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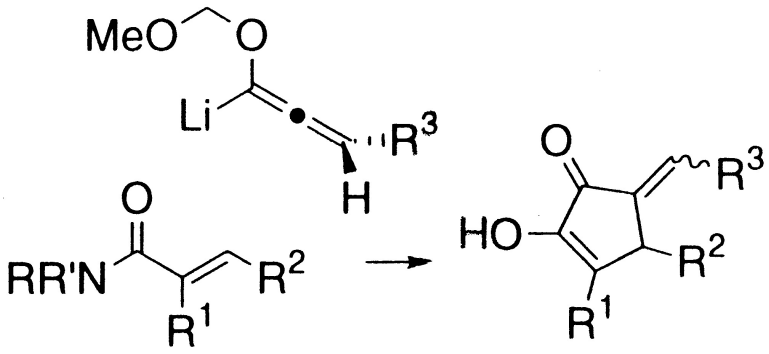

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Parallel Synthesis and Biocatalytic Amplification of a Cross-Conjugated Cyclopentenone Library

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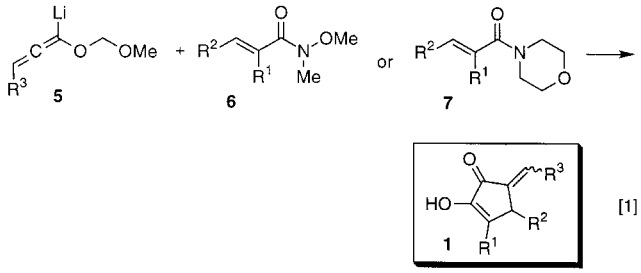
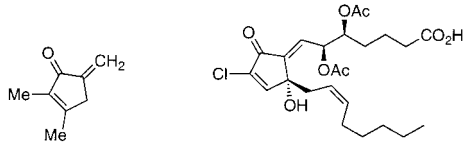
A combination of parallel chemical synthesis and biocatalysis has been used to prepare and amplify a library of cross-conjugated cyclopentenones. A number of marine and terrestrial natural products with antibiotic activity are known to incorporate this pharmacophore. The library was screened for anticancer, antimycobacterial, antifungal, and antibacterial activity. The positive results from the screens provide an indication of the structural features that are associated with activity in the various assays and suggest promising avenues for further inquiry.

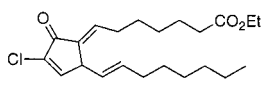
A simple method for the assembly of cross-conjugated cyclopentenones (**1**) was developed by Tius and co-workers.[1] The method, which has now been optimized, makes use of an unusual spontaneous cyclization, which appears to be a variant of the Nazarov reaction.[2] The synthetic method offers an appealing means of constructing a parallel library. The cyclopentenones are assembled from the combination of two very simple and easily accessible fragments, an allenyl ether and an α,β-unsaturated amide (eq 1). A single operation leads



[1]

to a small organic molecule that incorporates a high degree of complexity. Moreover, the cross-conjugated cyclopentenone substructure appears in both terrestrial (e.g., methylenomycin B,[1g,3] **2**) and marine natural products (e.g., punaglandin 4,[4] **3**) that have general antibiotic or antitumor activity. The activity is not limited to the naturally occurring compounds. For example, the simple prostaglandin analogue **4**, which has been prepared through an application of this technique, is active in the KB assay at 7 μM concentration.[5] Extensive investigations by Suzuki and Noyori into the mechanism of cytotoxic/cytostatic action of a series of cross-conjugated prostaglandins suggest that Michael addition to the cyclopentenone by an intracellular nucleophile is the process that





initiates a cascade of events that results in the arrest of cell growth.[6] It is not known whether the mechanism of the action of **4**, or **2**, follows the same paradigm.

Some years ago, Tius and co-workers examined the antibiotic properties of a small number of synthetic cross-conjugated cyclopentenones, similar to **1**, but lacking the α-hydroxyl substituent.[7] These materials were prepared by means of a two-step cyclization reaction. Promising activities were found to be associated with some of the compounds, but a systematic search for optimal structures was not carried out at that time. The decision to reexamine this problem stems from the current interest in parallel libraries of small organic molecules and also our ability to amplify the number of compounds by subjecting each library member to one or more biocatalytic transformations.[8] Combining chemical and biocatalytic reactions could provide the means to quickly access much larger numbers of compounds than would be available from synthesis alone. We also postulated that the biocatalytic steps would give us access to structural variants that are either inaccessible or accessible with difficulty by purely synthetic means, thus leading to greater structural diversity within the library. In what follows we describe the synthesis, biocatalytic amplification, and screening of the library.

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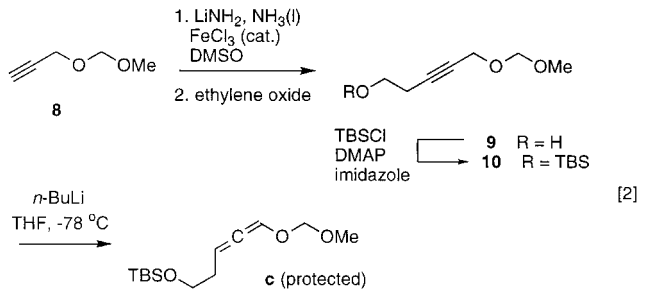
Chemical Synthesis

The reaction that was used to assemble the initial library is shown in eq 1. Addition of lithioallene **5** to Weinreb amide[9] **6** or morpholino amide[10] **7** leads to a stable, internally chelated tetrahedral intermediate that undergoes hydrolysis and spontaneous cyclization during workup with aqueous acid. When $R^3 \neq H$, cross-conjugated cyclopentenone **1** is isolated as a mixture of geometrical isomers at the exocyclic double bond. For reasons that have been discussed elsewhere,[5] the *Z* isomer always predominates. The reactions that assembled the library were performed in parallel in 15 mm test tubes, and the crude products were chromatographed in parallel. Yields varied between 23% and 86% and were not optimized. Weinreb and morpholino amides were prepared from the corresponding acids by treatment with carbon tetrabromide, triphenylphosphine, triethylamine, and *N*-methoxy-*N*-methylamine hydrochloride or morpholine.[11] Morpholino amide **F** was prepared from ethyl vinyl ether by first generating the vinyl anion,[12] followed by trapping with 4-morpholinecarbonyl chloride.[13] Ten amides were used in combination with three allenes (Figure 1). Allene **a** was prepared in the usual manner by isomerizing methoxymethyl propargyl ether with potassium *tert*-butoxide.[14] Allene **b** was prepared in a similar manner from the isomerization of the methoxymethyl ether of 2-buytn-1-ol with *n*-butyllithium in THF (89% yield). Several methods for the preparation of allene **c** were examined. Success was realized when methoxymethyl propargyl ether **8** was first homologated to **9** and protected as the *tert*-butyldimethylsilyl (TBS) ether **10** (eq 2). Isomerization of acetylene **10** to allene **c** (protected) was



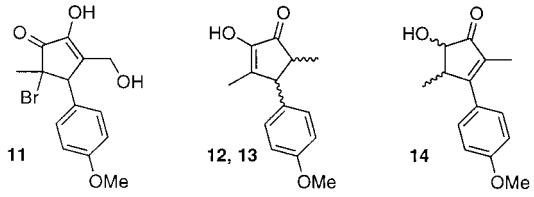
[2]

accomplished by treatment with *n*-butyllithium in THF. The silyl ether was cleaved from the cyclized products with TREAT·HF. In this way, a library of modest size was prepared. Of the 30 compounds shown in Figure 1, 28 were prepared. Cyclization of amide **F** was troublesome; therefore, **Fb** and **Fc** were never observed. We hypothesized that because we were proceeding from a substructure that is associated with potent pharmacological activity, the odds favored the discovery of more active leads even within a limited number of compounds.

Biocatalytic Amplification

Enzymes and whole cell biotransformations are beginning to play a more prominent role in drug candidate development. Recently, several examples of biocatalysis in the combinatorial synthesis and expansion of chemical libraries have been published.[8] An important advantage of biocatalysts in drug development is the ability to catalyze regio- and enantioselective reactions under mild operating conditions. In the present work, enzyme and microbial transformations were employed to expand on the original 28 cyclopentenones. To this end, Baker's yeast, peroxidases, oxidoreductases, and hydrolytic enzymes were examined for the reduction, halohydration, oxidation, and acylation of the initial library, respectively. The products were characterized by LC/MS. Several derivatives were further characterized by semi-preparative HPLC followed by ^{1}H NMR and chiral HPLC. The halogenated substrates (**B**, **G**, **I**) were not stable in aqueous solution and decomposed in the absence of enzyme to yield two compounds. These derivatives were not fully characterized, but LC/MS showed loss of the halogen substituent for both products.

Halohydration by Soybean Peroxidase. Soybean peroxidase was found to be an efficient catalyst for the halohydration of compounds **Aa**−**Ja** and **Ab**−**Jb**. The enzymatic synthesis of bromohydrins was confirmed by the presence of product peaks that indicated the expected molecular ion. Substrate **Ea** was used to optimize the reaction conditions. Multiple products were obtained, three of which were determined to be bromohydrins by LC/MS. These three bromohydrins were formed in a 12:5:1 ratio. Whereas the reaction conditions led to greater than 99% conversion of substrate, the bromohydrins were isolated in yields of 30%, 12.5%, and 2.5%, respectively. The two major bromohydrins were analyzed by LC/MS. The major product was determined to be **11** by ^{1}H NMR and LC/MS. To obtain more material



for ^{13}C NMR, a chemical synthesis using *N*-bromosuccinimide (NBS) was performed.[15] One equivalent of NBS was added to a DMSO solution containing substrate and a 2-fold excess of water. Several products were generated in this reaction, one of which matched the retention time and UV spectrum of the major enzymatically synthesized product. The ^{1}H NMR and mass spectrum for this compound were identical to those of enzymatically synthesized product **11**. Carbon NMR data were also consistent with this structure. Compound **11** is apparently derived from electrophilic attack of a bromonium species on the enol function of **Ea**. This generates a very reactive α-diketone that undergoes conjugate addition by water on the exocyclic double bond. Enolization of the product leads to **11**. This cascade of reactions suggests many opportunities for generating molecular complexity. The enantioselectivity of the enzyme-catalyzed reaction was determined by chiral HPLC. Bromohydrin **11** was formed in 80% ee. Enantiomeric excess was not determined for the other products. Earlier work has shown soybean peroxidase to be an effective bromination catalyst;[16] however, to our knowledge this is the first report of an enantioselective synthesis utilizing soybean peroxidase. The compounds **Ab**−**Jb** also successfully reacted with soybean peroxidase, although the product yields were lower. Similar to the

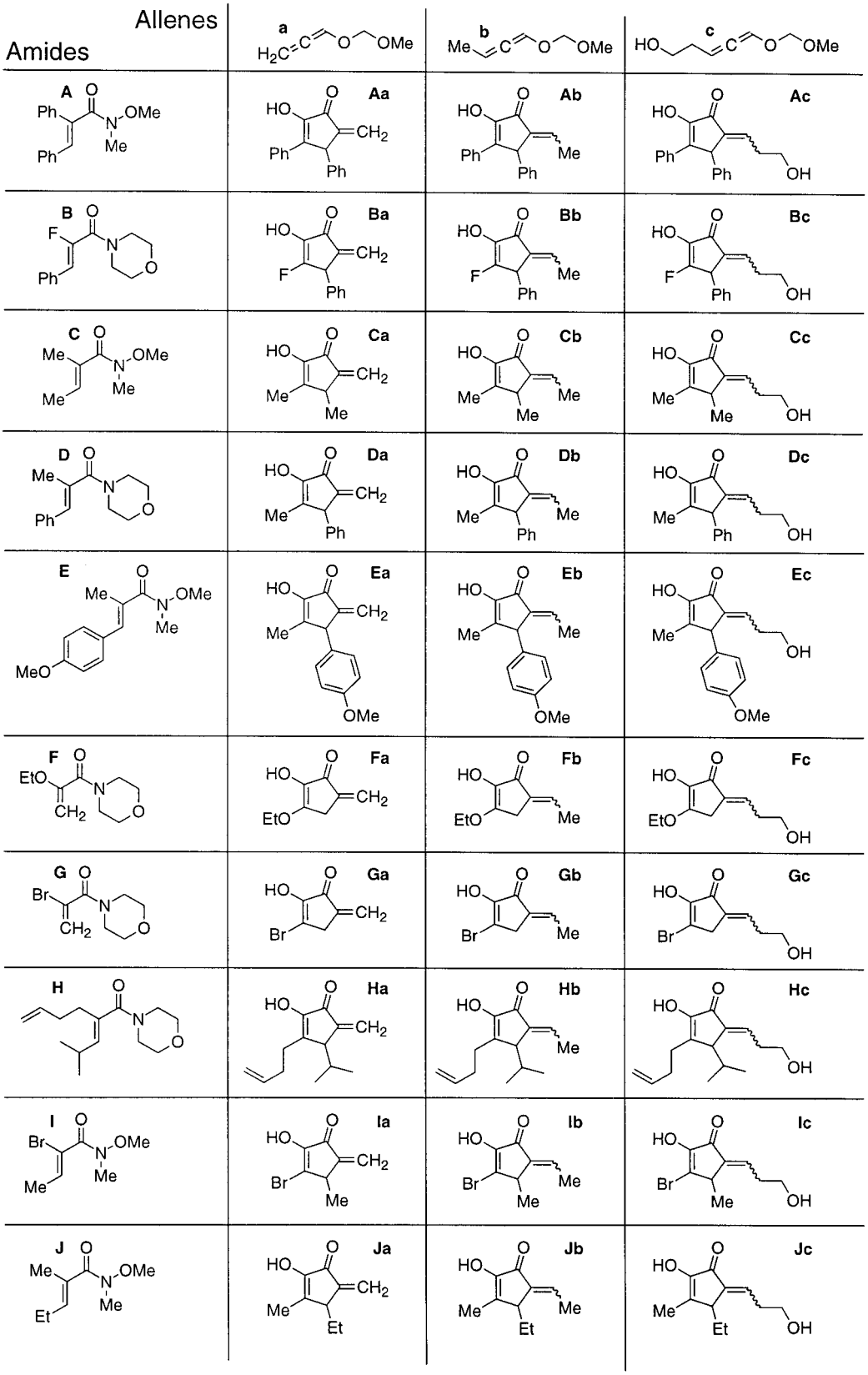


Figure 1. Cyclopentenones from synthesis.

reaction with **Aa**−**Ja**, multiple bromohydrin compounds were detected by LC/MS. Table 1 details the conversion of substrate to bromohydrin products for compounds **Aa**−**Jb**. Percent conversion of substrate varied as a function of substrate solubility, reaction rate, and selective substrate recognition by the enzyme.

Table 1. Percent Conversion of Substrates to Products with Soybean Peroxidase

	A	**C**	**D**	**E**	**H**	**J**
a	1	98	88	99	30	40
b	19	81	64	85	49	76

Table 2. Percent Conversion of Substrates with Baker's Yeast

	substrate					
	Aa	**Ca**	**Da**	**Ea**	**Ha**	**Ja**
conversion	5	95	99	97	72	98

Table 3. Yields of Butyrated Products Catalyzed by Lipase/Protease Mixtures

	compound					
	Ac	**Cc**	**Dc**	**Ec**	**Hc**	**Jc**
yield (%)	70	59	56	69	19	86

Reduction by Baker's Yeast. *Saccharomyces cerevisiae*, commonly known as Baker's yeast, has found wide use in organic synthesis, primarily for the reduction of aldehydes and ketones.[17] The reduction of the substrates **Aa**−**Ja**, except **Fa**, by Baker's yeast led to multiple products with an increase of molecular weight by 2 mass units. The reduction of **Ea** was used as a model reaction for product characterization. The conversion of **Ea** was greater than 97%, and two of the products, **12** and **13**, were determined to be diastereomers resulting from the reduction of the exocyclic double bond. The third product, **14**, accumulated with time. These three compounds were separated by reverse-phase HPLC, and the enantiomeric excess of each compound was determined. Compound **14** had an enantiomeric excess of 85%, while the diastereomers had enantiomeric excesses of 11% and 43%, respectively. The chemical reduction of the substrate **Ea** by $NaBH_4$ resulted in the production of racemic **12** and **13**. Thus, the biocatalytic tranformation produced products with some, although limited, enantioselectivity. For the remainder of the compounds, **Aa**−**Ja**, three products were produced, corresponding to analogues of **12**−**14**. The substrate conversions are shown in Table 2. Percent conversions were largely a function of substrate solubility. **Aa** was very insoluble in water and gave only 5% conversion.

Acylation by Protease/Lipase Mixtures. Compounds **Ac**−**Jc** were used as substrates in acylation reactions catalyzed by hydroxylase enzymes in organic solvents. Vinyl butyrate was used as the acyl donor. The acylation reactions produced products that had an increase in molecular weight of 70 mass units, the expected increase for a singly butyrated product. The position of butyration was expected to be the primary alcohol for several reasons. Previously reported acylations with these enzymes in organic solvents have shown selectivity for primary alcohols over enolic moieties.[18] Moreover, the UV spectra of the products exhibited no change, as would be expected if the enolic group had been butyrated. Interestingly, the compounds with a halide substituent were not acylated. In most cases, the enzyme mixture did not discriminate between the *E* and *Z* isomers, butyrating each equally. Moreover, all of the chromatograms were consistent with a single, regioselective acylation. The yields of the products are shown in Table 3.

Oxidation by Soybean Peroxidase. Multiple products resulted from the enzymatic oxidation of compounds **Ac**−**Jc**. Oxidation of the primary alcohol to the aldehyde was confirmed from the mass spectra of the new products. Two other products were detected that had longer retention times than the aldehyde, but the low yields prevented further characterization. Table 4 presents the yield of the aldehyde products.

Table 4. Yields of Aldehyde Products Catalyzed by Soybean Peroxidase

	compound					
	Ac	**Cc**	**Dc**	**Ec**	**Hc**	**Jc**
yield (%)	25	10	5	30	5	6

Table 5. Cytotoxicity (KB/LoVo)[a]

	allene		
	a	**b**	**c**
amide			
A	1.7/1.2[b]		_/2.4
B	4.5/1.0		9.4/2.9
C	_/4.0		
D	2.0/0.6		
E	1.5/1.3		
G	7.8/3.1	_/2.3	_/3.0
I	5.2/2.8	_/1.3	_/2.0
J	_/2.9		

[a] IC_{50}, μM. Compounds were dissolved in absolute ethanol and assayed at a final concentration of 0.5% in ethanol in KB and/or LoVo cells, unless otherwise indicated. Values greater than 10 μM are not shown.[21] [b] **Aa** was dissolved in DMSO and assayed at a final concentration of 0.1%. IC_{50} values were determined as described in the Experimental Section.

Alcohol oxidase (Sigma) and alcohol dehydrogenase from horse liver (Sigma) were also tested as oxidative catalysts; however, no aldehyde was detected in the products. The oxidation of the alcohol function in the **c** series extends previous work demonstrating chloroperoxidase from *Caldariomyces fumago*, lignin peroxidase, and soybean peroxidase as oxidase enzymes capable of oxidizing primary alcohols.[19,20]

Screening Results

The library was evaluated in standard screens for anticancer, antifungal, antibacterial, and antimycobacterial activities. Cytotoxicity was evaluated in the KB and LoVo cell lines (Table 5). Compounds active at $\leq$10 μM in one or both assays were considered active. Some trends were evident. Compounds derived from allene **a** had the best activity in the KB assay. Substitution on the exocyclic double bond eroded activity in the KB assay, whereas aryl substitution in the ring enhanced the activity (**Aa**, **Ba**, **Da**, and **Ea**), as did substitution by fluorine or bromine (amides **B**, **G**, and **I**). There were more hits in the LoVo assay, where similar trends were discerned. The biocatalytic derivatives were screened in the same assays and were found to be less active than the synthetic substrates from which they were derived.

The products from the biocatalytic expansion steps were screened as mixtures. The bromohydroxylation and reduction reactions both modified the exocyclic double bond, and the resulting derivatives were inactive. The acylation and oxidation of **Ac** lowered its activity appreciably; however, the presence of remaining substrate in the mixtures precluded accurate measurement of IC_{50} values. In all other cases, however, the cytotoxicity of the biocatalytic mixtures could be attributed entirely to remaining substrate (data not shown).

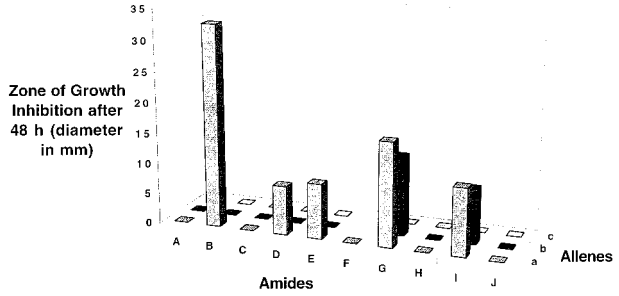


Figure 2. Antifungal disk susceptibility results for *C. albicans*.

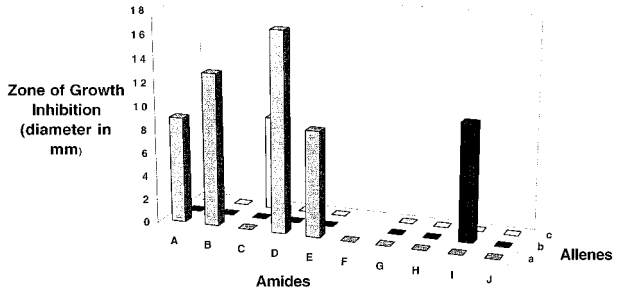


Figure 3. Antibacterial disk susceptibility results for *S. aureus*.

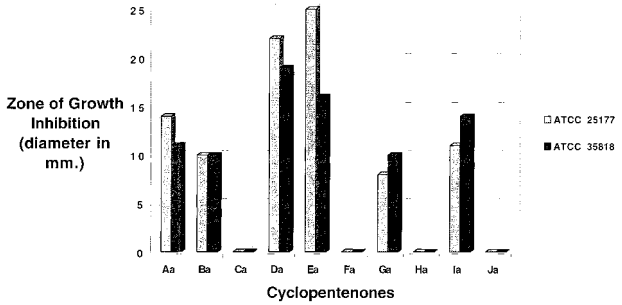


Figure 4. Antimycobacterial disk diffusion susceptibility results for *M. tuberculosis* strains.

A limited number of experiments were performed in order to probe the structural requirements for anticancer activity. The enolic hydroxyl group of **Ea** was converted to the methyl ether by treatment with iodomethane, potassium carbonate, and 18-crown-6 ether in DME. The IC_{50} values for this compound in the KB and LoVo assays were 3.0 and 0.7 μM, respectively, compared to 1.5 and 1.3 μM for the parent compound. This result indicates that a free α-hydroxyl group is not essential for the activity. The enolic hydroxyl group of **Ic** was converted to the butyrate by first treating the cyclic material with butyric anhydride and pyridine, then cleaving the silyl ether protecting group with TREAT·HF. The *E* and *Z* geometric isomers were separated from each other. In a separate experiment the dibutyrate of **Ic** was prepared (*E* + *Z* mixture). The three samples were evaluated independently in the KB and LoVo assays. Minor differences in activity were noted, suggesting that neither of the free hydroxyl groups of the series derived from allene **c** contributes substantially to the cytotoxicity.[22]

The library was screened against *C. albicans* in a disk-diffusion assay, using sample concentrations of 0.25 μmol/disk (Figure 2).[23] The trends in this assay showed parallels with the cytotoxicity screens. Most of the actives were derived from allene **a**; compounds derived from amides **G** and **I** were also active. The greatest zone of inhibition was observed for **Ba**, which inhibited growth in a 33 mm zone after 48 h. The methyl ether of **Ea** was also evaluated against *C. albicans*. The parent compound **Ea** created a 9 mm zone of inhibition after 48 h. The methyl ether was much more active, inhibiting a 28 mm zone after 48 h. The large difference between **Ea** and the methyl ether derived from it indicates that the free α-hydroxyl group may suppress the antifungal activity and suggests that chemical modification in this area may offer opportunities to improve the activity.

The library was also screened against *S. aureus* and *P. aeruginos*a. There was no activity against *P. aeruginosa* at the concentration tested (0.25 μmol/disk). A few members of the library were active against *S. aureus* (Figure 3). All cyclopentenones derived from allene **a** that also bear a γ aryl group were active. The methyl ether of **Ea** was screened, and once again, the zone of inhibition was greater than that of **Ea** (16 vs 9 mm).[24]

Antimycobacterial activity was assayed against two strains of *M. tuberculosis* (Figure 4).[25] Activity was detected only in compounds derived from allene **a**, and all active cyclopentenones inhibited both strains. Once again, a γ aryl group or a β halogen atom was associated with activity. In general, the cylopentenones bearing an aryl group were more active against ATCC 25177, whereas the compounds bearing a bromine atom were more effective against ATCC 35818. The methyl ether derivative of **Ea** had the same activity against ATCC 25177 as the parent compound but was significantly more active against ATCC 35818 than **Ea** (27 vs 16 mm zone of inhibition).

These results support our hypothesis that potentially useful activities can be discerned within a focused library of limited size. High activity in all screens, as was the case for **Ba**, may indicate nonspecific toxicity.

Conclusions

A parallel library of modest size was prepared and amplified biocatalytically and was screened. Actives were found in each of the screens. Substitution at the exocyclic carbon−carbon double bond generally led to a diminution of activity. It is tempting to interpret this result in terms of the probable mode of action, addition to the exocyclic double bond by a cellular nucleophile. The presence of an aryl ring at the γ carbon atom of the cyclopentenone in general potentiated the activity. The free α hydroxyl group in all the members of the synthetic library was expected to be reactive and to exert an effect on the activity. This was found to be the case especially in the antifungal screen in which a large difference in inhibition zones against *C. albicans* was found between **Ea** and the methyl ether derived from **Ea**. These trends will be exploited for the design and synthesis of a more focused second-generation library.

The structural diversity of the synthetic library is necessarily modest given its size. Nevertheless, promising leads were discovered presumably because the library was designed to incorporate a known pharmacophore. The isolation of **11** from the soybean peroxidase catalyzed bromohydration of **Ea** is a good example of how combining biocatalysis with synthesis can increase the size and structural diversity of a

library. It is worth noting that all synthetic compounds were prepared and evaluated as racemic mixtures. Therefore, it is possible that activity is vested in only one of the enantiomers.

Experimental Section

Chemical Synthesis. ^{1}H NMR and ^{13}C NMR spectra were recorded in deuteriochloroform ($CDCl_3$) with chloroform as an internal reference, at 300 MHz for ^{1}H NMR and at 75 MHz for ^{13}C NMR unless otherwise noted. Chemical shifts are referenced to the solvent standards and reported in δ units. Coupling constants are reported in hertz. IR spectra were recorded neat on a Perkin-Elmer IR 1430 spectrometer. MS data are reported in *m*/*z*. Thin-layer chromatography (TLC) was performed on Sigma-Aldrich precoated silica gel 60 F-354 analytical plates (0.25 mm). ICN silica gel (0.032−0.063 mm) was used for normal phase flash column chromatography. The purity and homogeneity of all materials were determined chromatographically and from ^{1}H NMR. THF was distilled from sodium benzophenone ketyl. Other reagents were obtained commercially and used as received.

Biocatalytic Syntheses. Products from all biotransformations were analyzed by a HP 1100 LC/MS system. The column was a reverse-phase ODS-A C18 column (250 mm × 4.6 mm) packed with 5 μm particles (YMC, Inc., Wilmington, NC). The mobile phase consisted of a 50:50 (v/v) mixture of acetonitrile and 20 mM NH_4OAc buffer, pH 4.5. The flow rate was 0.8 mL/min, and the column temperature was maintained at 30 °C. Mass spectra were acquired using the electrospray ionization module in positive ion mode. Purified products were analyzed for enantiomeric purity by HPLC with a Chiralcel OB-H column (150 mm × 4.6 mm) (Daicel Chemical Industries, Exton, PA). The mobile phase was a mixture of hexane and 2-propanol (9:1 v/v) with a flow rate of 1.0 mL/min at 30 °C. For the bromohydrins, the mobile phase was adjusted to 9.5:0.5 (v/v) hexane/2-propanol.

General Procedure for the Synthesis of the Amides. To a mixture of 0.288 g of 3-*p*-methoxyphenyl-2-methyl-2*E*-propenoic acid (1.5 mmol) and 0.596 g of carbon tetrabromide (1.8 mmol) in 7 mL of CH_2Cl_2 at 0 °C was added 0.5 mL of triethylamine (3.6 mmol), 0.176 g of *N*,*O*-dimethylhydroxylamine hydrochloride (1.8 mmol), and 0.472 g of triphenylphosphine (1.8 mmol). The reaction mixture was stirred for 30 min. Most of the solvent was evaporated, and the residue was chromatographed on silica gel to yield 0.293 g of amide **E** (83% yield) as a pale-yellow oil: $R_f = 0.23$ (30% EtOAc in hexanes); IR 2950, 1650, 1600 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 (d, $J = 8.5$ Hz, 2H), 6.89 (d, $J = 8.8$ Hz, 2H), 6.78 (br s, 1H), 3.81 (s, 3H), 3.68 (s, 3H), 3.27 (s, 3H), 2.12 (d, $J = 1.5$ Hz, 3H).

Synthesis of Allene b. To 2.50 mL of but-2-ynyloxymethoxymethane in 80 mL of THF at −78 °C was added 19.00 mL of *n*-BuLi (2.20 M solution in pentane, 41.80 mmol) dropwise. The reaction was stirred at −78 °C for 2 h, then poured into a mixture of 20 g of ice, 5 mL of methanol, and 0.10 g of K_2CO_3. The aqueous solution was extracted with ether (3×), and the combined organic layers were washed with water and brine and dried over sodium sulfate. Distillation (1 mmHg, ∼30 °C) provided 2.24 g of a mixture of allene and acetylene (89% yield, 4:1 ratio) as a colorless mobile oil: IR 2950, 1970, 1460 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.50 (m, 1H), 5.75 (dq, $J = 5.6$, 6.7 Hz, 1H), 4.76 (s, 2H), 3.39 (s, 3H), 1.79 (dd, $J = 7.1$, 2.5 Hz, 3H).

Synthesis of 9. To a mixture of 100 mL of ammonia (l) and catalytic $FeCl_3$ at −78 °C was added 0.70 g of lithium (100 mmol) in small pieces. After the reaction mixture turned from blue to gray, 10.00 g of **8** was added followed by 40 mL of DMSO. The −78 °C bath was removed, and the ammonia was evaporated. At −10 °C, 6.5 mL of ethylene oxide (130 mmol) was added. The temperature was continually adjusted so that the temperature of the exothermic reaction was never above 15 °C. The reaction was then stirred overnight at room temperature and quenched with ice−water (100 mL). The mixture was extracted with ether (10 × 50 mL), and the combined organic layers were washed with water and brine. Solvent was evaporated, and the residue was distilled under vacuum (105 °C/1 mmHg) to give 10.50 g of alcohol **9** (73% yield): IR 3450, 2900, 2230 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.70 (s, 2H), 4.22 (*t*, $J = 2.1$ Hz, 2H), 3.74 (q, $J = 6.1$ Hz, 2H), 3.38 (s, 3H), 2.51 (m, 2H), 1.86 (t, $J = 6.7$ Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 94.2, 83.4, 76.5, 60.4, 55.1, 54.3, 22.6.

Synthesis of 10. To 1.44 g (10 mmol) of **9**, 1.36 g (20 mmol) of imidazole, and 0.12 g (1 mmol) of DMAP in 20 mL of THF at 0 °C was added 1.95 g (13 mmol) of *tert*-butyldimethylchlorosilane as a solution in 5 mL of THF. After addition, the reaction mixture was warmed to room temperature and was stirred for 1 h. The reaction mixture was diluted with 30 mL of ether and filtered through a plug of silica gel. Solvent was evaporated to yield 2.42 g of **10** (99% yield) that was used without further purification: IR 2900, 2240 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.69 (s, 2H), 4.19 (t, $J = 2.2$ Hz, 2H), 3.72 (t, $J = 7.4$ Hz, 2H), 3.37 (s, 3H), 2.43 (m, 2H), 0.89 (s, 9H), 0.065 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 94.1, 83.2, 76.3, 61.5, 54.9, 54.1, 25.5, 22.8, 17.9, −5.6.

Synthesis of Allene c (Protected). To 2.58 g (10 mmol) of **10** in 15 mL of THF at −78 °C was added 8.1 mL (2.5 M solution in hexane, 20.3 mmol) of *n*-BuLi dropwise over 5 min. The reaction was stirred for 1.5 h and then quenched with ice, methanol, and potassium carbonate. The mixture was extracted with ether (3 × 30 mL), and the combined organic layers were washed with brine and dried over $MgSO_4$. Solvent was evaporated, and the residue was purified by flash column chromatography (10% EtOAc in hexanes) to yield 1.45 g of allene **c** (protected) (60% yield): IR 2900, 1970 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.55 (dt, $J = 5.6$, 2.5 Hz, 1H), 5.83 (q, $J = 6.4$ Hz, 1H), 4.79 (s, 2H), 3.71 (t, $J = 6.9$ Hz, 2H), 3.41 (s, 3H), 2.33 (dq, $J = 2.2$, 6.1 Hz, 2H), 0.89 (s, 9H), 0.055 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 194.2, 117.7, 102.8, 94.4, 62.1, 55.7, 34.4, 25.7, 18.1, −5.4.

Parallel Cyclizations. Standard 0.2 M solutions of the amides were prepared in THF and stored over 4 Å molecular sieves. Test tubes (13 mm × 100 mm) were dried, capped with septa, and purged with Ar. The amide solutions were added via syringe (1.06 mL of a 0.2 M solution of **E**, 50

mg, 0.212 mmol), and the test tubes were cooled to −78 °C. A standard solution of the allenyllithium was prepared in 25 mL of THF at −78 °C from allene **a** (1.00 g, 10 mmol) and *n*-BuLi (4.3 mL of a 2.2 M solution in hexanes, 9.5 mmol). Approximately 2.0 mL of the anion solution was added to each test tube via cannula. The test tubes were vortexed for 5 s, then kept at −78 °C for 15 min. The reactions were quenched with 5% HCl in EtOH (1.5 mL), washed with water (2×) and brine (2×) and dried over $MgSO_4$. Evaporation of solvent and purification using the ISCO CombiFlash Si1000s (5−20% EtOAc in hexanes) gave 41 mg of **Ea** (86% yield) as a white solid: $R_f = 0.14$ (15% EtOAc in hexanes); IR 3300, 1700, 1680 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.04 (d, $J = 8.8$ Hz, 2H), 6.86 (d, $J = 8.8$ Hz, 2H), 6.70 (br s, 1H), 6.13 (d, $J = 1.8$ Hz, 1H), 5.17 (s, 1H), 4.17 (s, 1H), 3.79 (s, 3H), 1.84 (d, $J = 1.5$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 189.8, 158.7, 151.3, 145.1, 142.1, 131.6, 128.9, 118.2, 114.1, 55.2, 49.1, 12.1. Mass spectrum: *m*/*z* 230 (M^+, 73), 172 (100); exact mass calcd for $C_{14}H_{14}O_3$ 230.0943, found 230.0940.

Biocatalytic Syntheses. Bromohydrins. A total of 10 mg of each substrate **Aa**−**Ja** and **Ab**−**Jb** was dissolved in a solution of *t*-BuOH and 0.15 M citrate/phosphate buffer (1:9 v/v), pH 2.5, containing 100 mM KBr. Soybean peroxidase (Sigma) was added three times in equal amounts (2 mg/mL) before the reaction and after 1 and 2 h. The biotransformation was initiated by adding 5 μL of a 3% (w/w) H_2O_2 solution. A total of 5 μL of the H_2O_2 solution was added every 20 min, and a total of 20 mg of enzyme was added at 1 and 2 h. The reaction was terminated at 4 h by extracting with EtOAc, and the emulsion was separated by centrifugation. The combined extracts were dried over $MgSO_4$ and reduced to dryness by rotary evaporation. **11**: yellow oil; UV−vis (ethanol) λ^{max} 225, 276 nm; mass spectrum (ESI) *m*/*z* (MH^+) 327, 329; ^1H NMR (500 MHz, $CDCl_3$) δ 7.04 (br d, 2H), 6.88 (d, $J = 8.5$ Hz, 2H), 4.53 (s, 1H), 4.55 (d, $J = 16.5$ Hz, 1H), 4.41 (d, $J = 16.5$ Hz, 1H), 3.81 (s, 3H), 1.25 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 197.4, 159.5, 147.3, 146.8, 139.7, 128.6, 114.4, 60.2, 58.8, 57.8, 55.3, 24.5.

Reduction by Baker's Yeast. Freeze-dried yeast cells (1 g) were activated by placing them in 24 mL of solution containing 5 g/L NaCl, 3 g/L sucrose, and 50 mM Tris buffer, pH 6.5. The yeast was incubated at 30 °C and shaken at 250 rpm for 1 day. The substrates (5 mg) were dissolved in EtOH (1 mL) and added to the yeast mixture. After 4 days, the reaction was stopped by extracting the mixture three times with EtOAc. The combined extracts were dried over $MgSO_4$ and reduced to dryness by rotary evaporation. **12**: light-yellow powder; UV−vis (ethanol) λ^{max} 228, 262 nm; mass spectrum (ESI) *m*/*z* (MH^+) 233; ^1H NMR (500 MHz, $CDCl_3$) δ 6.99 (br d, 2H), 6.84 (d, $J = 7.5$ Hz, 2H), 5.42 (br s, 1H), 3.94 (d, $J = 5.5$ Hz, 1H), 3.80 (s, 3H), 2.76 (quint, $J = 7.0$ Hz, 1H), 1.86 (s, 3H), 0.68 (d, $J = 7.0$ Hz, 3H). **13**: light-yellow powder; UV−vis (ethanol) λ^{max} 228, 262 nm; mass spectrum (ESI) *m*/*z* (MH^+) 233; ^1H NMR (500 MHz, $CDCl_3$) δ 7.04 (d, $J = 8.5$ Hz, 2H), 6.87 (d, $J = 8.5$ Hz, 2H), 5.33 (s, 1H), 3.81 (s, 3H), 3.25 (s, 1H), 2.25 (q, $J = 7.0$ Hz, 1H), 1.78 (s, 3H), 1.24 (d, $J = 7.5$ Hz, 3H). **14**: light-yellow powder; UV−vis (ethanol) λ^{max} 226, 300 nm; mass spectrum (ESI) *m*/*z* (MH^+) 233; ^1H NMR (500 MHz, $CDCl_3$) δ 7.50 (d, $J = 9.0$ Hz, 2H), 7.00 (d, $J = 9.0$ Hz, 2H), 4.40 (d, $J = 6.5$ Hz, 1H), 3.87 (s, 3H), 3.54 (quint, $J = 6.5$ Hz, 2H), 1.98 (s, 3H), 1.20 (d, $J = 7$ Hz, 3H).

Oxidation by Soybean Peroxidase. The same reaction conditions were used as for the halohydration reaction with the exception that the buffer solution contained no KBr. The substrates used for this reaction were also **Ac**−**Jc**.

Acylation by Protease/Lipase Mixtures. Compounds **Ac**−**Jc** (8 mg) were used as substrates in an acylation reaction catalyzed by hydrolase enzymes in organic solvents. Vinyl butyrate was used as the acyl donor in 100-fold molar excess of substrate. Substrate **Aa** was dissolved in 20 mL of benzene and chloroform (3:1 v/v) to which 5 mg of Subtilisin Carlsberg (SC) (Sigma) and 10 mg of lipase "M" (Amano) were added. Compounds **Bc**, **Gc**, and **Ic** were dissolved in 10 mL of benzene and THF (1:1 v/v) to which 10 mg of lipase "M" and 10 mg of protease type X (Sigma) were added. The remaining compounds were dissolved in 10 mL of benzene and chloroform (1:1 v/v) containing 10 mg of lipase "M" and 5 mg of SC. The reactions were performed in glass tubes with Teflon caps and were shaken at 250 rpm at 30 °C. The reactions were paused by centrifuging the enzyme after 4 days and restarted by adding an equivalent amount of fresh enzyme for 3 more days.

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Supporting Information Available. Cytotoxicity assay details and selected ^1H NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) In both assays cryptophycin 1 was used as the positive control: IC_{50}s (pM): KB 5.5−7.6; LoVo 8.0−17.7.
(22) *E*-**Ic** monobutyrate IC_{50}s (μM): KB 10.5; LoVo 2.75. *Z*-**Ic** monobutyrate: KB 7.8; LoVo 1.80. (*E* + *Z*)-**Ic** dibutyrate: KB 8.3; LoVo 1.70. *E*-**Ic**: KB 14.3; LoVo 3.15.
(23) Amphotericin B (0.027 μmol in DMF, 9 mm zone of inhibition after 48 h) and 5-fluorocytocine (0.039 μmol in water, 37 mm zone of inhibition after 48 h; 0.0077 μmol in water, 21 mm zone of inhibition after 48 h) were used as positive controls.
(24) Gentamicin dissolved in water was used as a positive control in the antibacterial assays, yielding zones of inhibition of 24 and 18 mm for 0.06 and 0.0025 μmol, respectively, against *S. aureus* and 28 and 17 mm for 0.06 and 0.0025 μmol, respectively, against *P. aeruginosa*.
(25) Streptomycin was used as a positive control [nanomoles of streptomycin/zone of inhibition for ATCC 25177 (mm)/zone of inhibition for ATCC 35818 (mm)]: 43/40/40; 8.6/30/25; 1.7/10/9.

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