w, br), 1690 (m), 1630 (vs, br), 1588 (s), 1510 (s), 1390 (vs), 1313 (s), 1245 (m), 1139 (vs) cm^{-1}; ^1H NMR (80 MHz, D_2O + DCl) δ4.56 (m, 2H, CH), 3.92 (m, 4H, CH_2SO_2); POSFAB-MS (glycerol/HCl) 241 (MH^+), 481 (M_2H^+).

L-, D- and meso-Lanthionine sulfones (24a, 24b and 24c).[40]

A performic acid solution was prepared by stirring together 1 vol. of 30% hydrogen peroxide and 9 vol. of 97% formic acid for 1 h at 25°C.[111] An aliquot (2.0 mL, 1.7 mmol) of this mixture was added to each pure lanthionine isomer (**16a** from **21a**, **16b** from **21b**, and **16c** from **21b**) (100 mg, 0.48 mmol) at 0°C and stirred 2 h. The solvent was evaporated at 30°C *in vacuo* and the syrupy residue was redissolved in H_2O (2 × 3 mL) and reconcentrated *in vacuo*. The solid residue was recrystallized from H_2O (1.0 mL) as described for lanthionine (see **16a**) above. In a typical oxidation 112 mg (90%) of **24a** (L) or **24b** (D) as monohydrates, or 105 mg (91%) of **24c** (*meso*, anhydrous) was

recovered after recrystallization.

For L-lanthionine sulfone monohydrate (24a): mp > 300°C (darkening at 200°C, 240°C black); $[\alpha]_D^{25}$ +25.9° (c 1.0, 1N HCl); IR (KBr disk) 3300-2150 (s, br), 2000 (w), 1663 (s), 1625 (s), 1541 (s), 1379 (s), 1301 (s), 1130 (s), 517 (m), 488 (m) cm^{-1}; ^1H NMR (400 MHz, D_2O + DCl) δ4.62 (dd, 2H, 4, 8 Hz, CH), 4.16 (dd, 2H, 4, 15 Hz, $CHHSO_2$), 3.97 (dd, 2H, 8, 15 Hz, $CHHSO_2$); Anal. Calc. for $C_6H_{12}N_2O_6S \cdot H_2O$: C, 27.91; H, 5.46; N, 10.84; S, 12.41. Found: C, 27.93; H, 5.44; N, 10.87; S, 12.39; POSFAB-MS (glycerol/HCl) 241 (MH^+), 481 (M_2H^+); R_f 0.27 (System C).

For D-lanthionine sulfone monohydrate (24b): mp > 300°C (darkening at 200°C, black by 240°C); $[\alpha]_D^{25}$ -25.6° (c 1.0, 1N HCl) (cf. **24a** above); IR, ^1H NMR, MS and chromatographic properties were identical to **24a**. Anal. Found: C, 27.87; H, 5.48; N, 10.80; S, 12.46.

For meso-lanthionine sulfone (24c): mp 187-188°C (dec) (lit.[67] 270-300°C (dec, browning at 210°C)); $[\alpha]_D^{25}$ 0.0° (c 1.0, 1N HCl); IR (KBr disk) 3300-2180 (s, br), 1670 (s), 1635 (s), 1548 (m), 1395 (s), 1310 (s), 1133 (s), 490 (m) cm^{-1}; ^1H NMR (200 MHz, D_2O + DCl) δ4.81 (dd, 2H, 4.0, 7.5 Hz, CH), 4.27 (dd, 2H, 4.0, 15 Hz, $CHHSO_2$), 4.09 (dd, 2H, 7.5, 15 Hz, $CHHSO_2$); Anal. Calc. for $C_6H_{12}N_2O_6S$: C, 30.00; H, 5.03; N, 11.66; S, 13.34. Found: C, 29.89; H, 5.22; N, 11.59; S, 13.14; POSFAB-MS (glycerol/HCl) 241 (MH^+); R_f ~0.3 (System C).

Z- and E-[N,N'-Bis(trifluoroacetyl)-L-dehydrolanthionine]-dimethyl esters (25 and 26).

The procedure of Janzen et al.[113a] was adapted. N,N'-Bis(trifluoroacetyl)-L-lanthionine dimethyl ester (**15**, $[\alpha]_D^{25}$ -9.8° (c 0.44, MeOH)) (1.30 g, 3.04 mmol) in dry CH_3CN (5.0 mL) was injected into a solution of XeF_2 (668 mg, 3.95 mmol) in CH_3CN (4.0 mL) at -23°C. The stirred solution was allowed to warm to 20°C and gas evolved in the reaction was collected in an inverted cylinder over hexane. After 40 min gas evolution ceased (~170 mL total generated, 100% theory Xe + HF ≡ 140 mL), and 1,1,1,3,3,3-hexamethyldisilazane (0.844 mmol, 4.0 mmol) was added and the solvent was removed in vacuo at 30°C. The residue was chromatographed (300 mg/run) on an MPLC silica column (40% EtOAc/60% hexanes, 3.0 mL/min) to yield **25** (972 mg, 75%) as the major product, followed by **26** (92 mg, 7%).

For Z-[N,N'-bis(trifluoroacetyl)-L-dehydrolanthionine]dimethyl ester (25): mp 94-96°C; $[\alpha]_D^{25}$ -1.8 (±0.1)° (c 1.0, MeOH); IR ($CHCl_3$ cast) 3300 (m, br), 3050 (w, br), 2958 (w), 1750 (m), 1718 (vs, br), 1597 (m), 1544 (m), 1439 (m), 1315 (m), 1253 (s), 1214 (s), 1173 (vs, br) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ8.06 (br s, 1H, C=CNH), 7.68 (br d, 1H, 7 Hz, CHNH), 7.47 (s, 1H, SCH=C), 4.94 (~d of t, 1H, 7, 4.5 Hz, CH), 3.84 (s, 3H, C=C-$COOCH_3$), 3.81 (s, 3H, CHCOOCH_3), 3.46 (~d, 2H, 4.5 Hz, CH_2S); An nOe enhancement of the 7.47 ppm resonance when irradiating at

3.84 ppm suggests Z-stereochemistry. Anal. Calc. for $C_{12}H_{12}N_2O_6SF_6$: C, 33.81; H, 2.84; N, 6.57. Found: C, 34.01; H, 3.15; N, 6.75; EI-MS: 426.0317 (426.0321 calcd.), 313.0227 ($M-CF_3C(O)NH_2$), 196.0221 (Base peak, $M-SCH_2CH(NHC(O)CF_3)COOCH_3$); CI-MS ($NH_3$) 444 ($M+NH_4^+$), 427 ($MH^+$); R_f 0.25 (40% EtOAc/hex).

For the E-isomer 26: IR (KBr disk) 3303 (m, br), 3060 (w, br), 2957 (m), 2920 (m), 2845 (m), 1722 (vs), 1659 (m), 1547 (s), 1440 (s), 1258 (vs), 1214 (vs), 1176 (vs, br), 1108 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ7.85 (br s, 1H, C=C-NH), 7.56 (br d, 1H, 7 Hz, NH), 7.26 (s, 1H, SCH=C), 4.94 (~d of t, 1H, 7, 4.5 Hz, CH), 3.87 (s, 3H, $C=C-COOCH_3$), 3.82 (s, 3H, $CHCOOCH_3$), .48 (~d, 2H, 4.5 Hz, CH_2S); no nOe enhancement of the 7.26 ppm resonance was observed on irradiation at 3.87 ppm; EI-MS 426.0305 (426.0321 calcd. for $C_{12}H_{12}N_2O_6SF_6$), 313.0213 ($M-CF_3C(O)NH_2$); CI-MS (NH_3) 444 ($M+NH_4^+$), 427 (MH^+); R_f 0.16 (40% EtOAc/hex).

L-Methionine methylsulfonium, iodide salt (27).

L-Methionine (1.00 g, 6.70 mmol) was dissolved in D_2O (27 mL) and the apparent pH adjusted to 3.5 by addition of CH_3COOH. Methyl iodide (0.90 mL, 14 mmol) was added to and the mixture was stirred in dark and monitored by ^1H NMR; after 4 h the reaction had proceeded 45% and after 23 h it was 87% complete by NMR. After 24 h the volume was reduced to 5 mL in vacuo at 30°C and MeOH (100 mL) was

added slowly to cause the crystallization of **27** as large silvery flakes (yield 1.25 g, 64%): mp 156-160°C (dec) (lit. mp ~150°C (dec) for DL (racemic)[56]; IR (KBr disk) 3660-3300 (m, br), 3200-2000 (s, br, mult), 1616 (s), 1567 (vs), 1542 (m), 1408 (m), 1371 (m), 1050 (w), 781 (m), 547 (s), 440 (m) cm^{-1}; ^1H NMR (80 MHz, D_2O) δ4.05 (~t, 1H, ~6.5 Hz, CH), 3.27 (m, 2H, CH_2S^+), 2.70 (s, 6H, $S^+(CH_3)_2$), 2.22 (m, 2H, $CHCH_2$); POSFAB-MS (glycerol) 164 (MH^+), 327 (M_2H^+), 102 (MH^+-Me_2S).

N,N'-Bis(tert-butoxycarbonyl)lanthionine (28).

The general method of Moroder et al.[265] was adapted. To a stirred solution of lanthionine (Sigma, mixture of all isomers) (2.00 g, 9.60 mmol) in dioxane/H_2O (2:1, 50 mL) with 1N NaOH (20 mL) was added di-tert-butyl pyrocarbonate (4.65 g, 21.1 mmol). The mixture was stirred 1.5 h, more di-tert-butyl pyrocarbonate (1.50 g, 7.2 mmol) was added, and stirring was continued 3 h longer. The volume was reduced 50% in vacuo at 40°C and the mixture was acidified to pH 2.25 with 2N H_3PO_4. Extraction with EtOAc (3 x 40 mL) followed by drying of organic phases over Na_2SO_4 and evaporation in vacuo provided 4.48 g of hygroscopic white foam. This material was recrystallized from EtOAc/hexane to obtain 3.17 g (88%) of **28** as a white crystalline solid: mp 128-129°C; IR ($CHCl_3$ cast) 3500-2200 (m, br, mult), 1722 (vs, br), 1658 (m), 1523 (m), 1393 (m), 1367 (m), 1250 (m), 1225

(m), 1163 (s) cm^{-1}; ^1H NMR (80 MHz, d_6-DMSO) δ7.00 (br d, 2H, 8 Hz, NH), 4.03 (br m, 2H, CH), 2.87 (m, 4H, CH_2S), 1.40 (s, 18H, tert-Bu); Anal. Calc. for $C_{16}H_{28}N_2O_8S$: C, 47.05; H, 6.91; N, 6.86; S, 7.85. Found: C, 47.05; H, 6.90; N, 6.76; S, 7.70. POSFAB-MS (glycerol) 409 (MH^+); R_f 0.57 (40 MeOH/60 EtOAc/1 HOAc)

N-(tert-Butoxycarbonyl)-S-methyl-L-cysteine (29).

The method of Paleveda et al.[118] was adapted. To a stirred solution of S-methyl-L-cysteine (Aldrich, $[\alpha]_D^{18}$ -28.9° (c 1.0, H_2O)) (3.00 g, 22.2 mmol) and triethylamine (4.7 mL, 67 mmol) in H_2O (14 mL)/dioxane (14 mL) was added 2-(tert-butoxycarbohyloxyimino)-2-phenylacetonitrile ("BOC-ON", Aldrich) (6.00 g, 24.4 mmol). The mixture was stirred 16 h at 25°C, diluted with H_2O (33 mL) and extracted with diethyl ether (6 × 45 mL). The aqueous phase was acidified to pH 2.5 with cold 2.5N HCl and extracted with CH_2Cl_2 (5 × 20 mL). The organic layers were dried over Na_2SO_4 and concentrated in vacuo to give 5.60 g of golden syrup. The syrup was recrystallized from EtOAc/hexane (50°C → -20°C) to yield 4.02 g (77%) of fine white needles of **29**: mp 76-78.5°C; $[\alpha]_D^{25}$ -17.9 (c 0.1)° (c 2.0, MeOH); IR ($CHCl_3$ cast) 3500-2200 s, br, multi), 1717 (vs, br), 1510 (s), 1394 (s), 1368 (s), 1248 (s), 1164 (vs), 1054 (m) cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ11.35 (s, 1H, COOH), 5.53 (br d, 1H, NH), 4.58 (br m, 1H, CH), 3.02 (~d, 2H, ~6 Hz, CH_2S), 2.18 (s, 3H, CH_3S), 1.47 (s,

9H, tert-Bu); Anal. Calc. for $C_9H_{17}NO_4S$: C, 45.94; H, 7.28; N, 5.95; S, 13.63. Found: C, 46.27; H, 7.39; N, 5.89; S, 13.53; EI-MS: 235.0875 (M^+, 235.0879 calcd.).

^{1}H NMR Study of S-Methylation of N,N'-Bis-(BOC)lanthionine:

Methyl iodide (156 μL, 2.50 mmol) was added to a solution of **28** (120 mg, 0.294 mmol) in d_7-DMF (1.5 mL), and the progress of the reaction was monitored by ^{1}H NMR. After 21.5 h at 25°C in dark, 45 (±4)% of **28** had been S-methylated (see **30** below), however neither prolonged reaction times nor further additions of CH_3I succeeded in driving the reaction past 45% completion. R_f ~0.13 for sulfonium salt (40 MeOH/60 EtOAc/1 HOAc; cf. R_f 0.57 for **28**).

N,N'-Bis(tert-butoxycarbonyl)lanthionine methylsulfonium, tetrafluoborate salt (30).

Silver tetrafluoborate (120 mg, 0.612 mmol) was added to **28** (250 mg, 0.612 mmol) in dry DMF (5.0 mL) followed by methyl iodide (228 μL, 3.67 mmol). The mixture was sealed under Ar and stirred in dark[illegible]8 h; TLC (see above) indicated most of **28** had bee[illegible]sumed. Excess CH_3I was removed in vacuo at 35°C and AgI was precipitated by addition of 2-propanol (25 mL) to the DMF solution. The mixture was stirred 10 min in the dark, solid AgI was removed by filtration, and the filtrate was concentrated

in vacuo to afford a yellow syrup. Trituration of the residue with dry ether removed unreacted **28**, and precipitated the methylsulfonium tetrafluoborate salt **30** as a hygroscopic yellow solid (179 mg, 47%) containing 1.5 mole equivalents of DMF. The DMF could not be removed *in vacuo* without substantial decomposition of **30**: ^{1}H NMR (80 MHz, CD_3CN) δ10.15 (br s, 2H, COOH), 8.01 (br s, 1.5 H, $(CH_3)_2NC(O)H$), 6.30 (br d, 2H, 8 Hz, NH), 4.67 (~dd, 2H, CH), 3.89 (m, 4H, CH_2S^+), 2.94 (s, 4.5H, $CH_3(CH_3)NC(O)H$), 2.79 (s, 4.5H, $CH_3(CH_3)NC(O)H$), 2.74 (s, 3H, S^+CH_3), 1.45 (s, 18H, *tert*-Bu); POSFAB-MS (glycerol) 423 (M^+ = [BOC-NHCH(COOH)CH_2]$_2S^+$Me), 424 (21% of M^+), 425 (8.5% of M^+), 845 ($M^+M^+-H^+$); R_f 0.13 (40 MeOH/60 EtOAc/1HOAc). This material decomposed before it could be fully characterized.

Dimethyl azodicarboxylate (34).

The modification of the method of MacKay and McIntyre[94] was employed. Dimethyl 1,2-hydrazinedicarboxylate[91,124] (154.8 g, 1.05 mol) was suspended in CH_2Cl_2 (1.4 L) with pyridine (84.5 mL, 1.05 mol). *N*-Bromosuccinimide (186.0 g, 1.05 mol) was added slowly over 15 min with rapid stirring. The solution was stirred 30 min and extracted with H_2O (3 × 1.5 L). The dichloromethane phase was dried over Na_2SO_4 and concentrated *in vacuo* at 30°C to produce 171.9 g of bright orange-red liquid. This liquid was distilled *in vacuo*, and early low boiling

fractions as well as the last 7-9% of the residue were discarded (Note, there is a danger of explosion in distillation[91]). The fraction of the distillate boiling at 47°C/0.35 mm Hg provided 126.3 g (83%) of **34** which was stored in dark under Ar at 4°C (solidifies, mp ~10°C): IR (film) 2960 (m), 1780 (vs), 1440 (s), 1244 (vs), 876 (m) cm^{-1}; ^1H NMR (80 MHz, neat with TMS) δ4.10 (s, OCH_3); EI-MS: 59.0182 (Base peak, $COOCH_3$), no M^+ observable.

Chromatographic properties of ROOC-N=N-COOR and ROOC-NHNH-COOR respectively in 45% EtOAc/55% hexane are: R_f 0.58 and 0.13 (±0.05) (R=Me); 0.71 and 0.23 (± 0.06) (R=Et); >0.8 and 0.41 (±0.05) (R=Bn). For comparison **36** has R_f 0.31 (±0.04) in this solvent.

N-(Benzyloxycarbonyl)-D-serine (35b).

This material was prepared according to the general procedure of Greenstein and Winitz.[267] Benzyl chloroformate (37.4 g, 0.22 mol) was added dropwise over 30 min to a vigorously stirred mixture of $NaHCO_3$ (42.0 g, 0.50 mol) and D-serine (Sigma) (21.0 g, 0.20 mol) in H_2O (250 mL). The mixture was stirred 1 h, extracted with Et_2O (50 mL), cooled on ice/H_2O, and acidified (pH 2.0) by careful addition of 5.7N HCl. The slurry was extracted with EtOAc (4 × 250 mL) and organic phases were pooled, dried over Na_2SO_4 and evaporated *in vacuo* to 32.5 g (68%) of white foam. This material was recrystallized from EtOAc/hexane to afford 30.0 g (62%) of Z-D-serine (**35b**): mp 117-119°C

(lit. mp 117-119°C[267b]); $[\alpha]_D^{25}$ -5.83° (c 7.0, HOAc) (lit. $[\alpha]_D^{25}$ -5.6° (c 7, HOAc),[267a] -5.8° (c 6, HOAc)[267b]); IR (KBr disk) 3440 (m, br), 3337 (s), 3318 (s), 3200 (m, br), 1747 (s), 1690 (vs), 1531 (s), 1247 (vs), 1059 (s), 1029 (s), 697 (s) cm^{-1}; ^1H NMR (300 MHz, d_6-acetone) δ7.38 (m, 5H, Ph), 6.43 (br, 1H, NH), 5.08 (s, 2H, $PhCH_2$), 4.30 (m, 1H, CH), 4.25 (br s, 1H, OH), 3.93 (m, 2H, CH_2OH); EI-MS: 239.0789 (239.0794 calcd. for $C_{11}H_{13}NO_5$); POSFAB-MS (glycerol) 240 (MH^+), 479 (M_2H^+).

N-(Benzyloxycarbonyl)serine β-lactones (36a, 36b, 36d) and benzyl N-vinylcarbamate (37).[268]

To a stirred suspension of Ph_3P (6.43 g, 24.5 mmol; dried *in vacuo* over P_2O_5) in dry CH_3CN/THF (10:1, 110 mL) at -50°C was added dimethyl azodicarboxylate (**34**) (2.70 mL, 3.58 g, 24.5 mmol) dropwise over 10 min. The mixture was stirred 10-15 min at -50°C until the orange color disappeared and a slurry of white solid formed. A solution of Z-serine (**35a**, **35b** or **35d**) (5.84 g, 24.4 mmol; dried *in vacuo* over P_2O_5) in CH_3CN/THF (8:1, 80 mL) was added dropwise over 15 min to the well-stirred slurry at -50°C. The mixture was stirred 20-30 min at -50°C, and 2 h at 25°C. Solvent was removed *in vacuo* at 35°C and the residue was flash chromatographed on silica gel[260] (45% EtOAc/55% hexane) to afford 4.10-4.37 g of **36**. This material could be recrystallized as fine white needles with 91-95% recovery by dissolving in a minimal volume of

EtOAc at 45-50°C, adding two volumes of warm CCl_4, followed by hexane to permanent cloudiness and cooling to -20°C.

Early fractions from the column yielded 0.56 to 0.73 g (13-17%) of moisture and acid sensitive benzyl vinylcarbamate (**37**) which could be freed of its decomposition products by bulb-to-bulb distillation (0.1 mm Hg/90°C); mp 41-43°C (lit. mp 43-44°C[269]); IR ($CHCl_3$ cast) 3320 (s, br), 1706 (vs), 1649 (s), 1520 (s), 1499 (s), 1450 (m), 1402 (s), 1260 (vs), 1090 (s), 696 (s) cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ7.34 (s, 5H, Ph), 7.30 (br s, 1H, NH), 6.90-6.48 (m, 1H, N-CH), 5.15 (s, 2H, CH_2Ph), 4.48 (~d, 1H, ~16 Hz, Z-CHH), 4.27 (~d, 1H, ~8 Hz, E-CHH); Anal. Calc. for $C_{10}H_{11}NO_2$: C, 67.77; H, 6.26; N, 7.90. Found: C, 67.27; H, 6.34; N, 8.00; EI-MS: 177.0792 (M^+, 177.0790 calcd.); R_f 0.70 (45% EtOAc/55% hexane).

For L-Z-serine β-lactone (36a):[120] mp 133-134°C (dec); $[\alpha]_D^{25}$ -26.8 (±0.1)° (c 1.0 to 5.0, CH_3CN); IR (CH_2Cl_2 cast) 3350 (m), 1845 (s, sh), 1830 (s), 1685 (vs), 1530 (vs), 1270 (s) cm^{-1}; ε_{1847} cm^{-1} (0.1 mm KBr, THF) 0.25 mL mg^{-1} mm^{-1}, 57 M^{-1} mm^{-1}; ^1H NMR (100 MHz, CD_2Cl_2) δ7.34 (s, 5H, Ph), 5.84-5.50 (br d, 1H, 8 Hz, NH), 5.14 (s, 2H, CH_2Ph), 5.02 (dd, 1H, 6, 8 Hz, CH), 4.43 (~d, 2H, 6 Hz, CH_2O); ^{13}C NMR (50.32 MHz, CD_2Cl_2) δ169.2, 155.8, 136.4, 129.0, 128.9, 128.7, 68.1, 66.6, 60.3; Anal. Calc. for $C_{11}H_{11}NO_4$: C, 59.72; H, 5.01; N, 6.33. Found: C, 59.60; H, 5.10; N, 6.21; EI-MS: 221.0691 (M^+, 221.0688

1227 (s, br), 1071 (m), 726 (m), 694 (m) cm^{-1}; ^1H NMR (80 MHz, d_6-DMSO) δ8.20 (d, 1H, 8 Hz, NH), 7.27 (s, 5H, Ph), 4.33 (m, 1H, CH), 3.71 (d , Hz, CH_2O), 3.55 (s, 2H, CH_2Ph); Anal. Calc. for $C_{11}H_{13}NO_4$: C, 59.19; H, 5.87; N, 6.27. Found: C, 59.04; H, 5.90; N, 6.40; EI-MS: 223.0845 (M^+, 223.0845 calcd.).

N-(Phenylacetyl)-L-serine β-lactone (39) and 2-(2-Phenylacetamido)ethylene (40).[268]

The lactonization of N-(phenylacetyl)-L-serine (**38**) (5.45 g, 24.4 mmol) was performed as outlined for the preparation of **36** above except that diethyl azodicarboxylate (3.86 mL, 24.5 mmol) was used in place of DMAD (**34**). Flash chromatography on silica gel[260] (35% EtOAc/65% hexane followed by 60% EtOAc/40% hexane) provided 3.82 g (76%) of β-lactone **39** and 905 mg (23%) of **40**. The β-lactone **39** was recrystallized from EtOAc/hexane, whereas **40** was recrystallized from CH_2Cl_2/hexane for analysis.

For 39: mp 118-119°C (lit. mp 122-123°C[271]); $[\alpha]_D^{25}$ -31.7 (±0.2)° (c 2.0, CH_3CN); IR (CH_2Cl_2 cast) 3320 (m), 1846 (m), 1819 (m), 1651 (vs), 1533 (s), 1102 (m), 892 (s), 697 (s) cm^{-1}; ^1H NMR (400 MHz, d_6-DMSO) δ8.68 (d, 1H, 7.6 Hz, NH), 7.13 (m, 5H, Ph), 5.09 (m, 1H, CH), 4.25 (m, 2H, CH_2O), 3.46 (s, 2H, CH_2Ph); ^{13}C NMR (100.6 MHz, d_6-DMSO) δ171.2, 169.9, 135.4, 129.1, 128.3, 126.6, 65.2, 58.0, 41.7; Anal. Calc. for $C_{11}H_{11}NO_3$: C, 64.38; H, 5.40;

N, 6.83. Found: C, 64.09; H, 5.45; N, 6.65; EI-MS: 205.0734 (M^+, 205.0739 calcd.), 175.0632 ($M-CH_2O$), 161.0837 ($M-CO_2$), 118.0418 ($PhCH=C=O$), 91.0532 (C_7H_7); R_f 0.35 (60% EtOAc/40% hexane).

For 40: mp 82-83°C; IR ($CHCl_3$ cast) 3235 (m), 1662 (m), 1635 (vs), 1530 (m), 1455 (w), 1422 (w), 1399 (w), 1263 (s), 1190 (m), 980 (m), 869 (m), 771 (m), 721 (m), 697 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ8.34 (d, 1H, 9.2 Hz, NH), 7.07 (m, 5H, Ph), 6.76 (m, 1H, CH_2CH), 4.47 (d, 1H, 15.4 Hz, Z-CHH), 4.26 (d, 1H, 8.4 Hz, E-CHH), 3.40 (s, 2H, $PhCH_2$); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ169.2 (s), 134.6 (s), 129.3 (d), 128.8 (d), 128.7 (d), 127.2 (d), 96.2 (t), 43.2 (t); Anal. Calc. for $C_{10}H_{11}NO$: C, 74.51; H, 6.87; N, 8.69. Found: C, 74.77; H, 6.87; N, 8.70; EI-MS: 161.0832 (M^+, 161.0841 calcd.), 118.0405 ($PhCH=C=O$); R_f 0.68 (60% EtOAc/40% hexane).

HPLC Studies of N-(Phenylacetyl)-L-Serine Lactonization:

These investigations were carried out using a Whatman Partisil M9 10/25 Silica column (25 x 9.4 cm) with a binary solvent gradient (A = hexane, B = EtOAc) and a detection at 260 nm (Program: 2.00 mL/min; 0 min (45% B), 18 min (75% B), 25 min (100% B), 40 min (100% B), 45 min (45% B), 60 min (45% B). Solutions of analytically pure **38**, **39** and **40** were used to calibrate the instrument on alternate runs and results were reproducible within ±2.0%. Reactions for HPLC analyses were carried out on

0.6 mmol scale according to the method used for **36** making appropriate changes as described in Table 2. Under the above conditions retention times for Ph_3P, DEAD, **40**, **39** and $Ph_3P{=}O$ were: 6.09 (±0.05), 6.95 (±0.09), 9.23 (±0.02), 17.94 (±0.10) and 34.9 (±0.4) min, respectively.

N-(tert-Butoxycarbonyl)-D-serine (41b).

The general method of Moroder et al.[265] was used. To a stirred solution of D-serine (Sigma) (12.5 g, 118 mmol) in dioxane/H_2O (2:1, 300 mL) and 1N NaOH (120 mL) was added di-tert-butyl pyrocarbonate (28.5 g, 130 mmol). The mixture was stirred 40 min and the volume reduced to 150 mL in vacuo at 35°C. After acidification (pH 2.25) with 1N H_2SO_4, EtOAc (3 × 100 mL) was used to extract the product. Organic layers were dried over Na_2SO_4 and concentrated in vacuo to a colorless oil which crystallized on treatment with hexane. This crude material was recrystallized from EtOAc/hexane to yield 14.7 g (61%) of **41b**: mp 88-89°C (lit. mp 75-78°C,[272a] 88-89°C[272b]); $[\alpha]_D^{25}$ -7.0° (c 1.0, CH_3CN); IR (CH_2Cl_2 cast) 1715 (vs), 1689 (vs), 1519 (m), 1395 (m), 1368 (m), 1168 (s) cm^{-1}; ^1H NMR (80 MHz, CD_3CN) δ10.1 (br s, 1H, COOH), 5.3-5.6 (br s, 2H, NH and OH), 4.0-4.3 (m, 1H, CH), 3.8-3.65 (m, 2H, CH_2OH), 1.43 (s, 9H, tert-Bu); Anal. Calc. for $C_8H_{15}NO_5$: C, 46.82; H, 7.37; N, 6.83. Found: C, 46.75; H, 7.25; N, 6.66.

N-(tert-Butoxycarbonyl)serine β-lactones (42a and 42b).[268]

These compounds were prepared from the corresponding L- and D-BOC-serines (**41a** and **41b**) (5.00 g, 24.4 mmol) in either THF at -78°C (60 ± 5% isolated yield), or CH_3CN/THF at -50°C (9:1; 70 ± 2% yield) according to the procedure outlined for **36**. Isolation by flash chromatography on silica[260] (35% EtOAc/65% hexane) provides 2.51 to 3.29 g of β-lactone which can be recrystallized as described for **36** above.

For the L-isomer (42a): mp 119.5-120.5°C (dec); $[\alpha]_D^{25}$ -26.7 (±0.2)° (c 1.0, CH_3CN); IR (CH_2Cl_2 cast) 3358 (s), 1836 (s), 1678 (vs), 1532 (s), 1291 (m), 1104 (s) cm^{-1}; $\varepsilon_{1847\ cm^{-1}}$ (0.1 mm KBr, THF or CH_3CN) 0.34 mL mg^{-1} mm^{-1}, 64 M^{-1} mm^{-1}; ^1H NMR (200 MHz, CD_2Cl_2) δ5.53 (br s, 1H, NH), 5.05 (dd, 1H, 8, 6 Hz, CH), 4.47 (~d, 2H, 6 Hz, CH_2O), 1.47 (s, 9H, tert-Bu); ^{13}C NMR (50.32 MHz, CD_2Cl_2) δ170.0 (s), 155.1 (s), 81.5 (s), 66.6 (t), 59.9 (d), 28.2 (q); Anal. Calc. for $C_8H_{13}NO_4$: C, 51.33; H, 7.00; N, 7.48. Found: C, 51.04; H, 6.97; N, 7.42; EI-MS: 188.0929 (MH^+, 188.0923 calcd.); CI-MS (NH_3) 205 ($M+NH_4^+$), 392 ($2M+NH_4^+$).

For the D-antipode (42b): mp 119-121°C (dec); $[\alpha]_D^{25}$ +26.6 (±0.2)° (c 1.0, CH_3CN); IR, NMR, MS, and chromatographic properties were identical to **42a**.

N-Benzylserines (43a and 43b).[268]

The procedure of Quitt et al.[273a] was used to produce

the L- (**43a**) and D- (**43b**) isomers from L- and D-serine (Sigma), respectively. Distilled benzaldehyde (20.3 mL, 200 mmol) was added to serine (21.2 g, 200 mmol) in 2N NaOH (100 mL). The solution was stirred 30 min under Ar, cooled to 4°C and $NaBH_4$ (2.28 g, 60.0 mmol) was added in small portions over 15 min. The mixture was stirred 1 h and the procedure was repeated with the same quantities of benzaldehyde and sodium borohydride. The mixture was stirred 2 h at 25°C and extracted with ether (3 × 75 mL). The aqueous phase was cooled on ice/H_2O and carefully acidified to pH 6-6.5 with 2N HCl. After 2 h at 4°C the white precipitate was filtered and recrystallized from water to give 13.9 g (36%) of N-benzylserine (**43a** or **43b**).

For L-N-benzylserine (43a): mp 220-222°C (dec, darkens at 216°C) (lit. mp 240°C [273a] 220-222°C (dec)[273b]; $[\alpha]_D^{25}$ +5.1 (±0.1)° (c 1.0, 6N HCl) (lit. $[\alpha]_D^{21}$ + 5.1° (c 1, 6N HCl)[273a]; IR (KBr disk) 3650-2200 (s, br, mult), 3000 (s), 1643 (vs), 1590 (s), 1555 (s), 1456 (m), 1400 (m), 1376 (m), 1330 (m), 1066 (s), 730 (m), 695 (s) cm^{-1}; ^1H NMR (300 MHz, D_2O + DCl) δ7.52 (s, 5H, Ph), 4.36 (s, 2H, CH_2Ph), 4.19 (m, 1H, CH), 4.14 (m, 2H, CH_2OH); EI-MS: 195.0883 (M^+, 195.0896 calcd. for $C_{10}H_{13}NO_3$); POSFAB-MS (glycerol/formic acid) 196 (MH^+), 391 (M_2H^+); R_f 0.62 (System D).

For the D-isomer (43b): mp 221-223°C (dec); $[\alpha]_D^{25}$ -5.1 (±0.1)° (c 1.0, 6N HCl); IR, NMR, MS, and

chromatographic properties were identical to **43a**.

N-Benzyl-N-(benzyloxycarbonyl)serines (44a and 44b).

Benzyl chloroformate (3.4 mL, 4.06 g, 23.8 mmol) was added dropwise over 30 min to a chilled (5 °C) solution of N-benzyl-D-serine (**43b**) (3.0 g, 15.4 mmol) in 2N NaOH (7.5 mL) and THF (2.5 mL) with vigorous stirring. Throughout the addition the apparent pH was maintained between 9.5-10.5 with 1N NaOH. The mixture was stirred 20 min, acidified to pH 2.0 with 2N HCl at 5 °C and extracted with EtOAc (3 x 75 mL). The crude product obtained on evaporation of the organic phases was purified by reverse phase MPLC (65% MeCN/H_2O, 3.0 mL/min) to afford 2.0-2.38 g (40-47%) of **44b** as a colorless syrup: $[\alpha]_D^{25}$ +25.2 (±.3)° (c 0.8, $CHCl_3$); IR ($CHCl_3$ cast) 3640-3100 (m, br), 1740 (m), 1702 (s), 1685 (s), 1454 (m), 1428 (m), 1247 (s), 699 (s) cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$)[202] δ7.27 (br s, 10H, Ph), 6.72 (br s, 2H, COOH, OH), 5.12 (s, 2H, OCH_2Ph), 4.65 (s, 0.75 x 2H) and 4.59 (s, 0.25 x 2H) (NCH_2), 4.30-3.50 (m, 3H, $CHCH_2OH$); Anal. Calc. for $C_{18}H_{19}NO_5$: C, 65.64; H, 5.81; N, 4.25. Found: C, 65.64; H, 5.75; N, 4.06; EI-MS: 329.1265 (M^+, 329.1263 calcd.); R_f 0.55 (10 MeOH/90 CH_2Cl_2/1 HOAc).

The L-isomer (**44a**) was prepared in an analogous manner from **43a** and possessed chromatographic and spectral properties identical to **43b**: $[\alpha]_D^{25}$ -24.4° (c 1.3, $CHCl_3$); Anal. Found: C, 65.42; H, 5.72; N, 4.25.

N-Benzyl-N-(benzyloxycarbonyl)serine β-lactones (45a and 45b).[268] These compounds were prepared in THF at -78°C from **44a** and **44b**, respectively, according to the previously outlined procedure for Z-serine β-lactones (**36**). Isolation by flash chromatography on silica[260] (25% EtOAc/hexanes) afforded β-lactone (71%) which was recrystallized as white needles from Et_2O or CCl_4/hexane: mp 73-74°C (L, **45a**), 75.5-76.0°C (D, **45b**); $[\alpha]_D^{25}$ -9.3° (**45a**), +9.5° (**45b**) (c 1.1, THF); IR ($CHCl_3$ cast) 1833 (vs), 1702 (vs), 1454 (m), 1423 (m), 1246 (s), 1107 (m), 699 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$)[202] δ7.50-7.10 (m, 10H, 2 Ph), 5.40-5.12 (m, 2H, $PhCH_2O$), 5.00-4.82 (m, 1H, CH), 4.58 (br s, 2H, $PhCH_2N$), 4.42 (~br s, 0.63H) and 4.27-4.08 (m, 1.37H, $CHCHHO$); ^{13}C NMR (75.5 MHz, $CDCl_3$)[202] 168.5 and 167.1, 155.4, 136.5, 129.1, 128.7, 128.5, 128.1, 127.5, 127.2, 69.0, 68.4, 65.8, 64.9, 51.9; Anal. Calc. for $C_{18}H_{17}NO_4$: C, 69.44; H, 5.50; N, 4.50. Found: C, 69.44; H, 5.49; N, 4.44 (for **45a**) and C, 69.57; H, 5.43; N, 4.73 (for **45b**); EI-MS: 311.1157 (M^+, 311.1157 calcd.); CI-MS (NH_3) 329 ($M+NH_4^+$), 312 (MH^+).

N-Acetyl-DL-serine β-lactone (46).

Dimethyl azodicarboxylate (**34**) (1.08 mL, 9.80 mmol) was added dropwise over 5 min to a rapidly stirred suspension of triphenylphosphine (2.57 g, 9.79 mmol; dried *in vacuo* over P_2O_5) in dry acetonitrile (60 mL) at -42°C. After stirring 10 min, a solution of anhydrous N-

acetyl-DL-serine (1.25 g, 8.49 mmol) in CH_3CN (50 mL)/HMPA (5.0 mL) was added dropwise over 10 min at -42°C. The mixture was stirred 10 min at -42°C and 3 h at 25°C, and then concentrated in vacuo at 30°C. Flash chromatography on silica[260] using 58% THF/42% toluene provided crude **46** (containing some $Ph_3P=O$ and HMPA) and 0.18 g (14%) of N-acetyl-DL-serine starting material. Rechromatography (flash) of the crude product using 75% THF/25% toluene afforded pure N-acetyl-DL-serine β-lactone (**46**) (0.56 g, 51% yield or 60% yield based on recovered starting material). This white solid was hygroscopic and quickly turned brown in air thereby making analysis difficult. Two-dimensional TLC indicates appreciable decomposition on silica. Substitution of n-Bu_3P (2.44 mL, 9.77 mmol) for Ph_3P in the above procedure results in retardation of the reaction (~8 h required) and reduced yields of β-lactone (0.36 g, 33%), but does facilitate purification (85% EtOAc/15% toluene, single flash column) from the phosphine oxide byproduct.

For N-acetyl-DL-serine β-lactone (46): IR (KBr disk) 3420 (s, br), 1845 (m), 1653 (s), 1540 (s), 1376 (m), 1369 (m), 1107 (m), 890 (m), 614 (m) cm^{-1}; ^1H NMR (400 MHz, d_6-DMSO) δ8.72 (br d, 1H, NH), 5.16 (m, 1H, CH), 4.34 (m, 2H, CH_2O), 1.87 (s, 3H, CH_3COO); Anal. Calc. for $C_5H_7NO_3$: C, 46.51; H, 5.46; N, 10.85. Found: C, 45.87; H, 5.60; N, 10.30 (sample rapidly takes on moisture); EI-MS: 129.0423 (M^+, 129.0426 calcd.), 114.0191 ($M-CH_3$),

101.0476 (M-CO), 85.0528 ($M-CO_2$).

D-Serine ethyl ester hydrochloride (47).

This compound was prepared analogous to D-serine methyl ester (**17**)[274] from D-serine (Sigma) (110 g, 1.05 mol) and HCl(g) (~0.91 kg) in anhydrous EtOH (3 L). Recrystallization from anhydrous EtOH/Et_2O provided 164 g (92%) of **47** after drying in vacuo over P_2O_5 and KOH pellets: mp 129-130°C (lit. mp 130-131°C,[274] for L-isomer); $[\alpha]_D^{25}$ +4.76 (±.05) (c 2.1, H_2O) (lit. $[\alpha]_D^{25}$ -4.8° (c 2.1, H_2O) for L-isomer[274]); IR (KBr disk) 3420 (s, br), 3250-2400 (m, br), 1970 (m, br), 1743 (s), 1599 (m), 1502 (m), 1477 (m), 1274 (s), 1247 (s), 1148 (m), 1116 (m), 1090 (s), 1023 (s), 552 (s) cm^{-1}; ^1H NMR (80 MHz, D_2O) δ4.50-4.00 (overlapping m's, 5H, $CHCH_2OH$, OCH_2CH_3 (q, 7 Hz, at 4.35)), 1.31 (t, 3H, 7 Hz, OCH_2CH_3); POSFAB-MS (glycerol) 134 (MH^+), 267 (M_2H^+), 400 (M_3H^+).

N-Trityl-D-serine ethyl ester (48).

The general procedure of Zervas and Theodoropoulos[126] was utilized. To a stirred suspension of D-serine ethyl ester hydrochloride (**47**) (100.0 g, 0.590 mol) in $CHCl_3$ (850 mL) at 5°C was added triethylamine (181 mL, 1.30 mol) followed by trityl chloride (165 g, 0.590 mol) in several portions over 10 min. The mixture was stirred 12 h at 25°C and the orange solution was washed with H_2O (2 × 1 L), dried over Na_2SO_4, and concentrated in vacuo at

45°C. After several days at 0.005 torr, 214 g (96%) of an orange foamy solid (**48**) was obtained which was used directly in the preparation of N-trityl-D-serine (**49**) below. ^{1}H NMR and TLC indicated ~5-7 mol% of trityl ethyl ether impurity was present.

For 48: IR ($CHCl_3$ cast) 3480 (m, br), 3100-2860 (m, mult), 1728 (vs), 1490 (m), 1470 (m), 1447 (s), 1329 (m), 1185 (vs), 1050 (m), 1030 (s), 759 (vs), 748 (vs), 707 (vvs) cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$) δ 7.62-7.00 (m, 15H, Ph), 3.83-3.39 (overlapping m's, 5H, $CHCH_2OH$, OCH_2CH_3), 2.70 (br s, 2H, OH, NH), 1.00 (t, 3H, 7 Hz, OCH_2CH_3); EI-MS: 375.1828 (M^+, 375.1835 calcd. for $C_{24}H_{25}NO_3$); R_f 0.47 (45% EtOAc/hexane).

N-Trityl-D-serine (49).

A modified procedure of Guttmann and Boissanoi[127] was used. N-Trityl-D-serine ethyl ester (**48**) (90.0 g, 0.240 mol) was heated to boiling for 2 minutes in 3.5N ethanolic KOH (180 mL). The mixture was cooled to 0°C and 1N H_3PO_4 (900 mL) was added slowly with stirring. H_2O (1 L) was added and the pH was adjusted to 3.5 with 1N H_3PO_4. The resulting white precipitate was filtered, and washed free of acid with H_2O (3 x 500 mL) by resuspending, stirring, and filtering. The residue (83.1 g, 99%) was dried *in vacuo* over P_2O_5 and then recrystallized from hot THF by addition of two volumes CCl_4, followed by hexane and cooling to -20°C to yield 72.0 g (86%) of N-trityl-D-

serine (**49**): mp 154-156°C (dec) (lit. mp 160°C[127] for L-isomer); $[\alpha]_D^{25}$ -8.9 (±.1)° (c 1.0, MeOH) (lit. $[\alpha]_D^{21}$ +9 (±2)° (c 1, MeOH)[127] for L-isomer); IR (CH_2Cl_2 cast) 3320 (m, br), 3100-3000 (s), 1650 (m), 1595 (s, br), 1492 (m), 1448 (s), 1385 (m), 1030 (m), 747 (m), 704 (vs) cm^{-1}; ^1H NMR (80 MHz, d_6-DMSO) δ7.63-7.10 (m, 15H, Ph), 4.18 (br s, 3H, NH_2^+, OH), 3.10-2.65 (overlapping m's, $CHCH_2OH$); Anal. Calc. for $C_{22}H_{21}NO_3$: C, 76.06; H, 6.09; N, 4.03. Found: C, 75.78; H, 6.01; N, 3.92; EI-MS: 243.1174 (Ph_3C, 243.1174 calcd.); POSFAB-MS (glycerol) 348 (MH^+), 243 (Ph_3C^+), 106 ($(MH^+-Ph_3C^+)H^+$); R_f 0.41 (10% MeOH/90 CH_2Cl_2).

N-(tert-Butoxycarbonyl)-L-threonine (50).

The general procedure of Paleveda et al.[118] was adapted. Triethylamine (42 mL, 30.5 g, 0.30 mol) was added to L-threonine (Sigma) (23.82 g, 0.200 mol) in H_2O/dioxane (1:1, 240 mL). The mixture was stirred 10 min at 25°C and N-(tert-butoxycarbonyloxyimino)-2-phenylacetonitrile ("BOC-ON"; 54.2 g, 0.22 mol) was added. The mixture was stirred 4.5 h, diluted with H_2O (300 mL), and extracted with ether (6 x 250 mL). The aqueous phase was cooled, acidified (pH 2.5) with cold 2.5N HCl, and extracted with CH_2Cl_2 (5 x 200 mL). The CH_2Cl_2 phases were dried over Na_2SO_4 and concentrated in vacuo to provide 25.1 g (57%) of crude white solid. This material was passed through a filtration column of silica in 85%

EtOAc/hexane. The recovered syrup crystallized on standing at -2 °C under hexane (yield 23.17 g, 53%): mp 77-81°C (lit. mp 76-80°C,[275a] 74-77°C[272a]); $[\alpha]_D^{25}$ -9.0 (±0.1)°, $[\alpha]_{578}^{25}$ -9.5 (±.1)° (c 1.0, HOAc) (lit. $[\alpha]_{578}$ -9.5° (c 1, HOAc)[272]); IR ($CHCl_3$ cast) 3600-2200 (m, br, mult), 1719 (vs), 1691 (vs), 1517 (s), 1392 (s), 1368 (s), 1165 (vs), 1068 (m) cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ7.83 (br s, 2H, OH, COOH), 5.80 (br s, 1H, NH), 4.38 (m, CHCHOH), 1.47 (s, 9H, tert-Bu); Anal. Calc. for $C_9H_{17}NO_5$: C, 49.31; H, 7.82; N, 6.39. Found: C, 49.53; H, 7.76; N, 6.39; EI-MS: 220.1176 (MH^+, 220.1186 calcd.); CI-MS (NH_3) 237 ($M+NH_4^+$), 220 (MH^+).

N-(tert-Butoxycarbonyl)-L-allo-threonine (51).

L-allo-Threonine (Aldrich, $[\alpha]_D$ +9.0° (c 2.0, H_2O)) (183 mg, 1.54 mmol) was dissolved in pH 10.0, 1.0 M sodium carbonate/bicarbonate (6 mL), and THF (3 mL) and di-tert-butyl pyrocarbonate (504 mg, 2.31 mmol) were added. The mixture was stirred vigorously for 2.5 h, carefully acidified to pH 2.5 with 3N HCl, and extracted with EtOAc (3 x 10 mL). Ethyl acetate phases were pooled, dried over Na_2SO_4 and evaporated in vacuo. The residue of colorless syrup was recrystallized from CCl_4/hexane to provide 292.3 mg (87%) of BOC-allo-L-threonine (**51**): mp 113-115°C (lit. mp 115°C,[276a] 118-120°C[276b]); $[\alpha]_D^{25}$ 0.0°, $[\alpha]_{365}^{25}$ +3.5 (±.1)° (c 1.0, MeOH) (lit. $[\alpha]_D^{28}$ 0.0°, $[\alpha]_{365}^{20}$ +3.1° (c 2.0, MeOH);[276a] $[\alpha]_D^{20}$ -7.5° (c [redacted] MeOH)[276b]); IR ($CHCl_3$

cast) 3340 (m, br), 2960 (s), 1715 (vs), 1695 (vs), 1512 (s), 1451 (m), 1392 (m), 1368 (s), 1250 (m), 1164 (vs), 754 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ7.20 (br s, 1H, COOH), 5.69 (d, 1H, 7.2 Hz, NH), 4.42-4.30 (m, 1H, CHOH), 4.27-4.15 (m, 1H, NCH), 4.10 (br s, 1H, OH), 1.53 (s, 9H, tert-Bu), 1.28 (m, 3H, $CHCH_3$); Anal. Calc. for $C_9H_{17}NO_5$: C, 49.31; H, 7.82; N, 6.39. Found: C, 49.03; H, 7.91; N, 6.13; CI-MS (NH_3) 220 (MH^+), 237 ($M+NH_4^+$), 163 ($MH^+-C_4H_8$).

Attempted Lactonizations of N-Protected Threonines: E-1-[N-(tert-Butoxycarbonyl)amino]propene (52).

Dimethyl azodicarboxylate (**34**) (0.60 mL, 5.5 mmol) was added dropwise over 5 min to a solution of Ph_3P (1.44 g, 5.5 mmol) in THF (25 mL) at -78°C. The mixture was stirred 15 min at -78°C until the orange color vanished and a slurry of white solid formed. BOC-L-Threonine (**50**) (1.10 g, 5.01 mmol) was added dropwise in THF (25 mL) over 10 min at -78°C, and the solution was stirred 20 min at -78°C and 1.5 h at 25°C. No β-lactone (λ_{max} = 1820 cm^{-1})[79b] could be detected at any time in the reaction by IR of the mixture, even after 16 h at 25°C. A single product (R_f 0.74, 25% EtOAc/hexane) running just behind Ph_3P (R_f 0.85) was observed. In order to ensure isolation of all product(s), unreacted Ph_3P was consumed by addition of a few μL of DMAD (**34**) and a filtration column (120 × 5 cm) of silica (40-63 μm) was employed. All eluant (25% EtOAc/hexane) was pooled until dimethyl 1,2-hydrazodi-

carboxylate emerged and concentrated in vacuo to afford 0.69 g (88%) of **52** as white needles. This material was chromatographically and analytically pure and all analyses were performed without recrystallization. The product (**52**) was sensitive to acid and moisture and decomposed slowly in $CDCl_3$ (50% in 3 days): mp 65.0-65.5°C; IR ($CHCl_3$ cast) 3325 (m), 2980 (m), 1710 (m), 1693 (s), 1679 (vs), 1521 (vs), 1367 (s), 1307 (vs), 1248 (s), 1167 (s), 1119 (s), 1018 (m), 950 (s), 861 (m), 680 (m) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ6.60 (br s, 1H, NH), 6.45 (t, 1H, 13 Hz, N-CH), 5.06-4.84 (d of q, 1H, 6.4, 13 Hz (J_{trans} HC=CH), E-CHCH$_3$), 1.62 (d, 3H, 6.4 Hz, CHCH$_3$), 1.47 (s, 9H, tert-Bu); ^{13}C NMR (90.56 MHz, $CDCl_3$) δ152.84 (s), 124.62 (d), 104.20 (d), 79.84 (s), 28.17 (q), 14.37 (q); Anal. Calc. for $C_8H_{15}NO_2$: C, 61.12; H, 9.62; N, 8.91. Found: C, 61.03; H, 9.59; N, 8.85; EI-MS: 157.1106 (M^+, 157.1103 calcd.), 101.0481 ($M-C_4H_8$); CI-MS (NH_3) 158 (MH^+), 175 ($M+NH_4^+$).

Z-1-[N-(tert-Butoxycarbonyl)amino]propene (53).

This material was obtained from the reaction of DMAD (**34**) (72.3 μL, 0.664 mmol), Ph_3P (174.1 mg, 0.664 mmol) and BOC-allo-L-threonine (**51**) (97.0 mg, 0.44 mmol) according to the procedure described for **52** above. No β-lactone was detected by IR or TLC in the reaction mixture at any point. A single product (R_f 0.78, 25% EtOAc/hexane (cf. R_f 0.74 for **52**)) was observed, and isolated as

outlined for **52**. Evaporation of the solvent *in vacuo* at 30°C provided 54.2 mg (78%) of **53** as white needles. This material was considerably more labile than **52**, and was decomposed rapidly by acid or moisture, and on standing in $CDCl_3$ (~50% in 8 h): mp 74.5-75.0°C (cf. mp 65.0-65.5°C for **52**); IR ($CHCl_3$ cast) 3300 (m), 1709 (m), 1677 (vs), 1515 (s), 1366 (m), 1250 (m), 1160 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ6.42 (t, 1H, 10.0 Hz, N-CH), 6.14 (s, 1H, NH), 4.70-4.56 (m, 1H, $CHCH_3$), 1.56 (dd, 3H, 7.3, 1.6 Hz (J_{trans} $HC=CCH_3$), CH_3), 1.49 (s, 9H, tert-Bu); ^{13}C NMR (75.46 MHz, $CDCl_3$) δ152.85 (s), 123.16 (d), 101.91 (d), 80.33 (s), 28.32 (q), 10.53 (q); EI-MS: 157.1102 (M^+, 157.1103 calcd.), 101.0477 ($M-C_4H_8$); CI-MS (NH_3) 158 (MH^+), 175 ($M+NH_4^+$).

L- and D-N-(Benzyloxycarbonyl)-β-bromoalanines (55a and 55b).[129,268]

$MgBr_2$-etherate was prepared by dropwise addition of Br_2(l) (dist. from P_2O_5) (1.0 mL, 19 mmol) to a suspension of excess Mg filings (1.0 g, 41 mmol) in Et_2O (20 mL) at 0°C in a flask equipped with an acetone/CO_2(s) condensor. Following the disappearance of Br_2, dry benzene (5 mL) was added and an aliquot (2 mL, 1.6 mmol $MgBr_2$) of this solution was added to Z-L-serine β-lactone (**36a**) (100 mg, 0.452 mmol) in Et_2O (20 mL)/THF (2 mL). After 5 min, the suspension was cooled to 5°C and 1N H_3PO_4 (20 mL) was added carefully. The Et_2O phase was separated

and the aqueous phase extracted with Et_2O (3 × 15 mL). The etheral extracts were dried over Na_2SO_4 and evaporated in vacuo to obtain 136 mg (99%) of chromatographically pure **55a**. This material could be recrystallized as light-sensitive white needles from EtOAc/hexane or CH_2Cl_2/hexane (67% recrystallized yield): mp 70-71°C; $[\alpha]_D^{25}$ +14.2 (±0.2)° (c 1.0, MeOH); IR (KBr disk) 3390 (s), 1730 (vs), 1648 (s), 1525 (s), 1433 (m), 1290 (m), 1180 (m), 1072 (m), 992 (m), 756 (m), 698 (m) cm^{-1}; ^1H NMR (80 MHz, CD_2Cl_2) δ8.63 (br s, 1H, COOH), 7.38 (s, 5H, Ph), 5.75 (br s, 1H, NH), 5.15 (s, 2H, CH_2Ph), 4.87 (m, 1H, CH), 3.83 (m, 2H, CH_2Br); Anal. Calc. for $C_{11}H_{12}NO_4Br$: C, 43.73; H, 4.00; N, 4.64; Br, 26.4. Found: C, 43.91; H, 4.10; N, 4.92; Br, 26.63; EI-MS: 302.9929 (302.9929 calcd. for $C_{11}H_{12}NO_4{}^{81}Br$); POSFAB-MS (glycerol) 302 (MH^+), 304 ($(M+2)H^+$, 99% of MH^+), 603, 605, 607 (M_2H^+ peaks in ~1:2:1 ratio); R_f 0.79 (40 $CHCl_3$/60 MeOH).

The D-antipode (**55b**) was obtained in an identical manner from Z-D-serine β-lactone (**36b**): mp 68-69.5°C; $[\alpha]_D^{25}$ -14.2 (±0.2)° (c 1.0, MeOH) (cf. **55a** above); IR, NMR and MS properties were identical to those of **55a**; Anal. Found: C, 43.50; H, 4.00; N, 4.48.

N-(Benzyloxycarbonyl)-β-chloro-D-alanine (56b).[268]

Into a stirred flas containing Mg filings (5 g, 206 mmol) suspended in Et_2O (50 mL), and equipped with an acetone/CO_2(s) condensor, was condensed Cl_2(l) (4.0 mL, 88

mmol) at -78°C in the dark. The mixture was allowed to react 2 h and 1 mL of the resulting suspension (~1.76 mmol of $MgCl_2$) was added dropwise with stirring to Z-D-serine β-lactone (**36b**) (150 mg, 0.678 mmol) in Et_2O (30 mL)/THF (2 mL). After 6.5 h at 22°C the suspension was cooled to 5°C and 1N H_3PO_4 (20 mL) was added carefully. The Et_2O phase was separated and the aqueous phase was extracted with Et_2O (3 × 20 mL). After drying over Na_2SO_4, solvent was removed in vacuo to obtain 165 mg of pure **56b** (94% yield),[72a] which could be recrystallized from EtOAc/hexane to yield 120 mg of a fluffy white solid (69%): mp 82-84°C (cf. L-isomer below); $[\alpha]_D^{25}$ -14.3 (±0.1)° (c 1.0, MeOH); IR (CH_2Cl_2 cast) 3320 (m, br), 1750 (s), 1724 (vs), 1525 (s), 1455 (m), 1439 (m), 1409 (m), 1215 (s), 1066 (s), 753 (m), 740 (m), 696 (m) cm^{-1}; ^1H NMR (80 MHz, CD_2Cl_2) δ8.84 (s, 1H, COOH), 7.41 (s, 5H, Ph), 5.85-5.70 (br, 1H, NH), 5.20 (s, 2H, CH_2Ph), 4.88 (m, 1H, CH), 4.02 (m, 2H, CH_2Cl); Anal. Calc. for $C_{11}H_{12}NO_4Cl$: C, 51.27; H, 4.69; N, 5.44; Cl, 13.76. Found: C, 51.26; H, 4.69; N, 5.25; Cl, 13.80; EI-MS: 257.0455 (M^+, 257.0455 calcd.), 259.0419 (30% of M^+); R_f 0.40 (20 MeOH/80 CH_2Cl_2).

N-(Benzyloxycarbonyl)-β-chloro-L-alanine (56a).

Titanium tetrachloride (74.5 μL, 0.678 mmol) was added to a solution of Z-L-serine β-lactone (**36a**) (150 mg, 0.678 mmol) in CH_2Cl_2 (10 mL) with stirring. Immediately a white precipitate began to form and after 30 min the

solvent was removed in vacuo. The residue was suspended in H_2O (20 mL) and the pH adjusted to 2.5. The TiO_2 precipitate was filtered and washed with EtOAc (3 x 10 mL). The phases were partitioned and the aqueous phase was further extracted with EtOAc (2 x 10 mL). Pooled ethyl acetate phases were dried over Na_2SO_4 and evaporated in vacuo to provide 173 mg (99%) of Z-β-chloro-L-alanine (**56a**), which was recrystallized from EtOAc/hexane: mp 88-89°C (lit. mp 89°C,[72a] 88°C[72c]); $[\alpha]_D^{25}$ +14.3 (±0.2)° (c 1.0, MeOH) (lit. $[\alpha]_D^{25}$ +14.25° (c 2.0, MeOH),[72a] + 27° (c 1.0, MeOH [72c]); IR, NMR, MS, and chromatographic properties were identical to **56b** above.

N-(Benzyloxycarbonyl)-O-acetyl-L-serine (57a).[268]

To 1.0 g of anhydrous NaOAc (dried 12 h at 120°C; 12 mmol) dissolved in glacial acetic acid (15 mL) was added Z-L-serine β-lactone (**36a**) (199.0 mg, 0.90 mmol), and the stirred mixture was heated to 45°C for 7 h. Solvent was removed in vacuo at 35°C, and the residue was acidified to pH 2 by solution in 1N HCl (~10 mL), and extracted with CH_2Cl_2 (3 x 30 mL). Organic phases were dried and evaporated in vacuo to afford a clear colorless syrup which solidified after successive trituration with, and evaporation of, toluene and ether to yield 246 mg of **57a** (97%). Recrystallization could be effected from $CHCl_3/Et_2O$: mp 88-89°C (lit. 87.5-88.5°C[277]); $[\alpha]_D^{25}$ -18.5 (±0.1)° (c 2.0, DMF) (lit. $[\alpha]_D$ -18.6° (c 2.0, DMF)[277];

IR (CH_2Cl_2 cast) 3600-2700 (m, br, mult), 1706 (vs, br), 1607 (vs), 1522 (m), 1407 (s), 1252 (vs, br), 1060 (m), 697 (m) cm^{-1}; ^1H NMR (80 MHz, $CHCl_3$) δ9.00 (br s, 1H, COOH), 7.31 (s, 5H, Ph), 5.91 (br d, 1H, 8 Hz, NH), 5.12 (s, 2H, CH_2Ph), 4.53 (m, 1H, CH), 4.45 (m, 2H, $CHCH_2O$), 1.98 (s, 3H, CH_3COO); EI-MS: 281.0899 (M^+, 281.0900 calcd. for $C_{13}H_{15}NO_6$), 221.0684 (M-CH_3COOH); R_f 0.68 (20 MeOH/80 CH_2Cl_2).

N-(Benzyloxycarbonyl)-DL-serine methyl ester (58d), N-(Benzyloxycarbonyl)dehydroalanine methyl ester (59), and N-(Benzyloxycarbonyl)-O-methyl-DL-serine methyl ester (60d).[268]

To a solution of NaOMe (1.36 mmol) in MeOH (5 mL) was added a solution of Z-serine β-lactone (**36a** or **36b**) (200.0 mg, 0.904 mmol) in THF (10 mL) dropwise with stirring over 5 min. After 25 min, HOAc (0.1 mL) was added to quench and the volume was reduced to 2 mL in vacuo at 30°C. The residue was partitioned between EtOAc/H_2O (3 × 30 mL/25 mL) and the ethyl acetate extract was dried over Na_2SO_4, concentrated in vacuo and flash chromatographed on silica[260] (55% EtOAc/hexane). This provided 201.9 mg of racemic Z-serine methyl ester (**58d**, 88%) and 25.1 mg of Z-dehydroalanine methyl ester (**59**, 12%) (R_f 0.23 and 0.80 (55% EtOAc/hex), respectively). If the reaction was allowed to proceed 25 min at 25°C and 50 min at 0-5°C before quenching, isolation as outlined above yields 153.6

mg (67%) of **58d**, 42.5 mg (20%) of **59**, and 31.2 mg (13%) of racemic Z-O-methyl-serine methyl ester (**60d**) (R_f 0.53 (55% EtOAc/hex)).

For 58d: oil (lit. mp 36-38°C[278]); $[\alpha]_D^{25}$ 0.0° (c 10, MeOH); IR ($CHCl_3$ cast) 3600-3200 (vs, br), 1721 (vs, br), 1526 (s), 1453 (m), 1437 (m), 1343 (s), 1260 (s), 1213 (vs), 1061 (vs), 699 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ7.36 (s, 5H, Ph), 6.03 (br d, 1H, 7.5 Hz, NH), 5.12 (s, 2H, CH_2Ph), 4.43 (m, 1H, CH), 3.97 (dd, 1H, 3, 11 Hz, CHHOH), 3.88 (dd, 1H, ~5, 11 Hz, CHHOH), 3.75 (s, 3H, $COOCH_3$), 2.77 (br s, 1H, OH); EI-MS: 253.0957 (253.0951 calcd. for $C_{12}H_{15}NO_5$).

For 59:[279] oil; IR ($CHCl_3$ cast) 3415 (m), 3360 (m), 1740 (s), 1716 (vs), 1638 (m), 1520 (vs), 1455 (m), 1441 (s), 1324 (s), 1222 (m), 1202 (s), 1068 (s), 895 (m), 698 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ7.36 (s, 5H, Ph), 7.30 (br s, 1H, NH), 6.23 (br s, 1H, E-CHH), 5.76 (d, 1H, ~1.5 Hz, Z-CHH), 5.15 (s, 2H, CH_2Ph), 3.83 (s, 3H, $COOCH_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ164.2 (s), 152.0 (s), 136.3 (s), 132.0 (s), 128.6 (d), 128.4 (d), 128.2 (d), 106.1 (t), 67.1 (t), 52.9 (q); EI-MS: 235.0845 (M^+, 235.0845 calcd. for $C_{12}H_{13}NO_4$), 176.0714 (M-$COOCH_3$); R_f 0.69 (35% EtOAc/hexane).

For 60d: $[\alpha]_D^{25}$ 0.0° (c 2.0, $CHCl_3$); IR (CH_2Cl_2 cast) 3340 (w, br), 1729 (s, br), 1518 (m), 1455 (m), 1292 (m), 1240 (m), 1210 (m), 1122 (m), 1070 (m), 1053 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$)[202] δ7.38 (m, 5H, Ph), 6.0 (br s,

0.33H) and 5.63 (br d, 0.67H, 8 Hz), (rotomeric NH), 5.15 (s, 2H, CH_2Ph), 4.50 (m, 1H, CH), 3.83 (br s, 1.0H) and 3.78 (br s, 2.0H) ($COOCH_3$), 3.87-3.78 (m, 1H, CHHOMe), 3.65-3.57 (dd, 1H, 4.12 Hz, CHHOMe), 3.35 (s, 2.0H) and 3.24 (s, 1.0H) (CH_2OCH_3); EI-MS: 267.1105 (267.1107 calcd. for $C_{13}H_{17}NO_5$).

N-(Benzyloxycarbonyl)-S-benzyl-D-cysteine (61b).[268]

Benzylmercaptan (1.20 mL, 9.95 mmol) was added dropwise under Ar to NaH (227 mg, 9.45 mmol) suspended in dry DMF (10 mL). The mixture was stirred 1 h and an aliquot of the benzyl thiolate solution (1.15 mL, 0.995 mmol) was added to Z-D-serine β-lactone (**36b**) (200.0 mg, 0.904 mmol) in DMF (7 mL). The mixture was stirred 30 min at 25°C, 0.05N H_3PO_4 (20 mL) was added (final pH 2.0), and the mixture was extracted with EtOAc (3 × 30 mL). Ethyl acetate phases were dried over Na_2SO_4 and concentrated in vacuo. The residue (0.33 g) was chromatographed on silica (CH_2Cl_2 → 9% MeOH/CH_2Cl_2) to provide 244 mg (78%) of **61b**, which could be recrystallized from EtOAc/hexane or Et_2O: mp 95-97°C (lit. mp 99°C for L-isomer[280]); $[\alpha]_D^{25}$ +45.0 (±0.2)° (c 1.0-2.0, acetone) (lit. $[\alpha]_D^{25}$ +45.1° (c 2, acetone)[280]); IR (KBr disk) 3267 (m), 1724 (vs), 1670 (s), 1544 (vs), 1495 (w), 1452 (m), 1424 (m), 1336 (m), 1321 (m), 1265 (vs), 1248 (s), 1054 (m), 696 (s) cm^{-1}; ^1H NMR (80 MHz, CD_2Cl_2) δ9.19 (br s, 1H, COOH), 7.37 (s, 5H, Ph), 7.31 (s, 5H, Ph), 5.63 (br s, 1H, NH), 5.15 (s, 2H,

PhC$\underline{H}_2$O), 4.60 (m, 1H, C$\underline{H}$), 3.75 (s, 2H, PhC$\underline{H}_2$S), 2.95 (m, 2H, CHC$\underline{H}_2$S); Anal. Calc. for $C_{18}H_{19}NO_4S$: C, 62.59; H, 5.54; N, 4.05; S, 9.28. Found: C, 62.55; H, 5.68; N, 3.92; S, 9.32; POSFAB-MS (glycerol) 346 (MH^+); R_f 0.21 (7.5% MeOH/CH_2Cl_2).

D- and DL-N^{α}-(Benzyloxycarbonyl)-β-isothiureido alanines (62b and 62d).[268]

Z-D-serine β-lactone (**36b**) (100 mg, 0.452 mmol) in THF (5 mL) was added to a solution of thiourea (263 mg, 3.45 mmol) in 50% aqueous THF (5 mL). The mixture was stirred 2 h at 25°C, the solvent was removed in vacuo, and the residue was recrystallized from MeOH/acetone to provide 106.1 mg (79%) of **62b**: mp 156-8°C (dec); $[\alpha]_D^{25}$ +24.6 (±0.2)° (c 1.0, 1N HCl); IR (KBr disk) 3600-2700 (s, br), 1689 (s), 1630 (m, br), 1518 (m), 1394 (m) cm^{-1}; ^1H NMR (80 MHz, D_2O + DCl) δ 7.37 (s, 5H, Ph), 5.18 (s, 2H, CH_2Ph), 4.60 (m, 1H, CH), 3.68 (m, 2H, CHCH_2S); Anal. Calc. for $C_{12}H_{15}N_3O_4S$: C, 48.48; H, 5.08; N, 14.12. Found: C, 48.72; H, 5.13; N, 13.72; POSFAB-MS (glycerol/HCl) 298 (MH^+), 595 (M_2H^+); R_f 0.65 (n-BuOH/HOAc/pyr/H_2O (4:1:1:2)).

Racemic **62d** was prepared analogously from Z-DL-serine β-lactone (**36d**) in 56% recrystallized yield: mp 173-175°C; IR (KBr disk) 3600-2700 (s, br), 1715 (vs), 1690 (vs), 1597 (s), 1582 (vs), 1528 (m), 1485 (s), 1462 (m), 1450 (m), 1430 (m), 1400 (m), 1200 (s), 1046 (s), 733 (m),

695 (s) cm^{-1}; ^1H NMR, MS and chromatographic properties were identical to **62b** above.

N^{α}-(Benzyloxycarbonyl)-β-(mercaptoethylamino)-D-alanine (63b).[268]

To Z-D-serine β-lactone (**36b**) (100.0 mg, 0.452 mmol) in CH_3CN or THF (5 mL) was added a solution of mercaptoethylamine hydrochloride (102 mg, 0.90 mmol) in degassed H_2O (5 mL). The pH of the mixture was raised to and maintained at pH 5.5 (±0.5) by the dropwise addition of 0.1N NaOH with rapid stirring. After 20 min no further addition of NaOH was required. The volume was reduced to half *in vacuo* at 35°C, the pH was adjusted to 6.8 with NH_4OH, and the mixture was extracted with CH_2Cl_2 (3 × 70 mL). The white solid 117 mg (87%) obtained on drying and evaporation of CH_2Cl_2 was recrystallized from CH_2Cl_2/hexane to yield 102 mg (76%) of **63b**[281]: mp 127.5-128.5°C; $[\alpha]_D^{22}$ +13.9 (±0.1)° (c 1, MeOH); IR (KBr disk) 3360 (br s), 3293 (s), 2560 (w), 1689 (vs), 1647 (vs), 1565 (m), 1544 (m), 1245 (m), 1020 (m) cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ7.28 (s, 5H, Ph), 6.85 (br s, 2H, CH_2NH, COOH), 5.86 (br d, 1H, 8 Hz, NHCH), 5.10 (s, 2H, CH_2Ph), ~4.20 (m, 1H, CH), 4.08 (dd, 1H, 12, 3 Hz, CHCHHS), 3.64 (dd, 1H, 12, 5.4 Hz, CHCHHS), 3.29 (t, 2H, 6 Hz, CH_2CH_2SH), 2.80-2.37 (m, 2H, CH_2SH), 1.38 (t, 1H, 8.4 Hz, CH_2SH). Absolute ^1H NMR assignments were made with the aid of decoupling experiments; Anal. Calc. for

$C_{13}H_{18}N_2O_4S$: C, 52.33; H, 6.08; N, 9.39; S, 10.75. Found: C, 52.06; H, 6.10; N, 9.29; S, 10.83; EI-MS: 298.0992 (M^+, 298.0987 calcd.); POSFAB-MS (glycerol) 299 (MH^+), 597 (M_2H^+); R_f 0.60 (60% acetone/CH_2Cl_2; ninhydrin and nitroprusside positive). After passing air through an acetone solution of **63b**, POSFAB-MS (glycerol) shows 595 ($(M_2-2H)H^+$) as base peak.

Reactions of β-lactones with ammonia.[268]

Dry NH_3(g) was bubbled (~100 mL/min) through a solution of the β-lactone (**36a**, **36b** or **42a**) (1 mmol) in 10-15 mL of anhydrous solvent at 0°C for either 15 min (CH_3CN solvent) or 1 h (THF), and the mixture was stoppered and allowed to react until all β-lactone was consumed (20 min-3 h at 0°C). Solvent was removed *in vacuo* at 35°C, and the residue was stirred with H_2O (25 mL) and extracted with Et_2O (4 × 30 mL). Evaporation of the aqueous phase provided amine products (eg., **65a**, **66**), while amides (eg., **64**) were obtained from the organic layers; these compounds could be recrystallized from MeOH/Et_2O and MeOH/H_2O, respectively. All reactions with ammonia were quantitative with the balance of product being amine or amide.

From the reaction of Z-L-serine β-lactone (**36a**) (221.2 mg, 1.00 mmol) with NH_3 in CH_3CN (0°C, 20 min) was obtained 183.6 mg (77%) of Z-L-serinamide (**64a**) and 54.5 mg (23%) of N^2-Z-L-2,3-diaminopropanoic acid (**65a**) (R_f

0.77 and 0.54 respectively in n-BuOH/HOAc/pyr/H_2O (4:1:1:2)). The analogous reaction of Z-D-serine β-lactone (**36b**) with ammonia in THF (3 h, 0°C) provided 59.1 mg (25%) of Z-D-serinamide (**64b**) and 178.9 mg (75%) of N^2-Z-D-2,3-diaminopropanoic acid (**65b**).

For L- and D-N-(Benzyloxycarbonyl)serine amides (64a and 64b): mp 131-132°C (L), 130-131°C (D) (lit. mp 132-133°C[282a] for L-isomer); $[\alpha]_D^{25}$ +14.8 (±0.1)° (c 1.0, EtOH) for L-isomer (lit. $[\alpha]_D^{25}$ +14.4,[282a] +14.9[282b] (c 5, EtOH)); IR (CH_2Cl_2 cast) 3500-3100 (s, br), 1690 (s), 1652 (vs, br), 1 97 (m), 1529 (s), 1278 (m), 1250 (s), 1234 (m), 1088 (m), 1057 (s), 960 (m), 764 (s), 702 (m) cm^{-1}; ^1H NMR (80 MHz, d_6-acetone) δ7.28 (s, 5H, Ph), 5.60 (br s, 1H, NH), 5.04 (s, 2H, CH_2Ph), 4.35 (m, 1H, CH), 3.75 (m, 2H, CH_2OH), 3.05 (br s, 3H, NH_2, OH); EI-MS: 238.0952 (M^+, 238.0954 calcd. for $C_{11}H_{14}N_2O_4$), 194.0816 ($M-H_2NCO$); POSFAB-MS (glycerol) 239 (MH^+).

For L- and D-N^2-(Benzyloxycarbonyl)-2,3-diaminopropanoic acids (65a and 65b): mp 229-231°C (dec) (L), 226-228°C (dec) (D) (lit. mp 228-230°C,[139a] 229-231°C (dec),[139b] 240-241°C (dec)[139c] for L-isomer); $[\alpha]_D^{25}$ -7.9 (±0.1)° (c 0.4, 1N NaOH) for L-isomer (lit. $[\alpha]_D^{25}$ -7.8°,[139a] -7.4°[139b] (c 0.4, 1N NaOH), -37 (±1)° (c 1.0, 1N HCl)[139c]; $[\alpha]_D^{25}$ +7.9 (±0.3)° (c 0.4, 1N NaOH), +37.5 (±0.5)° (c 1.0, 1N HCl) for the D-isomer; IR (KBr disk) 3375 (vs), 3318 (vs), 3205 (s), 1667 (vs), 1651 (vvs), 1632 (s), 1535 (s), 1467 (m), 1433 (m), 1309 (s), 1296

(s), 1255 (s), 1019 (s), 750 (m), 697 (s) cm^{-1}; ^1H NMR (80 MHz, D_2O + DCl) δ7.40 (s, 5H, Ph), 5.13 (s, 2H, CH_2Ph), 4.23 (m, 1H, CH), 3.85 (m, 2H, $CH_2NH_3^+$); Anal. Calc. for $C_{11}H_{14}N_2O_4$: C, 55.46; H, 5.92; N, 11.76. Found for D-isomer (**65b**): C, 55.41; H, 6.00; N, 11.37; EI-MS: 238.0952 (L), 238.0953 (D) (M^+, 238.0954 calcd.); POSFAB-MS (glycerol) 239 (MH^+) (L- or D-isomer).

N^2-(tert-Butoxycarbonyl)-L-2,3-diaminopropanoic acid (66a).[268]

From the reaction of BOC-L-serine β-lactone (**42a**) (163.0 mg, 0.871 mmol) with NH_3 in THF (15 mL) at 0°C for 3 h outlined above, was obtained 37.6 mg (21%) of BOC-L-serinamide (oil, R_f 0.71, not further characterized) and 141.1 mg (79%) of N^2-BOC-L-2,3-diaminopropanoic acid (**66a**) (R_f 0.47, n-BuOH/HOAc/pyr/H_2O (4:1:1:2)) as a white solid: mp 197-199°C (dec) (lit. mp 198-200°C[139a]); $[\alpha]_D^{25}$ -2.7 (±0.1)° (c 1.0, AcOH) (lit. $[\alpha]_D^{22}$ -2.7° (c 1, AcOH)); IR (KBr disk) 3388 (s), 3341 (s), 3210 (m), 1685 (s), 1647 (vs), 1527 (s), 1317 (m), 1299 (m), 1251 (m), 1008 (m) cm^{-1}; ^1H NMR (400 MHz, D_2O) δ4.12 (~t, 1H, 4.8 Hz, CH), 3.82 (~d, 2H, 4.8 Hz, $CH_2NH_3^+$), 1.43 (s, 9H, tert-Bu); Anal. Calc. for $C_8H_{16}N_2O_4$: C, 47.05; H, 7.90; N, 13.72. Found: C, 46.85; H, 7.91; N, 13.34; POSFAB-MS (glycerol) 205 (MH^+), 149 ($MH^+-C_4H_8$), 105 ($MH^+-C_4H_8$, CO_2), 409 (M_2H^+).

N^{α}-(Benzyloxycarbonyl)-β-(trimethylammonio)-L-alanine, inner salt (67a).[268]

To a solution of Z-L-serine β-lactone (**36a**) (200.0 mg, 0.904 mmol) in THF (5 mL) at 0°C was added liquid Me_3N (0.50 mL, 5.65 mmol). The mixture was stirred 2 h at 0-5°C and the solvent was removed in vacuo from the slurry at 35°C to provide a quantitative yield (254 mg) of **67a** as a white powder. This was recrystallized from MeOH by precipitation with Et_2O and stored over P_2O_5 in vacuo (recryst. yield 237 mg, 91%): mp 100-100.5°C (dec); $[\alpha]_D^{22}$ -7.9 (±0.2)° (c 1, MeOH); IR (MeOH cast) 3240 (w, br), 1710 (vs), 1625 (s), 1530 (m), 1490 (m), 1257 (m), 1058 (m) cm^{-1}; ^1H NMR (400 MHz, d_6-DMSO) δ7.38 (s, 5H, Ph), 7.11 (br d, 1H, 4 Hz, NH), 5.07 (s, 1H, CH_2Ph), 4.04 (m, 1H, CH), 3.86 (dd, 1H, 13.7, 3.1 Hz, $CHHN^+$), 3.40 (dd, 1H, 13.7, 8.4 Hz, $CHHN^+$), 3.10 (s, 9H, $N^+(CH_3)_3$); Anal. Calc. for $C_{14}H_{20}N_2O_4$: C, 59.99; H, 7.19; N, 9.99. Found: C, 59.80; H, 6.94; N, 9.69 (hygroscopic); POSFAB-MS (glycerol) 281 (MH^+), 561 (M_2H^+), 841 (M_3H^+); R_f 0.60 (n-BuOH/HOAc/pyr/H_2O (4:1:1:2)).

N^{α}-(Benzyloxycarbonyl)-β-(pyrazol-1-yl)-D-alanine (68b).

Pyrazole (81 mg, 1.2 mmol) in CH_3CN (4 mL) was added to a solution of Z-D-serine β-lactone (**36b**) (250 mg, 1.13 mmol) in CH_3CN (6 mL), and the mixture was heated to 50°C for 12 h. Solvent was removed in vacuo and the residue dissolved in hot MeOH (20 mL) and filtered.

Recrystallization of **68b** recovered from evaporation of the filtrate was achieved from MeOH/H_2O or EtOAc/hexane to yield 233 mg (71%) of white solid: mp 168.5-169.5°C (lit. mp 170-171°C for L-isomer[141]); $[\alpha]_D^{25}$ +53.1 (±0.3) (c 1.0, DMF) (lit. $[\alpha]_D^{28}$ -53.6° (c 1.0, DMF) for L-isomer[141]); IR (KBr disk) 3349 (m), 1970 (w), 1746 (m), 1696 (vs), 1534 (s), 1403 (m), 1334 (m), 1260 (m), 1068 (m), 773 (m), 749 (m), 699 (m), 667 (m), 659 (m), 613 (m) cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ7.54 (s, 1H, ArHHH), 7.50 (s, 1H, ArHHH), 7.33 (s, 5H, Ph), 6.26 (s, 1H, ArHHH), 5.08 (s, 2H, CH_2Ph), 4.75-4.60 (m, 2H, $CHCH_2N$), 4.56-4.35 (m, 1H, $NCHCH_2N$); Anal. Calc. for $C_{14}H_{15}N_3O_4$: C, 58.12; H, 5.23; N, 14.53. Found: C, 57.72; H, 5.32; N, 14.23; POSFAB-MS (glycerol) 290 (MH^+); R_f 0.35 (40% MeOH/$CHCl_3$).

Methyl (S)-2-[N-(tert-butoxycarbonyl)amino]-3-(dimethylphosphono)propanoate (69a).

BOC-L-serine β-lactone (**42a**) (250.0 mg, 1.34 mmol) was stirred in trimethylphosphite (3.0 mL, 25.4 mmol) at 50°C for 3 days, and 70°C for 2 days under an atmosphere of Ar. Excess $(CH_3O)_3P$ was removed in vacuo at 30°C and the residue (465 mg) was treated with boiling Et_2O (15 mL) and filtered. Evaporation of the filtrate afforded 407.8 mg (98%) of analytically pure **69a** as a clear colorless gum:[149] $[\alpha]_D^{25}$ +9.5 (±0.3)° (c 0.37, $CHCl_3$); IR ($CHCl_3$ cast) 3260 (w, br), 1742 (m), 1713 (s), 1523 (m), 1455 (m), 1367 (m), 1250 (s), 1164 (s), 1030 (vs), 840 (m)

cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, with and without ^{31}P-decoupling) δ5.66 (d, 1H, 8 Hz, NH), 4.65-4.40 (m, 1H, J(HCCP) = 22 Hz), 3.78 (s, 3H, $COOCH_3$), 3.75 (d, 6H, $J(H_3COP)$ = 11 Hz, $P(OCH_3)_2$), 2.39 (dd, 2H, J(HCCH) = 5 Hz, J(HCP) = 17.0 Hz, $CHCH_2P$), 1.47 (s, 9H, tert-Bu); ^{31}P NMR (161.96 MHz, $CDCl_3$) δ29.66 (br m, collapses to s with ^1H-decoupling);[149b] Anal. Calc. for $C_{11}H_{22}NO_7P$: C, 42.45; H, 7.12; N, 4.50. Found: C, 42.05; H, 7.10; N, 4.39; EI-MS: 252.0995 (calc. 252.1002 for $M-COOCH_3$), 196.0373 (M-tert-Bu, $COOCH_3$), 152.0478 (Base peak, $H_2\overset{+}{N}CHCH_2P(O)(OCH_3)_2$); CI-MS ($NH_3$) 329 ($M+NH_4^+$), 312 ($MH^+$). ^1H NMR results with 0.1 equivalent of hfc-Eu(III) chiral shift reagent in $CDCl_3$ suggest 92% (2S)/8% (2R)-isomers (i.e., 84% e.e.).

Methyl (S)-2-amino-3-phosphonopropanoate (70a) and (S)-2-amino-3-phosphonopropanoic acid (71a).

BOC-β-(dimethylphosphono)-L-alanine methyl ester (**69a**) (242 mg, 0.777 mmol) was dissolved in $CDCl_3$ (2.5 mL) and treated carefully with iodotrimethylsilane (553 μL, 3.89 mmol)[152] with cooling on ice/H_2O. After addition the reaction was monitored by ^1H NMR at 25°C. The reaction very rapidly (<10 min) generated the monomethyl ester which was consumed very slowly (~20% in 1 h). After 1 h at 25°C the reaction was quenched by addition to a mixture of Et_2O (5 mL) and 30% HOAc in H_2O (5 mL). The aqueous layer was separated and reextracted with Et_2O (2 x 5 mL),

and lyophilized. The residue was dissolved in H_2O and applied to a column of AG50-X8 (1 × 20 cm, H^+ form). Elution with H_2O (0.5 mL/min) provided first **71a** (23.4 mg, 18%, in 30-90 mL elution vol.) and then **70a** (101.3 mg, 71%, in 120-250 mL elution vol.) which were recovered by lyophilization. Recrystallization of the monomethyl ester **70a** could be effected from H_2O/MeOH/dioxane (90% recovery), whereas **71a** was recrystallized from H_2O/MeOH.

For 70a: mp 211-212°C; $[\alpha]_D^{25}$ +14.8 (±0.2)° (c 1.0, H_2O); IR (KBr disk) 3340 (s, br), 2920 (w), 1750 (s), 1630 (m), 1444 (w), 1384 (w), 1246 (m), 1140 (m), 1055 (m), 921 (m), 710 (m) cm^{-1}; ^1H NMR (400 MHz, D_2O, with/without ^{31}P-decoupling) δ4.32 (ddd, 1H, 4.3, 8.5 Hz, J(HCCP) = 17.5 Hz, CH), 3.83 (s, 3H, $COOCH_3$), 2.31 (ddd, 1H, 4.3, 16.0 Hz, J(HCP) = 17.0 Hz, CHCHHP), 2.15 (~d of t, 1H, 8.5 Hz, J(HCP) = 16.5 Hz, CHCHHP); ^{31}P NMR (161.96 MHz, D_2O) δ17.12 (~q, 16.5-17 Hz, collapses to s with ^1H-decoupling); Anal. Calc. for $C_4H_{10}NO_5P$: C, 26.24; H, 5.50; N, 7.65. Found: C, 26.04; H, 5.65; N, 7.62; POSFAB-MS (glycerol) 184 (MH^+), 169 (MH^+-CH_3), 125 ($MH^+-COOCH_3$); NEGFAB-MS (glycerol) 182 (M^-), 365 (M_2H^-), 168 ($M-CH_3$), 79 (Base peak, PO_3^-); R_f 0.20 (System A).

For the acid 71a: no distinct mp, darkens above 240°C, dec > 255°C; $[\alpha]_D^{25}$ +14 (±0.5)° (c 0.20, 1N NaOH) (lit. $[\alpha]_D^{25}$ +22° for ee = 86% (c 2.0, 1N NaOH);[149b] IR (KBr disk) 3430 (vs, br), 1730 (m, br), 1654 (s), 1630 (m), 1381 (w), 1150 (m), 1130 (m) cm^{-1}; ^1H NMR (400 MHz,

D_2O) δ4.22 (ddd, 1H, 4.2, 9.4 Hz, J(HCCP) = 15 Hz, CH), 2.37 (ddd, 1H, 4.2, 16 Hz, J(HCP) = 17 Hz, CHCHHP), 2.15 (~d of t, 1H, 9.4, ~16 Hz (J(HCP) = J(HCH)), CHCHHP); ^{31}P NMR (161.96 MHz, D_2O) δ18.21 (~dd, 15, ~16.5 Hz, collapses to s with ^1H-decoupling); POSFAB-MS (glycerol) 170 (MH^+), 339 (M_2H^+); NEGFAB-MS (glycerol) 168 (M^-), 337 (M_2H^-), 79 (Base peak, PO_3^-); R_f 0.24 (System A).

N-(Benzyloxycarbonyl)-L-asparagine (72a).

This material was prepared according to Ressler and Ratzkin.[169] L-Asparagine (15.0 g, 0.114 mol) was suspended by vigorous stirring in H_2O (79 mL) at 5°C and benzylchloroformate (15.3 mL, 0.136 mol) and 2N NaOH (115 mL) were added dropwise simultaneously over 90 min so as to maintain pH between 7.9-8.4. After 2 h the mixture was extracted with ether (4 × 150 mL) and the aqueous layer cooled and acidified to pH 1 with 5.7N HCl. The slurry was filtered, and the residue was washed with cold H_2O and recrystallized from MeOH/H_2O (1:1) to yield 21.4 g (71%) of **72a** as white needles: mp 166-167°C (lit. mp 164-165°C[169]); $[\alpha]_D^{25}$ -6.3 (±0.2)° (c 1.0, 1N $NaHCO_3$) (lit. $[\alpha]_D^{22}$ -6.5° (c 1, 1N $NaHCO_3$)[169]); IR ($CHCl_3$ cast) 3336 (s), 1694 (vs), 1642 (s), 1540 (m), 735 (m) cm^{-1}; ^1H NMR (80 MHz, CD_3CN) δ7.33 (s, 5H, Ph), ~6.0 (br s, 3H, NH, $C(O)NH_2$), 5.08 (s, 2H, CH_2Ph), 4.35 (m, 1H, CH), 2.85-2.67 (m, 2H, $CHCH_2$); EI-MS: 266.0880 (M^+, 266.0903 calcd. for $C_{12}H_{14}N_2O_5$), 249.0631 ($M-NH_3$).

N-(Benzyloxycarbonyl)-β-cyano-L-alanine (73a) from 72a.

According to Ressler and Ratzkin,[169] a solution of 1,3-dicyclohexylcarbodiimide (5.70 g, 27.6 mmol) in dry distilled pyridine (15 mL) was added dropwise over 30 min to Z-L-asparagine (**72a**) (7.00 g, 26.3 mmol) in pyridine (35 mL) at 15-20°C. After 1.5 h at 25°C, the white slurry of dicyclohexylurea was filtered, and the filtrate was concentrated in vacuo at 30°C to 25 mL and again filtered. The filtrate was concentrated to a thick syrup which was diluted with H_2O (50 mL) and cooled to 4°C for 1 h. Again the mixture was filtered, and the cold filtrate was acified to pH 2.0 with 5.7N HCl. The white crystals of **73a** which separated (4.45 g, 69%) were dried in vacuo over P_2O_5 and recrystallized from 1,2-dichloroethane (~50 mL) to provide 3.94 g (60%) of pure **73a**: mp 130-131°C (lit. mp 130-131°C[169]); $[\alpha]_D^{25}$ -45.1 (±0.2)° (c 1.0, DMF) (lit. $[\alpha]_D^{22}$ -45.2° (c 0.96, DMF)[169]); IR (MeOH/$CHCl_3$ cast) 3315 (s, br), 2265 (w), 1744 (vs), 1696 (vs, br), 1541 (s), 1404 (m, br), 1314 (m), 1268 (s), 1189 (m), 1060 (m), 747 (m), 694 (m) cm^{-1}; ^1H NMR (300 MHz, d_6-acetone) δ7.42-7.25 (m, 5H, Ph), 7.08 (br d, 1H, 7.5 Hz, NH), 5.12 (s, 2H, CH_2Ph), 4.67-4.57 (m, 1H, CH), 3.10 (dd, 1H, 5.6, 17.0 Hz, CHHCN), 3.05 (dd, 1H, 7.8, 17.0 Hz, CHHCN); EI-MS: 248.0794 (M^+, 248.0797 calcd. for $C_{12}H_{12}N_2O_4$); R_f 0.18 (10% MeOH/$CHCl_3$ on silica), 0.65 (60% CH_3CN/H_2O on RP-8).

N-(Benzyloxycarbonyl)-β-cyano-L-alanine (73a) and 2-[N-(Benzyloxycarbonyl)amino]propenoic acid (74) from 36a.

Z-L-Serine β-lactone (**36a**) (100.0 mg, 0.451 mmol) in dry CH_3CN (2.5 mL) was added dropwise over 5 min to a solution of anhydrous tetra-n-butylammonium cyanide (133.3 mg, 0.496 mmol)[241] in acetonitrile (5 mL) at -15°C. The solution was stirred 1 h at -15°C, and 1 h at RT, and the solvent was removed in vacuo. The residue was partitioned between pH 2, 2 M KCl/HCl buffer (10 mL) and EtOAc (3 × 10 mL), and the pooled organic layers were washed with saturated aqueous NaCl (2 × 10 mL), dried over Na_2SO_4, and concentrated in vacuo. Reverse-phase MPLC (60% CH_3CN/H_2O, 3.25 mL/min) yielded 72.1 mg (64%) of pure Z-β-cyano-L-alanine (**73a**) followed by 39.7 mg (35%) Z-dehydroalanine (**74**) which were recovered by evaporation of solvent in vacuo at 30°C. Recrystallization of **73a** was effected from 1,2-dichloroethane whereas **74** was recrystallized from EtOAc/hexane.

For 73a from 36a: mp 133-134°C (lit. 130-131°C,[169] 133-134°C[170]); $[\alpha]_D^{25}$ -45.1 (±0.1)° (c 1.0, DMF) (lit. $[\alpha]_D^{22}$ -45.2°,[169] -44.2°[170] (c 0.96, DMF)); IR, ^1H NMR and chromatographic properties were identical to authentic **73a** (prepared from **72a** above); EI-MS: 248.0798 (M^+, 248.0797 calcd. for $C_{12}H_{12}N_2O_4$).

For 2-[N-(benzyloxycarbonyl)amino]propenoic acid (74): mp 107-109°C (lit. mp 108-109°C,[187a] 109-110°C[187b]); IR ($CHCl_3$ cast) 3350 (m, br), 3000-2800 (mult,

m), 1720 (vs, br), 1610 (m), 1500 (m), 1390 (m), 1240 (m), 1180 (m), 1140 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ7.38 (s, 5H, Ph), 7.20 (br s, 1H, NH), 6.36 (s, 1H, E-CHH), 5.94 (s, 1H, Z-CHH), 5.18 (s, 2H, CH_2Ph); EI-MS: 221.0683 (M^+, 221.0688 calcd. for $C_{11}H_{11}NO_4$); CI-MS (NH_3) 239 ($M+NH_4$).

N-(Benzyloxycarbonyl)-O-([N-(benzyloxycarbonyl)amino]-propenoyl)-DL-serine methyl ester (75) and 59.

Z-Serine β-lactone (**36a** or **36b**) (100.0 mg, 0.452 mmol) was dissolved in THF (5.0 mL) and 1,8-diaza-bicyclo[5.4.0]undec-7-ene (DBU) (103 mg, 101 μL, 0.678 mmol) was added with stirring. The mixture was stirred 2 h at 25°C before workup. The products isolated depended upon the method used:

Method A: The mixture was added to Et_2O (35 mL) and extracted with 0.1N HCl (3 × 20 mL), and the etheral phase was dried over Na_2SO_4 and concentrated to 3-5 mL. This solution was treated with an excess of etheral diazomethane, and evaporated in vacuo. The oily residue was purified by MPLC (silica, 35% EtOAc/hexane, 3 mL/min) to afford 19.5 mg (18%) of Z-dehydroalanine methyl ester (**59**) and 70.2 mg (68%) of **75**; **Method B:** The reaction mixture was added slowly to pH 3 H_2O (20 mL) while maintaining the pH between 3 and 5 with 1N H_3PO_4. The aqueous mixture was acidified to pH 2.5 and extracted with EtOAc (3 × 15 mL), and the organic layers were dried over Na_2SO_4 and concentrated in vacuo to ~5 mL. Treatment with

excess diazomethane and chromatography as outlined in Method A produced Z-dehydroalanine methyl ester (**59**) in 87% isolated yield (i.e., 93.0 mg). The spectral and chromatographic properties of **59** were identical to those previously described.

For 75: white semisolid; $[\alpha]_D^{25}$ 0.0° (c 1.0, $CHCl_3$); IR ($CHCl_3$ cast) 3350 (m, br), 1740 (s, sh), 1718 (vs, br), 1635 (m), 1523 (vs), 1452 (m), 1317 (s), 1218 (s), 1068 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ7.36 (s, 5H, Ph), 7.34 (s, 5H, Ph'), 7.20 (br s, NHC), 6.25 (br s, 1H, E-CHH), 5.71 (d, 1H, ~1 Hz, Z-CHH), 5.61 (br d, 1H, 8 Hz, NHCH), 5.15 (s, 2H, CH_2Ph), 5.11 (s, 2H, CH_2Ph'), 4.71 (m, 1H, $CHCH_2O$), 4.53 (m, 2H, $CHCH_2O$), 3.78 (s, 3H, $COOCH_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ169.24, 162.99, 155.43, 152.85, 135.66, 135.52, 130.30, 128.38, 128.34, 128.17, 128.10, 128.02, 127.96, 106.81, 67.13, 66.90, 65.37, 52.99, 52.80; Anal. Calc. for $C_{23}H_{24}N_2O_8$: C, 60.52; H, 5.30; N, 6.13. Found: C, 60.00; H, 5.37; N, 5.95; EI-MS: 365.1048 ($M-C_7H_7$, 365.1051 calcd.), 321.1093 ($M-C_7H_7$, CO_2); CI-MS (NH_3) 474 ($M+NH_4^+$), 366 ($MH^+-C_7H_7$), 253 (Base peak, [$M+NH_4-(CH_2=C(NHZ)COOH$); R_f 0.39 (35% EtOAc/hexane).

Reactions of Z-Serine β-Lactones with Diazomethane:

A. Benzyl carbamate (76) and 58a from CF_3COOH quench:

Etheral diazomethane solution (5 mL, 1.9 mmol; distilled from KOH and titrated vs. C_6H_5COOH) was added to Z-L-serine β-lactone (100.0 mg, 0.452 mmol) in dry DMF (5

mL) and the mixture was stirred in the dark at 25°C. After 25 h all β-lactone was consumed (by IR and TLC) and the excess of CH_2N_2 was quenched by addition of CF_3COOH (~0.5 mL) with the generation of a transient blue color. Removal of the solvent in vacuo at 25°C, and flash chromatography[260] (45% EtOAc/hexane) of the CH_2Cl_2-soluble portion of the residue provided 50.3 mg (74%) of benzyl carbamate (**76**) and 28.6 mg (25%) of Z-L-serine methyl ester (**58a**). Recrystallization of **76** could be effected from EtOAc/hexane or CCl_4/hexane. Compound **58a** was recrystallized from diisopropyl ether/hexane.

For benzyl carbamate (76): mp 84.0-84.5°C (lit. mp 87-89°C[261]); IR ($CHCl_3$) 3420 (s), 3330 (m), 3270 (m) 1690 (vs), 1610 (m), 1447 (s), 1404 (m), 1344 (m), 1071 (s), 732 (m) cm^{-1}; ^1H NMR(300 MHz, $CDCl_3$) δ7.38 (s, 5H, Ph), 5.10 (s, 2H, CH_2Ph), 4.85 (br s, 2H, NH_2); Anal. Calc. for $C_8H_9NO_2$: C, 63.56; H, 6.00; N, 9.27. Found: C, 63.16; H, 6.02; N, 9.03; EI-MS: 151.0633 (M^+, 151.0634 calcd.); CI-MS (NH_3) 169 ($M+NH_4^+$); R_f 0.50 (45% EtOAc/hexane).

For 58a: mp 33-35°C (lit. mp 33-35°C[283]); $[\alpha]_D^{25}$ -13 (±0.5)° (c 0.9, MeOH) (lit. $[\alpha]_D^{25}$ -13.2° (c 10, MeOH)[283]); IR, ^1H NMR, and EI-MS characteristics were identical to that reported above for **58d**. In addition: ^{13}C NMR (75.5 MHz, $CDCl_3$) δ171.02, 156.23, 136.02, 128.50, 128.20, 128.07, 67.17, 63.14, 56.03, 52.66; CI-MS (NH_3) 271 ($M+NH_4^+$), 254 (MH^+).

B. 3-[N-(Benzyloxycarbonyl)amino]-2-methoxy-4,5-dihydrofuran (77), N-(Benzyloxycarbonyl)-L-homoserine (78a) and 58a.

Diazomethane was freshly prepared from Diazald®[261,284] with the substitution of CH_2Cl_2 for Et_2O as the solvent, and was distilled twice and dried over Na_2SO_4 at 4°C. Diazomethane (2.38 mmol) in CH_2Cl_2 (5.0 mL) was added to Z-L-serine β-lactone (**36a**) (120.0 mg, 0.542 mmol) in dry DMF (5.0 mL) and the mixture was stirred 19 h in the dark. The solvent was removed in vacuo at 25°C to yield a slightly yellow residue which was fractionated by MPLC (silica, 45% EtOAc/hexane, then 55% EtOAc/hexane, 3.0 mL/min) to afford 80.7 mg (60%) of **77**, 41.1 mg (30%) of **58a**, and 12.4 mg (9%) of **78a**. Z-L-Serine methyl ester (**58a**) possessed spectral and chromatographic properties identical with **58a** from **A** above. Treatment of **77** in CH_2Cl_2 with CF_3COOH (~3 eq) generated **76** as the only UV active product. Z-L-Homoserine was recrystallized from EtOAc/hexane.

For 3-[N-(benzyloxycarbonyl)amino]-2-methoxy-4,5-dihydrofuran (77): moisture and acid sensitive oil; IR ($CHCl_3$ solution) 3410 (m), 1747 (s, sh), 1726 (vs), 1564 (m), 1501 (s), 1450 (w), 1439 (w), 1298 (s), 1271 (m), 1241 (m), 1052 (s), 697 (m) cm^{-1}; ^1H and ^{13}C NMR assignments were verified by ^1H-decoupling and ^{13}C-^1H heteronuclear shift correlation experiments: ^1H NMR (300 MHz, $CDCl_3$) δ7.37 (br s, 5H, Ph), 6.82 (br s, 1H, NH),

5.15 (m, 1H, J(HCH) = 16 Hz, $CH_2\underline{CH}HO$), 5.10 (s, 2H, $\underline{CH_2}Ph$), 4.73 (ddd, 1H, 7, 8, 16 Hz, $CH_2\underline{CH}HO$), 3.83 (s, 3H, $O\underline{CH_3}$), 2.14 (m, 1H, $\underline{CH}HCH_2O$), 2.14 (m, 1H, $CH\underline{H}CH_2O$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ167.93 (s, C-2), 153.66 (s, $O\underline{C}(O)NH$), 135.62, C-1' of Ph), 128.49 (d), 128.25 (d), 128.06 (d) (CH's of Ph), 102.06 (s, C-3), 79.93 (t, C-5), 66.93 (t, $Ph\underline{CH_2}$), 53.71 (q, $O\underline{CH_3}$), 25.43 (t, C-4); EI-MS: 249.0999 (M^+, 249.1001 calcd. for $C_{13}H_{15}NO_4$), 158.0453 ($M-C_7H_7$), 114.0555 ($C_5H_8NO_2^+$, $M-C_7H_7$, CO_2); CI-MS (NH_3) 267 ($M+NH_4^+$), 250 (MH^+), 516 ($2M+NH_4^+$); R_f 0.45 (45% EtOAc/hexane).

For Z-L-homoserine (78a): mp 98-100°C (lit. mp 99-100°C[285]); IR ($CHCl_3$ cast) 3600-3140 (s, br), 3120-2800 (m, mult), 1750 (s, sh), 1722 (vs, br), 1526 (s, br), 1340 (m), 1260 (s), 1216 (s, br), 1084 (s, sh), 1064 (s), 697 (m) cm^{-1}; ^1H NMR (300 MHz, d_6-acetone) δ7.35 (s, 5H, Ph), 6.50 (br s, 1H, NH), 5.08 (s, 2H, $\underline{CH_2}Ph$), 4.30 (br s, 1H, OH), 4.40 (m, 1H, CH), 3.71 (m, 2H, $\underline{CH_2}OH$), 1.93 (m, 2H, $CH\underline{CH_2}CH_2OH$); EI-MS: 253.0954 (253.0951 calcd. for $C_{12}H_{15}NO_5$), 162.0683 ($M-C_7H_7$).

N-(Benzyloxycarbonyl)-O-toluenesulfonyl-DL-serine methyl ester (79d).

A modification of the procedure of Photaki[187a] was employed. Z-DL-serine (Aldrich; 2.39 g, 10.0 mmol) in CH_2Cl_2 (40 mL)/THF (15 mL) was treated with a solution of diazomethane in CH_2Cl_2 (20 mL, 10 mmol) until a faint

yellow color persisted and the evolution of N_2(g) ceased. Several drops of acetic acid were used to dispel the color, and the solvent was removed in vacuo to provide a quantitative yield of **58d** (2.53 g) as a syrup. To a solution of this Z-DL-serine methyl ester (**58d**) (2.53 g, 10.0 mmol) in pyridine (9.0 mL, 11 mmol) at -15°C was added p-toluenesulfonyl chloride (2.10 g, 11.0 mmol) in small portions over 5 min. The mixture was stirred 6 h at -10 to -5°C, and poured onto crushed ice (200 mL). Enough 20% aqueous citric acid was added to adjust pH to 5.0 and the mixture was allowed to stand 16 h at 0°C. The mixture was extracted with EtOAc (3 x 100 mL) and the combined organic extracts were washed with 50% saturated aqueous $CuSO_4$ (4 x 150 mL) (to remove pyridine), and saturated brine (3 x 150 mL). The EtOAc phases were dried over Na_2SO_4 and evaporated in vacuo at 35°C to provide 3.84 g (94%) of crude **79d** as a golden syrup. The syrup was recrystallized from a small volume of hot MeOH and dried in vacuo to yield 2.65 g (65%) of pure racemic **79d**: mp 97-99°C (lit. mp 119-120°C for L-isomer[187a]); IR ($CHCl_3$ cast) 3380 (m, br), 1752 (s, sh), 1725 (vs), 1523 (s), 1453 (m), 1438 (m), 1364 (vs), 1345 (vs), 1250 (m), 1214 (s), 1190 (s, sh), 1177 (vs) cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ7.64 (d, 2H, 8 Hz, o-ArH), 7.46 (d, 2H, 8 Hz, m-ArH), 7.37 (s, 5H, Ph), 5.60 (br d, 1H, ~ 6 Hz, NH), 5.10 (s, 2H, CH_2Ph), 4.58 (m, 1H, CH), 4.40 (m, 2H, CH_2OTs), 3.72 (s, 3H, $COOCH_3$), 2.43 (s, 3H, $ArCH_3$); CI-MS (NH_3) 425

($M+NH_4^+$); R_f 0.65 (50% EtOAc/hexane).

N-(Benzyloxycarbonyl)-γ-γ'-di-tert-butyl-DL-γ-carboxy-glutamic acid (80d).

This compound was prepared by a modification of the procedure of Boggs et al.[183a] Di-tert-butyl malonate (1.15 mL, 5.14 mmol) was added dropwise with stirring to a suspension of NaH (88.5 mg, 3.68 mmol, washed 3x with dry THF (3 mL)) in DMF (10 mL) at 0°C. After 30 min the NaH had dissolved and evolution of H_2(g) had ceased. This solution was added dropwise over 10 min to Z-O-Ts-DL-serine methyl ester (**79d**) (1.35 g, 3.31 mmol) in DMF (5.0 mL) at 25°C. The mixture was stirred 20 h at 25°C under Ar, and worked up by addition to H_2O while maintaining pH between 3 and 5 with 1N HCl. The pH was adjusted to 2.5, saturated brine (20 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 x 100 mL). Pooled organic phases were washed with saturated $NaHCO_3$ (20 mL) and brine (20 mL), dried, and concentrated in vacuo to yield 2.45 g of an oil containing Z-di-tert-butyl-γ-carboxyglutamic acid α-methyl ester (R_f 0.30, 20% EtOAc/hexane). This material was directly saponified by stirring 25 min at 25°C in 0.48N ethanolic KOH (40 mL, 19 mmol). The mixture was cooled on ice/H_2O and neutralized (pH 7.0) with cold 1N HCl, and the ethanol removed in vacuo. The aqueous mixture was acidified to pH 2.5 and extracted with EtOAc (3 x 100 mL). Organic phases were pooled, dried over

Na_2SO_4, and evaporated. The residue was either flash chromatographed on silica[260] (10% MeOH/$CHCl_3$), or subjected to reverse-phase MPLC (40% MeOH/30% CH_3CN/30% H_2O, 3.0 mL/min) to afford 1.28 g (88% yield) of **80d** as a syrup which crystallized from CCl_4/pentane: mp 74-76°C (lit. mp 64-65°C[183b]); IR ($CHCl_3$ cast) 3340 (m, br), 2970 (m), 1740 (s, sh), 1727 (vs), 1523 (m, br), 1369 (m), 1255 (s, br), 1162 (m, sh), 1143 (s) cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ10.70 (br s, 1H, COOH), 7.38 (s, 5H, Ph), 5.58 (br d, 1H, 8.4 Hz, NH), 5.12 (s, 2H, CH_2Ph), 4.68-4.30 (m, 1H, N-CH), 3.38 (~t, 1H, ~6.5 Hz, $CH_2CH(COO^tBu)_2$), 2.70-1.95 (m, 2H, $CHCH_2CH$), 1.45 (br s, 18H, tert-Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ175.67 (s), 168.29 (s), 168.00 (s), 156.21 (s), 136.11 (s), 128.42 (d), 128.23 (d), 128.04 (d), 82.16 (t), 67.18 (s), 52.53 (d), 50.81 (d), 30.82 (t), 27.80 (q); Anal. Calc. for $C_{22}H_{31}NO_8$: C, 60.40; H, 7.14; N, 3.20. Found: C, 60.34; H, 7.14; N, 3.17; CI-MS (NH_3) 455 ($M+NH_4^+$), 399 ($M+NH_4-C_4H_8$), 347 ($MH-C_7H_7$), 355 ($M+NH_4-C_4H_8$, CO_2); R_f 0.58 (0.5% HOAc in EtOAc).

Malonate Diester Additions to N-Protected Serine β-Lactones:

Reagents, solvents and conditions were as specified in Table 3. Typically the final concentration of N-protected serine β-lactone in the reaction mixtures was 0.4-0.5 mmol/5 mL. For reactions done under basic conditions (Entries 1-12), a solution of β-lactone (~0.25

M) was added dropwise to the malonate diester and base. Procedures involving the trimethylsilyl ketene acetal of di-tert-butyl malonate (**83**) were performed by addition of reagent to a solution of the β-lactone and **83**. Reactions not involving Ti(IV) were worked up by addition to H_2O (20 mL) while maintaining the pH between 3 and 5 with 1N HCl, lowering pH to 2.5, and extracting with EtOAc (3 × 30 mL). Reactions employing Ti(IV) reagents were quenched by addition to pH 3.0 aqueous saturated EDTA (30 mL) in a similar fashion, followed by filtration, washing of the residue with Et_2O, and extraction of the aqueous layer with Et_2O (3 × 30 mL). Organic layers were dried over Na_2SO_4 and concentrated in vacuo. Products were isolated by reverse-phase MPLC (60% CH_3CN/H_2O or 40% MeOH/30% CH_3CN/30% H_2O, 3.0 mL/min). Typical experimentals for both types of reactions are described below.

Data for Malonate Addition Products:

Spectral, physical, and chromatographic properties of Z-dehydroalanine (**74**) and benzyl carbamate (**76**) were as previously described.

For N-(benzyloxycarbonyl)-γ,γ'-di-tert-butyl-L-γ-carboxyglutamic acid (80a): mp 85-87°C (lit. mp 84-86°C,[183d] 87-89°C[183a]); $[\alpha]_D^{25}$ +11.8 (±0.1)° (c 1.2, $CHCl_3$) (lit. $[\alpha]_D^{25}$ +11.9° (c 1.2, $CHCl_3$),[183d] -11.2 and 12.4°[183a] (c 1.1, MeOH)); IR, NMR, MS, and chromatographic characteristics were essentially identical to **80d** above.

For tert-butyl (S)-4-[N-(benzlyoxycarbonyl)amino]-2-(tert-butoxycarbonyl)-5-hydroxy-3-oxopentanoate (81): gum; IR ($CHCl_3$) 3380 (m, br), 2979 (s), 2934 (m), 1750 (m, sh), 1725 (vs, br), 1516 (m), 1456 (m), 1394 (m), 1370 (s), 1310 (s, br), 1255 (s, br), 1155 (s, br), 1058 (m, br), 850 (m), 740 (m), 697 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.8 (br s, 0.35H, enolic OH), 7.42-7.26 (m, 5H, Ph), 5.[illegible] (d, 0.65H, 8.0 Hz, NH-keto), 5.54 (d, 0.35H, 8 Hz, NH-enol), 5.20-5.04 (m, 2H, CH_2Ph), 4.96-4.86 (m, 0.35H, $CHCH_2$-enol), 4.61-4.52 (m, 0.65H, $CHCH_2$-keto), 4.64 (br s, 0.65, $CH(CO^tBu)_2$), 4.15 (dd, 0.65H, 3.0, 12.0 Hz, CHHOH-keto), 3.78 (dd, 0.65H, 4.5, 12.0 Hz, CHHOH-keto), 3.88 (dd, 0.35H, 5.4, 11.0 Hz, CHHOH-enol), 3.76 (dd, 0.35H, 7, 11.0 Hz, CHHOH-enol), 3.16 (br s, 1H, OH), 1.49 (br s) and 1.45 (br s) (18H, 2 tert-Bu); ^{13}C NMR (75.46 MHz, $CDCl_3$) (~2:1 keto(k)/enol(e)) δ198.70 (k, s), 175.83 (e, s), 170.64 (e, s), 165.63 (e, s), 163.95 (k, s), 163.78 (k, s), 156.26 (k, s), 155.79 (e, s), 136.14 (s), 136.02 (s), 128.59 (d), 128.55 (d), 128.30 (d), 128.14 (d), 103.69 (e, s), 83.90 (k, s), 83.80 (e, s), 83.60 (k, s), 82.32 (e, s), 67.38 (k, t), 67.18 (e, t), 64.62 (k, d), 63.93 (e, t), 61.99 (k, t), 61.72 (e, d), 53.54 (k, d), 28.23 (e, q), 27.97 (e, q), 27.64 (k, q), 27.78 (k, q); Anal. Calc. for $C_{22}H_{31}NO_8$: C, 60.40; H, 7.14; N, 3.20. Found: C, 60.39; H, 7.39; N, 3.09; CI-MS (NH_3) 455 ($M+NH_4$), 437 ($M+NH_4-H_2O$), 337 ($M+NH_4-H_2O$, CO_2, C_4H_8), 237 ($M+NH_4-H_2O$, $2CO_2$, $2C_4H_8$, Base peak); R_f 0.26 (25%

EtOAc/toluene).

For tert-butyl (S)-4-[N-(benzyloxycarbonyl)amino]-5-hydroxy-3-oxopentanoate (82): oil; IR ($CHCl_3$ cast) 3330 (m, br), 1716 (vs, br), 1519 (m), 1455 (w), 1369 (m), 1330 (m), 1255 (s), 1151 (m), 1055 (m), 698 (m) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ7.37 (s, 5H, Ph), 5.91 (br d, 1H, 8 Hz, NH), 5.14 (s, 2H, CH_2Ph), 4.49 (m, 1H, $CHCH_2OH$), 4.14 (dd, 1H, 4, 12 Hz, $CHCHHOH$), 3.89 (dd, 1H, 6, 12 Hz, $CHCHHOH$), 3.58 (d, 1H, 18 Hz, $C(O)CHHCOO^tBu$), 3.54 (d, 1H, 18 Hz, $C(O)CHHCOO^tBu$), 2.8 (br s, 1H, OH), 1.4[illegible] s, 9H, tert-Bu); CI-MS (NH_3) 355 ($M+NH_4$, Base peak) 33[illegible] ($M+NH_4-H_2O$), 237 ($M+NH_4-H_2O$, CO_2, C_4H_9); R_f 0.80 (0.5% HOAc in EtOAc).

For N-(tert-butoxycarbonyl)-γ-γ'-dibenzyl-L-γ-carboxyglutamic acid (84): gum; IR ($CHCl_3$ cast) 3360 (m, br), 2970 (w), 1744 (s, sh), 1717 (vs), 1523 (m, br), 1373 (m), 1252 (s, br), 1162 (m), 1141 (m), 698 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ10.74 (br s, 1H, COOH), 7.43-7.28 (br m, 10H, Ph's), 5.50 (br, 1H, NH), 5.33-5.13 (m, 4H, CH_2PH's), 4.48-[illegible]8 (br m, 1H, CH), 3.74-3.67 (m, 1H, $CH(COOBn)_2$), 2.68-2.58 (m, 1H, $CHCHHCH(COOBn)_2$), 2.35-2.23 (m, 1H, $CHCHHCH(COOBn)_2$), 1.43 (s, 9H, tert-Bu); Anal. Calc. for $C_{25}H_{29}NO_8$: C, 63.68; H, 6.20; N, 2.97. Found: C, 63.49; H, 6.36; N, 3.03; CI-MS (NH_3) 489 ($M+NH_4^+$), 415 ($M+NH_4-H_2O$, C_4H_8), 381 ($MH-C_7H_7$), 337 ($MH-C_7H_7$, CO_2), 302 (Base peak, ($CH_2(COOBn)_2+NH_4$)).

For benzyl (S)-4-[N-(tert-butoxycarbonyl)amino]-2-(benzyloxycarbonyl)-5-hydroxy-3-oxopentanoate (85): mp

92-97°C (from $\underline{i}Pr_2O$/pentane); $[\alpha]_D^{25}$ +3.3 (±0.2)° (c 0.60, $CHCl_3$); IR ($CHCl_3$ cast) 3400 (w, br), 1749 (s), 1734 (s), 1718 (vs), 1660 (w, sh), 1500 (m), 1455 (m), 1392 (m), 1369 (m), 1265 (s, br), 1163 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ7.48-7.26 (m, 10H, 2Ph), 5.50 (br d, 1H, 8 Hz, NH), 5.30-5.04 (m, 4H, $2CH_2Ph$), 4.65 (s, 1H, $CH(COOBn)_2$), 4.63-4.50 (m, 1H, $CHCH_2OH$), 4.13 (dd, 1H, 3.3, 12.0 Hz, CHCHHOH), 3.79 (dd, 1H, 5, 12 Hz, CHCHHOH), 3.62 (br s, 1H, OH), 1.49 (s, 9H, tert-Bu) (cf. **81** above, <7% enol); ^{13}C NMR (100.57 MHz, $CDCl_3$) δ199.09 (s), 189.03 (s), 166.27 (s), 138.29 (s), 135.16 (s), 128.75 (d), 128.62 (d), 128.51 (d), 128.45 (d), 128.32 (d), 128.00 (d), 81.30 (s), 70.15 (t), 67.35 (t), 67.28 (t), 64.21 (d), 58.81 (d), 28.21 (q); Anal. Calc. for $C_{25}H_{29}NO_8$: C, 63.68; H, 6.20; N, 2.97. Found: C, 63.49; H, 6.21; N, 2.92; CI-MS (NH_3) 489 ($M+NH_4$), 471 ($M+NH_4-H_2O$), 381 ($MH-C_7H_7$), 337 ($MH-C_7H_7$, CO_2, Base peak), 302 ($CH_2(COOBn)_2+NH_4^+$).

For benzyl (S)-4-[N-(benzyloxycarbonyl)amino]-5-hydroxy-3-oxo-pentanoate (86): mp 104-105°C (from i-Pr_2O/pentane); $[\alpha]_D^{25}$ +4.5 (±0.1)° (c 1.30, $CHCl_3$); IR 3380 (m, br), 1749 (vs), 1719 (vs), 1695 (s, sh), 1500 (s), 1456 (m), 1392 (m), 1368 (m), 1264 (vs), 1163 (s), 1055 (m), 698 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ7.40-7.35 (m, 5H, Ph), 5.41 (br d, 1H, NH), 5.30-5.08 (m, 3H, CH_2Ph, OH), 4.66-4.56 (m, 1H, $CHCH_2OH$), 4.57 (dd, 1H, 4, 11 Hz, CHHOH), 4.39 (dd, 1H, 3.5, 11 Hz, CHHOH), 3.70 (d, 1H, 16 Hz, D_2O exchangeable, C(O)CHHCOOBn), 3.66 (d, 1H, 16 Hz,

D_2O exchangeable, C(O)CHHCOOBn), 1.43 (s, 9H, tert-Bu); ^{13}C NMR (100.13 MHz, $CDCl_3$) 199.59 (s), 166.78 (s), 155.00 (s), 135.07 (s), 128.89 (d), 128.80 (d), 128.72 (d), 128.64 (d), 128.60 (d), 81.5 (s), 70.11 (t), 67.29 (t), 66.14 (t), 58.80 (d), 27.99 (q); CI-MS (NH_3) 355 ($M+NH_4^+$), 337 ($M+NH_4-H_2O$, Base peak), 281 ($M+NH_4-H_2O$, C_4H_8), 229 ($M+NH_4-H_2O$, $PhCH_2OH$).

tert-Butyl 3-tert-butoxy-3-trimethylsiloxypropenoate (83).

A procedure modified from that of Ainsworth et al.[189] was employed. Sodium hydride (0.418 g, 17.4 mmol; washed with THF (3 × 5 mL)) was suspended in dry THF (20 mL) and di-tert-butyl malonate (3.0 mL, 13.4 mmol; distilled) was added dropwise and the mixture stirred 1 h at 25°C. Chlorotrimethylsilane (6.8 mL, 53.5 mmol) was added to dry Et_3N (7.5 mL, 53.5 mmol) in Et_2O (10 mL) and the mixture was centrifuged to remove triethylamine hydrochloride. The supernatant was added to the malonate solution and stirred 3 h at 25°C under Ar. The mixture was centrifuged under Ar and the supernatant was concentrated in vacuo at 30°C. The golden oily residue was stirred with Et_2O/pentane (1:3, 50 mL) and filtered. The filtrate was concentrated in vacuo at 30°C to provide 3.12 g (81%) of **83** as a yellow oil which was pure by ^1H NMR. This material was further purified by bulb-to-bulb distillation (0.025 torr/50 (±3)°C) to provide 2.94 g (76%) of clear colorless oil (**83**). This material decomposes on silica to

di-tert-butyl malonate: IR (film) 2979 (s), 1745 (m, sh), 1729 (s, sh), 1715 (vs), 1677 (m), 1606 (vs), 1392 (m), 1369 (s), 1251 (vs), 1125 (vs, br), 1064 (vs), 854 (vs) cm^{-1}; NMR suggests a ratio of approximately 1.5 (±0.1):1 for E- and Z-isomers each having major/minor rotomers; ^1H NMR (360 MHz, $CDCl_3$) δ4.31 (s, 0.34H, CH (Z)), 4.16 (s, 0.05H, CH (Z)), 3.18 (s, 0.35H, CH (E)), 3.09 (s, 0.26H, CH (E)), 1.47 (s, 10.7H, tert-Bu (E)), 1.46 (s, 7.3H, tert-Bu (Z)), 0.33-0.28 (m, 5.4H, $OSi(CH_3)_3$ (E)), 0.23-0.15 (m, 3.6H, $OSi(CH_3)_3$ (Z)); ^{13}C NMR (90 MHz, $CDCl_3$) δ168.14 (E, Z), 80.95 (Z), 79.87 (E), 49.61 (E), 44.51 (Z), 29.32, 28.76, 28.64, 28.32, 28.14, 0.73 (Z), -1.82 (E); Anal. Calc. for $C_{14}H_{28}O_4Si$: C, 58.29; H, 9.78. Found: C, 58.35; H, 9.76; EI-MS: no M^+, 232.1126 ($M-C_4H_8$, 232.1124 calcd. for $C_{10}H_{20}O_4Si$), 176.0504 ($(CH_3)_3SiO-C(O)CH_2CO_2H$), 75.0269 ($(CH_3)_2SiOH$); GC (RSL-300, 100°C, 13.9 mL/min N_2) single peak, t_R = 15.6 min.

Illustrative Example of a Reaction of Malonate Diester-Anion with N-Protected Serine β-Lactones (Entry 7, Table 3):

A dispersion of 35% KH in mineral oil (w/w) (100.5 mg, 35.2 mg KH, 0.877 mmol) was washed by suspension and settling in dry THF (3 × 2 mL). To a stirred suspension of the KH in THF (0.5 mL) at 0°C was added dibenzyl malonate (240 μL, 0.962 mmol) dropwise. The mixture was

warmed to 25°C and stirred 10 min until the evolution of H_2(g) ceased. DMF (2.5 mL) was added followed by dropwise addition of a solution of BOC-L-serine β-lactone (**42a**) (150 mg, 0.801 mmol) in DMF (5.0 mL) at 0°C over 10 min. After 39 h at 0 (±2)°C all β-lactone was consumed and workup and isolation as described in the general procedure provided 137.3 mg (36%) of **84**, 196.8 mg (52%) of **85**, and 23.9 mg (9%) of **86**.

Illustrative Example of a Reaction of 83 with Z-L-Serine β-Lactone (36a) (Entry 13, Table 3):

Titanium tetrachloride (26 μL, 0.24 mmol) was added dropwise to titanium(IV) isopropoxide (252 μL, 0.85 mmol) in dry CH_2Cl_2 (1.5 mL) at -78°C and the mixture warmed to 25°C. This solution was added dropwise over 25 min at -15°C to **83** (190 μL, 0.68 mmol) and Z-L-serine (**36a**) (75.0 mg, 0.339 mmol) in CH_2Cl_2 (5.0 mL). The mixture was stirred 3 h at -15°C and 1 h at 25°C. Workup and isolation as outlined in the general procedure afforded 128.6 mg (87%) of **81**.

(S)-2-Methoxy-2-(trifluoromethyl)phenylacetate, potassium salt (87).[268]

(S)-(-)-2-Methoxy-2-(trifluoromethyl)phenylacetic acid[196,286] (MTPA) (1.00 g, 4.27 mmol) was dissolved in THF/MeCN/H_2O (8 mL/1 mL/2 mL), and 2N aqueous KOH (~2.1 mL) was added dropwise to adjust the apparent pH to 6.5.

The solvent was removed *in vacuo* (30°C) and the solid residue was shaken with Et_2O (50 mL) and chilled (4°C). The salt **87** was obtained as a white powder (89% yield) by filtration, washing with chilled ether, and drying *in vacuo* over P_2O_5 and KOH: mp >330°C; $[\alpha]_D^{25}$ -69.2° (c 3.0, MeOH); IR (KBr disk) 1660 (m), 1650 (s), 1629 (vs), 1378 (s), 1265 (s), 1167 (vs), 1154 (vs), 801 (s), 718 (s), 697 (s) cm^{-1}; Anal. Calc. for $C_{10}H_8O_3F_3K$: C, 44.12; H, 2.96. Found: C, 44.13; H, 2.96; NEGFAB-MS (glycerol): 233 (M^-).

General Procedure for Determination of Optical Purity of β-Lactones 36a, 36b, 44a, and 44b as (S)-MTPA Derivatives 88a, 88b, 89a, and 89b:[268]

A solution of the β-lactone **36** or **44** (0.271 mmol) and MTPA salt **87** (147.7 mg, 0.542 mmol) was stirred 18 h in dry DMF at 3°C. The DMF was removed *in vacuo* and the residue was treated with an excess of etheral diazomethane. The syrup obtained after evaporation of the solvent was redissolved in $CHCl_3$, and an aliquot was submitted to analysis by HPLC (Beckman 5 μm Ultrasphere-Si; 254 nm detection). For ^{19}F NMR analysis, the remainder of the sample was purified by MPLC (silica, EtOAc/hexanes (35:65) for **88a**, **88b**, and **90**; (26:74) for **89a**, **89b**, and **91**) to yield the appropriate *N*-protected *O*-[(*S*)-2-methoxy-2-(trifluoromethyl)phenylacetyl]serine methyl ester (typically 63-68% isolated) and methyl (*S*)-2-

methoxy-2-(trifluoromethyl)phenylacetate (**92**)[196,287] (typically 64.6 mg, 48%) as liquids. For **92**: $[\alpha]_D^{25}$ -72.2° (c 0.34, acetone); IR ($CHCl_3$ cast) 1752 (vs), 1450 (m), 1273 (s), 1170 (vs), 1030 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ7.52 (m, 2H, o-Ph), 7.40 (m, 3H, m, p-Ph), 3.90 (s, 3H, $COOCH_3$), 3.55 (~q, 3H, ~1.5 Hz, OCH_3); ^{19}F NMR (376 MHz, $CDCl_3$) δ-72.31 (CF_3); EI-MS: 248.0661 (M^+, 248.0661 calcd. for $C_{11}H_{11}F_3O_3$).

Data for 88a (S,S-isomer) from Z-L-serine β-lactone (36a):[121] IR ($CHCl_3$ cast) 3470-3200 (w, br), 1754 (vs), 1728 (s), 1510 (m), 1271 (s), 1220 (s), 1170 (vs) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ7.46 (m, 2H, o-Ph), 7.37 (m, 3H, m, p-Ph), 7.35 (s, 5H, $PhCH_2O$), 5.51 (d, 1H, 7 Hz, NH), 5.12 (s, 2H, CH_2Ph), 4.72 (dd, 1H, 10.5, 3.5 Hz, CHCHHO), 4.68 (m, 1H, CH), 4.60 (dd, 1H, 2.5, 10.5 Hz, CHCHHO), 3.66 (s, 3H, $COOCH_3$), 3.47 (~q, 3H, ~1.5 Hz, OCH_3); ^{19}F NMR (376.5 MHz, $CDCl_3$) δ-72.76 (CF_3);[199] Anal. Calc. for $C_{22}H_{22}NO_7F_3$: C, 56.29; H, 4.72; N, 2.98. Found: C, 56.11; H, 4.76; N, 2.91; EI-MS: 469.1345 (M^+, 469.1349 calcd.).

Data for 88b (R,S-isomer) from Z-D-serine β-lactone (36b):[288] IR and EI-MS as described for **88a**. ^1H NMR (300 MHz, $CDCl_3$) was indistinguishable from **88a** except for δ3.73 (s, 3H, $COOCH_3$), 3.49 (~q, 3H, ~1.5 Hz, OCH_3); ^{19}F NMR (376.5 MHz, $CDCl_3$) δ-72.23 (CF_3);[199] Anal. Found: C,

56.20; H, 4.72; N, 2.94.

Data for 90: This standard was prepared by subjecting a mixture of **36a**[197] (65.22%, 0.1768 mmol) and **36b**[198] (34.78%, 0.0942 mmol) to the above general procedure. ^{19}F NMR (376.5 MHz, $CDCl_3$) δ -76.26 (65% (S,S)-CF_3), -76.23 (35% (R,S)-CF_3).[199] HPLC analysis (9% EtOAc/91% hexane, 0.8 mL/min) provided a ratio of 64.80 (±0.11)% S,S (t_R = 78 min) and 35.20% R,S-isomer (t_R = 85 min).

Data for 89a (S,S-isomer) from β-lactone 44a:[203] IR ($CHCl_3$ cast) 1754 (vs), 1706 (s), 1273 (s), 1244 (s), 1183 (s), 1172 (s), 1028 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$)[202] δ7.50-7.10 (m, 15H, 3 Ph), 5.13 (m, 2H, $PhCH_2O$), 4.82-4.00 (m, 5H, $CH-CH_2O$, $PhCH_2N$), 3.60 (s, 0.59 × 3 H) and 3.33 (s, 0.41 × 3 H), ($COOCH_3$ conformers), 3.40 (br s, 3H, OCH_3); ^{19}F NMR (376.5 MHz, $CDCl_3$) δ -72.14 (CF_3); Anal. Calc. for $C_{29}H_{28}NO_7F_3$: C, 62.25; H, 5.04; N, 2.50. Found: C, 62.43; H, 4.98%; N, 2.46%; CI-MS (NH_3) 577 ($M+NH_4^+$), 560 (MH^+).

Data for 89b (R,S-isomer) from 44b:[289] IR ($CHCl_3$ cast) 1754 (vs), 1705 (s), 1270 (m), 1237 (s), 1182 (s), 1171 (s), 1026 (s) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$)[202] δ7.53-7.05 (m, 15H, 3 Ph), 5.16 (m, 2H, $PhCH_2O$), 4.91-4.01 (m, 5H, $CH-CH_2O$, $PhCH_2N$), 3.61 (s, 0.58 × 3 H) and 3.34 (s, 0.42 × 3 H) ($COOCH_3$ conformers), 3.40 (br s, 3H, OCH_3); ^{19}F NMR

(376.5 MHz, $CDCl_3$) δ-72.04 (s, 0.58 × 3 F) and -71.96 (s, 0.42 × 3 F) ($\underline{CF_3}$ conformers); Anal. Found: C, 61.90; H, 5.03; N, 2.37; CI-MS (NH_3) 577 ($M+NH_4^+$), 560 (MH^+).

Data for 91: This reference sample was prepared by submitting a mixture of ($\underline{S}$)-β-lactone (**44a**)[200] (67.12%, 0.1292 mmol) and ($\underline{R}$)-β-lactone (**44b**)[201] (32.88%, 0.0633 mmol) to the above general procedure. ^1H NMR (200 MHz, $CDCl_3$)[202] δ3.46 (s, ($\underline{S,S}$)-OCH_3), 3.43 (s, ($\underline{R,S}$)-OCH_3) (total 3H, ~2:1, incomplete resolution) with the remainder of spectrum as described for **89a** and **89b** above; ^{19}F NMR (376.5 MHz, $CDCl_3$)[202] δ-72.14 (s, 67% ($\underline{S,S}$)-$\underline{CF_3}$), -71.96 (s), -72.04 (s) (58:42 ratio of conformers), 33% ($\underline{R,S}$)-$\underline{CF_3}$). HPLC analysis (6% EtOAc/94% hexane, 1.0 mL/min) provided a ratio of 67.4 (±0.3)% $\underline{S,S}$ (t_R = 79.5 min) and 32.6% $\underline{R,S}$-isomers (t_R = 73.8 min).

Reactions of Higher-Order Organocyanocuprates $R_2Cu(CN)Li_2$ with β-Lactones:[268]

These reagents were prepared immediately before use as outlined by Lipshutz et al.[221a] Reactions were routinely followed by spotting onto TLC silica plates which had previously been treated with HOAc. Following removal of HOAc in vacuo, the plate was developed (EtOAc/hexane), sprayed with alkaline bromocresol green spray,[290] and heated. When β-lactone could no longer be detected the reaction was terminated by addition to

degassed 0.5N HCl (~15 mol eq. relative to β-lactone) at 0-5°C, 0.25 volumes MeOH were added, and the mixture was stirred 20 min under Ar. The CuCl precipitate was removed by suction filtration and washed with Et_2O (1 volume). The filtrate was partitioned, and the aqueous layer further extracted with Et_2O (3 × 1 vol.). Ether phases were pooled and washed successively with saturated brine, pH 3.0 saturated EDTA solution, and again with brine (0.25 volumes of each), dried over Na_2SO_4 and evaporated *in vacuo*. Chromatographic purification of the residue afforded the results indicated below.

(S)-2-[(Benzyloxycarbonyl)amino]butanoic acid (93a, Table 4, Entry 1)

The cuprate $Me_2Cu(CN)Li_2$ was formed by addition of MeLi in Et_2O (7.23 mmol, 4.13 mL) to CuCN (417 mg, 4.65 mmol) in THF (8 mL) at -78°C.[221a] The mixture was stirred at -23°C for 20 min and β-lactone (**36a**)[208] (200 mg, 0.904 mmol) was added dropwise in THF (2.5 mL) over 5 min. The mixture was stirred 2 h at -23°C and 15 min at 0°C. The reaction mixture was then quenched and extracted as outlined above. Reverse phase MPLC (45% CH_3CN/H_2O, 3.0 mL/min) yielded 100.8 mg (47%) of **93a** as a syrup which crystallized from Et_2O/hexane: mp 78.5-79.0°C (lit.[267a] 78-79°C); $[\alpha]_D^{25}$ -31.3 (±.2)° (c 2.0, EtOH) (lit.[267a] $[\alpha]_D^{25}$ -32° (c 2, EtOH)); IR (CH_2Cl_2 cast) 3350-2200 (m, br), 1717 (vs), 1526 (s), 1456 (m), 1415 (m), 1345 (m),

1231 (m), 1216 (s), 1085 (m), 1054 (m), 697 (m) cm^{-1}; ^1H-NMR (200 MHz, $CDCl_3$)[202] δ7.70 (br s, 1H, COOH), 7.33 (s, 5H, Ph), 6.30 (br s, 0.2 H) and 5.35 (d, 0.8H, 8 Hz) (rotomeric NH), 5.11 (s, 2H, $PhCH_2O$), 4.46-4.15 (m, 1H, CH), 2.07-1.62 (m, 2H, CHHMe), 0.96 (t, 3H, 7.5 Hz, CH_3); EI-MS: 237.1004 (M^+, 237.1001 calcd. for $C_{12}H_{15}NO_4$); CI-MS (NH_3) 255 ($M+NH_4^+$). Deprotection to (S)-2-aminobutanoic acid (**111a**) and GC analysis as the camphanamide methyl ester derivative (**118a**) indicated 97.83 (±0.14)% enantiomeric excess (i.e., 1.08% D-isomer present).

(R)-2-[N-Benzyl-N-(benzyloxycarbonyl)amino]butanoic acid (94b, Entry 4, Table 4).

The cuprate $Me_2Cu(CN)Li_2$ was prepared from CuCN (59.3 mg, 0.636 mmol) in THF (3 mL), and MeLi in Et_2O (1.06 mmol, 0.95 mL).[221a] The (R)-β-lactone (**44b**)[201] (110 mg, 0.353 mmol) was added in THF (2 mL) dropwise over 5 min at -78°C, and the mixture was stirred at -78°C (1 h), and -45°C (30 min). Quenching and extraction in the usual fashion followed by reverse-phase MPLC (56% CH_3CN/H_2O, 3.0 mL/min) provided 24.0 mg of unreacted β-lactone (**44b**) (22%), 6.9 mg ketone (**95**) (6%), and 75.7 mg (72%) of the (R)-acid **94b**: $[\alpha]_D^{25}$ +37.3 (±0.7)° (c 0.46, $CHCl_3$); IR ($CHCl_3$ cast) 3030 (m, br), 1741 (m), 1705 (vs), 1670 (m), 1454 (m), 1420 (m), 1250 (m), 698 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$)[202] δ9.12 (br s, 1H, COOH), 7.27 (br s, 10H,

2Ph), 5.17 (s, 2H, $PhCH_2O$), 4.80-4.00 (m, 3H, CH, $PhCH_2N$), 2.14-1.60 (m, 2H, $CHCH_2CH_3$), 0.81 (m, 3H, CH_3); EI-MS: 327.1469 (M^+, 327.1471 calcd. for $C_{19}H_{21}NO_4$); CI-MS (NH_3) 345 ($M+NH_4^+$), 328 (MH^+). Optical purity analysis (GC) as derivative **118b** indicated 2.07 (±0.21)% of the S-isomer or 95.9 (±0.5)% e.e.

For 2-[N-benzyl-N-(benzyloxycarbonyl)amino]-1-hydroxybutan-3-one (95): IR ($CHCl_3$ cast) 3450 (m), 1697 (s), 1238 (s), 1127 (m), 700 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$)[202] δ7.30 (m, 10H, 2Ph), 5.20 (m, 2H, $PhCH_2O$), 4.56 (m, 2H, $PhCH_2N$), 4.12 (m, 1H, CH), 3.8-3.4 (m, 2H, $CHCH_2O$), 3.26 (br s, 0.6H) and 2.36 (br s, 0.4H), (CH_2OH) 2.00 (s, 1.8H) and 1.74 (s, 1.2H) ($C(O)CH_3$); Anal. Calc. for $C_{19}H_{21}NO_4$: C, 69.71; H, 6.47; N, 4.28. Found: C, 69.78; H, 6.42; N, 4.08; CI-MS (NH_3) 345 ($M+NH_4^+$), 328 (MH^+).

(R)-2-[N-(Benzyloxycarbonyl)amino]heptanoic acid (96b, Entry 5, Table 4).

The cuprate $n\text{-}Bu_2Cu(CN)Li_2$ was formed from CuCN (528 mg, 5.90 mmol) in THF (6.0 mL) and n-BuLi in hexanes (11.3 mmol, 4.30 mL).[221a] The β-lactone (**36b**)[198] (250 mg, 1.13 mmol) was introduced in THF (4 mL) dropwise over 7 min at -23°C, and the mixture was stirred 2 h. Workup in the usual manner and reverse phase MPLC (40 MeOH/25 MeCN/35 H_2O, 3 mL/min) yielded 196 mg of **96b** (62%) which was recrystallized from CCl_4/hexane: mp 63-64°C (lit.[225] mp

63-65°C for S-isomer); $[\alpha]_D^{25}$ +3.4 (±0.1)° (c 1.43, 95% EtOH) (lit.[225] $[\alpha]_D^{22}$ -3.5° (c 2, 95% EtOH) for S-isomer); IR ($CHCl_3$ cast) 3320 (m, br), 1717 (vs, br), 1521 (m), 1453 (m), 1412 (m), 1340 (m), 1230 (m), 1212 (m), 1053 (m), 695 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$)[202] δ10.20 (br s, 1H, COOH), 7.36 (s, 5H, Ph), 5.80 (br s, 0.3H) and 5.22 (d, 0.7H, 8.2 Hz) (NH), 5.18-5.07 (m, 2H, $PhCH_2O$), 4.45-4.34 (m, 0.7H) and 4.34-4.20 (m, 0.3H), (CH), 1.95-1.79 (m, 1H, CHCHH-Bu), 1.77-1.60 (m, 1H, CHCHH-Bu), 1.45-1.20 (m, 6H, $(CH_2)_3$), 0.88 (~t, 3H, CH_3). EI-MS: 279.1468 (M^+, 279.1470 calcd. for $C_{15}H_{21}NO_4$). Optical purity analysis (GC) as **121b** indicated 1.24 (±0.16)% S-isomer or 97.5 (±0.3)% e.e.

(S)-2-(N-Benzyl-N-(benzyloxycarbonyl)amino)heptanoic acid (97a, Entry 6, Table 4).

The cuprate was formed from CuCN (101 mg, 1.13 mmol) in THF (2.2 mL) and n-BuLi in hexanes (1.9 mmol, 1.6 mL).[221a] A solution of β-lactone (**44a**)[200] (171 mg, 0.548 mmol) in THF (3.3 mL) was added dropwise over 5 min at -78°C, the mixture was stirred 40 min at -78°C, warmed to -46°C and allowed to reach -36°C over 1 h. Workup and reverse phase MPLC (65% $MeCN/H_2O$, 3 mL/min) gave 154 mg (76%) of acid **97a** and 11 mg (5%) of ketone **98**. **For 97a:** $[\alpha]_D^{25}$ -32.3° (c 0.5, $CHCl_3$); IR ($CHCl_3$ cast) 3100 (m), 1706 (s), 1235 (m), 1100 (m), 698 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$)[202] δ9.84 (br s, 1H, COOH), 7.26 (m, 10H, 2

Ph), 5.18 (s, 2H, $PhCH_2O$), 4.64 (m, 1H, CH), 4.5-4.15 (m, 2H, $PhCH_2N$), 1.91 (m, 1H, CHCHH-Bu), 1.74 (m, 1H, CHCHH-Bu), 1.11 (m, 6H, $(CH_2)_3$), 0.78 (m, 3H, CH_3); Anal. Calc. for $C_{22}H_{27}NO_4$: C, 71.52; H, 7.37; N, 3.79. Found: C, 71.30; H, 7.43; N, 3.55; EI-MS: 369.1935 (M^+, 369.1940 calcd.). Optical purity analysis (GC) as derivative **121a** indicated 87.0 (±0.6)% e.e.

For 2-[N-benzyl-N-(benzyloxycarbonyl)amino]-1-hydroxyheptan-3-one (98): IR ($CHCl_3$ cast) 3440 (m, br), 1700 (s), 1233 (m), 1125 (m), 699 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$)[202] δ7.34 (m, 10H, 2 Ph), 5.20 (m, 2H, $PhCH_2O$), 4.53 (m, 2H, $PhCH_2N$), 4.11 (m, 1H, CH), 3.8-3.5 (m, 2H, $CHCH_2$), 3.24 (m, 0.6H) and 1.85 (m, 0.4H) (OH), 2.25 (m, 2H, $C(O)CH_2$-Pr), 1.40 (m, 1H, CHH), 1.17 (m, 2H, CH_2), 1.00 (m, 1H, CHH), 0.77 (m, 3H, CH_3); Anal. Calc. for $C_{22}H_{27}NO_4$: C, 71.52; H, 7.37; N, 3.79. Found: C, 71.52; H, 7.28; N, 3.67; EI-MS: 369.1943 (M^+, 369.1940 calcd.), 284.1286 (M-C(O)Bu).

N-(Benzyloxycarbonyl)-L-leucine (99a, Entry 7, Table 4).

Isopropylmagnesium chloride in Et_2O (5.42 mmol, 1.80 mL) was added dropwise over 5 min to β-lactone (**36a**)[208] (200 mg, 0.904 mmol) and $CuBr \cdot SMe_2$ (35.0 mg, 0.17 mmol) in THF (8 mL)/Me_2S (0.4 mL) at -23°C. The mixture was stirred 1.5 h at -23°C, and quenched by addition to cold degassed 0.5N HCl (20 mL). Extraction and washing in the usual fashion followed by reverse phase MPLC (46%

MeCN/H_2O, 3.5 mL/min) afforded 106 mg (44%) of **99a** as a syrup: $[\alpha]_D^{25}$ -16.8 (±0.2)° (c 1.0, 95% EtOH) (lit.[133] $[\alpha]_D$ -16.5 (±1)° (c 1.0, EtOH)); IR (acetone cast) 3320 (m, br), 2959 (s), 1716 (vs, br), 1528 (s), 1451 (m), 1410 (m, br), 1341 (m), 1260 (m), 1225 (m), 1047 (m), 692 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$)[202] δ9.21 (br s, 1H, COOH), 7.31 (s, 5H, Ph), 6.70 (br d, 0.23H) and 5.27 (d, 0.77H, 8 Hz) (NH, rotomers), 5.18-5.03 (m, 2H, OCH_2Ph), 4.40 (br m, 0.77H) and 4.25 (br m, 0.23H) (NCH, rotomers), 1.82-1.48 (m, 3H, CH_2CHMe_2), 1.04-0.82 (m, 6H, $2CH_3$); EI-MS: 265.1313 (M^+, 265.1314 calcd. for $C_{14}H_{19}NO_4$), 220.1336 ($M-CO_2H$); CI-MS (NH_3) 283 ($M+NH_4^+$), 266 (MH^+). Optical purity analysis (GC) as the camphanamide methyl ester derivative **123a** indicated no detectable R-isomer (i.e., >99.4% e.e.).

N-Benzyl-N-(benzyloxycarbonyl)-L-leucine (100a, Entry 8, Table 4).

Isopropylmagnesium chloride in Et_2O (3.0 mmol, 1.0 mL) was added dropwise over 5 min to β-lactone (**44a**)[200] (180 mg, 0.578 mmol) and $CuBr \cdot SMe_2$ (25 mg, 0.122 mmol) in THF (6 mL)/Me_2S(0.3 mL) at -23°C. The mixture was stirred 2 h at -23°C and quenched by addition to cold degassed 0.5N HCl (20 mL). Extraction and washing of the etheral phases followed by reverse phase MPLC (55% MeCN/H_2O, 3.3 mL/min) yielded 170 mg (83%) of **100a** as an oil: $[\alpha]_D^{25}$ -44.7° (c 2.5, $CHCl_3$); IR ($CHCl_3$ cast) 3160 (m br), 1740

(s), 1705 (vs), 1680 (s), 1498 (m), 1468 (s), 1454 (s), 1418 (s), 1315 (s), 1240 (vs), 1208 (s), 1179 (s), 699 (vs) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$)[202] δ9.75 (br s, 1H, COOH), 7.45-7.10 (m, 10H, 2 Ph), 5.19 (s, 2H, $PhCH_2O$), 4.87-4.62 (m, 1H, CH), 4.60-4.30 (m, 2H, $PhCH_2N$), 1.90-1.20 (m, 3H, CH_2CHMe_2), 0.94-0.53 (m, 6H, 2 CH_3); Anal. Calc. for $C_{21}H_{25}NO_4$: C, 70.97; H, 7.09; N, 3.94. Found: C, 70.68; H, 7.10; N, 3.87; EI-MS: 355.1785 (M^+, 355.1784 calcd.); CI-MS (NH_3) 373 ($M+NH_4$), 356 (MH^+). Optical purity analysis (GC) as **123a** showed no detectable R-isomer (ie., >99.4% e.e.).

(S)-[N-(Benzyloxycarbonyl)amino]-4,4-dimethylpentanoic acid (101a, Entry 10, Table 4).

The higher-order mixed organocuprate tert-$Bu(Me)Cu(CN)Li_2$ was formed from CuCN (267 mg, 2.98 mmol) in THF (7.5 mL), MeLi in Et_2O (2.80 mmol, 1.65 mL), and tert-BuLi in pentane (2.80 mmol, 1.55 mL).[221b] The β-lactone (**36a**)[208] (200 mg, 0.904 mmol) in THF (3.5 mL) was added dropwise over 5 min at -23°C, and the mixture was stirred 1 h. Workup in the usual fashion and reverse phase MPLC (57% $MeCN/H_2O$, 3 mL/min) provided 121 mg (48%) of **101a** which crystallized from Et_2O/hexane: mp 95-97°C; $[\alpha]_D^{25}$ -16.7 (±0.2°) (c 1.17, MeOH)[211b]; IR ($CHCl_3$, cast) 3320 (m br), 2957 (s), 1719 (vs), 1531 (s), 1245 (s), 1050 (m), 694 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$)[202] δ7.90 (br s, 1H, COOH), 7.30 (s, 5H, Ph), 6.10 (br d, 0.20H, 8Hz) and

5.33 (d, 0.80H, 8.5 Hz) (NH), 5.20-5.00 (m, 2H, $PhCH_2O$), 4.45-4.20 (m, 1H, CH), 1.85-1.70 (m, 1H, CHHBu-tert), 1.53-1.40 (dd, 1H, 9, 14 Hz, CHHBu-tert), 0.92 (br s, 9H, tert-Bu); Anal. Calc. for $C_{15}H_{21}NO_4$: C, 64.50; H, 7.58; N, 5.01. Found: C, 64.54; H, 7.33; N, 5.21. EI-MS: 279.1470 (M^+, 279.1470 calcd.), 234.1494 ($M-CO_2H$); CI-MS (NH_3) 297 ($M+NH_4^+$), 280 (MH^+). Optical purity analysis (GC) of the derivative **125a** indicated 99.3 (±0.4)% e.e.

(S)-2-[N-Benzyl-N-(benzyloxycarbonyl)amino]-4,4-dimethylpentanoic acid (102a), N-Benzyl-N-(benzyloxycarbonyl)-L-alanine (103a), and Benzyl N-benzyl carbamate (104).

This reaction was carried out by J.C.G. Drover.[124] tert-Butyllithium (3.0 mmol, 2.0 mL) was added dropwise to a suspension of $CuBr \cdot SMe_2$ (0.340 g, 1.66 mmol) in THF (3 mL) at -78°C and the mixture was stirred for 40 min at -78°C and 20 min at -45°C. A solution of β-lactone (**44a**)[200] (199 mg, 0.382 mmol) in THF (3 mL) was added dropwise over 15 min, and stirring was continued 7 h at -46°C, and 1 h at -10°C. Workup and reverse phase MPLC (60% CH_3CN/H_2O, 3 mL/min) afforded 71.9 mg (51%) of **102a**, 16.9 mg (18%) of urethane **104**, and 27.0 mg (23%) of alanine derivative **103a**.

For 102a, Entry 12, Table 4: mp 114-116°C; $[\alpha]_D^{25}$ -32.4° (c 1.0, $CHCl_3$); IR ($CHCl_3$ cast) 3100 (m, br), 2957 (s), 1742 (m), 1706 (vs), 1453 (m), 1367 (m), 1244 (m),

698 (s) cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ10.20 (br s, 1H, COOH), 7.30 (s, 10H, 2Ph), 5.22 (s, 2H, $PhCH_2O$), 4.74-4.27 (m, 3H, CH, $PhCH_2N$), 2.08 (dd, 1H, 5, 14 Hz, CHHBu-tert), 1.60 (dd, 1H, 5, 14 Hz, CHHBu-tert), 0.82 (s, 9H, tert-Bu); Anal. Calc. for $C_{22}H_{27}NO_4$: C, 71.52; H, 7.37; N, 3.79. Found: C, 71.25; H, 7.31; N, 3.74; EI-MS: 369.1938 (M^+, 369.1940 calcd.). Subsequent deprotection to (S)-2-amino-4,4-dimethylpentanoic acid (**114a**) and GC analysis as **125a** indicated 99.2 (±0.1)% enantiomeric excess.

For 103a: $[\alpha]_D^{25}$ -28.8° (c 0.88, $CHCl_3$); IR ($CHCl_3$ cast) 3100 (m), 1704 (s), 1260 (m), 1213 (m), 1070 (m), 1015 (m), 698 (s) cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ8.75 (br s, 1H, COOH), 7.27 (s, 10H, 2Ph), 5.22 (s, 2H, $PhCH_2O$), 4.92-4.2 (m, 3H, $PhCH_2N$, CH), 1.37 (d, 3H, 7 Hz, CH_3); EI-MS: 313.1311 (M^+, 313.1314 calcd. for $C_{18}H_{19}NO_4$).

For 104: mp 59-61°C (lit.[291] mp 60°C); IR ($CHCl_3$ cast) 3325 (m), 1690 (s), 1532 (m), 1454 (m), 1268 (s), 1140 (m), 748 (m), 697 (s) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ7.34 (m, 10H, 2Ph), 5.15 (s, 2H, $PhCH_2O$), 5.10 (br s, 1H, NH), 4.38 (d, 2H, 6 Hz, CH_2N); EI-MS: 241.1103 (M^+, 241.1102 calcd. for $C_{15}H_{15}NO_2$).

(S)-2-[N-(Benzyloxycarbonyl)amino]-4-pentenoic acid[226] (105a, Entry 13, Table 4).

To β-lactone (**36a**)[32] (74.0 mg, 0.334 mmol) and $CuBr \cdot SMe_2$ (17.2 mg, 0.084 mmol) in THF (3.0 mL) and Me_2S

(0.15 mL) was added vinylmagnesium chloride in THF (1.67 mmol, 1.10 mL) dropwise over 5 min at -23°C. The mixture was stirred 2 h at -23°C and worked up in the usual manner. Reverse phase MPLC (43% CH_3CN/H_2O, 3.0 mL/min) yielded 39.1 mg (47%) of **105a** as a white solid which was recrystallized from Et_2O/hexane: mp 63.5-64.5°C (lit.[292a] mp 65°C); $[\alpha]_D^{25}$ +17.5 (±0.2)° (c 2.0, $CHCl_3$) (lit.[292a] $[\alpha]_D^{25}$ +17.6 (±0.6)° (c 5.0, $CHCl_3$)); IR (acetone cast) 3400 (m, br), 1718 (vs), 1525 (s), 1415 (m), 1345 (m), 1210 (s), 1054 (s), 697 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ10.5 (br s, 1H, COOH), 7.34 (br s, 5H, Ph), 5.80-5.64 (m, 1H, $CH_2CH=CH_2$), 5.31-5.10 (m, 5H, NH, CH_2Ph, $C=CH_2$), 4.54-4.44 (m, 1H, CH), 2.70-2.50 (m, 2H, $CHCH_2CH$); EI-MS: 249.0995 (M^+, 249.1001 calcd. for $C_{13}H_{14}NO_4$), 208.0597 ($M-CH_2CH=CH_2$); CI-MS (NH_3) 268 ($M+NH_4$), 250 (MH^+).[292b] Optical purity analysis (GC) of the derivative **127a** indicated 98.40 (±0.20)% e.e.

(S)-2-[N-Benzyl-N-(benzyloxycarbohyl)amino]-4-pentenoic acid (106a, Entry 14, Table 4).

The cuprate $(CH_2CH)_2Cu(CN)Li_2$ was prepared from CuCN (103.6 mg, 1.16 mmol) in THF (5.0 mL) and vinyllithium in THF (1.93 mmol, 1.04 mL).[221a] The β-lactone (**44a**)[200] (200 mg, 0.642 mmol) in THF (2.5 mL) was added dropwise over 5 min at -78°C, and the mixture stirred 1 h at -78°C, 3 h at -46°C and 30 min at 0°C. Workup in the usual manner and reverse phase MPLC (55% CH_3CN/H_2O, 3 mL/min) afforded 122

mg (56%) of **106a** as an oil: $[\alpha]_D^{25}$ -33.3° (c 2.5, $CHCl_3$); IR ($CHCl_3$ cast) 3100 (m, br), 1742 (m), 1706 (vs), 1678 (m), 1498 (m), 1455 (m), 1421 (m), 1240 (s), 698 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ8.55 (br s, 1H, COOH), 7.27 (m, 10H, 2Ph), 5.71-5.44 (m, 1H, vinylic-CH), 5.18 (s, 2H, $PhCH_2O$), 5.07-4.81 (m, 2H, vinylic-CH_2), 4.69 (m, 1H, CH), 4.49-4.02 (m, 2H, $PhCH_2N$), 2.80-2.40 (m, 2H, $CHCH_2$); EI-MS: 298.1083 ($(M-CH_2CH=CH_2)$, 298.1080 calcd. for $C_{17}H_{16}NO_4$), 294.1491 ($M-CO_2H$, 294.1494 calcd.), 254.1180 ($M-C_3H_5$, CO_2), 204.1027 ($M-CO_2Bn$); CI-MS (NH_3) 357 ($M+NH_4^+$), 340 (MH^+). Optical purity analysis (GC) as **127a** indicated 71.5 (±0.5)% e.e.

N-(Benzyloxycarbonyl)-L-Phenylalanine (107a, Entry 15, Table 4) and (S)-2-[N-(Benzyloxycarbonyl)amino]-1,1-diphenylpropan-1,3-diol (108a).

Phenylmagnesium chloride in THF (5.42 mmol, 2.71 mL) was added dropwise to β-lactone (**36a**)[208] (200 mg, 0.904 mmol) and $CuBr \cdot SMe_2$ (55.8 mg, 0.271 mmol) in THF (8 mL) and Me_2S (0.4 mL) at -23°C over 5 min, and the mixture was stirred 2 h at -23°C. Workup and reverse phase MPLC (46% CH_3CN/H_2O, 3.5 mL/min) provided 149 mg (55%) of Z-L-phenylalanine (**107a**), and 147 mg (43%) of the tertiary alcohol **108a**, which was recrystallized from $CHCl_3$/hexane.

For 107a, Entry 15: mp 86-87°C (lit.[267a] mp 88-89°C); $[\alpha]_D^{25}$ +5.1 (±0.1)° (c 2.0, 98% EtOH) (lit.[267] $[\alpha]_D^{25}$ +5.1° (c 2.0, EtOH)); IR (acetone cast) 3320 (m), 2850

(m), 1720 (s, br), 1520 (m), 1498 (m), 1456 (m), 1417 (m, br), 1349 (m), 1260 (m, br), 1218 (m, br), 1056 (m), 689 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$)[202] δ8.74 (br s, 1H, COOH), 7.45-7.05 (m, 10H, 2Ph), 6.24 (d, 0.22H, 7 Hz) and 5.25 (d, 0.78H, 8 Hz) (NH, rotomers), 5.15-4.93 (m, 2H, OCH_2Ph), 4.68 (m, 0.78H) and 4.51 (m, 0.22H) (NCH), 3.19 (dd, 0.78H, 5.5, 14 Hz) and 3.08 (br dd, 0.22H) (CHHPh), 3.10 (dd, 0.78H, 6.5, 14 Hz) and 2.95 (br dd, 0.22H) (CHHPh); EI-MS: 299.1146 (M^+, 299.1158 calcd. for $C_{17}H_{17}NO_4$), 208.0603 ($M-C_7H_7$), 148.0524 ($M-PhCH_2O_2CNH_2$); CI-MS (NH_3) 317 ($M+NH_4^+$), 300 (MH^+). Optical purity determination (GC) as the derivative **129a** indicated no detectable R-isomer (i.e. >99.4% e.e.).

For 108a: mp 134.0-134.5°C; $[\alpha]_D^{25}$ -68.4 (±0.2)° (c 1.0, $CHCl_3$); IR ($CHCl_3$ cast) 3360 (m, br), 1692 (s), 1538 (m), 1492 (m), 1448 (m), 1258 (m), 1062 (s), 747 (s), 695 (vs) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$)[202] δ7.55-7.00 (m, 15H, 3Ph), 5.90 (d, 1H, 9 Hz, NH), 4.98 (d, 1H, 12.5 Hz, PhCHHO), 4.91 (d, 1H, 12.5 Hz, PhCHHO), 4.88 (m, 1H, $CHPh_2$), 4.70 (m, 0.9H) and 4.56 (m, 0.1H) (N-CH), 3.74 (m, 1H, CHHOH), 3.65 (m, 1H, CHHOH), 3.04 (br s, 2H, 2OH); Anal. Calc. for $C_{23}H_{23}NO_4$: C, 73.19; H, 6.14; N, 3.71. Found: C, 73.14; H, 6.31; N, 3.70; EI-MS: 183.0809 (Ph_2COH^+, Base peak); CI-MS (NH_3) 395 ($M+NH_4^+$), 378 (MH^+), 360 (MH^+-H_2O, Base peak).

N-(Benzyloxycarbonyl)-D-phenylalanine (107b, Entry 16, Table 4).

The cuprate $Ph_2Cu(CN)Li_2$ was prepared from CuCN (311.5 mg, 3.48 mmol) in THF (7.0 mL), and PhLi in cyclohexane-Et_2O (7:3) (6.75 mmol, 3.55 mL).[221a] The β-lactone (**36b**)[198] (150 mg, 0.5 mmol) in THF (2.5 mL) was added dropwise over 5 min at -15°C and the mixture was stirred 2 h. Workup in the usual fashion followed by reverse phase MPLC (62% MeOH/H_2O, 3 mL/min) afforded 93.5 mg (46%) of Z-D-phenylalanine (**107b**): mp 100-101°C (lit.[267a] mp 88-89°C; mp 103°C for DL); $[\alpha]_D^{25}$ -1.6 (±0.1)° (c 2.0, 95% EtOH) (lit.[267a] $[\alpha]_D^{25}$ +5.1 (c 2, EtOH) for L-isomer); IR, ^1H NMR and MS identical to **107a** above. Optical purity determination (GC) as the derivative **129b** indicated 29.6 (±0.1)% e.e.

N-Benzyl-N-(benzyloxycarbonyl)-L-phenylalanines (109a, Entry 17, Table 4).

This reaction was performed by J.C.G. Drover.[124] Phenylmagnesium bromide in THF (1.87 mmol, 3.55 mL) was added dropwise over 10 min to a stirred suspension of $CuBr \cdot SMe_2$ (197 mg, 0.957 mmol) in THF (5 mL) and Me_2S (0.2 mL) at -12°C. The mixture was stirred 2 h at -12°C and β-lactone (**44a**)[215] (120 mg, 0.384 mmol) in THF (3 mL) was introduced dropwise over 5 min. The mixture was stirred 4 h at -12°C and worked up in the usual fashion. Purification by reverse phase MPLC (60% CH_3CN/H_2O, 3

mL/min) afforded 24.6 mg (17%) of biphenyl (**110**) (mp 68-70°C; lit.[214] mp 69-71°C), and 89.5 mg (60%) of **109a**: $[\alpha]_D^{25}$ -107° (c 0.59, $CHCl_3$); IR ($CHCl_3$ cast) 3100 (m), 3025 (m), 1706 (s), 1238 (s), 1123 (m), 986 (m), 750 (m), 698 (s) cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ9.95 (br s, 1H, COOH), 7.25 (m, 15H, 3Ph), 5.26 (s, 2H, $PhCH_2O$), 4.7-3.72 (m, 3H, $PhCH_2N$, CH), 3.3 (m, 2H, CH_2Ph); Anal. Calc. for $C_{24}H_{23}NO_4$: C, 74.02; H, 5.95; N, 3.60. Found: C, 74.11; H, 6.03; N, 3.36; EI-MS: 389.1631 (M^+, 389.1627 calcd.). Optical purity determination (GC) as derivative **129a** indicated 88.5 (±0.2)% e.e.

General Procedures for Deprotection of Amino Acid Derivatives and Determination of Stereochemical Purity: Hydrogenolytic Deprotection of 93, 94, 96, 97, 99, 100, 101, 102, 107, and 109(ab) to Free Amino Acids (see Table 5).

Typically a solution of chromatographically pure but unrecrystallized N-protected amino acid (approx. 50 mg) in $HOAc/H_2O$ (2:1, ~7 mL) was stirred with 5% Pd on carbon under an atmosphere of H_2 for 12-16 h. The catalyst was removed by filtration, and washed with $HOAc/H_2O$ (2:1, 3 × 1 mL). The filtrate was evaporated to dryness in vacuo (35-40°C) and the residue was redissolved in H_2O and lyophilized. Further drying to constant weight in vacuo over P_2O_5 and KOH pellets afforded the free zwitterionic amino acids in 91-99% yield. Optical rotations were

measured when >10 mg of deprotected amino acid was generated.

For (S)-2-aminobutanoic acid (111a) from 93a: Deprotection of **93a** (Entry 1, Table 4) (16.90 mg, 71.2 μmol) produced **111a** (7.13 mg, 97%): IR (KBr disk) 3440 (vs, br), 1626 (s, br), 1474 (w), 1396 (w), 1115 (m) cm^{-1}; ^1H NMR (300 MHz, D_2O) δ3.87 (t, 1H, 6.0 Hz, CH), 2.0-1.85 (m, 2H, $CHCH_2CH_3$), 0.97 (t, 3H, 7.5 Hz, CH_3); POSFAB-MS (glycerol/HCl) 104 (MH^+), 207 (M_2H^+); R_f 0.18 (System B).

For (R)-2-aminobutanoic acid (111b) from 94b: Deprotection of **94b** (Entry 4, Table 4) (36.96 mg, 113 μmol) provided **111b** (11.24 mg, 97%): $[\alpha]_D^{25}$ -7.9 (±0.1)° (c 0.8, H_2O), -40.5 (±0.2)° (c 1.0, AcOH) (lit. $[\alpha]_D^{25}$ -7.94 (c 4.0, H_2O),[261] +42.0 (c 1.2, AcOH) for (S)-isomer[209]); IR, NMR, MS, and chromatographic properties were identical to **111a** above.

For (R)-2-aminoheptanoic acid (112b) from 96b: Deprotection of **96b** (Entry 5, Table 4) (32.83 mg, 117 μmol) yielded **112b** (16.38 mg, 96%): $[\alpha]_D^{25}$ -32.3 (±0.2)° (c 1.02, AcOH) (lit. $[\alpha]_D^{25}$ +33.0 (c 1 to 2, HOAc) for (S)-isomer[209]); IR (KBr disk) 3420 (m, br), 2980-2800 (m, mult), 1625 (m), 1585 (s), 1510 (m), 1405 (m), 1050 (m, br) cm^{-1}; ^1H NMR (300 MHz, D_2O) δ3.74 (t, 1H, 6.3 Hz, CH), 1.86 (br m, 2H, $CHCH_2Bu$), 1.35 (br m, 6H, $(CH_2)_3$), 0.87 (~t, 3H, ~7 Hz, CH_3); POSFAB-MS (glycerol/HCl) 146 (MH^+), 291 (M_2H^+); R_f 0.55 (System B). Analogous deprotection and optical purity analyses on recrystallized **96b** provided

112b with identical properties and e.e.

For (S)-2-aminoheptanoic acid (112a) from 97a: Deprotection of **97a** (Entry 6, Table 4) (39.66 mg, 107 μmol) provided **112a** (14.95 mg, 94%): $[\alpha]_D^{25}$ +28.5 (±0.2)° (c 0.97, HOAc) (lit. $[\alpha]_D^{25}$ +33.0° (c 1 to 2, HOAc)[209]; IR, NMR, MS, and chromatographic properties were identical to **112b** above.

For L-leucine (113a) from 99a: Liberation of L-leucine (**113a**) from **99a** (Entry 7, Table 4) (26.07 mg, 98 μmol) proceeded in 97% yield (12.46 mg): $[\alpha]_D^{25}$ +22.5 (±0.1)° (c 1.0, HOAc) (lit. $[\alpha]_D^{25}$ +22.49 (c 2.0, HOAc)[210]; IR (KBr disk) 3422 (m, br), 3075 (vs, br), 2957 (vs), 2929 (s, sh), 2872 (m), 2000 (w, br), 1690 (s, br), 1579 (m), 1509 (m), 1487 (s), 1388 (m), 1369 (m), 1139 (m), 1076 (m), 1024 (m), 821 (m) cm^{-1}; ^1H NMR (400 MHz, D_2O) δ4.04-3.92 (~t, 1H, C<u>H</u>), 1.90-1.67 (m, 3H, CHC<u>H</u>$_2$C<u>H</u>Me$_2$ 1.03-0.91 (m, 6H, 2C<u>H</u>$_3$); POSFAB-MS (glycerol/HCl) 132 (MH^+), 263 (M_2H^+), 86 (MH^+-H_2O, CO); R_f 0.32 (System B).

For 113a from 100a: Hydrogenolysis of **100a** (Entry 8, Table 4) (49.64 mg, 140 μmol) yielded 18.19 mg (99%) of **113a**: $[\alpha]_D^{25}$ +22.5 (±0.2)° (c 1.0, AcOH) (cf. above); IR, ^1H NMR, POSFAB-MS, and chromatographic properties were identical to **113a** above; EI-MS: 131.0946 (M^+, 132.1025 calcd. for $C_6H_{13}NO_2$).

For (S)-2-amino-4,4-dimethylpentanoic acid 114a from 101a: This compound was generated in 99% yield (14.01 mg)

from **101a** (Entry 10, Table 4) (27.11 mg, 97 μmol): $[\alpha]_D^{25}$ +16.0 (±0.2)° (c 1.0, AcOH) (lit. $[\alpha]_D$ + 14.7°,[211b] +16.3°[212] (c 1.0 to 1.2, HOAc)); IR (KBr disk) 3440 (s, br), 3100 (m, br), 2956 (m), 1624 (s), 1576 (s), 1410 (m), 1321 (m) cm^{-1}; ^1H NMR (300 MHz, D_2O) δ3.70 (dd, 1H, 5.0, 7.3 Hz, CH), 1.92 (dd, 1H, 5.0, 15 Hz, CHH^{t}Bu), 1.61 (dd, 1H, 7.3, 15.0 Hz, CHH^{t}Bu), 0.97 (s, 9H, tert-Bu); POSFAB-MS (glycerol/HCl) 146 (MH^+), 291 (M_2H^+), 57 ($C_4H_9^+$); R_f 0.43 (System B).

For 114a from 102a: Deprotection of **102a** (Entry 12, Table 4) (39.5 mg, 107 μmol) produced a 99% yield (15.50 mg) of **114a**: $[\alpha]_D^{25}$ +15.6 (±0.4)° (c 0.55, HOAc) (cf. above); IR, NMR, MS, and chromatographic properties were identical to **114a** above.

For L-phenylalanine (116a) from 107a: Deprotection of Z-L-phenylalanine (**107a**, Entry 15, Table 4) (42.80 mg, 143 μmol) provided 21.43 mg (91%) of **116a**: $[\alpha]_D^{25}$ -7.50° (c 1.8, HOAc), -35.0° (c 1.8, H_2O) (lit. $[\alpha]_D^{25}$ -7.5° (c 1-2, HOAc),[213] -34.5°,[213] -35.1°[214] (c 1-2, H_2O); IR (KBr disk) 3420 (m, br), 3030 (vs, br), 2100 (w), 1681 (s), 1561 (vs), 1494 (vs), 1478 (vs), 1409 (m), 1306 (m), 745 (s), 699 (vs) cm^{-1}; ^1H NMR (400 MHz, D_2O) δ7.50-7.26 (m, 5H, Ph), 4.14-4.03 (m, 1H, CH), 3.34-3.24 (dd, 1H, CHHPh), 3.19-3.10 (dd, 1H, CHHPh); POSFAB-MS (glycerol/HCl) 166 (MH^+), 331 (M_2H^+), 120 (MH^+-H_2O, CO), 91 (C_7H^+); R_f 0.38 (System B).

For D-phenylalanine (116b) from 107b: Deprotection of **107b** (Entry 16, Table 4) (5.09 mg, 17 μmol) provided 2.80 mg (99%) of **116b** with spectral and chromatographic characteristics identical to those for **116a** above. Recrystallized **107b** was similarily deprotected and optical purity analyses indicated exactly the same e.e. (29.6%).

For L-phenylalanine (116a) from 109a: Hydrogenolytic deprotection of **109a** (Entry 17, Table 4) (24.10 mg, 61.9 μmol) afforded 10.19 mg (99%) of **116a**: $[\alpha]_D^{25}$ -30.5 (±0.2)° (c 1.0, H_2O) (lit. $[\alpha]_D^{25}$ -34.5°,[213] -35.1°[214] (c 1-2, H_2O); IR, NMR, MS, and chromatographic properties were identical to those of **116a** above.

Deprotection of 105a and 106a to (S)-2-amino-4-pentenoic acid (115a).

Compound **105a** (16.9 mg) or **106a** (23.0 mg) (0.068 mmol) in THF (1.5 mL) was added to a blue solution of $Na_{(s)}$ (~1 mg) in $NH_{3(l)}$ (6 mL). Tiny shavings of sodium (~0.3 mg each) were added to the mixture until the blue color obtained on dissolution of the metal persisted for about 1 min. A crystal of NH_4OAc was added to decolorize the solution, and the solvents were evaporated in a stream of dry argon. The residue was dried briefly *in vacuo*, dissolved in H_2O (1.5 mL), and the pH adjusted to 6.0 with acetic acid. The aqueous solution was extracted with CH_2Cl_2 (3 mL) to remove residual organic impurities, and applied to a column of BioRad Ion Retardation Resin Ag11

A8 (30 g, 1 × 40 cm)[293] packed in H_2O. Elution with H_2O (0.4 mL/min) provided the amino acid free of salts. Lyophilization of these fractions afforded 7.4-7.25 mg (93-95%) of (S)-2-amino-4-pentenoic acid **115a**: IR (KBr disk) 3410 (m, br), 3140 (s, br), 2950 (s, br), 2100 (w), 1750 (w), 1695 (m), 1614 (vs), 1587 (s), 1560 (s), 1512 (vs), 1404 (s), 1305 (m), 910 (m) cm^{-1}; ^1H NMR (300 MHz, D_2O) δ5.84-5.68 (m, 1H, $CH_2\underline{CH}=CH_2$), 5.30-5.20 (m, 2H, $CH=\underline{CH_2}$), 3.81-3.75 (dd, 1H, 5.0, 7.0 Hz, $\underline{CH}$), 2.70-2.51 (m, 2H, $CH\underline{CH_2}$); POSFAB-MS (glycerol/HCl) 116 (MH^+), 231 (M_2H^+); R_f 0.80 (System C).

Preparation of N-(1S,4R)-ω-camphanoyl-amino acid methyl esters for Determination of Stereochemical Purity (see Table 5).

A modification of the procedure of Armarego et al.[205] was employed. In all cases the free amino acids obtained by deprotection (above) were directly derivatized without recrystallization to avoid possible enrichment of one antipode. Typically, (-)-(1S,4R)-camphanoyl chloride (46.9 mg, 0.216 mmol) was added to a mixture of the amino acid (0.108 mmol) in 1 M $NaHCO_3/Na_2CO_3$ buffer (pH 10, 2 mL) with toluene (0.4 mL). The mixture was stoppered and stirred vigorously for 2 h. Following acidification to pH 1 with 5.7N HCl and extraction with CH_2Cl_2 (4 × 5 mL), the organic phases were dried over Na_2SO_4 and evaporated in vacuo. The residue was treated with an excess of CH_2N_2 in

Et_2O, and the solvent and excess reagent were removed *in vacuo* to provide a crude sample for analytical GC separation of diastereomers. Analytically-pure samples were secured by removal of the side-product, methyl (-)-(1*S*,4*R*)-camphanoate (**117**), by sublimation (65°C, 0.01 mm Hg, ~6 h). *N*-camphanoyl amino acid methyl esters were obtained in yields of 78-95% in this manner, along with a sublimate of 22.0-33.4 mg (48-51%) of methyl (1*S*,4*R*)-4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]heptane-1-carboxylate (**117**): mp 108.0-108.5°C; $[\alpha]_D^{25}$ -12.1° (c 2.0, 95% EtOH) (lit.[294] mp 108.4-108.5°C; $[\alpha]_D^{25}$ -12.4° (c 2.2, EtOH)); IR ($CHCl_3$ cast) 1782 (vs), 1727 (s), 1277 (m), 1100 (m), 924 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ3.86 (s, 3H, $COOCH_3$), 2.47-2.36 (m, 1H, 6-H_{exo}), 2.10-1.99 (m, 1H, 6-H_{endo}) 1.98-1.88 (m, 1H, 5-H_{exo}), 1.73-1.62 (m, 1H, 5-H_{endo}), 1.13 (s, 3H, 10-CH_3), 1.07 (s, 3H, 9-CH_3), 0.97 (s, 3H, 8-CH_3), (Absolute ^1H NMR assignments were made on the basis of nOe enhancements and confirmed by ^1H-decoupling experiments. See **117** structure (Figure 20) for numbering system.); Anal. Calc. for $C_{11}H_{16}O_4$: C, 62.25; H, 7.60. Found: C, 62.14; H, 7.55; EI-MS: 212.1049 (M^+, 212.1049 calcd.).

The values reported in Table 4 are the result of the differences in the optical purity of amino acid products (determined as these derivatives) from that of the starting serine β-lactones (determined as the MTPA derivatives (**88**, **89**)).

Methyl 2-([(1S,4R)-4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]heptane-1-carbonyl]amino)butanoates (118a, 118b, and 119).

These compounds were prepared from (S)- and (R)-2-aminobutanoic acids, respectively (using products **111a** and **111b** from deprotection of **93a** and **94b** as well as authentic material[98]) as outlined above.

For the (2S)-isomer 118a: mp 74-76°C; $[\alpha]_D^{25}$ -16.5° (c 1.08, $CHCl_3$); IR ($CHCl_3$ cast) 3365 (m, br), 2960 (s), 1790 (vs), 1749 (s), 1672 (s), 1528 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$)[206] δ6.92 (br d, 1H, 8 Hz, NH), 4.59 (m, 1H, CH), 3.76 (s, 3H, $COOCH_3$), 2.56-2.43 (m, 1H, 6'-H_{exo}), 2.03-1.86 (m, 3H, 6'-H_{endo}, 5'-H_{exo}, $CHHCH_3$), 1.81-1.68 (m, 2H, 5'-H_{endo}, $CHHCH_3$), 1.13 (s, 3H, 10'-CH_3), 1.12 (s, 3H, 9'-CH_3), 0.93 (s, 3H, 8'-CH_3), 0.92 (m, 3H, CH_2CH_3); EI-MS: 297.1576 (M^+, 297.1577 calcd. for $C_{15}H_{23}NO_5$).

For the (2R)-isomer 118b: oil; $[\alpha]_D^{25}$ -13.8° (c 1.06, $CHCl_3$); IR ($CHCl_3$ cast) 3370 (m, br), 2968 (s), 1792 (vs), 1742 (s), 1675 (s), 1526 (s), 1265 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$)[206] δ6.79 (d, 1H, 8 Hz, NH), 4.61-4.52 (m, 1H, CH), 3.74 (s, 3H, OCH_3), 2.60-2.46 (m, 1H, 6'-H_{exo}), 2.02-1.86 (m, 3H, 6'-H_{endo}, 5'-H_{exo}, $CHHCH_3$), 1.78 (dd, 1H, 7, 14.5 Hz, $CHHCH_3$), 1.75-1.68 (m, 1H, 5'-H_{endo}), 1.13 (s, 3H, 10'-CH_3), 1.09 (s, 3H, 9'-CH_3), 0.98 (s, 3H, 8'-CH_3), 0.95 (t, 3H, 7 Hz, CH_2CH_3); EI-MS: 297.1576 (M^+, 297.1577 calcd. for $C_{15}H_{23}NO_5$).

Reference Standard 119: This material was prepared from commercial racemic 2-aminobutanoic acid[98] as an oil which possessed spectral properties consistent with an equimolar mixture of **118a** and **118b**. GC analysis (RSL-300, 160°C, 1.0 min, 1.5°C/min to 200°C, 50°C/min to 250°C, 6.6 psi) afforded a ratio of 48.25% to 51.75 (±0.08)% for the 2R- (t_R = 17.54 min) and 2S-isomers (t_R = 18.57 min), respectively, in **119**. Samples and standards established limits of detection at approximately 0.3%.

Methyl 2-([(1S,4R)-4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]heptane-1-carbonyl]amino)acetate (120).

To glycine (250 mg, 3.33 mmol) in 1 M $NaHCO_3/Na_2CO_3$ buffer (pH 10, 15 mL) was added (-)-(1S,4R)-camphanoyl chloride (1.44 g, 6.66 mmol) in toluene (3 mL). The mixture was stirred vigorously for 2 h, with maintenance of pH 10 ±0.5 with 2N KOH. The mixture was acidified to pH 1 by careful addition of 5.7N HCl and extracted with CH_2Cl_2 (4 × 8 mL). Organic extracts were dried over Na_2SO_4, concentrated *in vacuo* and treated with excess etheral diazomethane. Solvent and excess reagent were removed *in vacuo*, and the residue was fractionated by MPLC (silica, 50% EtOAc/hexane, 3 mL/min) to provide 0.49 g (51%) of methyl camphonoate (**117**) and 1.80 g (95%) of **120**. This material could be recrystallized from CCl_4/hexane for analysis: mp 85.5-86.0°C (lit.[205] mp 84°C); $[\alpha]_D^{25}$ -18.1 (±0.1)°, $[\alpha]_{578}^{25}$ -18.9 (±0.1)° (c 1.5,

MeOH) (lit. $[\alpha]_{578}^{20}$ -21.3° (c 1.5, MeOH) from ORD curve[205]); IR ($CHCl_3$ cast) 3380 (w, br), 1790 (vs), 1756 (s), 1675 (vs), 1530 (s), 1209 (m), 1179 (m), 920 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$)[206] δ6.95 (br s, 1H, NH), 4.20 (dd, 1H, 6, 18 Hz, 2-pro-S-CHHCOOCH$_3$), 4.00 (dd, 1H, 5, 18 Hz, 2-pro-R-CHHCOOCH$_3$), 3.77 (s, 3H, COOCH$_3$), 2.60-2.45 (m, 1H, 6'-H$_{exo}$), 2.03-1.87 (m, 2H, 6'-H$_{endo}$, 5'-H$_{exo}$), 1.78-1.62 (m, 1H, 5'-H$_{endo}$), 1.13 (s, 3H, 10'-CH$_3$), 1.11 (s, 3H, 9'-CH$_3$), 0.98 (s, 3H, 8'-CH$_3$); Anal. Calc. for $C_{13}H_{19}NO_5$: C, 57.98; H, 7.11; N, 5.20. Found: C, 57.74; H, 7.11; N, 5.03; EI-MS: 269.1263 (M^+, 269.1263 calcd.), 223.1207 (M-H_2O, CO).

(S) and (R)-Methyl 2-([(1S,4R)-4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]heptane-1-carbonyl]amino)heptanoates (121a) and (121b)

These compounds were produced by deprotection of **96b** and **97a** to (R)- and (S)-2-aminoheptanoic acids (**112b** and **112a** respectively) followed by derivatization as outlined above.

For the (2S)-isomer 121a from 112a: IR ($CHCl_3$ cast) 3360 (w, br), 2959 (s), 2926 (s), 2850 (m), 1796 (vs), 1746 (s), 1678 (s), 1527 (m), 1260 (m), 1060 (m), 1015 (m), 921 (m), 795 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$)[206] δ6.87 (d, 1H, 8 Hz, NH), 4.62 (m, 1H, CH), 3.75 (s, 3H, COOCH$_3$), 2.60-2.42 (m, 1H, 6'-H$_{exo}$), 2.04-1.79 (m, 3H, 6'-H$_{endo}$, 5'-H$_{exo}$, CHCHH-Bu), 1.78-1.60 (m, 2H, 5'-H$_{endo}$,

CHCHH-Bu), 1.40-1.21 (m, 6H, $(CH_2)_3$), 1.12 (s, 3H, 10'-CH_3), 1.11 (s, 3H, 9'-CH_3), 0.92 (s, 3H, 8'-CH_3), 0.90-0.82 (m, 3H, CH_2CH_3); EI-MS: 339.2042 (M^+, 339.2046 calcd. for $C_{18}H_{29}NO_5$); CI-MS (NH_3) 357 ($M+NH_4^+$), 340 (MH^+).

For the (2R)-isomer 121b from 112b: IR and MS behavior were identical to **121a**. ^1H NMR (300 MHz, $CDCl_3$)[206] was virtually identical to **121a** except for: δ6.75 (d, 1H, 8 Hz, NH), 3.73 (s, 3H, $COOCH_3$), 1.09 (s, 3H, 9'-CH_3), 0.98 (s, 3H, 8'-CH_3).

Reference Standard 122.

Since 2-aminoheptanoic acid was not commercially available, this compound was prepared by a diastereoselective alkylation of the corresponding glycine derivative **120** by an adaptation of the method of Piotrowska and Abramski.[216] To a solution of dry diisopropylamine (2.0 mmol, 282 μL) and tetramethylenediamine (2.0 mmol, 270 μL) in THF (5 mL) was added n-BuLi (2.0 mmol in 0.760 mL hexane). The mixture was stirred 20 min at 25°C and cooled to -78°C. A solution of **120** (269 mg, 1.0 mmol) in THF (6.0 mL) was added dropwise and the mixture stirred 15 min at -78°C. 1-Bromopentane (124 μL, 1.0 mmol) was injected and stirring continued 5 h at -78°C. The mixture was poured into 5% aqueous NH_4Cl (15 mL), pH was adjusted to 6.0, and the mixture was extracted with Et_2O (3 × 15 mL). Etheral phases were dried over

Na_2SO_4, concentrated *in vacuo* and subjected to MPLC (silica, 25% EtOAc/hexane, 3 mL/min) to provide 37.3 mg (11%) of **122** as a mixture of diastereomers: IR and MS properties were identical to **121a** and **121b** above; Anal. Calc. for $C_{18}H_{29}NO_5$: C, 63.69; H, 8.61; N, 4.13. Found: C, 63.57; H, 8.40; N, 4.16; ^1H NMR (300 MHz, $CDCl_3$)[206] indicated 70% of (2*S*)- and 30% (2*R*)-isomers from the ratio of 8'-$C\underline{H}_3$ (0.92 and 0.98 ppm)[207] and $COOC\underline{H}_3$ (3.75 and 3.73 ppm) integrals, respectively. GC analysis (DB 17^+, 170°C, 2.0 min, 2°C/min to 230°C, 7.12 psi) afforded a ratio of 69.79 (±0.10)% to 30.21% for the (2*S*)- (t_R = 24.65 min) and (2*R*)-isomers (t_R = 24.07 min), respectively. The estimated limit of detection is <0.5%.

Methyl 2-([(1*S*,4*R*)-4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]heptane-1-carbonyl]amino)-4-methylpentanoates (123a) and (124)

(2*S*)-isomer 123a. This compound was prepared using products **113a** of the deprotection of **99a** or **100a** as outlined above: mp 51-52°C; IR ($CHCl_3$ cast) 3438 (m, br), 3355 (m, br), 2955 (m), 1793 (vs), 1745 (m), 1675 (s), 1525 (m), 1167 (m), 1011 (m), 921 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$)[206] δ6.76 (br d, 1H, 8.4 Hz, N$\underline{H}$), 4.70-4.60 (m, 1H, C$\underline{H}$), 3.75 (s, 3H, $COOC\underline{H}_3$), 2.56-2.43 (m, 1H, 6'-$\underline{H}_{exo}$), 2.02-1.89 (m, 2H, 6'-$\underline{H}_{endo}$, 5'-$\underline{H}_{exo}$), 1.76-1.55 (m, 4H, -$C\underline{H}_2C\underline{H}Me_2$, 5'-$\underline{H}_{endo}$), 1.12 (s, 3H, 10'-$C\underline{H}_3$), 1.11 (s, 3H, 9'-$C\underline{H}_3$), 0.96 (d, 3H, 2.8 Hz, $CH(C\underline{H}_3)CH_3$), 0.93 (d, 3H, 3 Hz, $CH(CH_3)C\underline{H}_3$), 0.91 (s, 3H, 8'-$C\underline{H}_3$); Anal. Calc. for

$C_{17}H_{27}NO_5$: C, 62.75; H, 8.36; N, 4.30. Found: C, 62.80; H, 8.23; N, 4.19; EI-MS: 325.1889 (M^+, 325.1889 calcd.).

Reference Standard 124: This material was prepared as an oil from authentic L-leucine (8.42 mg) and D-leucine (10.67 mg)[98] (0.146 mmol total) according to the general procedure: IR and MS behavior was essentially identical to **123a**. ^1H NMR (300 MHz, $CDCl_3$)[206] indicated 44% (2S)- and 56% (2R)-isomers, with resolved peaks due to the (2R)-isomer at δ6.70 (br d, 1H, 8.4 Hz, NH), 3.73 (s, 3H, $COOCH_3$), 1.08 (s, 3H, 9'-CH_3), 0.99 (s, 3H, 8'-CH_3),[207] and all other peaks as described for **123a** above. Anal. Found: C, 62.38; H, 8.07; N, 4.29. GC analysis (RSL-300, 110°C, 1.0 min, 1.5°C/min to 210°C, 50°C/min to 250°C, 2.0 min, 6.7 psi) indicated 44.1 (±0.30)% and 55.9% of the (2S)- (t_R = 53.80 min) and (2R)-isomers (t_R = 52.95 min) respectively. Limits of detection were established with additional standards as <0.5% of the (2R)-isomer.

Methyl 2-([(1S,4R)-4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]-heptane-1-carbonyl]amino)-4,4-dimethylpentanoates (125a and 126).

(2S)-isomer 125a: This compound was prepared using product **114a** of the deprotection of **101a** or **102a** in the usual manner: IR ($CHCl_3$ cast) 3365 (m, br), 2957 (s), 1792 (vs), 1748 (s), 1675 (s), 1527 (s), 1274 (m), 1169 (m), 1060 (m), 923 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$)[206]

δ6.68 (d, 1H, 8 Hz, NH), 4.59 (~d of t, 1H, 8.4, 3.7 Hz, CH), 3.74 (s, 3H, $COOCH_3$), 2.54-2.38 (m, 1H, 6'-H_{exo}), 2.03-1.87 (m, 2H, 6'-H_{endo}, 5'-H_{exo}), 1.81 (dd, 1H, 14.6, 3.7 Hz, CHH-Bu-t), 1.76-1.66 (m, 1H, 5'-H_{endo}), 1.52 (dd, 1H, 14.5, 8.4 Hz, CHH-Bu-t), 1.13 (s, 3H, 10'-CH_3), 1.12 (s, 3H, 9'-CH_3), 0.97 (s, 9H, t-Bu), 0.92 (s, 3H, 8'-CH_3); EI-MS: 339.2045 (M^+, 339.2046 calcd. for $C_{18}H_{29}NO_5$);

Reference Standard 126. This material was prepared from a mixture of authentic D- and L-γ-methylleucine (58.90 mg and 33.70 mg, respectively)[98] as outlined in the general procedure: IR and MS behavior were as described for **125a**. ^1H NMR (300 MHz, $CDCl_3$)[206] indicated 64% (2R)- and 36% (2S)-isomers,[207] with resolved peaks due to the (2R)-isomer at δ4.67 (d of t, 1H, 3.0, 9.0 Hz, CH), 3.73 (s, 3H, $COOCH_3$), 1.10 (s, 3H, 9'-CH_3), 0.99 (s, 3H, 8'-CH_3), and all other peaks as described for **125a** above. GC analysis (RSL-300, 160°C, 1.0 min, 1.5°C/min to 210°C, 50°C/min to 250°C, 1.0 min, 6.6 psi) afforded a ratio of 64.43 (±0.04)% and 35.57% of the (2R)- (t_R = 24.0 min) and (2S)-isomers (t_R = 25.2 min) respectively. Limits of detection were established as <0.25% of the (2R)-isomer.

Methyl 2-([(1S,4R)-4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]-heptane-1-carbonyl]amino)-4-pentenoates (127a and 128).

For (2S)-isomer 127a: This compound was prepared from product **115a** of the deprotection of **105a** or **106a**

using the general procedure outlined above. Due to the volatility of **127a** under the usual sublimation conditions, purification by MPLC (silica, 35% EtOAc in hexane, 3 mL/min) was used to provide **127a** as an oil (91% yield): IR ($CHCl_3$ cast) 1793 (vs), 1746 (m), 1677 (s), 1524 (m), 920 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$)[206] δ6.94 (br d, 1H, 8 Hz, NH), 5.77-5.59 (m, 1H, vinylic-CH), 5.51-5.18 (m, 2H, vinylic-CH_2), 4.78-4.64 (m, 1H, CH), 3.77 (s, 3H, $COOCH_3$), 2.68-2.43 (m, 3H, 6'-H_{exo}, $CHCH_2$), 2.01-1.84 (m, 2H, 6'-H_{endo}, 5'-H_{exo}), 1.76-1.63 (m, 1H, 5'-H_{endo}), 1.11 (s, 3H, 10'-CH_3), 1.10 (s, 3H, 9'-CH_3), 0.91 (s, 3H, 8'-CH_3); Anal. Calc. for $C_{16}H_{23}NO_5$: C, 62.12; H, 7.49; N, 4.53. Found: C, 62.14; H, 7.30; N, 4.43; EI-MS: 309.1573 (M^+, 309.1576 calcd.).

Reference Standard 128: This material was prepared from authentic D-(7.06 mg) and L-allylglycine (12.00 mg)[98] as described for **127a**: IR and MS behavior was identical to **127a**. ^1H NMR (300 MHz, $CDCl_3$)[206] provided a ratio of 37% (2R)- and 63% (2S)-isomers, with resolved peaks due to the (2R)-isomer at δ6.83 (br d, 1H, 8 Hz, NH), 3.75 (s, 3H, $COOCH_3$), 1.12 (s, 3H, 10'-CH_3), 1.09 (s, 3H, 9'-CH_3), 0.98 (s, 3H, 8'-CH_3),[207] with all other peaks as described for **127a** above. GC analysis (RSL-300, 120°C, 2.0 min, 2.0°C/min to 220°C, 50°C/min to 250°C, 7.10 psi) afforded a ratio of 37.47 (±0.32)% and 62.53% of the (2R)- (t_R = 36.50 min) and (2S)-isomers (t_R = 37.26 min), respectively. Limits of detection were determined to be

<0.6% of (2R)-isomer.

Methyl 2-([(1S,4R)-4,7,7-trimethyl-3-oxo-2-oxabicyclo-[2.2.1]heptane-1-carbonyl]amino)-3-phenylpropionates (129a, 129b, and 130).

For (2S)-isomer 129a: This compound was prepared from the deprotection product **116** of **107a** or **109a** according to the general procedure, with purification by flash chromatography[260] (40% EtOAc in hexane) or sublimative removal of **117**: IR ($CHCl_3$ cast) 3360 (m, br), 2960 (m), 1789 (vs), 1752 (m), 1671 (s), 1523 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ7.34-7.18 (m, 3H, m, p-Ph), 7.18-7.10 (m, 2H, o-Ph), 6.81 (br d, 1H, 8.5 Hz, NH), 4.94 (m, 1H, CH), 3.73 (s, 3H, $COOCH_3$), 3.22 (dd, 1H, 5.5, 14 Hz, CHHPh), 3.02 (dd, 1H, 8.5, 14 Hz, CHHPh), 2.52-2.39 (m, 1H, 6'-H_{exo}), 1.97-1.84 (m, 2H, 5'-H_{exo}, 6'-H_{endo}), 1.72-1.57 (m, 1H, 5'-H_{endo}), 1.07 (s, 3H, 10'-CH_3), 1.01 (s, 3H, 9'-CH_3), 0.61 (s, 3H, 8'-CH_3)[207] (Absolute assignments are based on nOe and ^1H-decoupling results.); Anal. Calc. for $C_{20}H_{25}NO_5$: C, 66.84; H, 7.01; N, 3.90. Found: C, 66.63; H, 6.99; N, 3.87; EI-MS: 359.1735 (M^+, 359.1733 calcd.).

For (2R)-isomer 129b: This compound was prepared from the deprotection product of **107b** (Entry 16) exactly as described for **129a** above. Spectral characteristics of the resulting stereochemically impure material (64.8% (2R)) were essentially as described for **130** below.

Reference Standard 130: The procedure outlined for **129a** was employed to derivatize a mixture of D- (0.209 g) and L-phenylalanine[98] (0.100 g): IR and MS behavior was identical to **129a**. ^{1}H NMR (300 MHz, $CDCl_3$) provided a ratio of 66% (2R)- and 34% (2S)-isomers, with resolved peaks due to the (2R)-isomer[207] at δ3.72 (s, 3H, $COOCH_3$), 1.09 (s, 3H, 10'-CH_3), 1.06 (s, 3H, 9'-CH_3), 0.88 (s, 3H, 8'-CH_3) with all other peaks as described for **129a** above. Anal. Found: C, 66.47; H, 7.11; N, 3.89. GC analysis (RSL-300, 170°C, 2.0 min, 2.0°C/min to 250°C, 3.0 min, 6.8 psi) indicated 66.07 (±0.36)% and 33.93% of the (2R)- (t_R = 32.3 min) and (2S)-isomers (t_R = 33.1 min), respectively. Limits of detection were established as <0.4% under these conditions.

N-(Benzyloxycarbonyl)-3-(3,4-dimethoxyphenyl)-L-alanine (131a) and 3,3',4,4'-Tetramethoxy-1,1'-biphenyl (132).

To Mg (2.18 g, 89.5 mmol; 40-80 mesh) suspended in dry THF (8 mL) was added 4-bromoveratrole (2.0 mL) in THF (3 mL). While heating to reflux, more 4-bromoveratrole (10.0 mL, 20.4 g total, 94.0 mmol) in THF (15 mL) was added dropwise over 30 min. The mixture was heated to reflux 2 h and stirred 16 h at 25°C to produce a viscous brown solution which was diluted with THF (15 mL) to facilitate transfers. Titration against menthol/phenanthroline[258b] at -23°C indicated ~0.7 M (40% yield) in the corresponding Grignard reagent. An aliquot (6.0 mL) of

this solution (~4 mmol, ~5.7 mL) was added dropwise over 8 min to a solution of Z-L-serine β-lactone (**36a**) (175 mg, 0.791 mmol) and $CuBr \cdot SMe_2$ (40.65 mg, 0.198 mmol) in THF (5 mL)/Me_2S (0.3 mL) at -23°C. After stirring 1 h at -23°C the mixture was worked up in the usual fashion. Etheral extracts were washed with brine (2 x 25 mL) and H_2O, and concentrated to a red-orange oil which was subjected to reverse phase MPLC (46% CH_3CN/H_2O, 3 mL/min) to yield impure (colored) **131a** and 175.0 mg (~35%) of **132** (R_f 0.56 (40% EtOAc/hex), 0.73 (0.5% HOAc in EtOAc)). The colored **131a** was further purified by preparative TLC (0.5% HOAc in EtOAc) to yield 75.9 mg (27%) of chromatographically pure **131a** which was recrystallized from EtOH/hexane (92% recovery): mp 116-117°C (lit. mp 117°C[228]); $[\alpha]_D^{25}$ +13.3 (±0.2)° (c 1.0, EtOH) (lit. $[\alpha]_D^{25}$ +13.4° (c 1.0, EtOH)[228]); IR ($CHCl_3$ cast) 3330 (w, br), 2950 (m), 1719 (s, br), 1590 (w), 1516 (vs), 1453 (m), 1263 (s), 1237 (s), 1140 (m), 1025 (s), 755 (w), 695 (w) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 10.4 (br s, 1H, COOH), 7.40-7.28 (m, 5H, Ph), 6.78 (d, 1H, J_{ortho} = 8.0 Hz, ArH), 6.70 (dd, 1H, J_{ortho} = 8.0 Hz, J_{meta} = 2.0 Hz, ArH'), 6.68 (~d, 1H, J_{meta} = 2.0 Hz, ArH"), 5.17 (d, 1H, 8.0 Hz, NH), 5.13-5.08 (m, 2H, OCH_2Ph), 4.70-4.62 (m, 1H, CH), 3.86 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3'), 3.16 (dd, 1H, 5.5, 14.0 Hz, CHCHHAr), 3.09 (dd, 1H, 6.0, 14.0 Hz, CHCHHAr); EI-MS: 359.1375 (M^+, 359.1369 calcd. for $C_{19}H_{21}NO_6$), 151.0764 (Base peak, $(MeO)_2ArCH_2^+$); R_f 0.52 (0.5% HOAc in EtOAc).

For 132: mp 130-132°C (lit. mp 132-133°C[295]); IR ($CHCl_3$ cast) 1600 (m), 1502 (s), 1462 (m), 1434 (m), 1253 (s), 1230 (vs), 1175 (m), 1143 (s), 1058 (m), 1022 (vs), 79( ) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ7.15-7.03 (m, 4H, o-, m-ArH), 6.94 (d, 2H, ~9 Hz, o'-ArH), 3.96 (s, 6H, OCH_3), 3.93 (s, 6H, OCH_3'); EI-MS: 274.1204 (M^+, Base peak, 274.1205 calcd. for $C_{16}H_{18}O_4$), 259.0970 ($M-CH_3$).

(S)-3-Amino-2-oxetanone, trifluoroacetic acid salt (140).

Typically BOC-L-serine β-lactone (**42a**) (187.2 mg, 1.00 mmol) was treated with distilled CF_3COOH (3.0 mL) at 0-5°C for 10 min. The solvent was removed in vacuo by bulb-to-bulb distillation at 25°C (receiving bulb at -78°C) with the aid of a Kügelrohr apparatus and the residue of **140** was dissolved in the desired solvent and immediately reacted with a nucleophile. The yield of **140** is quantitative by ^1H NMR (360 MHz, CF_3COOD): δ5.51 (m, 1H, CH), 4.87 (m, 2H, CH_2); IR (H_2O solution) 1836 cm^{-1}; IR (CH_3CN solution) 1842 cm^{-1}; POSFAB-MS (glycerol) 88 (MH^+), 289 ($[(MH)_2 \cdot CF_3COO]^+$); R_f ~0.77 (System A, some hydrolysis on plate). Full characterization was only possible as the tosylate salt.

(S)-3-Amino-2-oxetanone, p-toluenesulfonic acid salt (141).

BOC-L-serine β-lactone (**42a**) (600.0 mg, 3.20 mmol) and anhydrous p-toluenesulfonic acid (578.6 mg, 3.36 mmol)

were treated with distilled CF_3COOH (10 mL) at 0-5°C for 10 min. Removal of TFA *in vacuo* (see **140**) provided a white crystalline residue which was triturated with dry Et_2O (20 mL) and filtered to yield **141** (806 mg, 97%). This material was analytically pure however if desired recrystallization could be effected from DMF/Et_2O (25°C→-20°C): mp (~4°/min) 135°C (darkening), 173°C (dec. rapid); $[\alpha]_D^{25}$ -15.9 (±0.1)° (c 2.2, DMF); IR (Fluorolube® mull) 3040 (s, vbr), 1838 (vs), 1600 (w), 1585 (w), 1550 (m) cm^{-1}; IR (pH 6.8, aqueous solution) 1820 cm^{-1}; IR (DMF solution) 1830 cm^{-1}; ^1H NMR (300 MHz, d_7-DMF) δ7.66 (d, 2H, 8.0 Hz, o-ArH), 7.15 (d, 2H, 8.0 Hz, m-ArH), 5.53 (dd, 1H, 4.6, 6.5 Hz, CH), 4.74 (m, 1H, CHHO), 4.68 (m, 1H, CHHO), 3.70 (vbr s, 3H, NH_3^+), 2.31 (s, 3H, $ArCH_3$); ^{13}C NMR (75.5 MHz, d_7-DMF) δ165.84, 145.12, 139.14, 128.67, 126.08, 64.70, 57.45, 20.70; Anal. Calc. for $C_{10}H_{13}NO_5S$: C, 46.32; H, 5.05; N, 5.04; S, 12.37. Found: C, 46.44, H, 5.14; N, 5.24; S, 12.41; POSFAB-MS (glycerol) 88 ($MH^+=C_3H_6NO_2$), 180 (MH^+(gly)), 260 (MH^+(TsOH)).

O-Trifluoroacetyl-L-serine, p-toluenesulfonic acid salt (142).

BOC-L-serine β-lactone (**42a**) (106.0 mg, 0.566 mmol) and AG1-X8 resin (240 mg, ~0.8 meq, CF_3COO^- form, dried *in vacuo* at 64°C) were treated with distilled TFA (4 mL) and the mixture was stirred 16 h under Ar. The resin was removed by filtration and washed with CF_3COOH (2 × 1

mL). p-Toluenesulfonic acid (97.5 mg, 0.566 mmol) was added to the combined filtrate and washings and TFA was removed by bulb-to-bulb distillation in vacuo (see **140**). The white solid residue was triturated with dry Et_2O (5 mL), filtered and washed well with ether to yield **142** (178.5 mg, 87%): mp 181.5-182.0°C (darkens at 178°C); $[\alpha]_D^{25}$ +10.0 (±0.4)° (c 0.45, DMF); IR (KBr disk) 3420 (m, vbr), 3300-2400 (s, vbr), 1799 (s), 1754 (s), 1621 (w), 1600 (w), 1532 (m), 1345 (w), 1229 (m), 1194 (s), 1156 (vs), 1129 (m), 1041 (s), 1014 (s), 812 (m), 691 (s) cm^{-1}; ^1H NMR (400 MHz, d_7-DMF) δ9.15 (br s, ~4H, COOH, NH_3^+), 7.66 (d, 2H, 8.0 Hz, o-ArH), 7.14 (d, 2H, 8.0 Hz, m-ArH), 5.14 (dd, 1H, 2.4, 12.3 Hz, CHHO), 5.01 (dd, 1H, 4.6, 12.3 Hz, CHHO), 4.88 (br m, 1H, CH), 2.30 (s, 3H, $ArCH_3$); ^{19}F NMR (376.5 MHz, d_7-DMF) δ-75.5 (s, CF_3COO); Anal. Calc. for $C_{12}H_{14}NO_7SF_3$: C, 38.61; H, 3.78; N, 3.75. Found: C, 38.96; H, 3.93; N, 3.99; EI-MS: 172.0195 (TsOH), 156.0274 ($(M-CO_2H)=C_4H_5NO_2F_3$); POSFAB-MS (glycerol) 202 ($MH^+=CF_3COOCH_2CH(NH_3^+)COOH$), 374 ($MH^+$·TsOH), 294 ($MH^+$·gly); R_f ~0.68 (System A, some hydrolysis on plate).

2-[(N-Trifluoroacetyl)amino]propenoic acid (143).

BOC-L-serine β-lactone (**42a**) (114.0 mg, 0.769 mmol) was dissolved in distilled TFA and allowed to stand 16 days under Ar. Bulb-to-bulb distillation in vacuo (0.1 torr) at 25°C (see **140**) first removed the TFA and subsequently caused the sublimation of a white solid which

was collected in a clean chilled receiving bulb. The last third of **143** sublimate was obtained on warming to 45°C for a total of 116.9 mg (83%) of **143**. ^{1}H NMR on the residue indicated it was primarily O-trifluoroacetyl-serine (**142**). For **143**: mp 126-128°C; IR (CH_3CN cast) 3380 (m), 3400-2200 (mult, br, w), 1744 (m), 1702 (vs), 1638 (w), 1552 (s), 1445 (s), 1300 (m), 1213 (s), 1188 (m), 1164 (s), 910 (m) cm^{-1}; ^{1}H NMR (300 MHz, CD_3CN) δ8.72 (br s, ~1H; NH), 6.46 (s, 1H, E-CHH), 6.11 (s, 1H, Z-CHH); Anal. Calc. for $C_5H_4NO_3F_3$: C, 32.80; H, 2.20; N, 7.65. Found: C, 32.52; H, 2.18; N, 7.62; EI-MS: 183.0134 (183.0144 calcd.); CI-MS (NH_3) 201 ($M+NH_4^+$); R_f ~0.87 (System B; UV active, pink-brown with ninhydrin).

O-(p-Toluenesulfonyl)-L-serine, p-toluenesulfonic acid salt (144).

To β-lactone **141** (50.0 mg, 0.192 mmol), anhydrous p-toluenesulfonic acid (69.0 mg, 0.40 mmol) and AG1-X8 resin (110 mg, ~0.35 meq, CF_3COO^- form, dried in vacuo at 64°C) was added distilled TFA (4 mL). The mixture was stirred at 25°C for 1 week under Ar. The resin was removed by filtration and washed with TFA (2 x 1 mL). The filtrate and washings were concentrated in vacuo to yield a hygroscopic white solid (82.6 mg). ^{1}H NMR indicated this product was 75 mol% pure, with the balance being serine (TsOH salt) which was presumably generated by hydrolysis in the reaction or isolation. Recrystallization from

DMF/Et_2O (2×) did not alter the product composition appreciably. For **144**: IR (MeOH cast) ~3000 (m, br), 2919 (m), 1750 (m), 1630 (m), 1495 (w), 1370 (m), 1211 (m), 1192 (s), 1178 (vs), 1125 (m), 1085 (m), 1011 (m), 685 (m), 570 (m) cm^{-1}; ^1H NMR (300 MHz, d_7-DMF) δ9.2 (br s, 1H, COOH), 7.86 (d, 2H, 8 Hz, o-$ArHSO_3R$), 7.66 (d, 2H, 8 Hz, o-$ArHSO_3^-$), 7.54 (d, 2H, 8 Hz, m-$ArHSO_3R$), 7.17 (d, 2H, 8 Hz, m-$ArHSO_3^-$), 4.85-4.80 (m, 1H, CH), 4.70-4.60 (m, 2H, CH_2OTs), 2.46 (s, 3H, p-CH_3ArSO_3R), 2.30 (p-$CH_3ArSO_3^-$), [25 mol% serine (TsOH salt): δ4.88 (m, 1H, CH), 4.34 (d, 2H, CH_2OH)]; POSFAB-MS (glycerol) 260 ($TsOCH_2CH(NH_3^+)COOH$), 173 ($TsOH_2^+$); R_f 0.80 (System A; serine R_f 0.55).

O-Phospho-L-serine (145).[233]

K_2HPO_4 (0.446 g, 3.28 mmol, dried 4 h at 130°C) and 18-crown-6 ether (0.867 g, 3.28 mmol) were stirred 16 h in anhydrous DMF (10 mL). BOC-L-serine β-lactone (**42a**) (169.0 mg, 0.902 mmol) was deprotected to **140** and added as a solution in DMF (3 mL). The mixture was stirred 3 days, diluted with H_2O (to 50 mL), and applied to a column of AG1-X8 (80 mL, 3 cm dia., OH^- form). Elution (2 mL/min) with a linear gradient (0→3 M over 1.0 L) of formic acid afforded O-phospho-L-serine (**145** (145.6 mg) in 87% yield after lyophilization: mp 170-171°C (dec) (lit.[296] mp 175-176°C (dec)); $[\alpha]_D^{25}$ +7.2° (c 1.0, H_2O) (lit.[296] $[\alpha]_D^{25}$ +7.2° (c 1.0, H_2O)); IR (KBr disk) 3420 (w, br), 3180 (w),

2700 (w), 2400-2260 (w), 1620 (w), 1560 (m), 1260 (m), 1089 (s), 1045 (s), 1000 (s), 970 (s), 740 (m), 810 (m) cm^{-1}; ^1H NMR (400 MHz, D_2O + DCl) δ4.37-4.27 (m, 2H, CH-CHH), 4.26-4.16 (m, 1H, CHH); ^{31}P NMR (161.96 MHz, D_2O + DCl) -0.45 (br s); Anal. Calc. for $C_3H_3NO_6P$: C, 19.47; H, 4.35; N, 7.57. Found: C, 19.27; H, 4.29; N, 7.82; POSFAB-MS (glycerol/HCl) 186 (MH^+); 371 (M_2H^+); R_f .43 (System A).

β-Chloro-L-alanine (hydrochloride (20a) and free base (21a)).[233]

Concentrated HCl (1.0 mL, ~12 mmol) was added to **140** produced from BOC-L-serine β-lactone (**42a**) (92.0 mg, 0.492 mmol). After 5 min H_2O (5 mL) was added and the solvent was removed in vacuo at 35°C. The residue was redissolved in H_2O (5 mL) and again the solvent was removed. Recrystallization of the solid residue from MeOH/Et_2O yields 78.5 mg (92%) of β-chloro-L-alanine, hydrochloride salt (**20a**). Since literature reports that **20a** has "no distinct mp" and $[\alpha]_D$ "close to zero",[102] the material was converted to the free base **21a** for complete characterization. Hence, **20a** was dissolved in a minimal amount of H_2O, one equivalent of 2N LiOH was added, and **21a** (56.5 mg, 93% recovery) was obtained by addition of excess EtOH with cooling to -10°C.

An identical yield of **20a** was also obtained simply by addition of conc. HCl to BOC-L-serine β-lactone (**42a**) (1.0

mL/100 mg).

For 20a from 140: $[\alpha]_D^{25}$ +0.80° (c 1.0, H_2O); IR (KBr disk) 3720-2200 (vs, br), 1980 (m), 1960 (m), 1745 (vs), 1600 (s), 1500 (vs), 1410 (s), 1350 (s), 1230 (s), 1200 (s), 1150 (m), 1070 (m), 890 (m), 850 (s), 790 (s), 680 (s) cm^{-1}; ^1H NMR (80 MHz, D_2O) δ4.60-4.44 (m, 1H, CH), 4.25-4.07 (m, 2H, CH_2Cl); Anal. Calc. for $C_3H_7NO_2Cl_2$: C, 22.52; H, 4.42; N, 8.75. Found: C, 22.09; H, 4.48; N, 8.63; POSFAB-MS (glycerol) 124 (MH^+); R_f 0.76 (System A).

For 21a: mp 156-157°C (lit.[110] mp 160°C); $[\alpha]_D^{25}$ -15.8° (c 1.0, H_2O) (lit.[102] $[\alpha]_D^{20}$ -15.5° (c 1, H_2O), -15° (c 9.9, H_2O)[42a]); IR (KBr disk) 3660-2160 (m, mult, br), 2080 (w), 1630 (s), 1600 (s), 1390 (s), 1300 (s), 860 (s), 640 (s), 540 (s), 450 (s) cm^{-1}; ^1H NMR (300 MHz, D_2O) δ4.62 (dd, 1H, 3.25, 4.5 Hz, CH), 4.21 (dd, 1H, 4.5, 13.0 Hz, CHHCl), 4.10 (dd, 1H, 3.25, 13.0 Hz, CHHCl); Anal. Calc. for $C_3H_6NO_2Cl$: C, 29.16; H, 4.90; N, 11.34; Cl, 28.69. Found: C, 28.99; H, 4.95; N, 11.08; Cl, 28.51; POSFAB-MS (glycerol) 124 (MH^+).

β-Cyano-L-alanine (146) from 140.

A solution of **140** (prepared from 79.0 mg, 0.422 mmol of BOC-L-serine β-lactone (**42a**)) in DMF (2 mL) was added dropwise to tetra-n-butylammonium cyanide (170 mg, 0.633 mmol) in DMF (3 mL) at -10°C over 10 min. The solution was stirred 30 min at -10°C and allowed to warm to 25°C over 30 min. The solvent was removed in vacuo at 25°C to

yield an orange syrup which was dissolved in H_2O (1 mL) and applied to a column of AG11 A8 (30 g, 1 × 40 cm).[297] Elution with H_2O (1 mL/min), and lyophilization of the fractions which produced the characteristic blue-green color with ninhydrin spray reagent provided 40.3 mg (84%) of **146** free of salts. For an improved melting point this solid was precipitated from pH 7.0 H_2O by addition of dioxane and dried *in vacuo* over P_2O_5 (34.5 mg, 72% yield after two precipitations): mp 213-216°C (dec, 1st ppt), 217-218°C (2nd ppt) (lit. mp 206°C, 208-209°C, 218-218.5°C[169]); $[\alpha]_D^{25}$ -2.9° (c 1.4, 1N HOAc) (lit. $[\alpha]_D^{25}$ -2.9° (c 1.4, 1N HOAc)[169]); IR (KBr disk) 3420 (m, br), 3020 (s, br), 2225 (w), 1630 (vs, br), 1610 (s), 1575 (m), 1528 (s), 1417 (s), 1330 (s), 1160 (w), 1070 (w), 880 (w) cm^{-1}; ^1H NMR (200 MHz, D_2O) δ4.10 (t, 1H, 5.8 Hz, CH), 3.08 (d, 2H, 5.8 Hz, CH_2CN); POSFAB-MS (glycerol/HCl) 115 (MH^+), 229 (M_2H^+), 343 (M_3H^+); R_f ~0.65 (System A, characteristic blue-green color with ninhydrin).

β-Cyano-L-alanine (146) from 73a.

N-(Benzyloxycarbonyl)-β-cyano-L-alanine (**73a**) (300.0 mg, 1.21 mmol) was dissolved in distilled CH_3CN (3.0 mL) and iodotrimethylsilane (170 μL, 1.21 mmol)[1] was added and the mixture was stirred 10 min. The reaction was quenched by addition to H_2O (5 mL) containing 1N NH_4OH (1.4 mL) and extracted with Et_2O (3 × 20 mL). If necessary the pH of the aqueous phase was adjusted to

6.0. Dioxane (~100 mL) was added to precipitate the product (**146**) (117.3 mg, 84%) which was filtered, washed with Et_2O and dried *in vacuo* over P_2O_5: mp 214-216°C; $[\alpha]_D^{25}$ -2.9° (c 1.4, 1N HOAc) (lit.[169] mp 218-218.5°C, $[\alpha]_D^{25}$ -2.9° (c 1.4, 1N HOAc)). Spectral and chromatographic properties were identical to those reported for **146** (from **140**) above.

β-(Pyrazol-1-yl)-L-alanine (147).

(S)-3-Amino-2-oxetanone salt **141** (100.0 mg, 0.385 mmol) was added to pyrazole (131.3 mg, 1.93 mmol) in distilled DMF (3.0 mL) and the mixture was stirred at 25°C for 2.5 h. The solvent was removed *in vacuo* at 25°C and the residue was dissolved in H_2O (3 mL) and applied to a column of AG50-X8 (1 × 10 cm, H^+ form). The resin was washed with H_2O (30 mL) and eluted with a gradient of aqueous NH_4OH (0→0.25N over 70 mL, then 100 mL of 0.25N NH_4OH). The product emerged chromatographically pure with 0.25N NH_4OH and was lyophilized (2×) and dried *in vacuo* over P_2O_5 to yield 77% (46.2 mg) of **147** (mp 234-236°C (dec)). For an improved melting point this material could be recrystallized from [redacted]EtOH: mp 241-243°C (lit. mp 236-238°C (dec),[142a] [redacted](dec)[142b]); $[\alpha]_D^{25}$ -72 (±1)° (c 0.54, H_2O) (lit. $[\alpha]_D^{25}$ [redacted]° (c 3.4, H_2O)[142a], -72.0° (c 1.0, H_2O)[142b]); IR (KBr disk) 3700-2200 (m, vbr), 1617 (s), 1580 (s), 1485 (m), 1395 (m), 767 (m) cm^{-1}; ^1H NMR (300 MHz, D_2O) δ7.65 (d, 1H, 2 Hz, ArHH'H"), 7.61 (d, 1H,

2 Hz, ArHH'H"), 6.38 (t, 1H, 2 Hz, ArHH'H"), 4.66 (d, 2H, 5 Hz, CH_2N), 4.18 (d, 1H, 5 Hz, CH); POSFAB-MS (glycerol/HCl) 156 (MH^+), 311 (M_2H^+); R_f 0.67 (Solvent A; characteristic blue-purple color with ninhydrin).

β-Azido-L-alanine (148).

A solution of **141** (53.0 mg, 0.204 mmol) in DMF (1 mL) was added to NaN_3 (14.61 mg, 0.225 mmol) in DMF (5 mL) and the mixture was stirred 3.5 h. The solvent was removed *in vacuo* at 25°C. The residue was dissolved in H_2O (0.3 mL) and applied to a column of Bio-Rad Ion Retardation Resin Ag11 A8 (15 g, 1 × 20 cm)[297] and eluted with H_2O (0.3 mL/min). Fractions containing amino acid were pooled and lyophilized to yield 25.6 mg (96%) of β-azido-L-alanine. For the optimum melting point this material was recrystallized by dissolving in a minimal volume of H_2O at 40°C, adding MeOH (3 vol.) and acetone (until cloudy) and cooling to -20°C:[242,243] mp 174-175.5°C (dec); $[\alpha]_D^{25}$ +37.2 (±0.5)° (c 0.5, H_2O); IR (KBr disk) 3420 (m, br), 3070 (s, br), 2113 (s), 1600 (vs, br), 1440 (s) cm^{-1}; ^1H NMR (300 MHz, d_4-MeOD) δ3.63 (dd, 1H, 4.5, 12 Hz, $CHHN_3$), 3.53 (dd, 1H, 7.2, 12 Hz, $CHHN_3$), 3.37 (dd, 1H, 4.5, 7.2 Hz, CH); ^1H NMR (300 MHz, D_2O) δ3.93 (dd, 1H, 5.0, 17.5 Hz, $CHHN_3$), 3.92 (m, 1H, CH), 3.84 (dd, 1H, 7.0, 17.5 Hz, $CHHN_3$); EI-MS: 131.0570 (MH^+, Calc. 131.0570 for $C_3H_7N_4O_2$), 88.0400 (MH^+-HN_3), 85.0515 ($M-CO_2H$), 74.0245 (Base peak, $M-CH_2N_3$); POSFAB-MS (glycerol) 131 (MH^+); R_f

0.80 (System A; UV active; brown-purple with ninhydrin).

L-Cysteine (9a) from 140.[233]

A suspension of LiSH (1.23 M) in THF was produced by bubbling H_2S(g) into THF containing 1.23 M n-BuLi at 0°C. To the suspension of LiSH (2.24 mmol, 1.82 mL of 1.23 M) was added **140** (produced from 210 mg, 1.12 mmol of **42a**) in CH_3CN (1 mL). The mixture was stirred 1 h under Ar, acidified with conc. HCl (0.15 mL, 1.8 mmol), and solvent was removed in vacuo at 35°C. The residue was dissolved in H_2O and applied to a column of AG50-X8 (80 mL, 3 cm dia., H^+ form). Elution with a linear gradient of degassed aqueous HCl (0→2 M over 1 L) provided L-cysteine hydrochloride free of cystine after removal of solvent in vacuo. This material was isolated as the zwitterion from a minimal amount of EtOH by adjusting the pH to 6.5 with conc. NH_3, cooling to -20°C, and immediate recrystallization of the precipitate from hot degassed H_2O to yield **9a** (120.1 mg, 88% overall): mp 240-241°C (dec) (lit.[298] mp 240°C (dec)); $[\alpha]_D^{25}$ +6.5 (±0.2)° (c 2.0, 5N HCl) (lit.[214] $[\alpha]_D^{25}$ +6.5° (c 2, 5N HCl)); IR (KBr disk) 3600-2650 (s, br), 2540 (m), 2160-1980 (w, br), 1610 (s), 1582 (s), 1519 (s), 1397 (s), 1291 (m), 658 (m), 528 (m), 510 (m) cm^{-1}; ^1H NMR (300 MHz, D_2O δ4.31 (dd, 1H, 4.5, 5.5 Hz, CH), 3.16 (dd, 1H, 5.5, 15.0 Hz, CHHS), 3.13 (dd, 1H, 4.5, 15.0 Hz, CHHS); Anal. Calc. for $C_3H_7NO_2S$: C, 29.74; H, 5.83; N, 11.56; S, 26.46. Found: C, 29.46; H, 5.85;

N, 11.55; S, 26.68; POSFAB-MS (glycerol/HCl) 122 (MH^+), 243 (M_2H^+); R_f 0.45 (System A).

S-(Aminoethyl)-L-cysteine hydrochloride (149) from 140.[233]

To 2-aminoethanethiol hydrochloride (201.0 mg, 1.77 mmol) in degassed H_2O (3.0 mL) was added **140** (produced from BOC-L-serine β-lactone (**42a**) (151.0 mg, 0.807 mmol)) in H_2O (1.0 mL). The pH of the stirred solution was maintained at 5.0-5.5 by dropwise addition of 1N NaOH. When additions of base were no longer required to maintain the pH at 5.5 (~35 min), the mixture was applied to a column of AG50-X8 (80 mL, 3 cm dia., H^+ form) and eluted with a linear gradient (0→2 M over 1.0 L) of aqueous HCl (2 mL/min). Lyophilization of the chromatographically pure fractions yielded 138.2 mg (85%) of **149**, which was recrystallized from EtOH/acetone (85% recovery) for elemental analysis: mp 193-194°C (dec) (lit. mp 192-192.5°C,[244a] 205-207°C[244b]); $[\alpha]_D^{25}$ +7.2° (c 1.0, H_2O) (lit.[244a] $[\alpha]_D$ +7.2° (c 1, H_2O)); IR (KBr disk) 3650-2100 (s, br), 2000 (w, br), 1622 (s), 1587 (s), 1516 (s), 1494 (s), 1463 (s), 1427 (s), 1415 (s), 1400 (s), 1348 (s), 1303 (s), 560 (m) cm^{-1}; ^1H NMR (300 MHz, D_2O) δ4.00 (dd, 1H, 4.8, 6.0 Hz, CH), 3.27 (t, 2H, 6.5 Hz, $CH_2NH_3^+$), 3.16 (dd, 1H, 4.8, 14 Hz, CHCHHS), 3.14 (d, 6.0, 14 Hz, CHCHHS), 2.94 (t of d, 1H, 6.5, 14 Hz, SCHH), 2.92 (t of d, 1H, 6.5, 14.0 Hz, SCHH); Anal. Calc. for $C_5H_{13}N_2O_2SCl$: C, 29.92; H, 6.54; N, 13.96; Cl, 17.66; S,

15.97. Found: C, 29.59; H, 6.44; N, 13.98; Cl, 17.40; S, 15.88; POSFAB-MS (glycerol/HCl) 165 (MH^+), 329 (M_2H^+); R_f ~0.20 (System A).

S-2-(Aminoethyl)-L-cysteine hydrochloride (149) from 3a.

This material was prepared according to Cavallini et al.[244a] To a solution of L-cysteine (**3a**) (5.61 g, 46.3 mmol) and KOH (8.05 g, 139 mmol) in degassed H_2O (25 mL) at 70°C was added 2-bromoethylamine hydrobromide (9.47 g, 46.2 mmol) over 10 min. The mixture was stirred 4 h at 25°C, conc. HBr (1.8 mL) was added, and the acidic mixture was applied to a column of AG50-X8 (300 mL, 5 cm dia., H^+ form). The resin was washed well with H_2O and S-2-(aminoethyl)-L-cysteine was eluted with 1.0 M NH_4OH. Lyophilization, acification (pH 3) with aqueous HCl, and recrystallization from $EtOH/Et_2O$ provided **149** (5.58 g, 60%): mp 193-194°C (dec) (lit. mp 192-192.5°C,[244a] 205-207°C[244b]); $[\alpha]_D^{25}$ +7.2° (c 1.0, H_2O) (lit.[244a] $[\alpha]_D^{25}$ +7.2 (H_2O)). Spectral characteristics were identical to those of **149** (from **140**) above.

L,L-Lanthionine (16a) from 140.[233]

To L-cysteine (**9a**) (0.473 g, 3.91 mmol) in degassed H_2O (5 mL) at pH 5.3 was added **140** (produced from 0.244 g, 1.30 mmol of BOC-L-serine β-lactone **42a**) in H_2O (5 mL). The pH of the mixture was maintained at 5.0-5.5 with dropwise addition of 1N NaOH. After 40 min the pH

remained constant and the material was applied to a column of AG50-X8 resin (80 mL, 3 cm dia., H^+ form). Elution with a linear gradient of aqueous HCl (0→5 M over 1.5 L) provided chromatographically pure **16a** which was recovered by removal of solvent in vacuo. This material was recrystallized by suspending in H_2O (2.5 mL), dissolving by addition of conc. ammonia, cooling to 0°C, and neutralization (pH 6) with formic acid. Cooling several hours at 4°C yielded **16a** (251 mg, 93% overall) as the zwitterion: mp 294-295°C (dec, darkens at 247°C) (lit.[214] mp 293-295 °C (dec)); $[\alpha]_D^{25}$ +8.6° (c 5.0, 2.4N NaOH) (lit. $[\alpha]_D^{22}$ +6 (±1)° (c 1.0, 1N NaOH),[42a] +7° (c 1, 1N NaOH),[42b] +8.4° (c 1.0, 1.0N NaOH),[42c] (c1.4, 2.4N NaOH),[42d] +8.6° (c 5.0, 2.4N NaOH),[42a,102] +9.4° (c 1.4, 2.4N NaOH)[71]); IR (KBr disk) 3400 (w, br), 3300-2250 (s, br), 2080 (w), 1608 (s), 1593 (s), 1512 (s), 1389 (s), 1347 (s), 539 (m) cm^{-1}; ^1H NMR (400 MHz, D_2O + DCl) δ4.45 (dd, 2H, 7.4, 4.4 Hz, 2CH), 3.38 (dd, 2H, 4.4, 15.0 Hz, 2CHHS), 3.26 (dd, 2H, 7.4, 15.0 Hz, 2CHHS); Anal. Calc. for $C_6H_{12}N_2O_4$: C, 34.61; H, 5.81; N, 13.45; S, 15.40. Found: C, 34.63; H, 5.84; N, 13.50; S, 15.43; POSFAB-MS (glycerol/HCl) 209 (MH^+), 417 (M_2H^+); R_f 0.33 (System A). HPLC analysis of **16a** from **140** according to Schuster[108] indicates no detectable meso-lanthionine (i.e. <1%) in the sample (t_R = 23.5 (±0.1) min (LL), 24.3 min (meso)).

S-Sulfo-L-cysteine, monosodium salt dihydrate (150).

To $Na_2S_2O_3$ (73.2 mg, 0.463 mmol) in H_2O (1 mL) at pH 5.0 was added [illegible] prepared from BOC-L-serine β-lactone (**42a**) (43.0 mg, [illegible] mmol)) in H_2O (1 mL). The pH was maintained at 5.0 [illegible] h and the solvent was removed *in vacuo* at 25°C. The residue was dissolved in H_2O (1.0 mL), applied to a column of Rexyn 102 (1 × 10 cm, H^+ form) and eluted with H_2O (0.25 mL/min). S-Sulfo-L-cysteine (R_f 0.81, System A; characteristic brown color with ninhydrin) eluted chromatographically pure as the monosodium salt after 15-20 mL. Lyophilization and recrystallization from H_2O/Et_2O (pH 5) provided 49.7 mg (83%) of **150** as a white solid: mp 135°C foams but remains white (loss of H_2O), 202-204°C (dec); $[\alpha]_D^{25}$ -83.7 (±0.2)° (c 2.5, H_2O) (lit. $[\alpha]_D^{25}$ -86.8° (c 4.73, H_2O)[299] for the 1 1/2 hydrate); IR (KBr disk) 3450 (m, br), 3150 (m, br), 1635 (s, br), 1515 (m), 1400 (m), 1358 (m), 1235 (s), 1217 (s), 1200 (s), 1137 (s), 1030 (s), 636 (s) cm^{-1}; ^1H NMR (300 MHz, D_2O) δ4.18 (dd, 1H, 3.7, 8.0 Hz, CH), 3.68 (dd, 1H, 3.7, 15.5 Hz, $CHHSSO_3^-$), 3.[illegible] (dd, 1H, 8.0, 15.5 Hz, $CHHSSO_3^-$); Anal. Calc. for $C_3H_6NO_5S_2Na \cdot 2H_2O$ (FW 259.2): C, 13.90; H, 3.89; N, 5.40; S, 24.74. Found: C, 14.11; H, 3.85; N, 5.21; S, 24.59; NEGFAB-MS (glycerol) 200 ($^-O_3SSCH_2CH(NH_2)COOH$).

L-Cysteine dimethylsulfonium, bis(p-toluenesulfonic acid) salt (151).

To (S)-3-amino-2-oxetanone p-toluenesulfonic acid salt (**141**) (100.0 mg, 0.386 mmol) and anhydrous p-toluenesulfonic acid (99.6 mg, 0.579 mmol) in TFA (3.0 mL) was added dimethylsulfide (113 μL, 1.54 mmol). After 15 min the solvent was removed in vacuo. The syrupy residue was dissolved in MeOH (5 mL) and **151** crystallized as shiny white needles (168.1 mg, 88%) following addition of Et_2O (20 mL) and cooling to -20°C: mp 141-142°C (dec); $[\alpha]_D^{25}$ +11.8 (±0.4)° (c 0.96, DMF); IR (MeOH cast) 3483 (m), 2930 (m, vbr), 1742 (m), 1193 (vs), 815 (m), 682 (m), 567 (s) cm^{-1}; ^1H NMR (300 MHz, d_4-MeOH) δ7.70 (d, 4H, 8 Hz, o-ArH), 7.24 (d, 4H, 8 Hz, m-ArH), 4.65 (dd, 1H, 5.8, 8.2 Hz, CH), 3.95 (dd, 1H, 8.2, 13.8 Hz, CHHS), 3.81 (dd, 1H, 5.8, 13.8 Hz, CHHS), 3.07 (s, 3H, $S(CH_3)CH_3$), 3.06 (s, 3H, $S(CH_3)CH_3$), 2.36 (s, 6H, $ArCH_3$); Anal. Calc. for $C_{19}H_{27}NO_8S_3$: C, 46.23; H, 5.51; N, 2.84; S, 19.48. Found: C, 46.15; H, 5.49; N, 2.91; S, 19.55; EI-MS: 172.0195 (TsOH), 62.0207 (Me_2S); POSFAB-MS (glycerol) 150 (100%, $Me_2S^+CH_2CH(NH_2)COOH$); R_f ~0.08 (System A).

General Methodology with Polystyrene Resins.

All glassware employed in reactions and handling of resins was pretreated with a 10% solution of Surfasil siliconizing agent (Pierce) in hexane and oven dried at least 4 h at 140°C in order to minimize loses of resin due

to adhesion. All resin manipulations, including drying in vacuo at elevated temperatures, were conveniently carried out in the apparatus illustrated in Figure 23. A positive pressure of Argon was used at the vacuum take-off port to maintain solvents in the top reactor vessel. For filtration, the Ar was replaced by a vacuum to remove solvent and solutes thereby leaving the resin behind in the reactor vessel. Reactions at low temperature were carried out with a dry ice/solvent mixture in the outer glass jacket. For drying the resin at elevated temperatures, water was placed in the jacket and heated with a thermostatted coil. The stir rod was withdrawn, the vessel stoppered and a vacuum (<0.05 torr) applied to both the vacuum take-off and a neck of the reactor vessel for drying in vacuo. In this fashion the resin could be left in the reactor vessel at all times and subjected to reactions, washes, drying, regeneration, etc. Gentle stirring (50-140 rpm) was used at all times to avoid mechanical destruction of resin particles. The term "washing" implies the suspension of the resin in solvent, stirring 15-20 min followed by removal of solvent by suction filtration. β-Lactonization reactions were conveniently monitored by solution IR on the supernatant or filtrate. IR characteristics of the resin were recorded as a Fluorolube® mull in the absorbance mode to allow direct comparison of relative intensities of bands. Elemental analyses are the result of redoubled

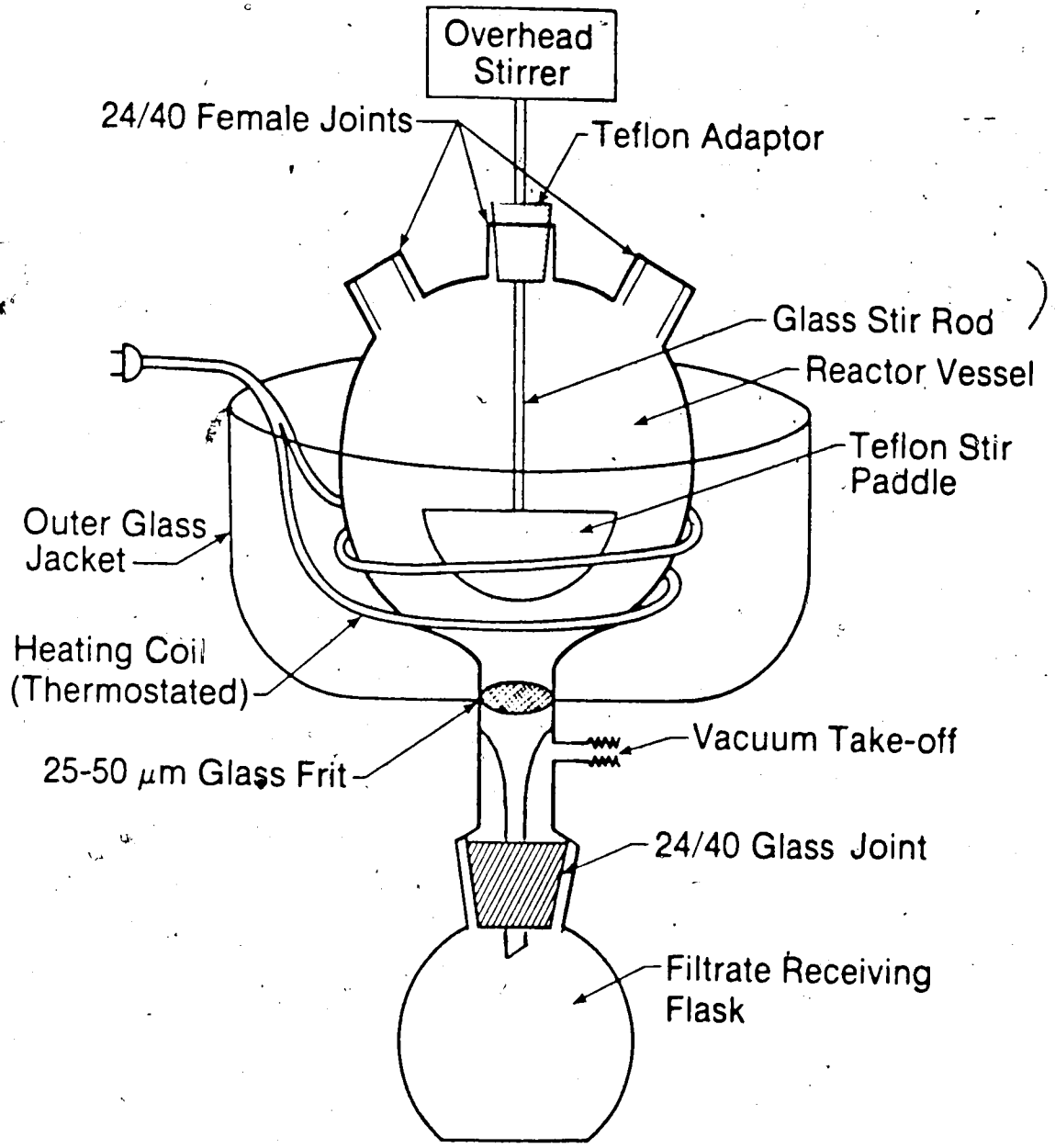


Figure 23. Reactor Vessel for Resin Reactions.

combustion.

Characterization of Commercial Hydroxymethyl Polystyrene Resin (152).

Commercial hydroxymethyl polystyrene resin (~1 meq/g or ~10 mol% of units; 1% crosslinked from Bachem Inc.) (20.0 g) was washed with THF/CH_2Cl_2 (1:1, 2 × 500 mL) and dried in vacuo at 64°C to constant weight: IR (Fluorolube® mull) 3575 (m), 3440 (m, vbr), 3080 (m), 3060 (m), 3030 (s), 2920 (vs), 2850 (m), 1993 (w), 1870 (w), 1805 (w), 1742 (w), 1601 (vs), 1583 (m), 1493 (s), 1451 (m) cm^{-1}; Anal. Calc. for $(C_{8.107}H_{8.215}O_{0.107})_x$ based on 1.00 meq/g or 10.7 mol% loading of units (ave. unit FW 107.38): C, 90.68; H, 7.71. Found: C, 90.13; H, 7.64.

Methyl Hydrazodicarboxylate-Derivatized Polystyrene Resin (153).

Hydroxymethyl resin (**152**) (19.0 g, ~19 meq) was stirred in CH_2Cl_2 (350 mL) with pyridine (1.53 mL, 19.0 mmol) in a dry atmosphere, and excess phosgene (8.0 mL at 0°C, 11.3 g, ~114 mmol) was bubbled into the stirred mixture at 25°C over 30 min. The mixture was stirred 1.5 h at 25°C, solvent was removed by filtration under dry Ar, and the chloroformate form of the resin was washed with dry CH_2Cl_2 (3 × 5 min, 250 mL) to remove excess phosgene and pyridinium hydrochloride. A mixture of triethylamine (7.95 mL, 57 mmol) and methyl hydrazinocarboxylate (5.14

g, 57 mmol) in HMPA (25 mL)/CH_2Cl_2 (250 mL) was added to the activated resin at 0-5°C, and stirred 16 h at 25°C. The solvent was removed by filtration and the resin was washed successively with MeOH (250 mL), MeOH/H_2O (1:1, 250 mL), MeOH (250 mL), and Et_2O (2 x 250 mL) and dried *in vacuo* at 60°C to provide approximately 20.3 g (~98%) of snowy-white derivatized resin **153**: IR (Fluorolube® mull) 3380 (s, br), 3310 (s, br), 3082 (s), 3060 (s), 3030 (s), 3002 (m), 2925 (vs, br), 2850 (m), 1945 (w), 1860 (w), 1800 (w), 1790-1680 (vs, vbr), 1601 (vs), 1583 (s), 1500 (m), 1450 (m, br) cm^{-1}; Anal. Calc. for 8.75 mol% loading or ~0.75 meq/g, $(C_{8.350}H_{8.524}N_{0.175}O_{0.349})_x$ (ave. unit FW 116.92): C, 85.77; H, 7.35; N, 2.09. Found: C, 85.33; H, 7.28; N, 2.07.

Oxidation of 153 to Methyl Azodicarboxylate-Derivatized Polystyrene Resin (154).

Methyl hydrazodicarboxylate resin **153** (17.0 g, ~12.7 meq at 0.75 meq/g) was gently stirred in CH_2Cl_2 or CH_3CN (250 mL) and pyridine (1.55 mL, 19.0 meq) was added followed by *N*-bromosuccinimide (18.4 mmol, 3.28 g). The mixture was stirred 1 h in the dark, and filtered. The resin was washed with CH_3CN (3 x 250 mL, until no orange color in filtrate) and Et_2O (2 x 250 mL) and dried *in vacuo* at 45°C to provide 16.84 g (~99%) of **154** as a bright orange resin: IR (Fluorolube® mull) 3540 (vw), 3360 (vw), 3100 (w), 3080 (m), 3055 (s), 3020 (vs), 2998 (m), 2920

(vs, br), 2845 (m), 1940 (w), 1920 (w), 1780 (vs, br), 1744 (m, sh), 1600 (s), 1581 (m), 1491 (vs), 1450 (s) cm^{-1}; From comparison of the absorbance ratios $A(\frac{3360}{3320})$ and $A(\frac{3540}{3320})$ with those of **152** and **153** an estimate of the percent of residual CH_2O-H and N-H units could be obtained. Typically this suggests 5 (±1)% of unoxidized hydrazo units (i.e., >94% yield in oxidation) and 8 (±3)% of underivatized hydroxymethyl units. Anal. Calc. for 8.75 mol% azodicarboxylate units (~0.74 meq/g azodicarboxylate units), $(C_{8.350}H_{8.350}N_{0.1748}O_{0.3296})_x$ (ave. unit FW 115.71): C, 85.90; H, 7.21; N, 2.09. Found: C, 85.61; H, 7.36; N, 1.91. Reaction with excess Ph_3P and BOC-serine (**41**), suggests 0.61 (±0.03) meq/g of usable azodicarboxylate units (86% of total azo units) based on chromatographic recoveries of unreacted triphenylphosphine, and Ph_3P=O byproduct. Both the elemental analysis (±0.3%) and this value of reducible azodicarboxylate units remained constant (±0.3 meq/g) over the five oxidation/Mitsunobu reaction cycles in which this resin was employed.

Resin-Mediated Formation of Benzyl Benzoate (155).

Methyl azodicarboxylate-derivatized resin (**154**) (6.55 g, 4.0 meq) was swollen in dry THF (100 mL, 15 min) and benzoic acid (366.4 mg, 3.0 mmol) in THF (50 mL) was added. To the stirred mixture at 25°C was added dropwise a solution of Ph_3P (786 mg, 3.0 mmol) and benzyl alcohol

(361 μL, 3.5 mmol) in THF (5 mL). After stirring 16 h the resin was filtered and washed with CH_2Cl_2 (4 × 150 mL). The filtrate was concentrated in vacuo at 30°C and flash chromatographed[260] (3.5% EtOAc/hexane) to yield 417 mg (65%) of benzyl benzoate (**155**): IR (film) 1720 (vs), 1451 (m), 1272 (vs), 1110 (m), 710 (s), 697 (m) cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ8.25-8.05 (m, 2H, o-PhCOO), 7.65-7.25 (m, 8H, m-, p-PhCOO, OCH_2Ph), 5.32 (s, 2H, OCH_2Ph); EI-MS: 212.0839 (M^+, 212.0837 calcd. for $C_{14}H_{12}O_2$), 105.0343 (Base peak, PhC=O).

Resin-Mediated Lactonization of BOC-L-Serine.

Typically, methyl azodicarboxylate-derivatized resin (**154**) (6.55 g, 4.0 meq) was swollen briefly (15 min) in dry THF (100 mL). The stirred suspension of beads was cooled to -45°C and BOC-L-serine (**41a**) (473.5 mg, 2.30 mmol) was added. To this mixture at -45°C was added a solution of triphenylphosphine (1.06 g, 4.0 mmol) in THF (5 mL) dropwise over 10 min. The suspension was stirred 30 min at -45°C, allowed to warm slowly to 0°C over 1 h, and stirred 2 h further. H_2O (36 μL) was added as a precautionary quench and the resin was filtered and washed with THF (2 × 100 mL) and CH_3CN (100 mL). The filtrate and washings were pooled and concentrated in vacuo at 35°C. The residue was flash chromatographed[260] (35% EtOAc/hexane) to yield 242.7 mg (56%) of BOC-L-serine β-lactone (**42a**) which possessed physical and spectral

properties identical to those previously described.

Alternatively BOC-L-serine β-lactone could be ured in 51% isolated yield (91% recovery) by selective crystallization as follows: The residue obtained from the filtrate (above) was treated with boiling anhydrous ether (60 mL) followed by cooling to 4°C (16 h). Precipitated triphenylphosphine oxide (1.08 g, ~95%) was removed by filtration. The etheral filtrate was concentrated in vacuo, and recrystallized by addition of hexane (~60 mL) to a solution of the residue in $CHCl_3$ (3 mL) and CCl_4 (7 mL) until persistent cloudiness at 45°C The mixture was filtered at 25°C and the filtrate was cooled to -20°C (48 h). Pure crystalline β-lactone (**42a**) (220.1 mg, 51% overall) was collected by filtration.

Acetoxymethyl Polystyrene Resin (156).

The procedure of Wang[300] was employed. Analyses on the chloromethylated polystyrene starting material (Bio-Beads S-X1 from Bio-Rad, 1% crosslinked, 200-400 mesh, 3.90 meq/g) indicated 50.1 mol% loading (Calc. 13.83% Cl. Found: 14.22, 13.79% Cl). Bio-Beads S-X1 (30.0 g, 117 mmol) were suspended in dimethylacetamide (700 mL) and stirred gently with potassium acetate (17.23 g, 176 mmol) at 85-90°C for 20 h. The resin was filtered and washed successively with H_2O/DMF (1:1, 2 × 500 mL), dioxane (3 × 250 mL), MeOH (2 × 300 mL) and Et_2O (2 × 300 mL). The white resin was dried in vacuo at 50°C to constant weight

to provide 32.28 g (98.5%) of acetoxymethyl resin **156**. Chlorine analysis indicates 0.37% Cl suggesting >97% conversion: IR (Fluorolube® mull) 2924 (s), 1736 (vs), 1601 (m), 1493 (m), 1452 (m), 1378 (m), 1361 (m) cm^{-1}; A (1736 cm^{-1}/2924 cm^{-1}) = 1.26, A (1736/1601) = 2.72; Solid State ^{13}C NMR (50.30 MHz) δ171 ($CH_3\underline{C}OO$), 147 (C-1 of Ar, $ArCH_2OAc$), 137 (C-4 of $ArCH_2OAc$), 127 (Ar's), 67 ($\underline{C}H_2OAc$), 52-35 ($\underline{C}H(Ar)\underline{C}H_2$), 22 ($\underline{C}H_3COO$) (see Figure 22); Anal. Calc. for 50.1 mol% acetoxymethyl residues (3.57 meq/g) or an average residue formula of $C_{9.503}H_{10.004}O_{1.002}$ (ave. FW 140.25/unit): C, 81.38; H, 7.19; Cl, 0. Found: C, 80.38; H, 6.92; Cl, 0.37.

Heavy-Loaded Hydroxymethyl Polystyrene Resin (157).

The procedure for reductive cleavage of **156** was adapted from Wang.[300] Acetoxymethyl resin **156** (32.0 g, 114 mmol) was suspended in dry ether (800 mL) and carefully treated with $LiAlH_4$ (15.75 g, 415 mmol) added in small portions over 30 min. The mixture was stirred 4 h, the vessel was equipped with a reflux condensor, and EtOAc (250 mL) was added slowly to quench. The mixture was stirred 30 min, and the resin was filtered and washed with EtOAc (200 mL), EtOAc/MeOH (1:1, 2 × 200 mL), and H_2O (2 L). Liberation of the resin from aluminates required extensive washing. The resin was gently stirred 2 days in (1:1:1) ethylene glycol/MeOH/pH 3, 20% aqueous citric acid (2 L) and filtered. The remaining grey color was removed

by washing successively with (1:2) 1N H_2SO_4/dioxane (3 × 2 L, 8 h), 0.1 M EDTA at pH 7 (1.5 L), 1N H_2SO_4 (2 × 1 L), MeOH (3 × 300 mL), THF (2 × 500 mL) and Et_2O (2 × 400 mL). The resulting snowy white resin was filtered and dried in vacuo at 60°C to constant weight (21.70 g, 99.7%). The complete absence of the carbonyl band at ~1730 cm^{-1} in IR suggests >99% of acetyl groups have been removed, and replaced with the genertion of hydroxyl (3350 cm^{-1}) groups: IR (Fluorolube® mull) 3350 (s, br), 2920 (vs), 1610 (m), 1492 (m), 1450 (m) cm^{-1}; Solid State ^{13}C NMR (50.30 MHz) δ147 (C-1 of Ar, $ArCH_2OH$), 137 (C-4 of $ArCH_2OH$), 127 (Ar's), 65 ($\underline{C}H_2OH$), 52-35 ($\underline{C}H(Ar)\underline{C}H_2$) (see Figure 22); Anal. Calc. for 50.1 mol% hydroxymethyl residues (4.2 meq/g) or an average unit formula of $C_{8.501}H_{9.002}O_{0.501}$ (ave. FW 119.19/unit): C, 85.66; H, 7.61; N, 0; Cl, 0. Found: C, 83.21; H, 7.34; N, 0.09; Cl, 0.42.

Methyl Hydrazodicarboxylate-Polystyrene Resin (158).

Dry hydroxymethyl resin **157** (25.0 g, ~105 meq) was added to CH_2Cl_2 (400 mL) containing phosgene (35.8 g, 362 mmol) at 0°C. Pyridine (8.50 mL, 105 mmol) was carefully added to this stirred mixture at 0°C. The mixture was stirred 2.5 h at 25°C and the resin was filtered under dry Ar and washed with CH_2Cl_2 (2 × 500 mL). Triethylamine (43.9 mL, 315 mmol) and methylcarbazate (28.4 g, 315 mmol) in HPMA (100 mL) were added slowly to this chloroformate

form of the resin in CH_2Cl_2 (400 mL) at 0°C. The mixture was stirred 16 h at 25°C, and the resin was filtered and washed successively with MeOH (500 mL), MeOH/H_2O (1:1, 2 × 400 mL), MeOH (400 mL) and Et_2O (2 × 500 mL) before drying in vacuo at 60°C. Analyses suggest ~60% incorporation of methylhydrazodicarboxylate residues (from %N) and ~40% unreacted chloroformate residues (from Cl analysis): IR (Fluorolube® mull) 3300 (w), 2920 (s), 1723 (vs, br), 1600 (m) cm^{-1}; Anal. Calc. for 50.1 mol% methyl hydrazodicarboxylate units: C, 67.75; H, 6.26; N, 7.91. Found: C, 73.08; H, 6.85; N, 4.96; Cl, 4.50.

(Phenyloxycarbonyl)oxymethyl-Polystyrene resin (159).

Hydroxymethyl resin **157** (1.38 g, 5.79 meq) was suspended in CH_2Cl_2 (15 mL) and phenylchloroformate (1.09 mL, 8.70 mmol) was added at 0°C. Pyridine (0.75 mL, 9.3 mmol) was added carefully at 0°C to this stirred mixture. After gentle stirring 16 h at 0°C, the resin was filtered and washed with CH_2Cl_2, acetone (3×), THF and Et_2O (2×) (25 mL) each and dried in vacuo at 50°C: IR (Fluorolube® mull) 2920 (s), 1760 (vs), no detectable OH stretch; Solid State ^{13}C NMR (50.30 MHz), δ153 (C=O), 146, 138, 123 (Ar), 71 ($ArCH_2CO_2Ph$), 52-35 ($CH(Ar)CH_2$) (see Figure 22); Anal. Calc. for 50.1 mol% phenylcarbonate residues (~2.8 meq/g): C, 80.42; H, 6.19; O, 13.39. Found: C, 79.36; H, 6.17; O, 13.10.

Methyl Hydrazodicarboxylate Resin 160 from 159.

Phenylcarbonate resin **159** (2.00 g, ~5.57 meq) was suspended in DMF (20 mL) and treated with methyl carbazate (1.57 g, 17.4 mmol) and 4-(dimethylamino)pyridine (1.07 g, 8.7 mmol). After stirring 5 days at 25°C the mixture was diluted with H_2O (20 mL), and filtered. The resin was washed with (1:1) MeOH/H_2O (3 × 50 mL), MeOH (2 × 50 mL), and ether (2 × 50 mL) and dried <u>in vacuo</u> at 60°C. Analyses indicate ~70 mol% of derivatized units are in the methyl hydrazodicarboxylate form and ~30% in the phenylcarbonate form: IR (Fluorolube® mull) 3300 (s, br), 2920 (s), 1720 (vs), 1601 (m) cm^{-1}; Comparison of IR band intensity ratios (NH, Ar(CH), C=O) with those of **159** and **153** suggests 30% phenylcarbonate/70% hydrazodicarboxylate functionalities; Solid State ^{13}C NMR (50.30 MHz) δ158 (carbamate C=O), 153 (carbonate C=O), 146 (C-1 of Ar), 128 (Ar), 68 ($Ar\underline{C}H_2O_2CNH$), 53 ($COO\underline{C}H_3$), 52-35 ($\underline{C}H(Ar)\underline{C}H_2$) (see Figure 22); Anal. Calc. for 35 mol% of <u>total</u> units as methyl hydrazodicarboxylate residues and 15 mol% in phenyl carbonate form: C, 71.23; H, 6.23; N, 5.53. Found: C, 69.32; H, 6.16; N, 5.36. From the N analysis this suggsts 1.91 meq/g hydrazo units.

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200. Compound **45a** determined to be 99% optically pure by ^{19}F NMR analyses on **89a**

201. Compound **45b** determined to be 97% optically pure by ^{19}F NMR analyses on **89b**.

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289. Compound **89b** prepared from D-serine[98] contained 1.5 (S,S)-isomer by ^{19}F NMR. HPLC suggests 1-2%.

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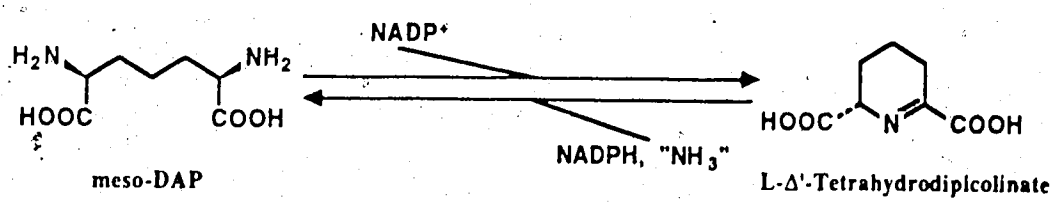
APPENDIX 1

The following enzymological results on amino acids prepared in the thesis have been obtained by Dr. M. Palcic and Dr. L. Lam. In all cases initial velocity data were analyzed by the method of Wilkinson.[301]

Diaminopimelate-Associated Enzymes:

The L-(2S,6S), D-(2R,6R) and meso-(2S,6R) isomers of lanthionine (**16a**, **16b**, **16c** respectively) and the corresponding sulfoxides (**22a**, **22b**, **22c**) and sulfones (**24a**, **24b**, **24c**, respectively) were tested for inhibition of diaminopimelate-associated enzymes. In no case was time-dependent inactivation characteristic of a suicide substrate observed.

meso-Diaminopimelate D-dehydrogenase (EC 1.4.1.16) catalyzes the biosynthesis of meso-diaminopimelic acid from L-Δ^1-tetrahydrodipicolinate.[65]



The enzyme occurs in various bacteria[65a] and has previously been noted to exhibit absolute specificity for the meso-isomer of diaminopimelate.[65a,c] meso-

Diaminopimelate D-dehydrogenase was isolated from Bacillus sphaericus IFO 3526 and enzyme activity was monitored spectrophotometrically by monitoring NADPH formation at 340 nm with meso-DAP as a substrate.[65] Incubations of the resolved L-(**4a**) and D-DAP (**4b**) preparations with the enzyme were useful in estimating stereochemical purity. Of the various lanthionine derivatives, only the meso-isomers **16c**, **22c** and **24c** show any appreciable interaction with the enzyme. The K_m for meso-lanthionine **16c** is 5.8 mM compared with 1.1 mM for meso-DAP, with a relative V_{max} of 1.1% that of the natural substrate. The meso-sulfoxide **22c** and sulfone **24c** are progressively poorer substrates, however some turnover is detected in the assay (Table 7).

Table 7. Lanthionine Derivatives as Substrates for Diaminopimelate Dehydrogenase

Substrate	Relative Velocity
meso-diaminopimelate (**4c**) (10 mM)	100% (K_m=1.0 mM)
meso-lanthionine (**16c** (10 mM)	1.08% (K_m=5.8 mM)
meso-lanthionine sulfoxide (**22c**) (14 mM)	0.09%
meso-lanthionine sulfone (**24c**) (10 mM)	0.02%

These results reflect the extremely high specificity of this enzyme and its poor tolerance for substitutions in the carbon chain.

meso-Diaminopimelate decarboxylase (EC 4.1.1.20) was isolated from Bacillus sphaericus and wheatgerm (Triticum vulgaris) and tested for inhibition of release of $^{14}CO_2$ from [1,7-^{14}C]-diaminopimelate (1.2 mM [DAP] total) by lanthionine derivatives (**16a→c**, **22a→c**, **24a→c**) (Table 8).[40] Lanthionine sufoxides (**22a→c**) are good competitive inhibitors (~50% inhibition at 1 mM) of both decarboxylases. The meso- and L-isomers (**24c** and **24a**, respectively) and lanthionine (**16c** and **16a**, respectively) are weaker competitive inhibitors (~50% inhibition at 10-20 mM). The corresponding D-isomers of the sulfone (**24b**) and sulfide (**16b**) are less effective. For comparison the observed K_m for the natural substrate meso-diaminopimelate (DAP) is 1.7 mM and 0.14 mM for the enzyme from B. sphaericus and T. vulgaris, respectively. Turnover of meso-lanthionine to produce thialysine (**149**) was verified by synthesis of authentic material. The lanthionine derivatives were not competitive inhibitors of lysine decarboxylase which does occur in mammals.

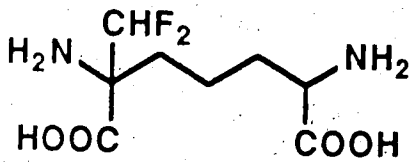
L-Diaminopimelate epimerase (EC 5.1.1.7) catalyzes the interconversion of L- and meso-DAP without the aid of pyridoxal phosphate (PLP).[64] The enzyme was isolated from Escherichia coli,[64b] and activity was measured by monitoring the conversion of optically pure L-DAP (**4a**) (K_m = 0.26 (±0.02) mM) to the meso-isomer (**4c**) with the aid of meso-DAP D-dehydrogenase[64b] in a coupled assay. The meso-

Table 8. Inhibition of Diaminopimelate Decarboxylase by Lanthionine Derivatives

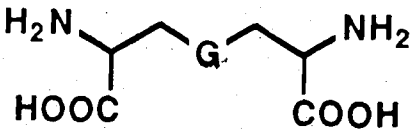
Analog[a]	B. sphaericus enzyme		T. vulgaris enzyme	
	Concentration of Analog (mM)	% Activity Remaining	Concentration of Analog (mM)	% Activity Remaining
α,α-diF-DAP[b]	10	64	7	88
Lanthionine sulfoxides				
22c	0.90	61	0.90	46
22a	0.90	56	0.90	49
22b	0.90	69	0.90	87
Lanthionine sulfones				
24c	10	60	10	57
24a	10	71	11	64
24b	10	91	10	86
Lanthionines				
16c	10	52	14	55
16a	10	74	16	49
16b	10	100	14	81

[a]Designators: a = L-(2S,6S), b = D-(2R,6R), c = meso-(2S,6R).

[b]α,α-Difluoromethyldiaminopimelate (α,α-diF-DAP) was prepared by Dr. J. Kelland.[40]



α,α-di-F-DAP



16a → c (G=S)
22a → c (G=SO)
24a → c (G=SO2)

isomer of lanthionine (**16c**) exhibited mixed inhibition of the enzymic epimerization (K_i = 0.18 mM, K_i' = 0.67 mM) whereas the L-isomer **16a** acted primarily as a competitive inhibitor (K_i = 0.42 mM, K_i' = 7.9 mM) and was not a substrate. The remaining lanthionine derivatives were poor inhibitors of the epimerase (Table 9).

Studies with Other Enzymes

Aspartate aminotransferase (EC 2.6.1.1) plays a central role in the intermediary metabolism of most organisms including mammals.[2,82,255,264] β-Phosphono-L-alanine (**71a**) acts as a good competitive inhibitor of this enzyme (K_i ~2 mM, compared with K_m ~6-8 mM for aspartate). β-Azido-L-alanine (**148**) was neither a suicide substrate or a competitive inhibitor.

Alanine aminotransferase (EC 2.6.1.2)[2,168a] was not inactivated or competitively inhibited by either β-phosphono-L-alanine (**71a**) or β-azido-L-alanine. In accordance with a previous report,[168a] β-cyano-L-alanine (**146**) exhibited time dependent inactivation of this enzyme which is characteristic of a suicide substrate.

Studies with aspartate α-decarboxylase (EC 4.1.1.11)[68] are still in progress. β-Cyano-L-alanine (**146**) acts only as a competitive inhibitor and not a suicide substrate of this pyruvoyl-dependent enzyme. Early results indicate that β-chloro-D-alanine (**21b**) and O-trifluoroacetyl-L-serine (**142**) are suicide substrates for this enzyme.

Table 9. Interaction of Lanthionine Derivatives with Diaminopimelate Epimerase

Lanthionine Derivative	K_i (Competitive) (mM)	K_i' (Noncompetitive) (mM)
16c (meso, X = S)	0.18	0.67
16a (L, G = S)	0.42	7.9
16b (D, G = S)	9	~19
22c (meso, G = SO)	11	>20
22a (L, G = SO)	>100	--
22b (D, G = SO)	--	--
24c (meso, G = SO_2)	21	--
24a (L, G = SO_2)	--	--
24b (D, G = SO_2)	--	--

NOTE: K_i and K_i' values which are not reported were too high to be reliable.