Synthesis, Stability, and Antimicrobial Activity of (+)-Obafluorin and Related β-Lactone Antibiotics

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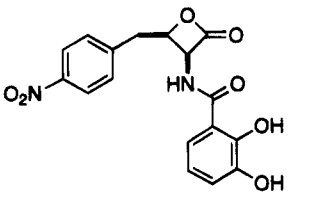
Optically pure obafluorin (**1**), an antibacterial agent from *Pseudomonas fluorescens*, was synthesized in six steps via lactonization of *N*-[(2-nitrophenyl)sulfenyl]-(2*S*,3*R*)-2-amino-3-hydroxy-4-(4-nitrophenyl)butanoic acid (**12a**), which was prepared in a stereospecific manner from 4-nitrophenylacetaldehyde (**9a**) and (*S*)-1-benzoyl-2-*tert*-butyl-3-methyl-4-imidazolidinone (**7**). A series of analogues was then synthesized in order to probe structural features required for antibacterial activity as well as those responsible for the hydrolytic decomposition of **1** to the corresponding hydroxy acid **23a**. Analogues **22b** and **22c** wherein the nitro group of **1** is replaced with hydrogen and chlorine, respectively, were prepared in a fashion similar to **1**, as were the *N*-acetyl, *N*-benzoyl, and *N*-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetyl (ATMO) derivatives **24a–c**. The tosylate salt of L-threonine-β-lactone (**21**) was transformed to a series of N-acylated derivatives including the following: **22d** (2,3-dihydroxybenzoyl), **25** (2-hydroxybenzoyl), **27** (3,4-dihydroxybenzoyl), **29** (4′-methyl-2,2′-bipyridine-4-carbonyl), **31** (ε-(L-α-aminoadipoyl)), **34** ((*N*′-2,3-dihydroxybenzoyl)-β-alanyl), **35** (bromoacetyl), **36** ((6-purinylthio)acetyl), and **37** ((4-pyridylthio)acetyl). The results show that α-amino β-lactones bearing an N-acyl group with an *o*- or *p*-hydroxybenzoyl moiety are especially prone to decomposition under aqueous conditions and that this effect is enhanced by replacement of the 4-nitrobenzyl group on the oxetanone ring of **1** with a methyl. The *N*-(3,4-dihydroxybenzoyl)-L-threonine β-lactone (**27**) converts slowly in the solid state to (4*S*,5*S*)-2-(3,4-dihydroxybenzoyl)-5-methyl-2-oxazoline-4-carboxylic acid (**39b**), which hydrolyzes rapidly in 4:1 CD_3CN:D_2O to *O*-(3,4-dihydroxybenzoyl)-L-allothreonine (**38b**). Direct hydrolysis of **27** to **38b** under the same conditions has a half-life of 2.4 days. Preliminary assays for antibacterial activity indicate that **29** has nearly comparable activity to obafluorin (**1**) but is much more stable. The (2-nitrophenyl)sulfenyl β-lactones **14** and **41**, as well as the *N*-(phenylsulfenyl)-L-threonine β-lactone (**44**), are the most active agents in the biological assays.

Introduction

Obafluorin (**1**), isolated by researchers at Squibb from *Pseudomonas fluorescens* (ATCC 39502),[1] is representative of a class of unusual antibacterial agents which contain an N-acylated α-amino β-lactone ring and bear a substituent at the β-carbon. Other examples include antibiotics such as oxazolomycin (**3**),[2] curromycins A (**4**) and B (**5**),[3] *N*-acetyl-L-threonine β-lactone (SQ 26,517) (**2**),[4] and related compounds formally derived from cyclization of β-hydroxy α-amino acid derivatives. Biosynthetic investigations on obafluorin (**1**) support possible involvement of such a lactonization process in its formation.[5]



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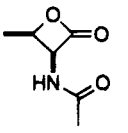

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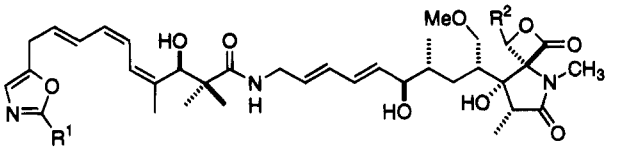

3 $R^1 = R^2 = H$

4 R^1 = Me $R^2 = CH_2OCH_3$

5 $R^1 = R^2$ = Me

Although the simpler naturally-occurring β-lactones bear some structural similarity to monobactams, their biological mode of action and structure–activity relationships have not yet been reported. This is probably due to the chemical instability of obafluorin (**1**) and the low yields obtained in attempts to directly cyclize compounds such as *N*-acetylthreonine to the corresponding β-lactone **2** (0.8%).[4b] Recently, we developed methodology to circumvent this synthetic problem[6] and reported in preliminary

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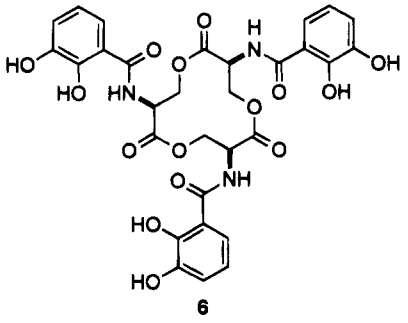
form the first total synthesis of optically pure **1**.[7] Rao et al. reported a synthesis of racemic diacetylobafluorin[8] using an approach similar to that previously described by us,[6] but it appears unlikely that the *O*-acetyl groups can be removed successfully because of the general instability of **1**[1b] and the sensitivity of such α-amino β-lactone derivatives to base and nucleophiles.[6,9] Since our route[7] permits facile variation of both the β-substituent and the acyl group on nitrogen, more stable and potentially more active analogues of **1** appeared accessible. In addition, derivatives bearing metal-complexing acyl groups (e.g., bipyridyl[10]) which can label the site of action of such β-lactone antibiotics *in vivo* are of interest. In the present work we report the full details of the first synthesis of natural (+)-obafluorin (**1**), the construction of a variety of optically pure analogues for probing structure–activity relationships and *in vivo* binding, the comparison of chemical stability of the β-lactones and their mode of decomposition, and the antimicrobial activity of these compounds against a limited selection of bacteria.

Classical structure–activity relationships with chloramphenicol analogues[11] suggest that alteration of the substituent at C-4 of the β-lactone ring of **1** could affect its antibiotic potency through changes in lipophilicity. The acidic 2,3-dihydroxybenzoyl substituent on nitrogen also alters water solubility, in this case as a function of pH. In addition, this N-acyl group is a powerful iron-chelating moiety which occurs as a key structural unit in natural siderophores such as enterobactin **6**, which has in fact



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been synthesized via an α-amino β-lactone.[12] Recent studies demonstrate that enhanced import of β-lactam antibiotics into bacterial cells is possible through covalent linking to iron-chelating groups, such as ferrichrome derivatives,[13] that are recognized by specific receptors of active transport systems.[14] Hence, part of this investigation also qualitatively examines the influence of lipophilicity and iron-chelating ability on the antimicrobial activity of the obafluorin analogues. Finally, structural features contributing to the instability of obafluorin (**1**) are identified.

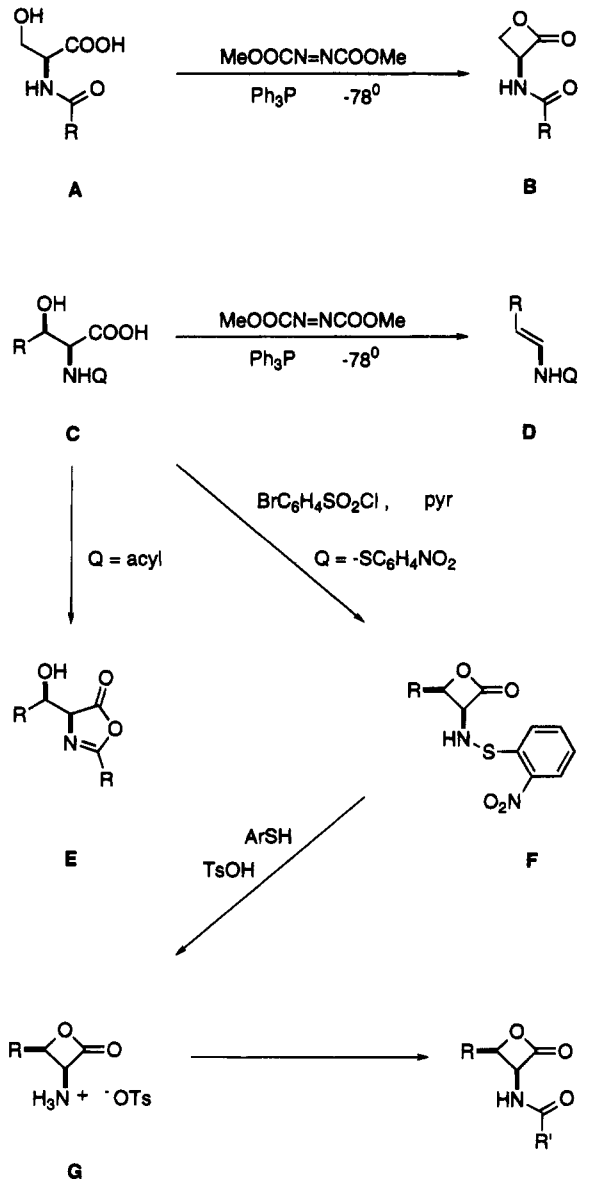

Figure 1. Synthetic routes to unsubstituted and β-substituted α-amino β-lactone derivatives.

Results and Discussion

Although serine β-lactones **B**, which are useful intermediates for synthesis of optically pure α-amino acids[15] or biopolymers,[16] are readily obtained by cyclization of N-protected serine derivatives **A** under modified Mitsunobu conditions, such hydroxyl group activation of the corresponding β-substituted analogues **C** leads to rapid stereospecific decarboxylative elimination to **D** (Figure 1).[9] Lactonization through carboxyl group activation is complicated by the tendency to form unstable azalactones

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E if the nitrogen has a carbonyl directly attached as part of the protecting group. An effective way to circumvent this problem is N-protection with an easily removable[17] (*o*-nitrophenyl)sulfenyl group, followed by carboxyl group activation with an arylsulfonyl chloride to afford the N-protected α-amino β-lactone **F**.[6] The ability to deprotect these β-lactones under acidic conditions and to N-acylate the resulting salts **G** allows independent variation of either the lactone moiety or the acyl group.

Syntheses of *N*-((2-Nitrophenyl)sulfenyl) Derivatives and Tosylate Salts of α-Amino β-Lactones. In order to understand the influence of the 4-nitrophenyl moiety of **1** on biological activity, initial targets included analogues wherein the nitro group on the aromatic functionality is replaced. The N-protected β-lactones could be synthesized as shown in Figure 2. Thus, treatment of the imidazolidinone **7** with lithium hexamethyldisilylamide, using methodology developed by Seebach and coworkers,[18] followed by *in situ* addition of chloro[tris(diethylamino)]titanium generates the titanium enolate **8**. The use of titanium was shown by the Schöllkopf group to enhance *threo* vs *erythro* diastereoselectivity in aldol condensations of bis-lactim ether enolates.[19] Initial attempts to react the lithium enolate of **7** directly with 4-nitrophenylacetaldehyde (**9a**) typically led to a 4:1 mixture of *threo* and *erythro* aldol adducts, apparently because of epimerization in the basic reaction mixture. In contrast, condensation of the titanium enolate **8** at −100 °C with **9a**, as well as the unsubstituted, 4-chloro-, and 4-methoxyphenylacetaldehydes (**9b**, **9c**, **9d**), gave only the corresponding *threo* aldol adducts as a mixture of rearranged isomers **10a−d** and **11a−d**. The adducts **10** and **11** exhibit different chemical shifts and coupling patterns for the hydrogens on the imidazolidinone ring and aldol carbon. Separation of these isomers, whose ratio depends on the structure of the aldehyde (Table 1), is difficult, but acidic hydrolysis of the mixture of **10** and **11** followed by ion-exchange chromatography in each case produced a pure *threo* β-hydroxy α-amino acid **12a−d**. There were no detectable traces (by ^{1}H NMR, TLC) of the *erythro* diastereomer. In the case of the 4-nitro compound **12a**, the optical purity was verified by preparation of the methyl ester of its (1*S*)-camphanamide **18** and comparison of the spectral data with the corresponding (1*S*)-camphanamide ester derivative **19** of (2*R*,3*S*)-2-amino-3-hydroxy-4-(4-nitrophenyl)butanoic acid (**17**) (Figure 3). This was prepared analogously to **12a** from the enantiomeric imidazolidinone **16**. The ^{1}H NMR spectra of the diastereomeric derivatives **18** and **19** are easily distinguishable and show that within detection limits (ca. 1%) both are stereochemically pure.

Each of the amino acids **12a−d** was protected on nitrogen with (2-nitrophenyl)sulfenyl chloride to form **13a−d**, respectively.[20] Carboxyl group activation of these compounds with (4-bromophenyl)sulfonyl chloride in pyridine afforded the corresponding N-protected β-lactones **14a−d**. Although the cyclization yields for the compounds bearing an arylmethyl group are disappointingly low (Table 1), the desired β-lactone is the only material easily isolable by chromatographic purification of the reaction mixture.

Removal of the (2-nitrophenyl)sulfenyl protecting group from these oxetanones proceeds with thiocresol and *p*-toluenesulfonic acid under carefully controlled condi-

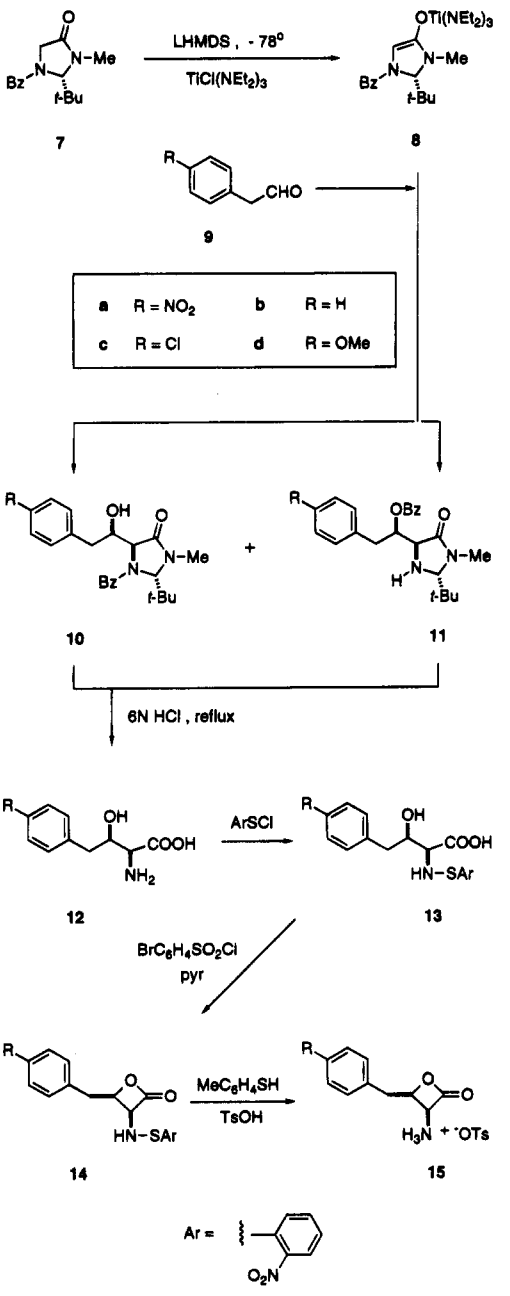

Figure 2. Syntheses of β-hydroxy α-amino acids and β-lactone tosylate salts related to **1**.

Table 1. Yields (%) of Intermediates for Obafluorin Analogues[a]

compd	**10−11** (ratio)[b]	**12**	**13**	**14**	**15**
a	61 (>99:1)	85	90	24	80
b	55 (60:40)	78	83	41	91
c	57 (55:45)	64	59	25	99
d	41 (72:28)	69	45	34	84

[a] Isolated yields of pure material. [b] Yield is given for mixture of **10** and **11**; ratio is determined by ^{1}H NMR.

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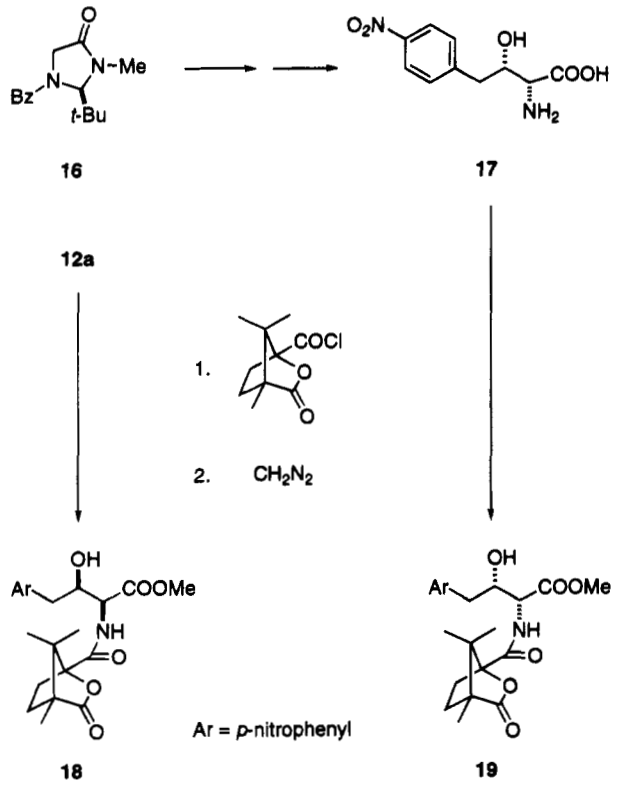

Figure 3. Derivatization for stereochemical analyses of **12a** and **17**.

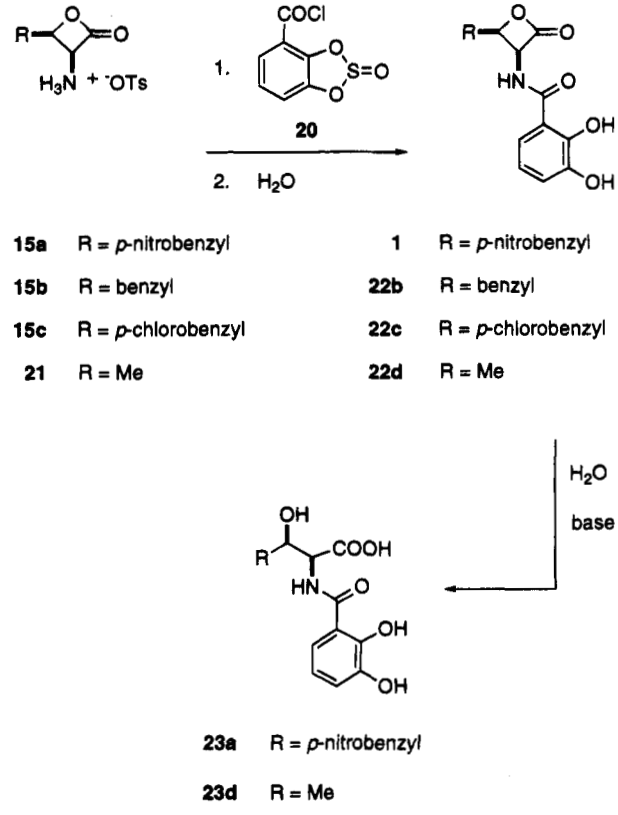

Figure 4. Preparation of obafluorin (**1**) and analogues **22b–d** and hydrolysis.

tions to provide the corresponding tosylate salts **15a–d**. This reaction is surprisingly sensitive to minor impurities and reagent concentration. Recent work on the mechanism of thiolysis of (*o*-nitrophenyl)sulfenamides suggests a two-step process involving nucleophilic attack by the aromatic thiol on the protonated sulfenamide to generate a sulfuranide intermediate which then decomposes to products.[17c] Generation of all possible disulfides during this process indicates that thiol–disulfide exchange may also complicate the mechanism of this deprotection reaction.[17a] As expected from our earlier studies on L-threonine β-lactone **21**,[6] the tosylate salts **15a–d** are much more stable than the corresponding β-unsubstituted salt of serine β-lactone,[15b] but dilute base or attempted chromatography on silica gel destroys these compounds very rapidly.

Syntheses of (+)-Obafluorin and Analogues Modified at the β-Substituent. In previous work we had demonstrated that the tosylate salts of α-amino β-lactones having a β-methyl group (e.g., L-threonine β-lactone (**21**)) can be N-acylated by a variety of reagents.[6] Since obafluorin is a very sensitive compound (see below), completion of its synthesis by N-acylation of the β-lactone salt **15a** with a 2,3-dihydroxybenzoyl moiety required an approach in which further transformations would be minimal and could proceed under very mild non-nucleophilic conditions. The acid chloride **20**, developed by Corey and Bhattacharyya,[21] has both phenolic hydroxy groups protected with a cyclic sulfite that hydrolyzes very readily upon exposure to water (Figure 4). Thus, reaction of the salt **15a** with **20** followed by aqueous workup produces optically pure (+)-obafluorin (**1**) in 57% yield after reversed-phase HPLC purification. As expected,[1b] obafluorin (**1**) prepared in this way decomposes rapidly upon standing at room temperature in aqueous acetonitrile to the corresponding β-hydroxy acid **23a**. The partly decomposed material can be repurified by rapid reversed-phase HPLC (isocratic elution with 55% MeCN–H_2O) to give pure **1**. Interestingly, the rigorously repurified material can be stored satisfactorily for many weeks at −15 °C in a dry inert atmosphere (e.g., Ar) and is much more stable to hydrolytic decomposition (see stability studies below). The optical rotation of synthetic obafluorin ($[\alpha]_D$ +70° (*c* = 0.1, MeCN), initially reported[7] as +43° (*c* = 0.03, MeCN)) is lower than the literature value[1b] for the natural compound ($[\alpha]_D$ +116° (*c* = 0.1 MeCN), and it was thus necessary to confirm the stereochemical integrity of our synthetic material. To achieve this, synthetic **1** was hydrolyzed under acidic conditions to the free amino acid (i.e., **12a**). Purification by ion-exchange chromatography, derivatization to the (1*S*)-camphanamide methyl ester as described above (cf. Figure 3), and ^1H NMR analysis as before revealed that the resulting derivative corresponded solely to **18**, thereby establishing the optical purity of synthetic **1**. Similar hydrolysis and derivatization of the β-hydroxy acid **23a**, obtained from aqueous decomposition of the initial sample of **1**, confirmed its stereochemistry and demonstrated that ring opening of **1** had occurred by attack at the carbonyl.

The tosylate salts **15b** and **15c**, as well as the L-threonine β-lactone salt **21**,[6] were individually acylated with acid chloride **20** to generate the obafluorin analogues **22b–d**, respectively (Figure 4). Like obafluorin (**1**), these compounds are quite sensitive until rigorously purified and decompose under aqueous conditions, primarily with hydrolysis of the β-lactone ring. As expected,[9] basic hydrolysis of **22d** gives the corresponding N-acyl-L-threonine derivative **23d**. Experiments on the mode of

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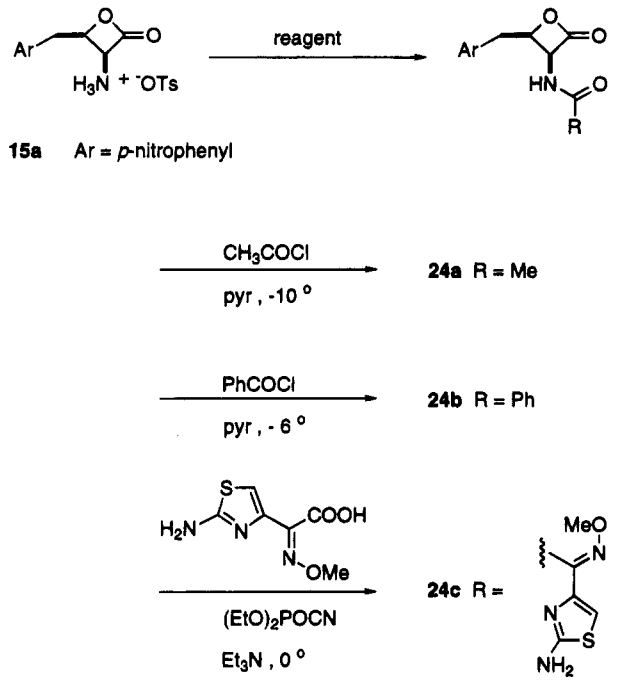

Figure 5. Preparation of obafluorin analogues **24a–c**.

decomposition of the β-lactones are discussed below in a separate section, as is their antibiotic activity.

Syntheses of Analogues of Obafluorin and SQ 26,-517 Bearing Modified N-Acyl Groups. Since *N*-acetyl-L-threonine β-lactone (SQ 26,517) (**2**) is antimicrobial and much more difficult to hydrolyze than **1**, an attempt to construct a more easily handled antibiotic focused on replacement of the *N*-(2,3-dihydroxybenzoyl) group. Reaction of the tosylate salt **15a** with acetyl chloride or with benzoyl chloride afforded the expected derivatives **24a** (61% yield) and **24b** (81%), respectively (Figure 5). Attachment of a 2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetyl (ATMO) group[22] to **15a** utilized diethylphosphoryl cyanide for coupling the corresponding acid to generate **24c** (25% yield, not optimized). All of these compounds were much more stable than obafluorin (**1**) or the analogues **22b–d** having the dihydroxybenzoyl group, but unfortunately none of them (i.e., **24a–c**) showed significant levels of antibiotic activity (see below).

The accessibility of threonine β-lactone tosylate salt (**21**)[6] and the antibiotic activity of both its *N*-acetyl derivative, SQ 26,517 (**2**),[4] and *N*-(2,3-dihydroxybenzoyl) compound **22d** encouraged use of this simple β-lactone core for further studies. It appeared likely that iron-chelating ability as well as hydrophilicity and bioavailability could be important for the antimicrobial action of **1**. These considerations, together with the need to improve the chemical stability and attach easily detectable groups for future studies on the site of action, suggested L-threonine β-lactone derivatives **25**, **27**, **29**, **31**, **34**, **36**, and **37** as potentially useful analogues (Figures 6 and 7). In order to clarify the importance of the *ortho* phenolic group for activity and stability, the monohydroxy derivative **25** and the 3,4-dihydroxybenzoyl analogue **27** were prepared by direct acylation. The cyclic sulfite acid chloride **26**

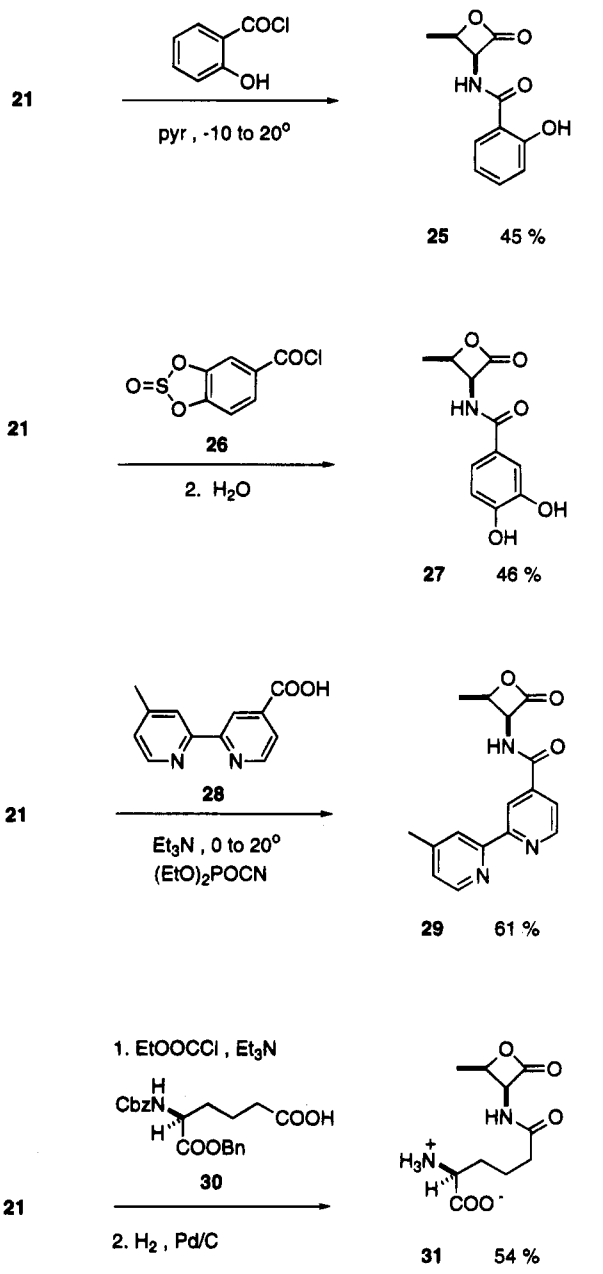

Figure 6. Syntheses of L-threonine β-lactone derivatives by direct acylation.

required for the latter process is readily available by treatment of 3,4-dihydroxybenzoic acid with thionyl chloride in analogy to production of **20**. As expected from the work with obafluorin (**1**) and its analogues **22b–d** having the 2,3-dihydroxybenzoyl moiety, the 3,4-cyclic sulfite hydrolyzes readily upon aqueous treatment to give **27**.

Direct acylation procedures with subsequent mild deprotection also afford access to **29** and **31**. The bipyridyl group is a potent metal chelator that has been coupled to peptides and can serve as a labeling device in biological systems.[10,23] Reaction of **21** with 4′-methyl-2,2′-bipyridinecarboxylic acid (**28**)[24] and diethylphosphoryl cyanide generates the corresponding oxetanone **29** in reasonable yield (61%). The hydrophilic α-aminoadipoyl functionality

(22) The ATMO group has been extensively used as an N-acyl group in β-lactam antibiotics: (a) Webber, J. A.; Wheeler, W. J. In *The Chemistry and Biology of β-Lactam Antibiotics*; Morin, R. B., Gorman, M., Eds.; Academic Press: New York, 1982; pp 371–436. (b) Boyd, D. B.; Elzey, T. K.; Hatfield, L. D.; Kinnick, M. P.; Morin, J. M. *Tetrahedron Lett.* **1986**, 3453–3456.

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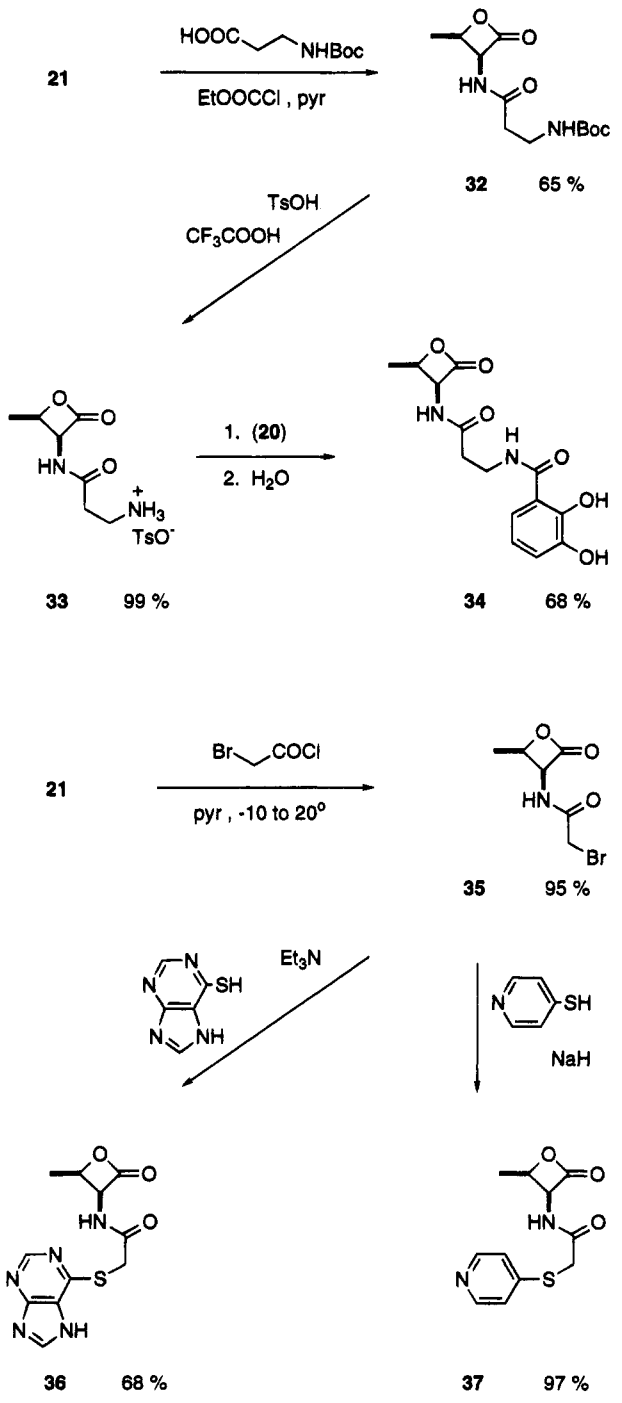

Figure 7. Multistep syntheses of L-threonine β-lactone derivatives by acylation followed by further transformation.

of **31** occurs in isopenicillin N[25] and can be readily attached by condensation of **21** with the mixed anhydride of the protected α-aminoadipic acid derivative **30**[26] and ethyl chloroformate (64% yield), followed by subsequent deprotection by hydrogenolysis. The latter step proceeds in good yield (84%) without significant destruction of the β-lactone.

The *N*-benzoyl β-lactone **24b** lacking the phenolic hydroxyls appears much more stable than obafluorin (**1**) (see also below). Hence, it seemed probable that insertion of a β-alanine linking arm between the *N*-(2,3-dihydroxybenzoyl) moiety and the β-lactone core as in **34** (Figure 7) could hinder interaction between these two entities and thereby increase resistance to decomposition while still maintaining potent iron-chelating ability and potential recognition by *in vivo* siderophore transport systems.[13,14] The most effective approach for its synthesis involves initial coupling of *N*-(*tert*-butoxycarbonyl) (Boc) β-alanine and L-threonine β-lactone tosylate salt (**21**) using ethyl chloroformate and pyridine to give **32**, followed by acidic deprotection to **33** and condensation with acid chloride **20**.

The (4-pyridylthio)acetyl group of **37** is present in a variety of highly potent β-lactam antibiotics, for example, cephaprin,[27] and bears some structural similarity to the (6-purinylthio)acetyl functionality of **36**. These analogues are also best made in two-stage processes wherein the β-lactone **21** is initially acylated with bromoacetyl chloride to form **35**, and then bromide displacement with the corresponding thiolate (i.e., 4-thiopyridine or 6-thiopurine) is utilized as the second step. Although the displacement with 6-thiopurine works reasonably well in the presence of triethylamine, the analogous reaction with 4-thiopyridine fails unless the thiolate anion is first formed with sodium hydride, presumably due to the predominance of the weakly nucleophilic thione form. The initial yield of **37** is nearly quantitative, but this compound is unstable and polymerizes too rapidly for biological evaluation. This is probably due to the mutual incompatibility of the sensitive β-lactone ring and the nucleophilic pyridine nitrogen over prolonged periods. Interestingly, the bipyridyl analogue **29** is much more stable than **37**. The reasons for this remain speculative, but electronic and steric factors may reduce the nucleophilicity of the nitrogens.

This work demonstrates that standard acylation methods will attach a large variety of functionalized acyl groups to the nitrogen of α-amino β-lactone tosylate salts, which are themselves readily available from the corresponding optically pure β-hydroxy α-amino acids. Although this β-lactone moiety is quite sensitive to nucleophiles and is rapidly destroyed by aqueous base, it survives a large variety of conditions commonly used in peptide coupling and deprotection. For example, reactions such as hydrogenation, acidic hydrolysis, and nucleophilic displacements can be successful provided conditions are kept sufficiently mild.

Stability and Hydrolytic Decomposition of 1 and Its Analogues. As described above, obafluorin (**1**) hydrolyzes spontaneously in water–acetonitrile to the β-hydroxy acid **23a** when it is first prepared and isolated from basic medium (pyridine). The stereochemistry of **23a** clearly demonstrates that attack by water proceeds at the carbonyl of **1**; this is typical behavior for β-substituted α-amino β-lactones under basic aqueous conditions.[9] However, once **1** has been repurified by repeated HPLC, NMR studies show that it is surprisingly stable in aqueous acetonitrile (D_2O:CD_3CN, 1:4) with only 8% decomposition over 5 weeks at 20 °C. Moreover, the sole detectable product of this prolonged treatment is the unexpected O-acyl derivative **38a** (Figure 8, R = *p*-nitrobenzyl, X = Y = OH, Z = H). Probably minute traces of basic impurities, such as pyridine or its salts with the

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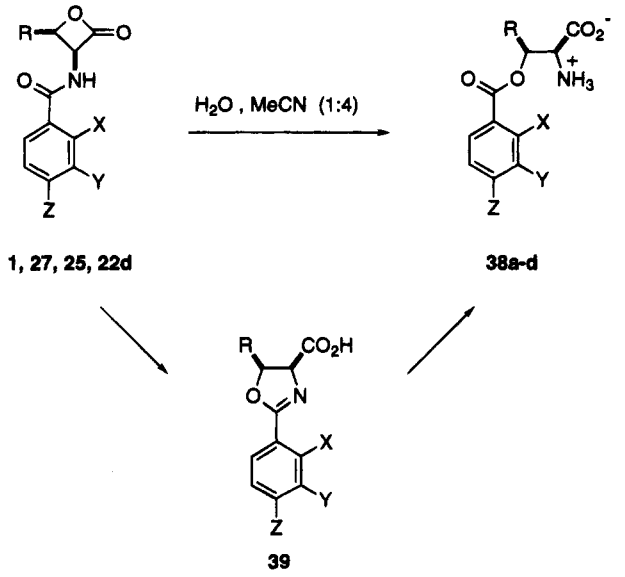

Figure 8. Aqueous decomposition of β-lactones bearing phenolic N-acyl groups. See text for structures.

catechol moiety, catalyze attack by water at the carbonyl in the initially isolated sample of **1**. This may involve intramolecular catalysis by a catecholate anion to enhance attack by water to form a tetrahedral intermediate. Once the traces of impurity have been removed by repeated HPLC, the antibiotic is much more stable and decomposes slowly under acidic conditions (catechol group) by an intramolecular pathway, possibly involving formation of an intermediate oxazoline **39a** (R = *p*-nitrobenzyl, X = Y = OH, Z = H).

Evidence for the generation of oxazoline **39** appeared upon prolonged storage (ca. 12 months) of dry solid *N*-(3,4-dihydroxybenzoyl)-L-threonine β-lactone (**27**) at −20 °C under argon. These conditions quantitatively convert β-lactone **27** to oxazoline **39b** (R = Me, X = H, Y = Z = OH), which upon exposure at room temperature to aqueous acetonitrile (D_2O:CD_3CN, 1:4) rapidly (approximate $t_{1/2}$ = 2.5 h) and quantitatively hydrolyzes to the corresponding O-acyl derivative **38b**. In contrast, the direct conversion of **27** to **38b** in the identical solvent and at the same temperature has a half-life of 2.4 days. As expected, no accumulation of intermediate oxazoline **39b** could be observed by NMR spectrometry during this transformation. Similar exposure of the *N*-(2,3-dihydroxybenzoyl)-L-threonine β-lactone (**22d**) and the 2-hydroxybenzoyl β-lactone **25** to aqueous acetonitrile (D_2O:CD_3CN, 1:4) shows that decomposition to the corresponding O-acyl derivatives **38d** (R = Me, X = Y = OH, Z = H) and **38c** (R = Me, X = OH, Y = Z = H) has a half-life of 9 days in each case. The structural assignments of oxazoline **39b** and O-acyl compounds **38a–d** are supported by spectral comparison (IR and NMR) to their N-acyl isomers **23a** and **23d**, as well as to related oxazolines[28] and substituted *O*-benzoyl derivatives[29] of threonine or allothreonine. Furthermore, acidic hydrolysis of pure oxazoline **39b** in refluxing 6 N HCl gives a 92:8 mixture (by NMR analysis) of L-allothreonine and threonine, thereby confirming the stereochemistry at the β-carbon. The origin of threonine as a minor component is uncertain, but it may be due to partial epimerization at the α-carbon during the vigorous hydrolytic procedure.

Examination of the N-acyl β-lactones prepared thus far clearly shows that the presence of an *o*- or *p*-hydroxybenzoyl moiety as the N-acyl substituent greatly decreases the stability under aqueous conditions. For such compounds, replacement of a substituted benzyl group on the oxetanone (e.g., 4-nitrobenzyl in **1**) with a less bulky methyl further increases the rate of hydrolytic decomposition of the pure β-lactone (e.g., **1** vs **22d** or **25**). This observation, together with the transformations of the 3,4-dihydroxy analogue **27**, support the proposal that the electron-rich aromatic ring assists rate-limiting attack by the amide oxygen at the β-position (C-4 of the oxetanone) to form an intermediate oxazoline **39** which then hydrolyzes more quickly to the O-acyl derivative **38**. This may occur most rapidly with **27** because the lack of an *ortho* substituent further reduces steric interactions and may favor a conformation wherein the electron-rich aromatic ring and the amide functionality are coplanar and the amide carbonyl is not hydrogen-bonded. Surprisingly, the presence of a linking arm between the dihydroxybenzoyl and β-lactone moieties in **34** fails to suppress ring cleavage, and solid samples stored in air decompose within a few weeks. The reason for this is presently unknown, but it may be due to intramolecular interaction (e.g., 12-membered ring) of a catechol hydroxyl with the β-lactone. Compounds **24a–c**, **29**, **31**, **35**, and **36**, which lack the hydroxybenzoyl group, are relatively stable under aqueous nonbasic conditions and display the standard reactivity of β-lactones.

Preliminary Examination for Antibiotic Activity. A number of the synthetic N-acylated α-amino β-lactones, deprotected α-amino β-lactone tosylate salts, and corresponding *N*-(2-nitrophenyl)sulfenyl derivatives were assayed for antimicrobial activity against several bacterial strains (Table 2). Two methods were used: agar plate diffusion with inhibition zone detection and test tube dilutions for quantitative estimation of minimum inhibitory concentration (MIC) by turbidity.[30] The results indicate that among the 4-benzyl-substituted derivatives, obafluorin (**1**) displays marginally greater potency than either the unsubstituted compound **22b** or the 4-chloro analogue **22c**. However, replacement of the 2,3-dihydroxybenzoyl moiety of **1** with acetyl, benzoyl, or ATMO (**24a**, **24b**, and **24c**) gives compounds which are completely inactive in any of our assays (data not shown).

In the series derived from L-threonine β-lactone, the *N*-(2,3-dihydroxybenzoyl) analogue **22d** displays activity similar to the corresponding *N*-acetyl derivative, SQ 26,517 (**2**), but is less effective than obafluorin (**1**). Nevertheless, the ready availability of the threonine β-lactone salt **21** makes it ideal for examination of the influence of the N-acyl group on antimicrobial action and stability. Unexpectedly, the analogues having the N-acyl group modified to 2-hydroxybenzoyl, 3,4-dihydroxybenzoyl, α-aminoadipoyl, or *N'*-(2,3-dihydroxybenzoyl)-β-alanyl (compounds **25**, **27**, **31**, **34**, respectively) show no significant antibiotic activity in our assays. In contrast, the bipyridyl derivative **29** and the (purinylthio)acetyl lactone **36** inhibit many of the test organisms. The (pyridylthio)acetyl analogue **37** decomposed too rapidly

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(30) *Antibiotics in Laboratory Medicine*; Lorian, V., Ed.; Williams and Wilkins: Baltimore, 1980; pp 1–113.

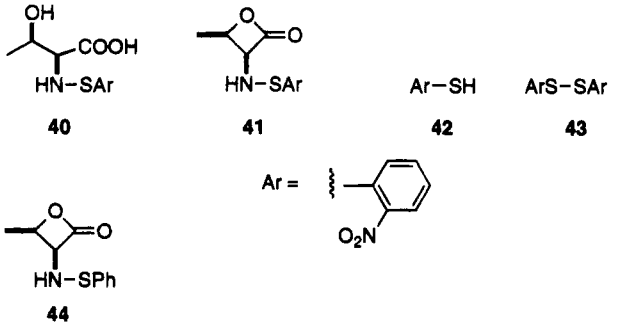
Table 2. Antibacterial Tests: Minimum Inhibitory Concentrations[a]

organism[b]	compd																
	1	**2**	**22b**	**22c**	**22d**	**25**	**27**	**29**	**31**	**35**	**36**	**14a**	**14c**	**40**	**41**	**42**	**44**
Staphylococcus aureus 25923	125 25[d]	125	250	250	N[c]	N	N	60	N	N	125			30			32
S. aureus 13565	125 50[d]	250	250	125	250	N	N	125	N	250	125			30			32
S. aureus 6538	125	250	N	N	N 125[d]	N	N	250 125[d]	N	N 250[d]	125	4	3	30	12	10	32
Streptococcus faecalis 7080	125	N	N	N	N 125[d]	N	N	N	N	N 250[d]	N 125[d]			250			32
Klebsiella pneumoniae 11296	125	N	125	250	125	N	N	125	N	125	N			125			32
Proteus vulgaris 13315	125	N	N	N	N	N	N	125	N	60	N			250			65

[a] All tests done by dilution method unless noted with results in μg/mL; see Experimental Section. [b] Organisms from American Type Culture Collection. [c] N indicates no inhibition at 250 μg/mL. [d] Agar diffusion method; see Experimental Section.

to make biological testing practical. The results indicate that among the N-acyl β-lactones examined thus far, obafluorin (**1**) possesses the most potent and broad antimicrobial spectrum against the organisms tested, although the much more stable bipyridyl compound **29** approaches its activity.

Surprisingly, the *N*-[(2-nitrophenyl)sulfenyl] β-lactones **14** and **41**,[6] which are synthetic precursors to the tosylate salts **15** and **21**, are remarkably active in our bioassay system. Examination of the literature indicated that some (2-nitrophenyl)sulfenamides possess potent antifungal and antibacterial properties, but unfortunately they also exhibit high mammalian toxicity and poor solubility.[31] To



clarify whether the β-lactone ring plays a significant role in the antibiotic effects of **14** and **41**, the activities of unlactonized *N*-[(2-nitrophenyl)sulfenyl]-L-threonine (**40**),[6] 2-nitrothiophenol (**42**), its symmetrical disulfide **43**, and *N*-(phenylsulfenyl)-L-threonine β-lactone (**44**) were compared. Compound **44** is available by treatment of tosylate salt **21** with phenylsulfenyl chloride. The results of antibacterial tests show that although free 2-nitrothiophenol (**42**) is a powerful antibiotic, and its possible formation *in vivo* through cleavage of the N–S bond of (2-nitrophenyl)sulfenamides **14**, **41**, and **40** may account for the bulk of their activity, the presence of the β-lactone moiety may enhance the antibacterial properties. For example, the β-lactones **14a** and **14c** are about three times more potent than **42**, and the threonine β-lactone derivative **41** is comparable to **42**, whereas the uncyclized *N*-[(2-nitrophenyl)sulfenyl]-L-threonine (**40**) is somewhat less active. The disulfide **43** is essentially inactive (data not shown), suggesting that it is not easily cleaved to thiol **42** under the assay conditions. Interestingly, β-lactone **44**, which is missing the nitro group, is also a potent antimicrobial agent.

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Conclusions

This work describes the first synthesis of optically pure obafluorin (**1**) in six steps (7.2% overall yield from **7**). All of the steps leading to this sensitive molecule proceed in good yield (>60%, most ≥80%) except for the lactonization (24%). The present study also demonstrates that the route is very versatile and allows preparation of a large variety of obafluorin analogues using many reagents common in peptide coupling and deprotection. Although the β-lactone moiety is inherently unstable to aqueous base and strong nucleophiles, the enhanced tendency of **1** to decompose, even under neutral or slightly acidic conditions, is primarily due to the catechol moiety which catalyzes hydrolysis of the lactone ring in the presence of trace impurities. Once these are removed by repeated HPLC separation, **1** is much more stable under slightly acidic aqueous conditions, and slow decomposition proceeds by intramolecular attack of the amide oxygen on C-4 of the oxetanone to generate an O-acyl derivative **38a**. This probably occurs via intermediate oxazoline formation, as can be demonstrated in the conversion of **27** to **38b**.

Replacement of the 4-nitrophenyl ring of **1** with hydrogen gives the more readily accessible derivatives of L-threonine β-lactone, some of which display significant antibacterial activity. For example, the bipyridyl analogue **29**, which also has potential as a biological tool, inhibits bacterial strains to nearly the same extent as **1**, but is much more stable and easily prepared. It is also interesting to note that the (2-nitrophenyl)sulfenamide derivatives are even more potent antimicrobial agents, although they may be toxic to mammalian cells. However, the *N*-phenylsulfenyl β-lactone **44**, which lacks the nitro group, still displays much better activity than the naturally-occurring obafluorin (**1**). Current investigations on incorporation of N-acyl-α-amino β-lactones into peptides for inhibition of specific enzymes (e.g., proteases) will be reported later.

Experimental Section

General Methods. Most general experimental procedures and instrumentation have been described previously[32] and are fully updated in the supplementary material. All reagents were purchased from Sigma or Aldrich and were used without further purification unless otherwise stated. All solvents were dried and distilled prior to use according to standard procedures.[33]

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(2*R*,5*S*,1′*R*)-1-Benzoyl-5-[1′-hydroxy-2′-(4″-nitrophenyl)ethyl]-2-*tert*-butyl-3-methylimidazolidin-4-one (10a). *n*-Butyllithium (1.95 M in hexanes, 3.56 mL, 6.95 mmol) was added to hexamethyldisilazane (1.47 mL, 6.97 mmol) in THF (4.0 mL) at −78 °C. The solution was stirred at −78 °C for 15 min and then at 20 °C for 10 min. The solvent was removed under vacuum (<0.1 Torr) over 30 min. The residue was dissolved in THF (40 mL) and cooled to −78 °C; to this solution was added dropwise a solution of (*S*)-1-benzoyl-2-*tert*-butyl-3-methyl-4-imidazolidinone (**7**)[18] (1.64 g, 6.32 mmol) in THF (21 mL), and the resulting orange/red solution was stirred at −78 °C for 30 min. A solution of chlorotris(diethylamino)titanium (2.06 g, 7.58 mmol) in dry hexane (6.3 mL) was added dropwise over 10 min, and the mixture was stirred at −78 °C for 1 h. The mixture was then cooled to −100 °C, and a solution of 4-nitrophenylacetaldehyde (**9a**)[34] (1.25 g, 7.57 mmol) in THF (21 mL) was added dropwise over 20 min. After an additional 10 min at −100 °C, the mixture was stirred 3.5 h at −78 °C. The reaction was quenched by the addition of saturated aqueous NH_4Cl solution (80 mL) with vigorous stirring, and the mixture was allowed to warm to 20 °C over ca. 30 min. The mixture was diluted with water (80 mL) and extracted with diethyl ether (3 × 150 mL). The combined organic layers were dried ($MgSO_4$) and evaporated *in vacuo*. The crude product was purified by flash chromatography (SiO_2, 40−60% EtOAc/hexane) to give **10a** (1.64 g, 61%): $[\alpha]_D$ +120.5° (*c* = 1.0, CH_2Cl_2); IR ($CHCl_3$ cast) 3375 (br), 2960, 1721, 1694, 1520 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.14 (d, 2 H, *J* = 12 Hz), 7.95 (d, 2 H, *J* = 10 Hz), 7.58 (t, 1 H, *J* = 10 Hz), 7.50 (d, 2 H, *J* = 12 Hz), 7.45 (t, 2 H, *J* = 10 Hz), 5.55 (dt, 1 H, *J* = 5, 7 Hz), 4.22 (d, 1 H, *J* = 2 Hz), 3.72 (dd, 1 H, *J* = 5, 2 Hz), 3.33 (d, 2 H, *J* = 7 Hz), 2.94 (s, 3 H), 0.98 (s, 9 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 25.6 (q), 31.3 (q), 36.9 (s), 37.5 (t), 59.9 (d), 74.5 (d), 83.7 (d), 123.7 (d), 128.5 (d), 129.6 (d), 129.7 (s), 130.6 (d), 133.4 (d), 144.7 (s), 147.0 (s), 165.5 (s), 172.7 (s); MS (CI, NH_3), 426 (MH^+, 100). Anal. Calcd for $C_{23}H_{27}N_3O_5$: C, 64.93; H, 6.40; N, 9.88. Found: C, 64.69; H, 6.38; N, 9.52.

(2*S*,5*S*,1′*R*)-1-Benzoyl-2-*tert*-butyl-5-(1′-hydroxy-2′-phenylethyl)-3-methylimidazolidin-4-one (10b) and (2*R*,5*S*,1′*R*)-5-[1′-(Benzoyloxy)-2′-phenylethyl]-2-*tert*-butyl-3-methylimidazolidin-4-one (11b). The mixture of aldol adducts was prepared analogously to **10a** using phenylacetaldehyde (**9b**) except that the lithium enolate of **7** was employed directly. The crude product was purified by flash chromatography (SiO_2, 40% EtOAc/hexane) to give a 60:40 mixture (by ^{1}H NMR) of **10b** and **11b** (55% yield): IR ($CHCl_3$ cast) 3380 (br), 2960, 1716, 1692, 1452 cm^{-1}; ^{1}H NMR for **10b** ($CDCl_3$, 200 MHz) δ 7.75 (d, 2 H, *J* = 7 Hz), 7.62−7.40 (m, 6 H), 7.10 (d, 2 H, *J* = 8 Hz), 5.78 (br s, 1 H), 4.70−4.60 (m, 2 H), 3.51−3.39 (m, 1 H), 3.16 (s, 3 H), 2.65 (d, 1 H, *J* = 12 Hz), 2.41−2.27 (m, 1 H), 1.09 (s, 9 H); ^{1}H NMR for **11b** δ 8.14−7.95 (m, 6 H), 7.62−7.40 (m, 4 H), 5.55−5.45 (m, 1 H), 4.01 (d, 1 H, *J* = 2 Hz), 3.90−3.86 (m, 1 H), 3.34 (d, 2 H, *J* = 10 Hz), 2.90 (s, 3 H), 0.89 (s, 9 H); exact mass 323.1398 (M^+ − ^{t}Bu, 49) (323.1395 calcd for $C_{19}H_{19}N_2O_3$); MS (CI) 381 (MH^+, 100).

(2*S*,5*S*,1′*R*)-1-Benzoyl-2-*tert*-butyl-5-[1′-hydroxy-2′-(4″-chlorophenyl)ethyl]-3-methylimidazolidin-4-one (10c) and (2*R*,5*S*,1′*R*)-5-[1′-(Benzoyloxy)-2′-(4″-chlorophenyl)ethyl]-2-*tert*-butyl-3-methylimidazolidin-4-one (11c). The condensation of **7** (1.56 g, 6.00 mmol) and (4-chlorophenyl)-acetaldehyde (**9c**)[34] (1.66 g, 10.7 mmol) using the procedure to prepare **10b** and **11b** gave a 55:45 mixture of **10c** and **11c** (1.43 g, 57%): mp 143−145 °C; IR (KBr, disk) 3400 (br), 2930, 1680, 1635, 1492 cm^{-1}; ^{1}H NMR for **10c** ($CDCl_3$, 400 MHz) δ 7.72 (d, 2 H, *J* = 7 Hz), 7.48−7.40 (m, 3 H), 7.15 (d, 2 H, *J* = 8 Hz), 6.89 (d, 2 H, *J* = 8 Hz), 5.74 (br s, 1 H), 4.59 (d, 1 H, *J* = 4 Hz), 4.51 (d, 1 H, *J* = 12 Hz), 3.43−3.33 (m, 1 H), 3.11 (s, 3 H), 2.53 (d, 1 H, *J* = 14.5 Hz), 2.24−2.17 (m, 1 H), 1.09 (s, 9 H); ^{1}H NMR for **11c** δ 7.95 (d, 2 H, *J* = 7 Hz), 7.58−7.51 (m, 3 H), 7.28−7.22 (m, 4 H), 5.52 (m, 1 H), 4.24−4.20 (m, 1 H), 3.71−3.66 (m, 1 H), 3.24−3.14 (m, 2 H), 2.91 (s, 3 H), 0.98 (s, 9 H); exact mass 359.0990 (M^+ − ^{t}Bu, 7) (359.0986 calcd for $C_{19}H_{18}N_2O_3{}^{37}Cl$), 357.1015 ($M^+$ − ^{t}Bu, 7) (357.1006 calcd for $C_{19}H_{18}N_2O_3{}^{35}Cl$).

(2*S*,5*S*,1′*R*)-1-Benzoyl-2-*tert*-butyl-5-[1′-hydroxy-2′-(4″-methoxyphenyl)ethyl]-3-methylimidazolidin-4-one (10d) and (2*R*,5*S*,1′*R*)-5-[1′-(Benzoyloxy)-2′-(4″-methoxyphenyl)ethyl]-2-*tert*-butyl-3-methylimidazolidin-4-one (11d). Condensation of **7** (2.60 g, 10.0 mmol) and 4-methoxyphenylacetaldehyde (**9d**)[35] (2.30 g, 16.0 mmol) using the procedure to prepare **10b** and **11b** gave a 72:28 mixture of **10d** and **11d** (1.70 g, 41%): mp 155−158 °C; IR (KBr) 3413, 2958, 1717, 1683, 1632, 1615 cm^{-1}; ^{1}H NMR for **10d** ($CDCl_3$, 400 MHz) δ 7.73 (d, 2 H, *J* = 7.1 Hz), 7.49 (m, 3 H), 6.88 (d, 2 H, *J* = 8.4 Hz), 6.74 (d, 2 H, *J* = 8.4 Hz), 5.74 (br s, 1 H), 4.59 (d, 1 H, *J* = 4.4 Hz), 4.42 (d, 1 H, *J* = 10.5 Hz), 3.74 (s, 3 H), 3.39 (m, 1 H), 3.10 (s, 3 H), 2.53 (d, 1 H, *J* = 14.1 Hz), 2.07 (m, 1 H), 1.09 (s, 9 H); ^{1}H NMR for **11d** δ 7.95 (m, 2 H), 7.49 (m, 3 H), 7.25 (d, 1 H, *J* = 8.0 Hz), 5.52 (m, 1 H), 4.20 (m, 1 H), 3.70 (m, 1 H), 3.15 (m, 2 H), 2.91 (s, 3 H), 0.98 (s, 9 H); MS (CI) 411 (MH^+, 100). Anal. Calcd for $C_{24}H_{30}N_2O_4$: C, 70.22; H, 7.37; N, 6.82. Found: C, 70.25; H, 7.15; N, 6.67.

(2*S*,3*R*)-2-Amino-3-hydroxy-4-(4-nitrophenyl)butanoic Acid (12a). Hydrochloric acid (6 N, 110 mL) was added to **10a** (978 mg, 2.3 mmol), and the mixture was heated to reflux overnight. The solution was cooled to 20 °C, diluted with water (50 mL), and washed with CH_2Cl_2 (3 × 100 mL). The aqueous phase was filtered through glass wool and evaporated *in vacuo*. The residue was purified by ion-exchange chromatography on AG 50W-X8 (H^+) resin (BioRad, Mississauga, ON) by elution with water followed with 1 N NH_4OH solution. The fractions containing the amino acid (ninhydrin positive) were first concentrated *in vacuo* and then lyophilized to yield **12a** (467 mg, 85%) as a powder: mp 233 °C dec; $[\alpha]_D$ +50° (*c* = 0.18, H_2O); IR (KBr) 3420, 1620, 1604, 1595 cm^{-1}; ^{1}H NMR (D_2O, 500 MHz) δ 8.06 (d, 2 H, *J* = 8 Hz), 7.22 (d, 2 H, *J* = 8 Hz), 4.24−4.20 (m, 1 H), 3.61 (d, 1 H, *J* = 5.5 Hz), 3.08 (dd, 1 H, *J* = 14, 3.5 Hz,), 2.84 (dd, 1 H, *J* = 14, 10 Hz); ^{13}C NMR (D_2O/DCl, 75.5 MHz) δ 37.2 (t), 55.2 (d), 67.3 (d), 122.0 (d), 128.4 (d), 143.0 (s), 144.7 (s), 167.4 (s); MS (CI, NH_3) 241 (MH^+, 90).

(2*S*,3*R*)-2-Amino-3-hydroxy-4-phenylbutanoic Acid (12b). This compound was prepared analogously to **12a** from the mixture of **10b** and **11b** in 78% yield: mp 195−198 °C; IR (KBr) 3418, 1617, 1602, 1585 cm^{-1}; ^{1}H NMR (D_2O/DCl, 200 MHz) δ 7.30−7.14 (m, 5 H), 4.20−4.11 (m, 1 H), 3.50 (d, 1 H, *J* = 4.5 Hz), 2.87, 2.68 (ABX, 2 H, J_{AX} = 4.5 Hz, J_{BX} = 9.5 Hz, J_{AB} = 14 Hz); MS (CI) 196 (MH^+, 100). Anal. Calcd for $C_{10}H_{13}NO_3$: C, 61.53; H, 6.71; N, 7.17. Found: C, 61.42; H, 6.76; N, 7.15.

(2*S*,3*R*)-2-Amino-3-hydroxy-4-(4-chlorophenyl)butanoic Acid (12c). This material was prepared analogously to **12a** from the mixture of **10c** and **11c** (1.30 g, 3.13 mmol) in 64% yield (0.46 g): mp 202−205 °C; IR (KBr) 3422, 1656, 1641, 1632, 1613, 1599 cm^{-1}; ^{1}H NMR (D_2O/DCl, 200 MHz) δ 6.70 (d, 2 H, *J* = 8.0 Hz), 6.62 (d, 2 H, *J* = 8.0 Hz), 3.85−3.76 (m, 1 H), 3.47 (d, 1 H, *J* = 4.5 Hz), 2.35, 2.19 (ABX, 2 H, J_{AX} = 4.5 Hz, J_{BX} = 9.5 Hz, J_{AB} = 14 Hz); exact mass 184.0530 (M^+ − CO_2H) (184.0529 calcd for $C_9H_{11}ClNO$). Anal. Calcd for $C_{10}H_{12}ClNO_3$: C, 52.30; H, 5.27; N, 6.10. Found: C, 52.17; H, 5.16; N, 5.96.

(2*S*,3*R*)-2-Amino-3-hydroxy-4-(4-methoxyphenyl)butanoic Acid (12d). This material was prepared analogously to **12a** from the mixture of **10d** and **11d** (1.48 g, 3.60 mmol) except that the acid hydrolysis was done for only 5.5 h because heating overnight hydrolyzed the methyl ether and generated the corresponding phenol (**12**, R = OH) quantitatively. Using the shorter reaction time gave **12d** (0.56 g, 69%): mp 190−191 °C dec; IR (KBr) 3437, 1656, 1631, 1613 cm^{-1}; ^{1}H NMR (D_2O/DCl, 200 MHz) δ 7.13 (d, 2 H, *J* = 8.0 Hz), 6.84 (d, 2 H, *J* = 8.0 Hz), 4.12 (m, 1 H), 3.68 (s, 3 H), 3.52 (d, 1 H, *J* = 4.5 Hz), 2.82 (dd, 1 H, *J* = 5.0, 14 Hz), 2.63 (dd, 1 H, *J* = 10, 14 Hz); MS (FAB) 226.07 (MH^+) (226.10 calcd for $C_{11}H_{16}NO_4$).

The corresponding phenol (**12**, R = OH) had the following properties: mp 214−216 °C dec; IR (KBr) 3360, 1637, 1613, 1515 cm^{-1}; ^{1}H NMR (D_2O, 200 MHz) δ 7.06 (d, 2 H, *J* = 8.0 Hz), 6.74 (d, 2 H, *J* = 8.0 Hz), 4.14 (m, 1 H), 3.53 (d, 1 H, *J* = 4.5

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Hz), 2.80 (dd, 1 H, J = 5.0, 14 Hz), 2.62 (dd, 1 H, J = 10, 14 Hz); MS (FAB, glycerol) 212.08 (MH^+) (212.09 calcd for $C_{10}H_{14}NO_4$).

(2S,3R)-2-[[(2-Nitrophenyl)sulfenyl]amino]-3-hydroxy-4-(4-nitrophenyl)butanoic Acid (13a). Small portions of (*o*-nitrophenyl)sulfenyl chloride (total 436 mg, 2.3 mmol) were added to a vigorously stirred solution of **12a** (497 mg, 2.07 mmol) in 1 N NaOH (2.3 mL) and dioxane (5 mL) while 1 N NaOH solution was added to maintain the reaction mixture at pH 8–9. The mixture was then stirred an additional 20 min, diluted with water (10 mL), acidified with 10% $KHSO_4$ to pH 2.5, and immediately extracted with EtOAc (3 × 15 mL). The combined organic extracts were dried and concentrated *in vacuo*. The residue was purified by flash chromatography (1% AcOH/EtOAc) to afford **13a** (736 mg, 90%) as an oil: IR (KBr) 3421, 1714, 1593, 1514 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 8.30–8.25 (m, 2 H), 8.15 (d, 2 H, J = 10 Hz), 7.76–7.71 (m, 1 H), 7.52 (d, 2 H, J = 10 Hz), 7.36–7.31 (m, 1 H), 4.30–4.25 (m, 2 H), 3.50 (d, 1 H, J = 5 Hz), 3.16, 3.12 (ABX, 2 H, J_{AB} = 14 Hz, J_{AX} = 6 Hz, J_{BX} = 9 Hz); ^{13}C NMR (CD_3OD, 75.5 MHz) δ 43.7 (t), 72.4 (d), 76.8 (d), 126.7 (d), 128.3 (d), 128.7 (d), 128.9 (d), 134.0 (d), 131.3 (d), 146.3 (s), 149.3 (s), 150.4 (s), 150.9 (s), 177.6 (s); MS (FAB^+, glycerol) 394 (MH^+).

(2S,3R)-2-[[(2-Nitrophenyl)sulfenyl]amino]-3-hydroxy-4-phenylbutanoic Acid (13b). Conversion of **12b** (241 mg, 1.24 mmol) to **13b** (302 mg) proceeded in 83% yield using the procedure for N-protection of **12a**. Compound **13b** had the following properties: IR (CH_3OH cast) 3600–3200, 1717, 1700, 1507 cm^{-1}; ^1H NMR (CD_3OD, 200 MHz) δ 8.37–8.24 (m, 2 H), 7.78–7.69 (m, 1 H), 7.37–7.16 (m, 6 H), 4.30–4.22 (m, 1 H), 3.43 (d, 1 H, J = 4 Hz), 3.07, 2.98 (ABX, 2 H, J_{AX} = 6 Hz, J_{BX} = 8 Hz, J_{AB} = 13 Hz); MS (FAB) 349 (MH^+).

(2S,3R)-2-[[(2-Nitrophenyl)sulfenyl]amino]-3-hydroxy-4-(4-chlorophenyl)butanoic Acid (13c). Conversion of **12c** (400. mg, 1.74 mmol) to **13c** (394 mg) proceeded in 59% yield using the procedure for N-protection of **12a**. Compound **13c** had the following properties: mp 68–75 °C; IR (KBr) 3434 (br), 1718, 1709, 1636, 1631, 1592, 1508 cm^{-1}; ^1H NMR ($(CD_3)_2CO$, 200 MHz) δ 8.40 (d, 1 H, J = 8 Hz), 8.28 (d, 1 H, J = 8 Hz), 7.81 (t, 1 H, J = 8 Hz), 7.47–7.20 (m, 5 H), 4.40–4.22 (m, 2 H), 3.64–3.53 (m, 1 H), 3.20–3.00 (m, 2 H); MS (FAB) 383 (MH^+).

(2S,3R)-2-[[(2-Nitrophenyl)sulfenyl]amino]-3-hydroxy-4-(4-methoxyphenyl)butanoic Acid (13d). Conversion of **12d** (600 mg, 2.60 mmol) to **13d** (453 mg) proceeded in 45% yield using the procedure for N-protection of **12a**. Compound **13d** had the following properties: mp 168–170 °C; IR (acetone cast) 3600–2800, 1712, 1592, 1566, 1512 cm^{-1}; ^1H NMR ($(CD_3)_2CO$, 200 MHz) δ 8.50 (d, 1 H, J = 8.0 Hz), 8.32 (d, 1 H, J = 8 Hz), 7.88 (t, 1 H, J = 8 Hz), 7.45(t, 1 H, J = 8 Hz), 7.28 (d, 2 H, J = 8.5 Hz), 6.89 (d, 2 H, J = 8.5 Hz), 4.40 (m, 2 H), 3.80 (s, 3 H), 3.62 (m, 1 H), 3.13 (m, 2 H); MS (FAB) 379.08 (MH^+) (379.10 calcd for $C_{17}H_{19}N_2O_6S$).

(3S,4R)-3-[[(2-Nitrophenyl)sulfenyl]amino]-4-[(4-nitrophenyl)methyl]-2-oxetanone (14a). A solution of (4-bromophenyl)sulfonyl chloride (731 mg, 2.86 mmol) in pyridine (4.0 mL) at 0 °C was added dropwise to a solution of **13a** (450 mg, 1.14 mmol) in pyridine (4.0 mL) at −45 °C. The mixture was stirred at −45 °C for 1 h and then at 0 °C for 4 h. Crushed ice (100 mL) was added, and the mixture was acidified with concentrated HCl to pH 2 with vigorous stirring. The mixture was then immediately extracted with EtOAc (3 × 50 mL), and the combined extracts were dried ($MgSO_4$) and concentrated *in vacuo*. Purification of the residue by flash chromatography (SiO_2, 30% EtOAc/hexane) gave **14a** as an oil (100 mg, 24%): IR ($CHCl_3$ cast) 3370, 1822, 1513, 1344, 737 cm^{-1}; ^1H NMR (CD_3CN, 200 MHz) δ 8.27 (d, 1 H, J = 8 Hz), 8.20 (d, 2 H, J = 9 Hz), 8.05 (d, 1 H, J = 8 Hz), 7.80–7.73 (m, 1 H), 7.56 (d, 2 H, J = 9 Hz), 7.42–7.33 (m, 1 H), 5.00–4.89 (m, 2 H), 4.49 (d, 1 H, J = 9 Hz), 3.43–3.21 (m, 2 H); MS (CI, NH_3) 349 ($MNH_4^+ - CO_2$, 23), 331 ($M^+ - CO_2$, 13).

(3S,4R)-3-[[(2-Nitrophenyl)sulfenyl]amino]-4-benzyl-2-oxetanone (14b). The procedure used to obtain **14a** was employed to convert **13b** (296 mg, 0.85 mmol) to **14b** (116 mg) in 41% yield: IR ($CHCl_3$ cast) 3365, 1824, 1590, 1567, 1512 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 8.32 (dd, 1 H, J = 1.2, 8 Hz), 8.04 (dd, 1 H, J = 1.2, 8 Hz), 7.80–7.72 (m, 1 H), 7.46–7.24 (m, 6 H), 4.98–4.79 (m, 2 H), 3.42 (d, 1 H, J = 8 Hz), 3.28–3.19 (m, 2 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 36.0 (t), 71.0 (d), 79.5 (d), 123.6 (d), 125.5 (d), 125.7 (d), 127.3 (d), 128.9 (d), 129.2 (d), 134.5 (d), 135.1 (s), 143.9 (s), 169.4 (s); MS (FAB) 331 (MH^+).

(3S,4R)-3-[[(2-Nitrophenyl)sulfenyl]amino]-4-[(4-chlorophenyl)methyl]-2-oxetanone (14c). The procedure used to obtain **14a** was employed to convert **13c** (594 mg, 2.30 mmol) to **14c** (85 mg) in 25% yield: mp 148–150 °C; IR (KBr) 3368, 1810, 1590, 1567, 1509 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 8.35 (dd, 1 H, J = 1.2, 8 Hz), 8.03 (dd, 1 H, J = 1.2, 8 Hz), 7.80–7.72 (m, 1 H), 7.41–7.22 (m, 5 H), 4.83–4.78 (m, 2 H), 3.44 (d, 1 H, J = 8 Hz), 3.30–3.07 (m, 2 H); MS (FAB) 365 (MH^+, ^{35}Cl), 367 (MH^+, ^{37}Cl).

(3S,4R)-3-[[(2-Nitrophenyl)sulfenyl]amino]-4-[(4-methoxyphenyl)methyl]-2-oxetanone (14d). The procedure used to obtain **14a** was employed to convert **13d** (188 mg, 0.57 mmol) to **14d** (62 mg) in 34% yield: mp 67–68 °C; IR ($CHCl_3$ cast) 3366, 1824, 1611, 1593, 1567, 1513 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 8.28 (dd, 1 H, J = 1.2, 8.0 Hz), 8.02 (dd, 1 H, J = 1.2, 8.0 Hz), 7.71 (m, 1 H), 7.32 (m, 1 H), 7.21 (d, 2 H, J = 8.0 Hz), 6.89 (d, 2 H, J = 8.0 Hz), 4.72–4.92 (m, 2 H), 3.80 (s, 3 H), 3.56 (d, 1 H, J = 8.0 Hz), 3.19 (m, 2 H); exact mass 360.0785 (360.0780 calcd for $C_{17}H_{16}N_2O_5S$), 316.0880 ($M^+ - CO_2$, 11) (316.0881 calcd for $C_{16}H_{16}N_2O_3S$). Anal. Calcd for $C_{17}H_{16}N_2O_5S$: C, 56.66; H, 4.48; N, 7.77. Found: C, 56.23; H, 4.40; N, 7.33.

(3S,4R)-3-Amino-4-[(4-nitrophenyl)methyl]-2-oxetanone, 4-Toluenesulfonate Salt (15a). Anhydrous 4-toluenesulfonic acid (135 mg, 0.784 mmol) and 4-thiocresol (185 mg, 1.49 mmol) were added to a stirred suspension of **14a** (280 mg, 0.746 mmol) in CH_2Cl_2 (8.0 mL). The mixture was stirred at 20 °C for 5 h, the solvent was evaporated *in vacuo*, and the residue was triturated five times with diethyl ether. The residue was then dried to give **15a** (235 mg, 80%) as a powder: mp 174 °C dec; $[\alpha]_D$ +59.2° (c = 0.5, DMF); IR (KBr) 3436, 1831, 1519, 1202 cm^{-1}; ^1H NMR (DMF-d_7, 400 MHz) δ 8.27, 7.65 (2 × d, 4 H, J = 11 Hz), 7.68, 7.15 (2 × d, 4 H, J = 9 Hz), 5.67 (d, 1 H, J = 7 Hz), 5.36–5.31 (m, 1 H), 3.57–3.54 (m, 2 H), 2.30 (s, 3 H); MS (FAB, glycerol) 395 (MH^+).

(3S,4R)-4-Amino-4-benzyl-2-oxetanone, 4-Toluenesulfonate Salt (15b). This compound was prepared analogously to **15a** using **14b** (32.6 mg, 0.099 mmol) except that the reaction mixture was stirred overnight at room temperature. Trituration as before gave **15b** (31.3 mg) in 91% yield: IR (KBr) 3440, 1830, 1208 cm^{-1}; ^1H NMR (DMF-d_7, 200 MHz) δ 7.67 (d, 2 H, J = 8 Hz), 7.40–7.25 (m, 5 H), 7.17 (d, 2 H, J = 8 Hz), 5.64 (d, 1 H, J = 7 Hz), 5.28–5.16 (m, 1 H), 3.54–3.37 (m, 2 H), 2.33 (s, 3 H); MS (FAB, glycerol) 350 (MH^+).

(3S,4R)-3-Amino-4-[(4-chlorophenyl)methyl]-2-oxetanone, 4-Toluenesulfonate Salt (15c). This compound was prepared analogously to **15a** using **14c** (51.0 mg, 0.14 mmol). After trituration with diethyl ether, **15c** (53.6 mg) was obtained in quantitative yield: mp 158–162 °C dec; IR (KBr) 3425, 1830, 1518, 1494, 1337, 1205 cm^{-1}; ^1H NMR (CD_3OD, 200 MHz) δ 7.71 (d, 2 H, J = 8 Hz), 7.40–7.18 (m, 6 H), 5.24 (d, 1 H, J = 6 Hz), 5.06–4.96 (m, 1 H), 3.23–3.02 (m, 2 H), 2.36 (s, 3 H); MS (FAB, glycerol) 384 (MH^+, ^{35}Cl).

(3S,4R)-3-Amino-4-[(4-methoxyphenyl)methyl]-2-oxetanone, 4-Toluenesulfonate Salt (15d). This compound was prepared analogously to **15a** using **14d** (20.1 mg, 0.056 mmol). After trituration with diethyl ether, **15d** (17.8 mg) was obtained in 84% yield: mp 143–45 °C dec; IR (KBr) 3424, 1827, 1516, 1462, 1252, 1206 cm^{-1}; ^1H NMR (CD_3OD, 200 MHz) δ 7.71 (d, 2 H, J = 8.0 Hz), 7.22, 7.16 (2 × d, 4 H, J = 8.0 Hz), 6.88 (d, 2 H, J = 8.0 Hz), 5.20 (d, 1 H, J = 7.0 Hz), 4.97 (m, 1 H), 3.77 (s, 3 H), 3.10 (m, 2 H), 2.35 (s, 3 H); MS (FAB, glycerol) 380 (MH^+).

(2R,3S)-2-Amino-3-hydroxy-4-(4-nitrophenyl)butanoic Acid (17). This was prepared analogously to **12a** by condensation of (*R*)-1-benzoyl-2-*tert*-butyl-3-methylimidazolidin-4-one (**16**)[18] (330 mg, 1.27 mmol) and 4-nitrophenylacetaldehyde (**9a**)[32] (250 mg, 1.51 mmol), followed by acidic hydrolysis (6 N HCl) and ion-exchange chromatography, to give **17** (51%): $[\alpha]_D$ −68° (c = 0.18, H_2O); remaining spectral data as for **12a**.

Methyl (2S,3R)-2-[(S)-Camphanamido]-3-hydroxy-4-(4-nitrophenyl)butanoate (18). Amino acid **12a** (10 mg, 0.042 mmol) was dissolved in pH 10 $NaHCO_3/Na_2CO_3$ buffer (1 *M*,

1.0 mL). A solution of (*S*)-camphanic acid chloride (18 mg, 0.083 mmol) in toluene (0.3 mL) was added at 20 °C. The mixture was sealed and stirred vigorously for 2.5 h. The mixture was acidified to pH 1 with 6 N HCl and extracted with CH_2Cl_2 (4 × 3 mL). The organic extracts were dried ($MgSO_4$), filtered, and evaporated *in vacuo*. This residue was dissolved in ether (2 mL) and esterified by adding ethereal diazomethane until a yellow coloration persisted. The excess diazomethane was blown off with argon for 10 min, and the solution was evaporated *in vacuo* to afford an oil. Methyl camphanoate was then removed by sublimation under vacuum (75 °C, 6 h, 0.3 mmHg) to leave pure **18** (16.2 mg, 90%): IR (CH_2Cl_2 cast) 3425, 1793, 1751, 1680, 1521, 1347 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.19 (d, 2 H, J = 11 Hz), 7.41 (d, 2 H, J = 11 Hz), 7.24 (d, 1 H, J = 12 Hz), 4.75 (dd, 1 H, J = 12, 2 Hz), 4.46–4.42 (m, 1 H), 3.78 (s, 3 H), 2.90, 2.83 (ABX system, 2 H, J_{AX} = 4.5 Hz, J_{BX} = 9 Hz, J_{AB} = 14 Hz), 2.49–2.56 (m, 1 H), 1.96–2.05 (m, 2 H), 1.71–1.77 (m, 1 H), 1.16, 1.15, 1.04 (3 × s, 9 H); exact mass 435.1772 (MH^+) (435.1767 calcd for $C_{21}H_{27}N_2O_8$).

Methyl (2*R*,3*S*)-2-[(*S*)-Camphanamido]-3-hydroxy-4-(4-nitrophenyl)butanoate (19). This was prepared from **17** (10 mg, 0.042 mmol) analogously to generation of **18**. Compound **19** (17.3 mg, 96%) had the following properties: IR (CH_2Cl_2 cast) 3427, 1793, 1752, 1681, 1521, 1347 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.19 (d, 2 H, J = 11 Hz), 7.42 (d, 2 H, J = 11 Hz), 7.23 (d, 1 H, J = 12 Hz), 4.75 (d, 2 H, J = 12, 2 Hz), 4.54–4.48 (m, 1 H), 3.76 (s, 3 H), 2.89 (d, 2 H, J = 9 Hz), 2.45 (d, 1 H, J = 4 Hz), 2.56–2.63 (m, 1 H), 1.95–2.05 (m, 2 H), 1.70–1.77 (m, 1 H), 1.15, 1.11, 1.02 (3 × s, 9 H); MS (EI) 435 (MH^+, 1); exact mass 416.1598 ($M^+ - H_2O$) (416.1583 calcd for $C_{21}H_{24}N_2O_7$).

(+)-Obafluorin (1) and Its Hydrolysis Product 23a. Acid chloride **20**[21] (83 mg, 0.381 mmol) was added to a suspension of salt **15a** (100 mg, 0.254 mmol) in dry CH_2Cl_2 (2.0 mL) at −12 °C under an argon atmosphere. This was followed immediately by pyridine (41 μL, 0.508 mmol). The mixture was stirred at −10 °C for 30 min and then warmed to 20 °C over 2 h and stirred for a further 2 h at 20 °C. The mixture was partitioned between water (20 mL) and EtOAc (30 mL). The aqueous layer was extracted with EtOAc (25 mL). The combined organic phases were washed with water (20 mL), dried ($MgSO_4$), filtered, and evaporated *in vacuo* to yield the crude product (126 mg). A portion (16 mg) of the crude product was purified by HPLC (Waters radial compression 8 × 200 mm μ-Bondapak 125Å, C_{18} reversed-phase, gradient elution, 28%–38% acetonitrile/water) to yield pure **1** (9.3 mg, 80% by extrapolation) as a white powder. With the exception of the extent of optical rotation, the properties of obafluorin (**1**) were consistent with those reported in the literature:[1b] $[\alpha]_D$ +70° (c = 0.1, CH_3CN); IR (CH_3CN cast) 1835, 1648, 1519, 1348 cm^{-1}; ^1H NMR (CD_3CN, 500 MHz) δ 8.10 (d, 2 H, J = 9 Hz), 7.44 (d, 2 H, J = 9 Hz), 7.18 (d, 1 H, J = 8 Hz), 7.04 (d, 1 H, J = 7 Hz), 6.84 (t, 1 H, J = 8 Hz), 6.77 (br s, 1 H), 5.74 (dd, 1 H, J = 8, 6 Hz), 5.07–5.01 (m, 1 H), 3.37, 3.21 (ABX system, 2 H, J_{AB} = 14 Hz, J_{AX} = 9 Hz, J_{BX} = 5 Hz); exact mass 340.0692 ($M^+ - H_2O$, 7.5) (340.0695 calcd for $C_{17}H_{12}N_2O_6$), 314.0889 ($M^+ - CO_2$, 5.0) (314.0902 calcd for $C_{16}H_{14}N_2O_5$).

Upon standing in aqueous acetonitrile over several hours, some hydrolysis of this sample of the β-lactone ring occurs to give (2*S*,3*R*)-2-[(2,3-dihydroxybenzoyl)amino]-3-hydroxy-4-(4-nitrophenyl)butanoic acid (**23a**), which can be repurified under the same HPLC conditions: IR (KBr) 3420, 1727, 1643, 1518, 1347 cm^{-1}; ^1H NMR ($(CD_3)_2CO$, 400 MHz) δ 8.17 (d, 2 H, J = 9 Hz), 7.96 (br s, 1 H), 7.60 (d, 2 H, J = 9 Hz), 7.45–7.40 (m, 1 H), 7.04–6.99 (m, 1 H), 6.82–6.75 (m, 1 H), 4.86–4.80, 4.65–4.58 (2 × br m, 2 H), 3.17–3.00 (m, 2 H); exact mass 376.0941 (M^+, 3) (376.0907 calcd for $C_{17}H_{16}N_2O_8$).

Hydrolysis and Stereochemical Analysis of (2*S*,3*R*)-2-[(2,3-dihydroxybenzoyl)amino]-3-hydroxy-4-(4-nitrophenyl)butanoic Acid (23a). The hydroxy acid **23a** (0.9 mg) obtained from hydrolysis of **1** was heated to reflux in aqueous hydrochloric acid (6 N, 1 mL) for 22 h. The mixture was concentrated *in vacuo* and purified by ion-exchange chromatography on AG50W-X8 (H^+) resin (0.5 cm × 4 cm) by elution with water followed with 1 N NH_4OH solution. The ammonia fractions were lyophilized and the residue was derivatized using (*S*)-camphanic acid chloride and diazomethane as described above for **12a** and **17**. ^1H NMR ($CDCl_3$, 500 MHz) analysis produced data identical to the derivative **18** prepared from amino acid **12a**.

Hydrolysis and Stereochemical Analysis of 1. Obafluorin (**1**) (2.0 mg, 0.0056 mmol) was heated to reflux in 6 N HCl (5 mL) for 12 h. The product was isolated and derivatized as described above for hydrolysis of **23a**. ^1H NMR ($CDCl_3$, 360 MHz) analysis produced data identical to the derivative **18** prepared from amino acid **12a**.

(3*S*,4*R*)-3-[(2,3-Dihydroxybenzoyl)amino]-4-benzyl-2-oxetanone (22b). Salt **15b** (50 mg, 0.143 mmol) was condensed with **20**[21] (47 mg, 0.215 mmol) using pyridine (23 μL, 0.286 mmol) at −10 °C and following the general procedure to prepare **1** to afford crude **22b** (58 mg) as a clear oil. A portion (18 mg) of this was purified by HPLC as before to yield pure **22b** (7 mg, 50% by extrapolation): IR ($CHCl_3$ cast) 3378, 3064, 1817, 1647, 1539, 1458, 1264, 1218, 747 cm^{-1}; ^1H NMR (CD_3CN, 200 MHz) δ 8.08 (d, 1 H, J = 8 Hz), 7.27–7.17 (m, 6 H), 7.04 (dd, 1 H, J = 8, 1.5 Hz), 6.82 (t, 1 H, J = 8 Hz), 5.75 (dd, 1 H, J = 8, 6 Hz), 5.04–4.94 (m, 1 H), 3.22, 3.10 (ABX, 2 H, J_{AX} = 8.5 Hz, J_{BX} = 5 Hz, J_{AB} = 14.5 Hz); exact mass 313.0953 (M^+, 29) (313.0950 calcd for $C_{17}H_{15}NO_5$).

(3*S*,4*R*)-3-[(2,3-Dihydroxybenzoyl)amino]-4-[(4-chlorophenyl)methyl]-2-oxetanone (22c). Salt **15c** (47.5 mg, 0.124 mmol) was condensed with **20**[21] (41 mg, 0.186 mmol) using pyridine (20 μL, 0.248 mmol) at −12 °C and following the general procedure to prepare **1** to afford crude **22c** (70 mg). A portion (18 mg) of this was purified by HPLC as before to yield pure **22b** (5.5 mg, 69% by extrapolation): IR ($CHCl_3$ cast) 3387, 1819, 1646, 1538, 1493, 1460, 1240, 751 cm^{-1}; ^1H NMR (CD_3CN, 400 MHz) δ 8.06 (br s, 1 H), 7.28–7.16 (m, 5 H), 7.04 (d, 1 H, J = 8 Hz), 6.82 (t, 1 H, J = 8 Hz), 5.72 (dd, 1 H, J = 9, 6.5 Hz), 5.00–4.94 (m, 1 H), 3.22, 3.07 (ABX, 2 H, J_{AX} = 9 Hz, J_{BX} = 5 Hz, J_{AB} = 15 Hz); exact mass 349.0538 (M^+, ^{37}Cl) (349.0531 calcd for $C_{17}H_{14}NO_5{}^{37}Cl$), 347.0563 ($M^+$, ^{35}Cl) (347.0560 calcd for $C_{17}H_{14}NO_5{}^{35}Cl$).

(3*S*,4*R*)-3-[(2,3-Dihydroxybenzoyl)amino]-4-methyl-2-oxetanone (22d). To a suspension of the L-threonine β-lactone tosylate salt (**21**)[6] (100 mg, 0.366 mmol) in dry CH_2Cl_2 (3 mL) cooled to −10 °C under Ar were added acid chloride **20** (125 mg, 0.572 mmol) and pyridine (60 μL, 0.732 mmol). The mixture was stirred at −10 °C for 30 min, allowed to warm to 20 °C over 1.5 h, and then stirred for a further 1.5 h. The mixture was partitioned between water (15 mL) and EtOAc (25 mL). The organic phase was washed with water (15 mL), and the combined aqueous layers were extracted with EtOAc (25 mL). The organic phases were dried and concentrated *in vacuo*. The crude product was purified by flash chromatography (SiO_2, 60% EtOAc/hexane) to yield **22d** (74 mg, 85%) as a solid: IR ($CHCl_3$ cast) 3380, 2922, 1816, 1646, 1540, 1459, 1336, 1268, 1020, 751 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.56 (d, 1 H, J = 7.5 Hz), 7.11–7.07 (m, 2 H), 6.81 (t, 1 H, J = 8 Hz), 5.78 (dd, 1 H, J = 7.5, 6 Hz), 5.03 (quint, 1 H, J = 6 Hz), 1.53 (d, 3 H, J = 6 Hz); exact mass 237.0640 (M^+, 50) (237.0638 calcd for $C_{11}H_{11}NO_5$). Anal. Calcd for $C_{11}H_{11}NO_5$: C, 55.70; H, 4.68; N, 5.90. Found: C, 56.30; H, 4.83; N, 5.53.

Hydrolysis of 22d to (2*S*,3*R*)-2-[(2,3-Dihydroxybenzoyl)-amino]-3-hydroxybutanoic Acid (23d). A solution of β-lactone **22d** (7.6 mg, 0.032 mmol) in THF (0.2 mL) and water (0.1 mL) was added to a cooled (0 °C) solution of 1 M NaOH (0.1 mL) in THF:H_2O (1:1, 1 mL). The mixture was stirred for 10 min and then concentrated *in vacuo*. The residue was dissolved in 1 N HCl (1 mL) and extracted with EtOAc (3 × 2 mL). The solution was dried and evaporated to give pure **23d** (5.5 mg, 67%): IR (MeOH cast) 3342, 2924, 1732, 1637, 1587, 1538, 1241 cm^{-1}; ^1H NMR (acetone-d_6, 200 MHz) δ 7.79 (br, 1 H), 7.39 (dd, 1 H, J = 8, 1 Hz), 7.00 (dd, 1 H, J = 8, 1 Hz), 6.78 (t, 1 H, J = 8 Hz), 4.70 (dd, 1 H, J = 8, 3 Hz), 4.47 (dq, 1 H, J = 7, 3 Hz), 1.26 (d, 3 H, J = 7 Hz); exact mass 255.0735 (255.0743 calcd for $C_{11}H_{13}NO_6$).

(3*S*,4*R*)-3-(Acetylamino)-4-[(4-nitrophenyl)methyl]-2-oxetanone (24a). A suspension of **15a** (10.0 mg, 0.025 mmol) in CH_2Cl_2 (0.5 mL) under Ar was cooled to −10 °C and treated with pyridine (4 μL, 0.049 mmol) and acetyl chloride (2.7 μL,

0.038 mmol). The mixture was stirred at −10 °C for 30 min and allowed to warm to 20 °C overnight. The mixture was diluted with EtOAc (5 mL) and washed with water (5 × 5 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated *in vacuo*. The solid residue was triturated with ether (3 × 5 mL) to afford pure **24a** (4.6 mg, 66%) as a white powder: IR (KBr) 3280, 1850, 1833, 1822, 1666, 1598, 1546 cm^{-1}; ^1H NMR ($(CD_3)_2CO$, 500 MHz) δ 8.21 (d, 2 H, J = 8 Hz), 8.16 (br d, 1 H, J = 8 Hz), 7.60 (d, 2 H, J = 8 Hz), 5.76 (dd, J = 8.5, 6 Hz), 5.07−5.03 (m, 1 H), 3.34, 3.28 (ABX, 2 H, J_{AX} = 9 Hz, J_{BX} = 5 Hz, J_{AB} = 15 Hz), 1.99 (s, 3 H); exact mass 246.0638 ($M^+ - H_2O$, 6) (246.0641 calcd for $C_{12}H_{10}N_2O_4$), 220.0846 ($M^+ - CO_2$, 37) (220.0848 calcd for $C_{11}H_{12}N_2O_3$); MS (CI) 265 (MH^+, 69).

(3*S*,4*R*)-3-(Benzoylamino)-4-[(4-nitrophenyl)methyl]-2-oxetanone (24b). A suspension of **15a** (10.0 mg, 0.025 mmol) was acylated with benzoyl chloride (4.5 μL, 0.038 mmol) and pyridine (4 μL, 0.049 mmol) as described for the preparation of **24a** to afford **24b** (6.7 mg, 81%): IR (KBr) 3435 (br), 3266 (br), 1841, 1649, 1537 cm^{-1}; ^1H NMR ($(CD_3)_2CO$, 400 MHz) δ 8.78 (d, 1 H, J = 8 Hz), 8.17 (d, 2 H, J = 8 Hz), 7.95 (d, 2 H, J = 8 Hz), 7.62−7.57 (m, 3 H), 7.53−7.48 (m, 2 H), 6.0 (dd, 1 H, J = 8, 6 Hz), 5.20−5.15 (m, 1 H), 3.51, 3.39 (ABX, 2 H, J_{AX} = 9.5 Hz, J_{BX} = 5 Hz, J_{AB} =15 Hz); exact mass 281.0925 ($M^+ - CO_2H$, 20) (281.0925 calcd for $C_{16}H_{13}N_2O_3$); MS (CI, NH_3) 343 (MNH_3^+).

(3*S*,4*R*)-3-[[2-(2-Aminothiazol-4-yl)-2-(methoxyimino)]-acetyl]amino]-4-[(4-nitrophenyl)methyl]-2-oxetanone (24c). To a stirred solution of 2-amino-α-(methoxyimino)-4-thiazoleacetic acid (7.0 mg, 0.035 mmol) and **15a** (12.0 mg, 0.030 mmol) in DMF (0.1 mL) at 0 °C were added diethylphosphoryl cyanide (5.0 μL, 0.033 mmol) and triethylamine (5.0 μL, 0.037 mmol) over 5 min. The mixture was stirred at 0 °C for 30 min and then at 20 °C for 2 h. The mixture was diluted with EtOAc (2 mL)/benzene (1 mL) and washed with water (1.5 mL) and saturated NaCl solution (2 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification by preparative TLC (EtOAc) provided **24c** (3.0 mg, 25%): IR (CH_2Cl_2−MeOH cast) 3286, 1832, 1672. 1519 cm^{-1}; ^1H NMR ($(CD_3)_2CO$, 500 MHz) δ 8.62 (br d, J = 9 Hz), 8.21 (d, 2 H, J = 8 Hz), 7.63 (d, 2 H, J = 8 Hz), 6.87 (s, 1 H), 6.52 (d, 1 H, J = 8 Hz), 5.96−5.94 (m, 1 H), 5.15−5.10 (q, 1 H, J = 7 Hz), 3.89 (s, 3 H), 3.40 (d, 2 H, J = 7 Hz); MS (EI) 428 (MNa^+).

(3*S*,4*R*)-3-[(2-Hydroxybenzoyl)amino]-4-methyl-2-oxetanone (25). A stirred suspension of the tosylate salt **21** (100 mg, 0.366 mmol) in dry CH_2Cl_2 (2.5 mL) cooled to −10 °C under argon was treated with pyridine (60 μL, 0.732 mmol), and a solution of salicyloyl chloride (57 mg, 0.366 mmol) in dry CH_2Cl_2 (2.5 mL) was added dropwise over 10 min. This procedure avoids polymerization of salicyloyl chloride. The mixture was stirred at −10 °C for 10 min, allowed to warm to room temperature over 2.5 h, and stirred at room temperature overnight. The mixture was partitioned between EtOAc (25 mL) and water (20 mL). The organic phase was washed with water (20 mL), and the combined aqueous phases were reextracted with EtOAc (25 mL). The combined organic phases were dried ($MgSO_4$), filtered, and evaporated *in vacuo*. The residue was purified by flash chromatography (SiO_2, gradient elution 40−50% EtOAc/hexane) followed by trituration with hexane to yield **25** (36 mg, 45%) as a powder: IR (KBr) 3392, 1811, 1648, 1532 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.55−7.42 (m, 2 H), 7.36 (d, 1 H, J = 7 Hz), 7.03−6.86 (m, 2 H), 5.77 (dd, 1 H, J = 7.5, 6 Hz), 5.03 (quint, 1 H, J = 6 Hz), 1.53 (d, 3 H, J = 6 Hz); exact mass 221.0689 (221.0688 calcd for $C_{11}H_{11}NO_4$).

(3,4-Dioxosulfinyl)benzoyl Chloride (26). A mixture of 3,4-dihydroxybenzoic acid (2.00 g, 13.0 mmol) and thionyl chloride (7.0 mL, 96.0 mmol) was heated to reflux for 5 h to give a clear, brownish solution. Excess thionyl chloride was removed *in vacuo*, and the residue was distilled to provide **26** (2.48 g, 87%) as a colorless liquid: bp 110−112 °C (0.25 Torr); IR ($CHCl_3$ cast) 1785, 1745, 1608, 1483 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.04 (dd, 1 H, J = 8.5, 2.5 Hz), 7.96 (d, 1 H, J = 2.5 Hz), 7.31 (d, 1 H, J = 8.5 Hz); exact mass 219.9413 (M^+, ^{37}Cl, 6) (219.9411 calcd for $C_7H_3O_4{}^{37}ClS$), 217.9440 (M^+, ^{35}Cl, 17) (217.9440 calcd for $C_7H_3O_4{}^{35}ClS$). Anal. Calcd for $C_7H_3ClO_4S$: C, 38.46; H, 1.38; Cl, 16.22; S, 14.67. Found: C, 38.49 H, 1.23; Cl, 15.89; S, 14.75.

(3*S*,4*R*)-3-[(3,4-Dihydroxybenzoyl)amino]-4-methyl-2-oxetanone (27). The procedure used to prepare **22d** was employed to acylate tosylate salt **21** (100 mg, 0.366 mmol) with acid choride **26** (120 mg, 0.549 mmol) using pyridine (60 μL, 0.732 mmol) and dry CH_2Cl_2 (3 mL). After flash chromatography, trituration with diethyl ether furnished pure **27** (40 mg, 46%) as a powder: IR (KBr) 3401, 1817, 1639, 1601, 1515 cm^{-1}; ^1H NMR ($(CD_3)_2CO$, 400 MHz) δ 8.60 (d, 1 H, J = 8 Hz), 7.47 (d, 1 H, J = 3 Hz), 7.38 (dd, 1 H, J = 8, 3 Hz), 6.88 (d, 1 H, J = 6 Hz), 5.83 (dd, 1 H, J = 8, 6 Hz), 4.95 (quint, 1 H, J = 6 Hz), 1.49 (d, 3 H, J = 6 Hz); exact mass 237.0638 (M^+, 5) (237.0637 calcd for $C_{11}H_{11}NO_5$).

(3*S*,4*R*)-3-[[(4′-Methyl-2,2′-bipyridinyl)-4-carbonyl]amino]-4-methyl-2-oxetanone (29). Diethylphosphoryl cyanide (19.4 mg, 0.11 mmol) and triethylamine (22.3 mg, 0.22 mmol) were added over 5 min to a stirred solution of acid **28**[24] (21.4 mg, 0.10 mmol) and tosylate salt **21** (27.3 mg, 0.10 mmol) in DMF (2 mL) at 0 °C. The solution was stirred at 0 °C for 30 min and then at room temperature for 23 h. The mixture was diluted with EtOAc (20 mL)/benzene (10 mL) and then washed with water (7 mL) and saturated NaCl solution (2 × 8 mL). The organic phase was dried (Na_2SO_4) and concentrated *in vacuo*. The crude product was dissolved in a minimum volume of EtOAc, quickly filtered through a small SiO_2 column, and concentrated *in vacuo* to provide **29** (18.0 mg, 61%) as a solid: mp 135−138 °C; IR (KBr) 3438, 3248, 1816, 1650, 1608, 1596, 1533 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 8.82 (d, 1 H, J = 5 Hz), 8.71 (s, 1 H), 8.55 (d, 1 H, J = 5 Hz), 8.27 (s, 1 H), 7.76 (dd, 1 H, J = 5, 1.5 Hz), 7.59 (d, 1 H, J = 7 Hz), 7.20 (d, 1 H, J = 5 Hz), 5.85 (dd, 1 H, J = 6, 7 Hz), 5.04 (quint, 1 H, J = 6 Hz), 2.47 (s, 3 H), 1.55 (d, 3 H, J = 6 Hz); exact mass 297.1111 (297.1114 calcd for $C_{16}H_{15}N_3O_3$).

(3*S*,4*R*)-3-[(5-Aminoadipo-1-yl)amino]-4-methyl-2-oxetanone (31). Triethylamine (37 μL, 0.263 mmol) and ethyl chloroformate (25 μL, 0.263 mmol) were added to a solution of α-aminoadipic acid derivative **30**[26] (97 mg, 0.263 mmol) in CH_2Cl_2 (9 mL) cooled to −5 °C under Ar. The solution was stirred for 30 min, and then **21** (72 mg, 0.263 mmol) and pyridine (43 μL, 0.526 mmol) were added. After an additional 30 min at −5 °C, the solution was allowed to warm to 20 °C overnight. The solvent was removed *in vacuo*, and the residue was partitioned between EtOAc (40 mL) and water (40 mL). The organic phase was dried ($MgSO_4$), filtered, and evaporated *in vacuo*. The product was purified by flash chromatography (SiO_2, 60−70% EtOAc/hexane) to give (3*S*,4*R*)-3-[[*N*-[(benzoyloxy)carbonyl]-α-benzyl-δ-[(*S*)-α-aminoadipoyl]]]amino]-4-methyl-2-oxetanone (79 mg, 64%) as a powder: mp 144−145 °C; IR ($CHCl_3$ cast) 3310, 1824, 1739, 1691, 1655, 1532 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.40−7.28 (m, 10 H), 7.06 (d, 1 H, J = 8 Hz), 5.64−5.54 (m, 2 H), 5.17 (s, 2 H), 5.10, 5.07 (AB, 2 H, J_{AB} = 14 Hz), 4.83 (quint, 1 H, J = 6 Hz), 4.47−4.36 (m, 1 H), 2.45−2.13 (m, 2 H), 1.93−1.62 (m, 4 H), 1.38 (d, 3 H, J = 6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 135.2 (s), 128.9−128.0 (m), 74.7 (d), 67.6 (t), 67.5 (t), 58.7 (d), 52.9 (d), 34.8 (t), 32.8 (t), 22.0 (t), 15.1 (q); exact mass 468.1888 (468.1896 calcd for $C_{25}H_{28}N_2O_7$). Anal. Calcd for $C_{25}H_{28}N_2O_7$: C, 64.09; H, 6.02; N, 5.98. Found: C, 64.44; H, 6.08; N, 5.98.

A solution of this protected derivative (30 mg, 0.064 mmol) in MeOH (4.5 mL) was hydrogenated at 20 °C over 10% Pd on carbon (5 mg) for 2 h, which produced a white precipitate. The mixture was evaporated to dryness *in vacuo*, and the residue was dissolved in water (10 mL). The solution was filtered through glass wool and extracted with diethyl ether (20 mL). The aqueous layer was lyophilized to yield **31** (13.2 mg, 84%) as a powder: mp 207−209 °C dec; IR (KBr) 3438, 1824, 1658, 1607, 1586, 1536 cm^{-1}; ^1H NMR (D_2O/$(CD_3)_2CO$, 400 MHz) δ 5.32 (d, 1 H, J = 6 Hz), 4.87 (quint, 1 H, J = 6 Hz), 3.58 (t, 1 H, J = 6 Hz), 2.26 (t, 2 H, J = 7 Hz), 1.78−1.50 (m, 4 H), 1.27 (d, 3 H, J = 6 Hz); MS (FAB) 245.05 (MH^+).

(3*S*,4*R*)-3-[[3′-[(*tert*-Butyloxycarbonyl)amino]propanoyl]-amino]-4-methyl-2-oxetanone (32). A solution of *N*-(*tert*-butoxycarbonyl)-β-alanine (37.8 mg, 0.20 mmol) in CH_2Cl_2 (6.0 mL) at −5 °C was treated with triethylamine (20.3 mg, 0.20 mmol) and ethyl chloroformate (22.0 mg, 0.20 mmol). The solution was stirred for 20 min before addition of the tosylate salt **21** (54.6 mg, 0.20 mmol) and triethylamine (40.6, 0.40

mmol). After 30 min at −5 °C, the solution was allowed to warm to 20 °C overnight. The solvent was removed *in vacuo*, and the residue was partitioned between EtOAc (40 mL) and water (15 mL). The organic phase was washed with water (10 mL) and saturated NaCl solution (10 mL), dried (Na_2SO_4), and concentrated *in vacuo*. It was then triturated twice with diethyl ether to yield **32** (35.4 mg, 65%) as a white solid: mp 114−116 °C; IR (KBr) 3360, 1833, 1687, 1660, 1537 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.40 (d, 1 H, J = 8 Hz), 5.62 (dd, 1 H, J = 8, 6 Hz), 5.16 (br s, 1 H), 4.89 (quint, 1 H, J = 6 Hz), 3.49−3.34 (m, 2 H), 2.53 (t, 2 H, J = 6 Hz), 1.50−1.40 (m, 12 H); exact mass 272.1371 (272.1372 calcd for $C_{12}H_{20}N_2O_5$), 228.1475 ($M^+ - CO_2$) (228.1475 calcd for $C_{11}H_{20}N_2O_3$). Anal. Calcd for $C_{12}H_{20}N_2O_5$: C, 52.92; H, 7.40; N, 10.29. Found: C, 52.87; H, 6.96; N, 10.07.

(3*S*,4*R*)-3-[(3-Aminopropanoyl)amino]-4-methyl-2-oxetanone, 4-Toluenesulfonate Salt (33). A solution of **32** (26.1 mg, 0.096 mmol) and 4-toluenesulfonic acid (19.0 mg, 0.10 mmol) in trifluoroacetic acid (1 mL) at 0 °C was stirred for 20 min. The solvent was removed under high vacuum, and the residue was triturated with ether to yield pure **33** (33 mg, 99%): IR (MeOH/$CHCl_3$ cast) 1821, 1666, 1638, 1632, cm^{-1}; ^1H NMR (DMF-d_7, 200 MHz) δ 9.18 (d, 1 H, J = 8 Hz), 7.65 (d, 2 H, J = 8 Hz), 7.15 (d, 2 H, J = 8 Hz), 5.70 (dd, 1 H, J = 6, 8 Hz), 4.92 (quint, 1 H, J = 6 Hz), 3.43−3.28 (m, 2 H), 2.86 (t, 2 H, J = 6 Hz), 2.28 (s, 3 H), 1.39 (d, 3 H, J = 6 Hz); MS (FAB) 345 (MH^+).

(3*S*,4*R*)-3-[[3-[(2,3-Dihydroxybenzoyl)amino]propanoyl]-amino]-4-methyl-2-oxetanone (34). Acid chloride **20**[21] (25.3 mg, 0.12 mmol) and triethylamine (20.3 mg, 0.20 mmol) were added to a suspension of **33** (31.0 mg, 0.09 mmol) in CH_2Cl_2 (2 mL) at −18 °C. After 20 min at −18 °C, the temperature was raised to −10 °C for 1 h and then to room temperature for 10 h. The solvent was removed *in vacuo*, and the residue was partitioned between EtOAc (40 mL) and water (20 mL). The organic phase was washed with water (20 mL) and saturated NaCl solution (10 mL), dried (Na_2SO_4), and concentrated *in vacuo* to furnish nearly pure **34** (20.1 mg, 68%). For biological tests, a sample (3.5 mg) was purified by HPLC (C_{18} reversed phase, isocratic elution with 13% acetonitrile/water): IR (KBr) 3440 (br), 1817, 1636, 1541 cm^{-1}; ^1H NMR ($(CD_3)_2CO$, 400 MHz) δ 8.27 (br d, 1 H, J = 8 Hz), 7.23 (d, 1 H, J = 8 Hz), 6.97 (dd, 1 H, J = 8, 1 Hz), 6.72 (t, 1 H, J = 8 Hz), 5.70−5.66 (m, 1 H), 4.89 (quint, 1 H, J = 6 Hz), 3.71−3.66 (m, 2 H), 2.69−2.65 (m, 2 H), 1.39 (d, 3 H, J = 6.5 Hz); ^{13}C NMR ($(CD_3)_2CO$, 100 MHz) δ 170.0, 169.4, 150.5, 145.8, 142.6, 119.5, 119.1, 117.6, 113.0, 75.1, 59.5, 36.5, 35.4, 15.2; exact mass 308.1006 (308.1008 calcd for $C_{14}H_{16}N_2O_6$), 290.0901 ($M^+ - H_2O$) (290.0899 calcd for $C_{14}H_{14}N_2O_5$).

(3*S*,4*R*)-3-[(Bromoacetyl)amino]-4-methyl-2-oxetanone (35). A mixture of **21** (47.0 mg, 0.17 mmol) in CH_2Cl_2 (7 mL) at −10 °C was treated with pyridine (33.3 mg, 0.42 mmol) and bromoacetyl chloride (33.1 mg, 0.20 mmol). After being stirred at −10 °C for 1 h, the mixture was allowed to warm to 0 °C over 2 h and then stirred at 20 °C for 8 h. The solvent was removed *in vacuo* and the residue was partitioned between EtOAc (60 mL) and water (50 mL). The aqueous layer was further extracted with EtOAc (30 mL). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo* to afford **35** (35.8 mg, 95%) as an oil which crystallized on standing. This compound exists as a mixture of rotamers in approximately a 4:1 (A:B) ratio as indicated by ^1H and ^{13}C NMR: mp 107−110 °C; IR ($CHCl_3$ cast) 3280 (br), 1850, 1810, 1675, 1664, 1541 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.39 (br, 0.8 H, A), 7.31 (br, 0.2 H, B), 5.62 (dd, 0.8 H, J = 8, 6 Hz, A), 5.60 (dd, 0.2 H, J = 8, 6 Hz, B), 4.94 (quint, 1 H, J = 6 Hz), 4.13 (s, 1.6 Hz, A), 3.96, 3.92 (AB, 0.4 H, J_{AB} = 13.5 Hz, B), 1.48 (d, 3 H, J = 6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.2, 166.4 (A), 166.1 (B), 76.6 (B), 76.4 (A), 59.1 (B), 58.8 (A), 42.1 (A), 27.8 (B), 14.9; exact mass 176.9789 ($M^+ - CO_2$) (176.9790 calcd for $C_5H_8{}^{79}BrNO$), 142.0504 ($M^+ - Br$) (142.0504 calcd for $C_6H_8NO_3$).

(3*S*,4*R*)-3-[[(6-Purinylthio)acetyl]amino]-4-methyl-2-oxetanone (36). A solution of **35** (24 mg, 0.108 mmol) and 6-mercaptopurine monohydrate (20 mg, 0.118 mmol) in DMF (2 mL) was treated with triethylamine (23 μL, 0.165 mmol). The mixture was stirred overnight at 20 °C. The solvent was removed *in vacuo*, and the residue was partitioned between EtOAc (25 mL) and water (10 mL). The organic phase was washed with water (15 mL), and the combined aqueous phases were extracted with further EtOAc (25 mL). The combined organic phases were dried ($MgSO_4$), filtered, and evaporated *in vacuo* to give **36** (21.5 mg, 68%) as a solid: IR (KBr) 3440, 1821, 1665, 1572, 1547 cm^{-1}; ^1H NMR ($(CD_3)_2CO$, 200 MHz) δ 8.68 (s, 1 H,), 8.53 (br s, 1 H), 8.40 (s, 1 H), 5.63 (dd, 1 H, J = 8.5, 6 Hz), 4.86 (quint, 1 H, J = 6 Hz), 4.20, 4.16 (AB, 2 H, J_{AB} = 14 Hz), 1.35 (d, 3 H, J = 6 Hz); exact mass 275.0471 ($M^+ - H_2O$) (275.0477 calcd for $C_{11}H_9N_5O_2S$).

(3*S*,4*R*)-3-[[(4-Pyridylthio)acetyl]amino]-4-methyl-2-oxetanone (37). Sodium 4-pyridylthiolate was prepared by the reaction of 4-mercaptopyridine (10 mg, 0.09 mmol) in DMF (3 mL) with sodium hydride (2.5 mg, 0.10 mmol) for 10 min at 20 °C. This solution was immediately added dropwise to a solution of **35** (20.1 mg, 0.09 mmol) in CH_2Cl_2 (2 mL) with vigorous stirring. After an additional 10 min, the mixture was partitioned between EtOAc (20 mL)/benzene (10 mL) and water (10 mL). The organic layer was washed with water (10 mL) and saturated NaCl solution (10 mL) and dried (Na_2SO_4), and the solvent was evaporated *in vacuo* to yield **37** (22.0 mg, 97%). The product was unstable and polymerized rapidly (several hours) to a solid. For **37**: IR ($CHCl_3$ cast) 1830, 1665 cm^{-1}; ^1H NMR (CD_3OD, 200 MHz) δ 8.35 (dd, 2 H, J = 4.5, 1.5 Hz), 7.39 (dd, 2 H, J = 4.5, 1.5 Hz), 5.54 (d, 1 H, J = 6, 3 Hz), 4.88 (quint, 1 H, J = 6 Hz), 3.89 (s, 2 H), 1.35 (d, 3 H, J = 6 Hz).

Procedure for NMR Studies on Aqueous Decomposition of β-Lactones to O-Acyl Derivatives 38. Samples of β-lactones (ca. 0.7−1.0 mg) were dissolved in CD_3CN (ca. 0.5 mL), and the ^1H NMR spectrum (360 MHz) was measured. D_2O was then added such that the ratio of D_2O:CD_3CN was 1:4. The ^1H NMR spectrum was acquired immediately [time (t) = 0] and then was measured again at t = 5 h, t = 24 h, and then daily or as necessary to determine the half-life or approximate rate of conversion to the O-acyl derivatives **38**.

For **(2*S*,3*S*)-2-Amino-3-[[(2,3-dihydroxy)benzoyl]oxy]-4-(4-nitrophenyl)butanoic acid (38a)** from obafluorin (**1**): 20% hydrolyzed in 5 weeks at 20 °C; ^1H NMR (D_2O:CD_3CN, 1:4, 360 MHz) new peaks seen at δ 5.73 (ddd, 1 H, J = 11, 4, 3 Hz), 4.10 (d, 1 H, J = 3 Hz).

For **(2*S*,3*S*)-2-amino-3-[(3,4-dihydroxybenzoyl)oxy]butanoic acid (38b)** from **27**: $t_{1/2}$ = 2.4 days; IR (MeOH cast) 3194, 1694, 1602, 1292, 1219 cm^{-1}; ^1H NMR (D_2O:CD_3CN, 1:4, 360 MHz) δ 7.43 (d, 1 H, J = 8 Hz), 7.40 (s, 1 H), 6.85 (d, 1 H, J = 8 Hz), 5.40 (dq, 1 H, J = 6, 3 Hz), 3.94 (d, 1 H, J = 3 Hz), 1.31 (d, 3 H, J = 6 Hz).

For **(2*S*,3*S*)-2-amino-3-[(2-hydroxybenzoyl)oxy]butanoic acid (38c)** from **25**: $t_{1/2}$ = 9.0 days; ^1H NMR (D_2O:CD_3CN, 1:4, 360 MHz) δ 7.91 (d, 1 H, J = 7 Hz), 7.50 (t, 1 H, J = 7 Hz), 6.93 (m, 2 H), 5.57 (dq, 1 H, J = 6.5, 3 Hz), 3.94 (d, 1 H, J = 3 Hz), 1.35 (d, 3 H, J = 6.5 Hz).

For **(2*S*,3*S*)-2-amino-3-[(2,3-dihydroxybenzoyl)oxy]butanoic acid (38d)** from **22d**: $t_{1/2}$ = 9.0 days; ^1H NMR (D_2O:CD_3CN, 1:4, 360 MHz) δ 7.41 (d, 1 H, J = 8 Hz), 7.05 (d, 1 H, J = 8 Hz), 6.77 (m, 1 H), 5.55 (dq, 1 H, J = 6.5, 3 Hz), 4.07 (d, 1 H, J = 3 Hz), 1.38 (d, 3 H, J = 6.5 Hz).

Conversion of β-Lactone 27 to (4*S*,5*S*)-2-(3,4-Dihydroxybenzoyl)-5-methyl-2-oxazoline-4-carboxylic Acid (39b) and Hydrolysis to 38b. The pure β-lactone **27** was sealed under Ar and stored as a dry solid in a freezer at −20 °C for ca. 12 months. Analysis of the resulting sample showed quantitative conversion to **39**: mp 107−108 °C dec; IR (KBr) 3427, 1680, 1659, 1602, 1548 cm^{-1}; ^1H NMR (CD_3CN, 360 MHz) δ 7.46−7.38 (m, 2 H), 6.84 (d, 1 H, J = 8 Hz), 5.08 (dq, 1 H, J = 10, 6 Hz), 4.86 (d, 1 H, J = 10 Hz), 1.32 (d, 3 H, J = 6 Hz); exact mass 237.0634 (237.0637 calcd for $C_{11}H_{11}O_5N$); MS (CI, NH_3) 238 (MH^+, 70).

Treatment of either **27** or **39** with aqueous acetonitrile as described above for hydrolysis studies using NMR spectrometry gave conversion to the O-acyl derivative **38b** (approximate $t_{1/2}$ = 2.5 h for **39b** → **38b**; $t_{1/2}$ = 2.4 days for **27** → **38b**).

Hydrolysis of Oxazoline 39b to L-Allothreonine. Oxazoline **39b** was heated at 115 °C in 6 N HCl (5 mL) for 22 h. The mixture was then diluted with water (5 mL) and lyophilyzed. The residue was purified on cation-exchange resin

(BioRad AG50W-X8, H^+ form) using elution first with water and then with 1 M aqueous ammonia. The ammonia eluate was lyophilized, and the product (1 mg) was analyzed by ^1H NMR (1 M DCl in D_2O, 360 MHz). Comparison to spectra of authentic samples of L-threonine and L-allothreonine acquired under identical conditions showed that the major hydrolysis product (92%) corresponded exactly to L-allothreonine and the sole other component (8%) corresponded to threonine.

(3*S*,4*R*)-3-[(Phenylsulfenyl)amino]-4-methyl-2-oxetanone (44). Pyridine (30 μL, 0.371 mmol) was added to a suspension of the tosylate salt **21**[6] (49 mg, 0.179 mmol) in dry CH_2Cl_2 (1 mL) at −10 °C under Ar. A solution of phenylsulfenyl chloride (32 mg, 0.221 mmol) was then added dropwise. The mixture was stirred at −10 °C for 40 min and then allowed to warm to room temperature over 1.5 h. The mixture was partitioned between EtOAc (20 mL) and water (20 mL). The organic phase was washed with water (2 × 20 mL), dried ($MgSO_4$), filtered, and evaporated *in vacuo*. The product was purified twice by flash chromatography (SiO_2, 10−15% EtOAc/hexane) to yield **44** (15.3 mg, 41%) as an oil: IR ($CHCl_3$ cast) 3360, 1816, 1021, 740 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.44−7.32 (m, 5 H), 4.86−4.70 (m, 2 H), 3.50 (br d, 1 H, J = 6.5 Hz), 1.49 (d, 3 H, J = 6 Hz); exact mass 209.0509 (209.0511 calcd for $C_{10}H_{11}NO_2S$).

Biological Assays. Bacterial strains are originally from the American Type Culture Collection (Rockville, MD) and were obtained from Prof. Michael Stiles, Department of Food Science, University of Alberta. Preliminary assays for antimicrobial activity employed the agar diffusion (spot on lawn) method.[30] Initial solutions of potential antibiotics were prepared at a concentration of 500 μg/mL in DMSO/water (1:1). Each of the solutions was repeatedly diluted 2-fold giving concentrations of 250, 125, and 62 μg/mL. Aliquots (5 μL) of each sample were applied evenly to a growing plate of the organisms, and the solvent was also spotted as a control. The inhibition zone was observed after 24 h incubation at 28 °C. Solutions of *N*-(2-nitrophenyl)sulfenyl α-amino β-lactones, 2-nitrothiophenol, and bis(2-nitrophenyl) disulfide were prepared with an initial concentration of 100 μg/mL in DMSO/water (1:1), diluted, and tested similarly. The minimum inhibitory concentration (MIC) of each sample was estimated using the test tube dilution method.[30] The stock solutions were sterilized by filtration. A standardized inoculum (12 h old) was added to the tubes (1 mL) containing several dilutions (with trypticase soy broth, final volume 0.4 mL) of each sample. The growth of the test organisms was monitored as a change in turbidity after 16 h incubation at 37 °C.

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Supplementary Material Available: Spectral data for compounds **1**, **12**, **12a,d**, **13a−d**, **14a−c**, **15a−d**, **17−19**, **22b−c**, **23a,d**, **24a−c**, **25**, **27**, **29**, **31**, **33−37**, **38b**, **39b**, and **44** (37 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.