Parallel Synthesis and Yeast Growth Inhibition Screening of Succinamic Acid Libraries

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Libraries of succinamic acid derivatives resulting from the condensation of a series of succinic acid derivatives with amines are reported as putative khafrefungin analogues. A total of 480 compounds derived from the initial condensation of 8 scaffolds with 60 different amines have been synthesized using automated technology with the help of scavenger resins. A simple acetate hydrolysis of five of the above sublibraries afforded additional 300 compounds for a total of 780 compounds. Around 55% of the library members showed purities higher than 70% (HPLC-ELS-MS) thus proving the generality of this approach. Results on growth inhibition of the yeast *Saccharomyces cerevisiae* in the presence of selected library members are also reported as a preliminary evaluation of the antifungal activity.

Introduction

Sphingolipids are components of cellular membranes that have also been shown to take part in the regulation of signal transduction pathways.[1−4] The proposed pathway for the biosynthesis of sphingolipids starts with the condensation of L-serine and palmitoyl CoA to provide 3-ketosphinganine, which is converted to sphinganine by the action of a specific reductase. However, the observed biosynthetic pathways from sphinganine show noticeable differences between mammals and fungi. Thus, while sphinganine is further transformed into ceramide in mammals, C4-hydroxylation followed by *N*-acylation leads to phytoceramide in fungi. Condensation of phytoceramide with inositol phosphate (IP), by means of inositol phosphorylceramide synthase (IPC synthase), which is encoded by the AUR1 gene, provides inositol phosphorylceramide (IPC), a reaction that occurs in the lumen of the Golgi apparatus. IPC is further modified to give complex fungal sphingolipids[5] (Scheme 1). The reaccion catalyzed by IPC synthase has been suggest to play a role in the transition from the G1 to the S phase of the cell cycle, and because this step is essential for sphingolipid biosynthesis in fungi, specific IPC synthase inhibitors are attractive targets for the design of new, nontoxic antifungal agents.[6−8]

The antifungal activity of the natural product khafrefungin has been associated with the inhibition of IPC synthase and the impairment of sphingolipid biosynthesis in fungi.[9] Khafrefungin (Scheme 2) is an aldonic acid derivative esterified with a C22 complex linear polyketide acid bearing four stereogenic centers, whose stereochemistry has been unambiguously assigned by total synthesis.[10] In addition, biological evaluation of some closely related synthetic analogues revealed the strict structural requirements imposed by IPC synthase for enzyme inhibition by this natural product.[11,12] It has not been reported yet the type of inhibition caused by khafrefungin, which would help to understand its characteristic activity profile. Moreover, IPC synthase is a membrane-bound enzyme located at the Golgi apparatus,[13] whose three-dimensional structure remains elusive, thus precluding the use of molecular modeling approaches to delineate the topology at the active site and to identify putative regulatory domains. This scenario seemed appropriate for the development of combinatorial strategies to the synthesis of potential IPC synthase inhibitors with high degree of diversity which it was hoped would shed light on the structural requirements of the catalytic site. In this paper, libraries of succinamic acid derivatives resulting from the condensation of a series of succinic anhydride derivatives with amines are reported as putative khafrefungin analogues. Results on growth inhibition of the yeast *Saccharomyces cerevisiae* in the presence of selected library members are also reported as a preliminary evaluation of the antifungal activity.

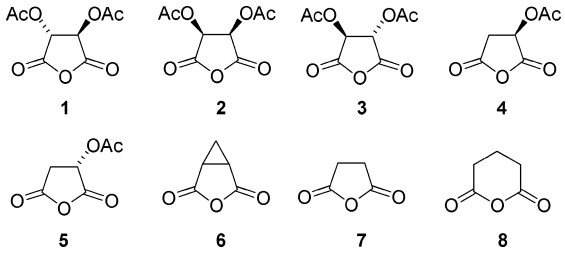

Figure 1. Anhydrides used as building blocks for library production.

Results and Discussion

Library Design. In the light of the above precedents, we designed a series of succinamic acid libraries as structural khafrefungin analogues, as shown in Scheme 2. From a retrosynthetic standpoint, the library was envisioned as the result of the condensation of a series of primary amines with suitable cyclic anhydrides. Anhydrides **1**−**3** (Figure 1) were

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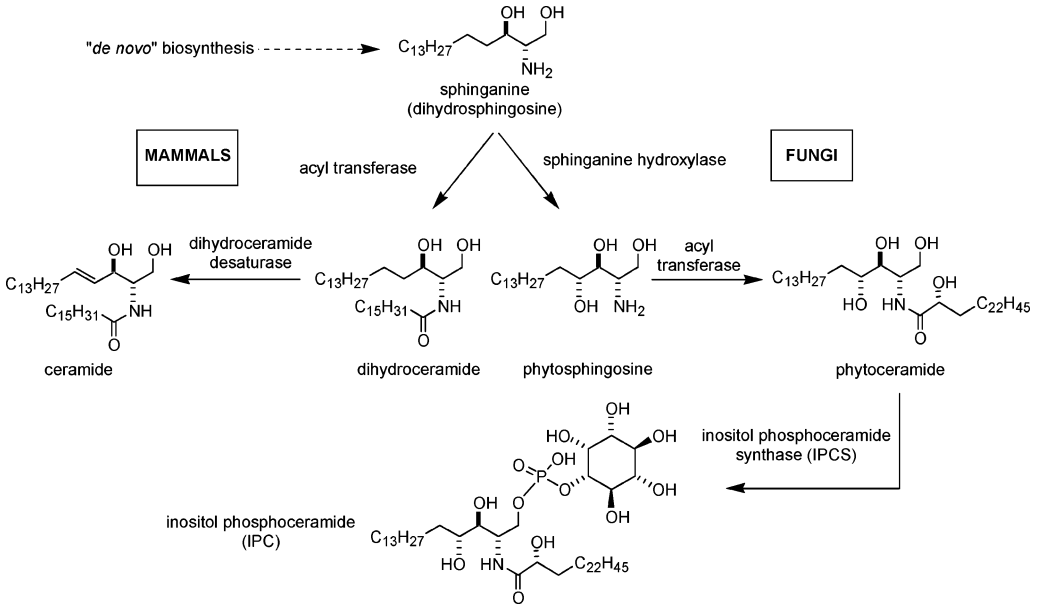
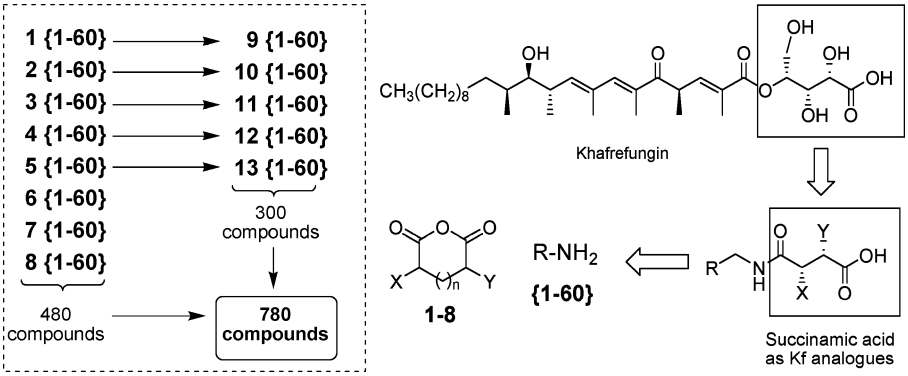
Scheme 1. Sphingolipid Biosynthesis in Mammals and Fungi



Scheme 2. Library Design of Succinamic Acids as Khafrefungin Analogues



selected for their structural similarity with the aldonic acid moiety present in khafrefungin. Moreover, to evaluate the influence of the functionality around the terminal carboxylate group of the aldonic fragment, C2-monosubstituted anhydrides **4**−**7** and anhydride **8** were also included as building blocks. Amines were selected from among the several thousands of commercially available and "in-house"-synthesized possibilities by generation of a virtual library. The library thus obtained was filtered to remove incompatibilities with the proposed reaction pathway, and classified into different families according to structural criteria. Each family was further filtered according to modified Lipinski's rules.[14] Final amine selection was made by hand on the basis of structural diversity and commercial availability.

Following the above considerations, condensation of anhydrides **1**−**8** (Figure 1) with selected amines (**1**−**60**) (Figure 2) afforded 8 sublibraries for a total 480 compounds. Deprotection of the corresponding *O*-acyl libraries afforded 5 additional sublibraries of 60 members each to give a total of 780 compounds.

Synthesis. A straightforward strategy, easily amenable to automated protocols, based on the combination of parallel synthesis and purification techniques was designed (Scheme 3). Reactions were optimized from anhydrides **1** and **5** and four selected amines. Suitable reaction conditions were achieved by treatment of the starting anhydride overnight with a slight excess of amine (1.1 equiv mol^{-1}) in 1,2-dichloroethane (DCE) at 45 °C. The excess amine was next removed with the help of a scavenger resin.[15]

The noncovalent interaction between the amine and the scavenger resin required the use of excess resin. For this reason, it was necessary to carefully optimize the reaction conditions to reach the highest conversions with the minimum of excess reagents. Amberlyst15 (10 equiv mol^{-1}), a reticular strong sulfonic acid resin, was used as scavenger resin, because of its compatibility with nonaqueous reaction conditions. In our hands, this approach proved to be superior to the classical acidic aqueous washing.[16,17] Adjustment of these optimized reaction conditions to the automated library production required further technical adjustments. Thus, glass plates (0.9 mL $well^{-1}$) instead of the standard plastic plates (1.7 mL $well^{-1}$) were required for the required reaction temperature. The higher reaction concentration imposed by this technical constraint made necessary the use of a small amount of DMF as cosolvent in some instances to improve solubilization. This modified protocol was successfully applied to the production of libraries **1**−**8**. Model experiments with anhydride **5** and amines **14**, **16**, **19**, and **21** (see Experimental Section) showed the regioselective formation of the expected adducts, resulting from amine attack upon the more reactive electron-deficient C1-carbonyl.[18,19] How-

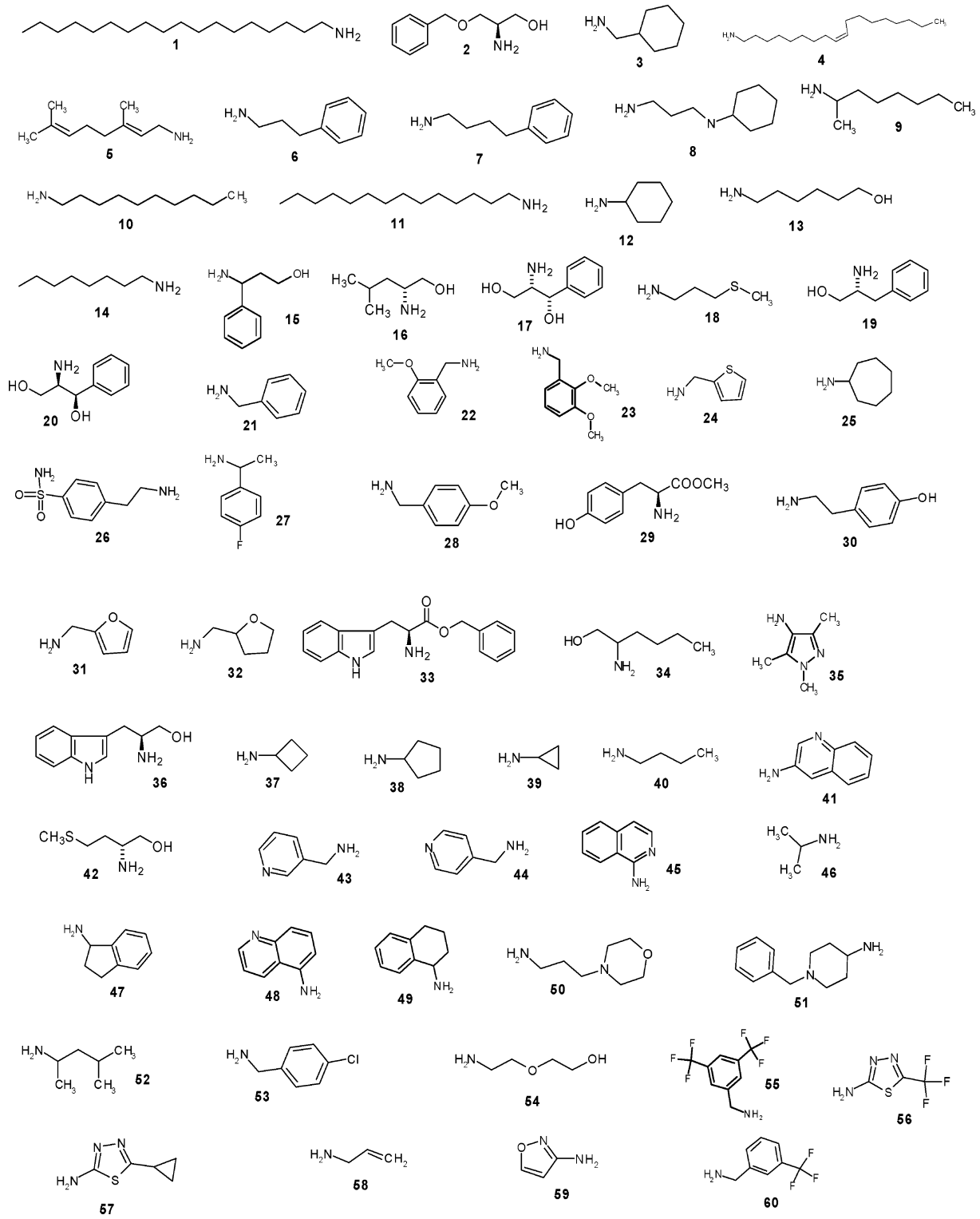


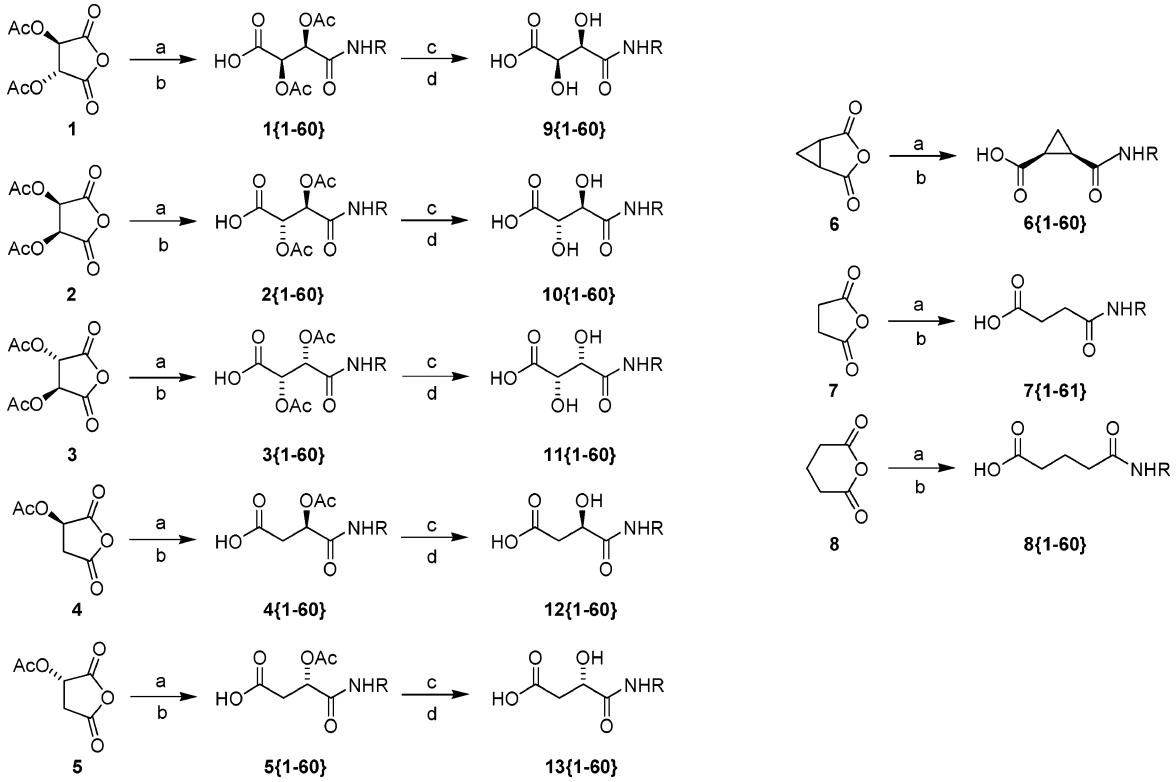
Figure 2. Amines used as building blocks for library production.

ever, minor amounts of regioisomeric adducts arising from attack to the less reactive C4-carbonyl were also observed (^{1}H NMR and or HPLC analysis) under our optimized reaction conditions.[20]

Libraries **1**−**5** were duplicated, and one set was submitted to acetate cleavage in the presence of NaOMe/MeOH, followed by quenching with excess Amberlyst15 as a proton source to give libraries **9**−**13**.

Plate Design. Reactions were carried out in a 96-well plate format (8 anhydrides × 11 amines in each plate). Reagents were added from stock solutions by means of a four-needle robot. A different anhydride was used for each row and a different amine for each column for a total of 6 reaction plates. The empty last row in all plates was used as blank control for biological screening. This plate format avoided errors derived from sample handling, since manipulation was reduced to a minimum. After the addition of the reactants, the plates were shaken at 800 rpm for 16 h at 45 °C. The solvent was removed under reduced pressure, and Amberlyst15 was added manually to each plate, followed

Scheme 3. Synthesis of Succinamic Acid Libraries[a]



[a] (a) RNH_2 (1.1 equiv), DCE, 45 °C, 16 h; (b) Amberlyst15 (10 equiv), MeOH, 25 °C, 1 h, (c) 30% NaOMe in MeOH (3 equiv mol^{-1}), 19 h, 25 °C; (d) Amberlyst15 (2.0 equiv), MeOH, 25 °C, 5 h.

by a fixed volume of MeOH. After the plates were stirred for 1 h, the resin was allowed to settle to the bottom of the plate, and the supernatants were transferred into another plate by means of an eight-needle robot. Following solvent evaporation, each plate containing the expected succinamic acids (libraries **1**−**8**) was portioned into different stock plates for analytical purposes and biological screening. In addition, libraries **1**−**5** were further treated with NaOMe in MeOH, followed by Amberlyst15, to afford deprotected libraries **9**−**13**.

Purities. All library members were analyzed by HPLC coupled to mass spectrometry (MS+ and MS−) using evaporative light scattering (ELS), UV (260 nm) detection, or both. Some of the compounds were also checked by ^{1}H NMR to verify the above routine analyses. Analytical data were processed with MassLynx diversity software. It is worth noting that, as a general trend, ^{1}H NMR analyses showed that UV results are not as reliable as those obtained with ELS because most of the library members had little or no absorption in their UV spectra. For this reason, HPLC-ELS-MS was used routinely as purity criterion. Around 40% compounds met our purity standards of higher than 80% purity, with an additional 15% ranging between 70 and 80% purities (Figure 3).

Anhydrides **1**−**8** afforded similar results in terms of purities of the resulting libraries, in the range of 40−50%, as indicated in Figure 4.[21]

The acetate hydrolysis of libraries **1**−**5** (see Scheme 2) was very efficient because the average purities of the resulting libraries **9**−**13** paralleled those of the corresponding *O*-acylated precursors (Figure 5).[22]

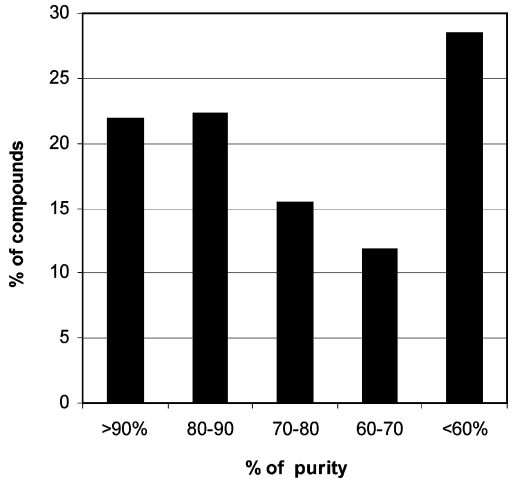


Figure 3. Average purities (based on ELS) of the final 780 compound library.

The amines showed some marked differences in terms of average purities (Figure 6). Although no clear correlations could be deduced from this study, the aliphatic amines showed somewhat higher efficiencies.

Biological Screening. Library members with purities higher than 80% were evaluated as growth inhibitors of the yeast *S. cerevisiae*. Compounds were tested at a 400 μM final concentration in YPD (yeast extract, peptone, dextrose) medium in duplicate runs, with or without 0.5% tergitol to improve the solubility of the more hydrophobic compounds (see Supporting Information for complete data of percent of yeast growth). We have observed that this detergent concentration did not interfere with yeast development.

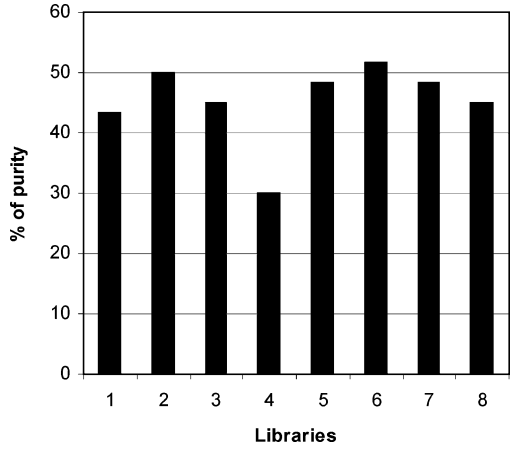


Figure 4. Average purities for libraries **1**−**8**.

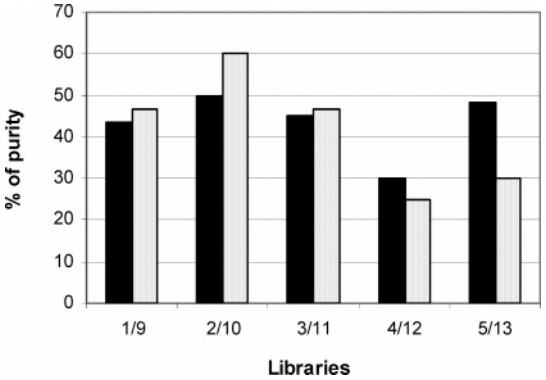


Figure 5. Efficency of the deprotection step in the synthesis of libraries **9**−**13** (dark bars) from libraries **1**−**5** (light bars).

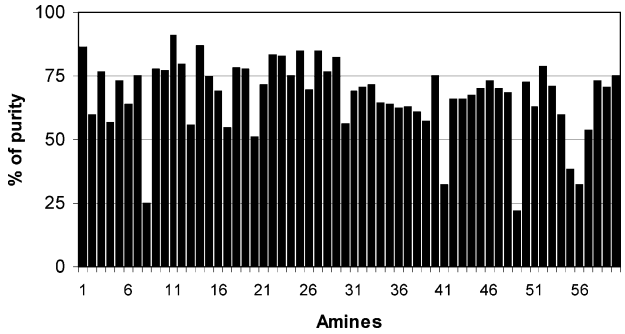


Figure 6. Average purities of library compounds based on the starting amine.

Khafrefungin was also tested as positive control and exhibited a minimal inhibitory concentration (MIC) value in the low micromolar range (10 μM), as previously reported for *S. cerevisiae*.[9−12] Despite the fact that none of the 330 tested compounds completely inhibited yeast growth at 400 μM, the observed results deserve some comments.

As a general trend, the addition of 0.5% tergitol in the incubation medium slightly improved the inhibitory activity of the tested compounds (see Supporting Information for percent growth data for all tested library members). The more active compounds found in this study are shown in Table 1. Some of them inhibited yeast growth up to 40−50% at 400 μM.

As shown in Figure 7, a comparison of activities between acetylated (**1**−**5**) and deacetylated (**9**−**13**) libraries derived from a given amine seem to be favorable to the latter, although no clear differences can be observed.

Table 1. Yeast Growth Inhibition (%) in the Presence of Selected Compounds at 400 μM. Incubations were Performed in YPD Medium Containing 0.5% Tergitol.

compound	growth inhibition	compound	growth inhibition
1{*33*}	45	**10**{*44*}	48
3{*33*}	41	**10**{*46*}	50
5{*53*}	48	**10**{*47*}	40
7{*44*}	44	**10**{*48*}	43
8{*46*}	41	**10**{*53*}	46
8{*47*}	45	**10**{*60*}	43
9{*46*}	50	**12**{*47*}	54
9{*47*}	46	**13**{*47*}	42
9{*53*}	47	**13**{*48*}	46
		13{*49*}	48

Interestingly, compounds from library **9**, having a 2,3-dihydroxy carboxylic acid framework with the same spatial arrangement as in khafrefungin, were generally more active than those arising from the dideoxo analogues (library **7**). However, compounds with an extra methylene group with respect to library **7**, that is, libraries **6** and **8,** showed higher growth inhibitory activity (Figure 8).

Among the amines used for library construction, some of them were consistently found in the active compounds (see Figure 9 and Supporting Information for comparison data of all amines). Those containing the more lipophilic amine **1** were slight inhibitors, while introduction of a double bond (amine **4**) or chain-length shortening (amines **10**, **11**, **14**, and **40**) led to a loss of the inhibitory activity. On the other hand, succinamic acid derivatives obtained by condensation with isopropylamine (**46**) exhibited a uniform inhibition pattern (Figure 9), and some of the resulting compounds were among the most active of this study (Table 1). Amines containing a heterocyclic or an aromatic moiety afforded interesting results. Thus, compounds arising from amines **33**, **44**, **47**, **48**, **49**, **50**, and **55** showed a moderate inhibitory activity (Figure 9).

In conclusion, none of the tested compounds initially designed as khafrefungin analogues inhibited *S. cerevisiae* growth in the same extent than the parent compound. Although a large number of compounds have been tested and some of them have shown a moderate activity, it has not been possible to define a structure−activity profile to design new antifungal compounds based on the present results. Moreover, the reported lack of activity of other khafrefungin analogues[11,12] and the noncompetitive IPC synthase inhibition shown by khafrefungin,[23] suggest a very specific allosteric interaction with the enzyme to generate a conformational change not achievable with the analogues reported so far. New strategies based on IPC synthase inhibition are currently underway in our laboratory, and the results will be published in the near future.

Experimental Section

General Methods. Optimization reactions were carried out using standard "on-bench" protocols. Solvents were distilled prior to use and dried by standard methods. Melting points were uncorrected. FT-IR spectra were reported in

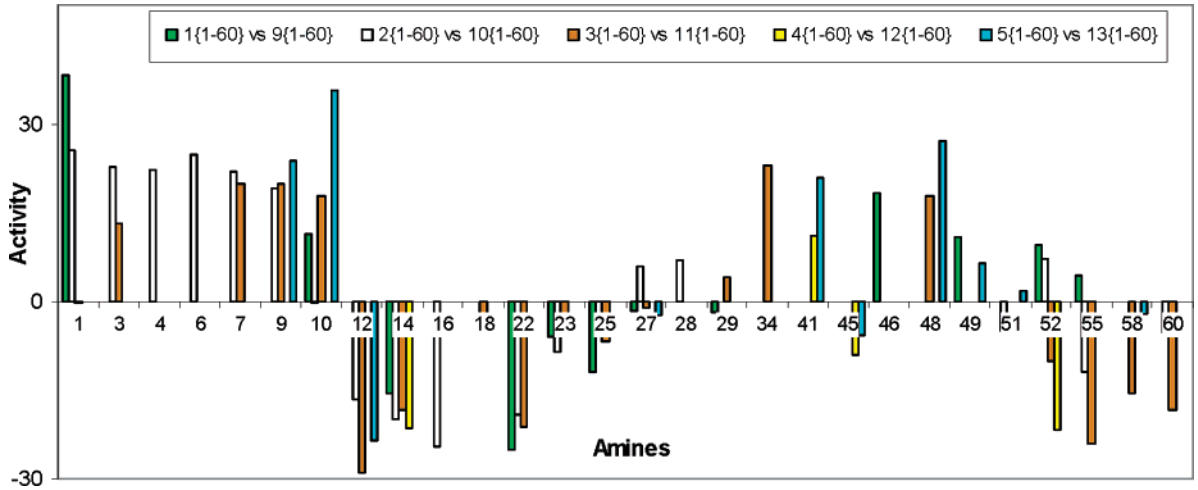


Figure 7. Differences on activity between acetylated (**1**−**5**) and deacetylated (**9**−**13**) libraries incubated overnight with *S. cerevisiae* in YPD medium. In all cases, activity is defined as "a versus b" and indicates percent growth of a minus percent growth of b. A positive result indicates that the corresponding deacetylated compound is better yeast growth inhibitor than the acetylated counterpart

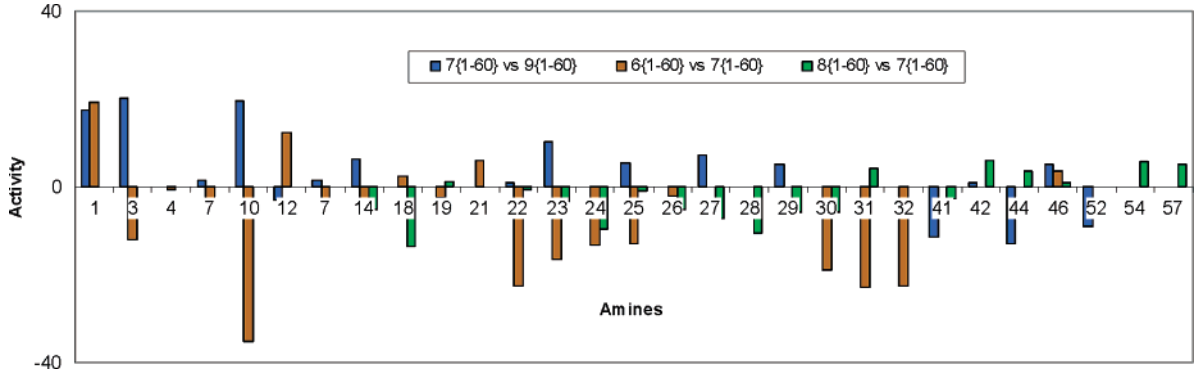


Figure 8. Relative activity of library **7** compared with libraries **6**, **8**, and **9** In all cases, activity is defined as "a versus b" and indicates percent growth of a minus percent growth of b. Compounds were incubated overnight with *S. cerevisiae* in YPD medium.

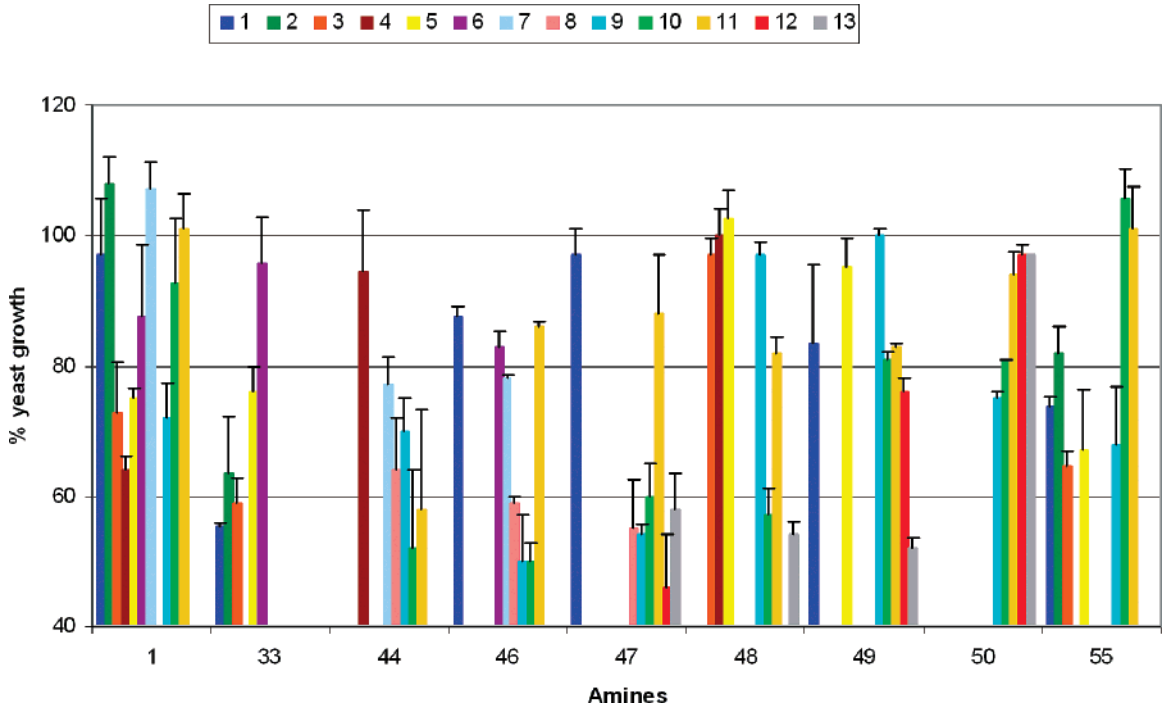


Figure 9. Yeast growth (%) as a function of the amine present in the succinamic acid derivative. Incubations were performed in YPD medium containing 0.5% tergitol.

cm^{-1}. ^{1}H and ^{13}CNMR spectra were obtained in $CDCl_3$ solutions at 500 or 300 MHz (for ^{1}H) and 125 or 75 MHz (for ^{13}C), respectively, unless otherwise indicated. Chemical shifts were reported in (δ) parts per million (ppm) relative

to the singlet at 7.24 ppm of $CDCl_3$ for ^1H and to the center line of a triplet at 77.0 ppm of $CDCl_3$ for ^{13}C. Amberlyst15 was activated prior to use as follows: A batch of 20 g was washed successively with water (200 mL), aqueous 1 N HCl (300 mL), water (100 mL), MeOH (300 mL), CH_2Cl_2 (300 mL), and THF (300 mL). The resin was dried under vacuum for 12 h and used as such in the reaction.

Biological Assay. The isogenic *S. cerevisiae* strain W303a (leu2, ura3, trp1, ade2, his3) was grown in YPD medium, composed of 1% yeast extract, 2% peptone, and 2% glucose. The yeasts were grown with aeration at 30 °C, and the growth was followed turbidimetrically at 600 nm. A yeast suspension in YPD medium was prepared with a final concentration of 0.15 OD per 0.1 mL. Tested compounds were dispensed in the corresponding wells of a 96-well polystyrene microtitre plate to a final concentration of 0.4 mM and inoculated with 0.1 mL of yeast suspension. Growth in liquid YPD with or without 0.5% tergitol in the presence of the synthesized compounds was monitored over a 16 h period. All experiments were repeated at least three times.

Optimization Reactions. Synthesis of Succinamic Acids by Condensation of Anhydrides 1 and 5 with Amines 14, 16, 19, and 21. A solution of the amine (1.1 mmol) in DCE was added dropwise over a solution of the starting anhydride (1 mmol) in DCE (3 mL) at room temperature (RT) . After the mixture was stirred for 12 h at 45 °C, the solvent was removed in vacuo. The resulting residue was taken up in MeOH (3 mL), and Amberlyst15 (100 mg) was added portionwise. The mixture was stirred at RT for 1 h, and a second batch of Amberlyst15 (100 mg) was added. After it was stirred for an additional 2 h, the mixture was filtered; the residue was thoroughly washed with MeOH (3 × 3 mL), and the combined extracts were evaporated to dryness.

Compound 1{*14*}.[24] IR (film, cm^{-1}): ν 3380, 2930, 2850, 1767, 1670, 1542. ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.0, 169.7, 169.3, 166.5, 72.1, 71.4, 39.8, 31.9, 29.5, 29.4 (2×), 26.9, 22.8, 20.7, 20.5, 14.2. ^1H NMR (500 MHz, $CDCl_3$): δ 0.85 (t, 3H), 1.25 (broad, 10H), 1.48 (broad, 2H), 2.11 (s, 3H), 2.17 (s, 3H), 3.19 (m, 1H), 3.31 (m, 1H), 5.59 (m, 1H), 5.57 (m, 1H), 6.65 (t, 1H, amide), 8.40 (broad, 1H, acid). EM: 346.2 $(M + 1)^+$, 368.2 $(M + 23)^+$. $[\alpha]_D$: −6.2 (c = 6, acetone).

Compound 1{*16*}. IR (film, cm^{-1}): ν 3510, 2960−2870, 1760, 1670, 1540. ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.2, 169.9, 168.8, 166.8, 72.1, 71.6, 64.8, 50.0, 39.7, 24.8, 23.1, 21.9, 20.5, 20.3. ^1H NMR (500 MHz, $CDCl_3$): δ 0.90 (dd, 6H), 1.33 (m, 1H), 1.42 (m, 1H), 1.54 (m, 1H), 2.16 (s, 3H), 2.19 (s, 3H), 3.54 (m, 1H), 3.70 (m, 1H), 4.03 (broad, 1H), 5.70 (m, 2H + OH). EM: 332.4 $(M - 1)^+$. $[\alpha]_D$: −26.1 (c = 1, acetone).

Compound 1{*19*}. IR (film, cm^{-1}): ν 3029, 2930, 1756, 1690, 1550. ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.7, 170,5, 168.9, 166.9, 137.1, 129.4, 128.9, 127.1, 72.2, 71.4, 63.4, 51.2, 38.7, 20.6 (2×). ^1H NMR (500 MHz, $CDCl_3$, some signals are duplicated because of rotamers): δ 2.11 (s, 3H), 2.19 (s, 3H), 2.84 (m, 2H), 3.58 (m, 1H), 3.68 (m, 1H), 4.22 (m, 1H), 5.56-5.70 (broad m, 2H), 7.25 (m, 5H). EM: 366.4 $(M - 1)^+$. $[\alpha]_D$: −28.6 (c = 1, acetone).

Compound 1{*21*}.[25] IR (film, cm^{-1}): ν 3360, 2950, 1760, 1670, 1540. ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.8 (2×), 169.5, 166.2, 72.0, 71.4, 43.7, 20.8, 20.4. ^1H NMR (500 MHz, $CDCl_3$): δ 2.05 (s, 3H), 2.17 (s, 3H), 4.33 (dd, J = 15 Hz, J'= 5 Hz, 1H), 4.63 (dd, J = 15 Hz, J'= 6.5 Hz, 1H), 5.67 (broad s, 1H), 5.86 (broad s, 1H), 6.65 (broad, 1H, amide), 7.33 (m, 5H). EM: 322.3 $(M - 1)^+$. $[\alpha]_D$: −15.1 (c = 1, acetone).

Compound 5{*14*}. (Data for the major isomer of a 80/20 mixture of regioisomers by ^1H NMR.) IR (film, cm^{-1}): 3500−3300 (broad), 2920, 2850, 1750, 1715, 1660,1560. ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.9, 169.8, 168.7, 69.6, 39.6, 36.1, 31.7, 29.2, 29.1, 26.7, 22.6, 20.8, 14.0. ^1H NMR (500 MHz, $CDCl_3$): δ 0.82 (t, 3H), 1.20 (broad, 10H), 1.45 (broad, 2H), 2.08 (s, 3H), 2.88 (m, 2H), 3.17 (t, 2H), 5.39 (m, 1H), 6.67 (m, 1H, amide), 10.05 (broad, 1H, acid). EM: 288.2 $(M + 1)^+$, 310.1 $(M + 23)^+$.

Compound 5{*16*}. (Data for the major isomer of a 75/25 mixture of regioisomers by ^1H NMR.) IR (film, cm^{-1}): ν 3500−3200 (broad), 2960, 2870, 1750, 1666, 1544. ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.7, 170.4, 169.6, 69.9, 64.4, 49.7, 39.5, 36.3, 24.6, 22.8 (2×), 20.6. ^1H NMR (500 MHz, $CDCl_3$): 0.82−0.90 (dd, 6H), 1.25 (m, 1H), 1.38 (m, 1H), 1.57 (m, 1H), 2.00 (s, 3H), 2.85 (m, 2H), 3.40 (m, 1H), 3.61 (m, 1H), 3.98 (m, 1H), 5.33 (t, 1H). 6.95 (d, 1H, amide). EM: 276.1 $(M + 1)^+$, 297.3 $(M + 23)^+$.

Compound 5{*19*}. (Data for the major isomer of a 85/15 mixture of regioisomers by ^1H NMR.) IR (film, cm^{-1}): ν 3800−3350 (broad), 3025, 2930, 1750, 1668, 1540. ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.6, 170.6, 169.6, 137.3, 129.2, 128.4, 126.5, 69.8, 62.6, 49.9, 36.4, 36.0, 20.5. ^1H NMR (500 MHz, $CDCl_3$): δ 2.05 (s, 3H), 2.65−2.90 (m, 4H), 3.80 (dd, 2H), 4.15 (broad, 1H), 5.35 (m, 1H), 7.10−7.30 (m, 5H). EM: 310.1 $(M + 1)^+$, 332.1 $(M + 23)^+$.

Compound 5{*21*}. (Data for the major isomer of a 90/10 mixture of regioisomers by ^1H NMR.) IR (film, cm^{-1}): ν 3370, 3029, 2930, 1745, 1664, 1540. ^{13}C NMR (125 MHz, $CDCl_3$): 173.0, 170.6, 169.7, 137.7, 137.6, 128.9, 127.8, 70.2, 43.6, 36.4, 20.9. ^1H NMR (500 MHz, $CDCl_3$): 2.03 (s, 3H), 2.86 (m, 2H), 4.37 (m, 2H), 5.44 (t, 1H), 6.35 (broad), 7.25 (m, 5H). EM: 266.1 $(M + 1)^+$, 288.1 $(M + 23)^+$.

Acetate Hydrolysis. A solution of the intermediate *O*-acetyl succinamic acid in MeOH (3 mL) was treated with 0.5 mL of a 30% MeOH solution of NaOMe. After it was stirred at at RT for 20 h, the reaction mixture was treated twice with 200 mg of Amberlyst15 and stirred for a total of 4 h (2 h for each cycle). The clear solution was filtered; the resin was washed with MeOH (3 × 3 mL), and the combined organic extracts were concentrated to dryness.

Compound 9{*14*}.[26] IR (KBr, cm^{-1}): ν 3450 (broad), 2930, 1645, 1579. 1424, 1330. ^{13}C NMR (125 MHz, D_2O): δ 177.8, 176.8, 73.9, 73.7, 49.0, 39.7, 31.3, 28.6, 26.2, 23.5, 22.3, 13.7. ^1H NMR (500 MHz, D_2O): δ 0.72 (broad, 3H), 1.17 (broad, 10H), 1.39 (broad, 2H), 3.12 (m, 2H), 4.21 (broad, 1H), 4.34 (broad, 1H). $[\alpha]_D$: +47.2 (c = 1, CH_3OH).

Compound 9{*16*}. IR (KBr, cm^{-1}): ν 3600−3300 (broad), 2950, 1650, 1580, 1420. ^{13}C NMR (125 MHz,

D_2O): δ 178.5, 175.8, 73.9, 73.8, 64.2, 49.7, 39.4, 23.4, 22.6 (2×). ^{1}H NMR (500 MHz, D_2O): δ 0.72 (dd, 6H), 1.20 (m, 1H), 1.29 (m, 1H), 1.44 (m, 1H), 3.45 (m, 2H), 4.15 (broad, 1H), 4.30 (broad, 1H). EM: 250.1 $(M + 1)^+$. $[\alpha]_D$: +10.3 ($c = 1$, acetone).

Compound 9{*19*}. IR (KBr, cm^{-1}): ν 3300−3100 (broad), 1640, 1580 1423. ^{13}C NMR (125 MHz, D_2O): δ 178.6, 176.2, 138.4, 129.5, 128.7, 126.7, 74.0, 73.8, 63.3, 53.0, 36.7. ^{1}H NMR (500 MHz, D_2O): δ 2.60 (dd, $J = 14$ Hz, $J' = 9$ Hz, 1H), 2.81 (dd, $J = 14$ Hz, $J' = 5.5$ Hz, 1H), 3.47 (dd, $J = 11.5$ Hz, $J' = 6$ Hz, 1H), 3.53 (dd, $J = 11.5$ Hz, $J' = 4.5$ Hz, 1H), 4.02 (m, 1H), 4.07 (broad s, 1H), 4.15 (broad s, 1H), 7.15 (m, 5H). EM: 282.1 $(M - 1)^+$. $[\alpha]_D$: +6.8 ($c = 1$, acetone).

Compound 9{*21*}. IR (KBr, cm^{-1}): ν 3500−3200 (broad), 3000, 1650, 1580, 1425, 1330. ^{13}C NMR (125 MHz, D_2O): δ 178.6 (2×), 138.1, 128.9, 127.5, 127.2, 42.8. ^{1}H NMR (500 MHz, D_2O): δ 3.38 (broad, 1H), 3.45 (broad, 1H), 4.25 (broad s, 1H), 4.40 (broad s, 1H), 7.25 (m, 5H). EM: 238.2 $(M - 1)^+$. $[\alpha]_D$: +7.5 ($c = 0.5$, H_2O).

Compound 13{*14*}. (Major isomer.)[18] IR (KBr, cm^{-1}): ν 3450 (broad), 2920, 2854, 1640, 1580, 1450. ^{13}C NMR (125 MHz, CD_3OD): δ 178.3, 175.4, 69.8, 41.3, 38.9, 31.8, 29.4, 29.3, 26.8, 22.5, 13.3. ^{1}H NMR (500 MHz, CD_3OD): δ 0.89 (t, 3H), 1.32 (broad, 10H), 1.54 (m, 2H), 2.41 (dd, 1H, $J = 15.5$ Hz, $J' = 8.5$ Hz), 2.64 (dd, 1H, $J = 15.5$ Hz, $J' = 4.0$ Hz), 3.22 (m, 2H), 4.31 (m, 1H). EM: 246.1 $(M + 1)^+$.

Compound 13{*16*}. (Major isomer.) IR (KBr, cm^{-1}): ν 3800−3100 (broad), 1680 (broad), 1322 (broad). ^{13}C NMR (125 MHz, D_2O): δ 177.2, 173.5, 74.9, 64.1, 50.2, 41.9, 39.1, 24.4, 23.5. ^{1}H NMR (300 MHz, D_2O): δ 0.73−0.80 (dd, 6H), 1.19 (m, 1H), 1.25 (m, 1H), 1.46 (m, 1H), 2.51 (m, 1H), 2.65 (m, 1H), 3.37 (m, 1H), 3.48 (m, 1H), 3.81 (broad, 1H), 4.17 (m, 1H). EM: 232.3 $(M - 1)^+$.

Compound 13{*19*}. (Major isomer.) IR (KBr, cm^{-1}): ν 3450 (broad), 1670−1630 (broad), 1414, 1320. ^{13}C NMR (125 MHz, D_2O): δ 171.2, 165.0, 138.3, 129.5, 128.7, 126.7, 70.5, 63.1, 53.5, 42.8, 36.5. ^{1}H NMR (500 MHz, CD_3OD): δ 2.21 (m, 1H), 2.58 (m, 1H), 2.81 (m, 1H), 2.95 (m, 1H), 3.50 (m, 2H), 4.10 (m, 1H), 4.35 (m, 1H), 7.35 (m, 5H). EM: 268.0 $(M + 1)^+$.

Compound 13{*21*}. (Major isomer). IR (KBr, cm^{-1}): ν 3400 (broad), 1670−1650 (broad), 1440−1420, 1330. ^{13}C NMR (125 MHz, CD_3OD): δ 179.5, 176.9, 140.0, 129.5, 128.4, 128.1, 71.0, 43.6, 42.7. ^{1}H NMR (500 MHz, CD_3OD): δ 2.45 (m, 1H), 2.70 (m, 1H), 4.45 (m, 2H), 7.30 (m, 5H). EM: 224.0 $(M + 1)^+$.

Automated Process. All liquid handling for the reactions was performed using a 4 needle MultiProbe HT from Perkin-Elmer using specifically designed racks to hold the reagent tubes and the reaction plates. An eight-needle MultiProbe HT from Perkin-Elmer was use for all plate replication.

The reactions were performed in glass reaction plates from Zinsser which were held in Charybdis frames. A septum was placed on the top of each plate thus enabling heating without evaporation of solvent. After the reaction, plate evaporation was achieved with a HT12-series II system from Genevac. All HPLC-ELS-MS analytical spectra were recorded for each sample using the following conditions: MS Micromass MUX 9 way, ESP+ve, CV 25V, 100−1000 amu (PT1-3 Fractionlynx, ESP±, ELS + DAD available); HPLC Luna C18 (2), 50 × 4.6 mm ID, 3 μm [(A) acetonitrile + 0.1% formic acid (v/v), (B) water + 0.1% formic acid (v/v)], 10% A (1 min) to 95% A in 4 min, hold 1 min, RT 6.0 min, 2.0 mL/min; UV 260 nm; sample solvent, DMSO. Analytical data were processed with MassLynx diversity software (Waters Corporation). HRMS were obtained with a Waters Micromass LCT Premier apparatus equipped with a dual electrospray (ESI) LockSpray ion source, and data were acquired in positive ESI. Compounds were analyzed by flow inject analysis using acetonitrile/water (75:25) as the mobile phase.

Synthesis of Libraries 1{*1*−*60*}−9{*1*−*60*} by Reaction of Amines 1−60 with Anhydrides 1−9. Solutions of 0.09 mmol of the corresponding anhydride (**1**−**9**) in 300 μL DCE were loaded into a glass plate. Subsequently, aliquots of 1.1 equiv mol^{-1} of the corresponding amines, **1**−**61**, in 300 μL DCE were added. The plates were shaken at 300 rpm overnight at 45 °C. Then, the solvent was removed, and activated Amberlist-15 (80 mg) and methanol (1 mL) were added. The plates were shaken at 800 rpm at RT for 1 h. The suspensions were filtered, and the resin was thoroughly washed with methanol (300 μL). The combined solutions were concentrated under reduced pressure to afford the required libraries, which were submitted to HPLC-MS analysis.

Synthesis of Libraries 9{*1*−*60*}−13{*1*−*60*} from Libraries 1{*1*−*60*}−5{*1*−*60*}. Each plate position was treated with MeOH (400 μL) and 3 equiv mol^{-1} of a 30% MeOH solution of NaOMe. The plates were shaken for 19 h at RT. Subsequently, 160 mg of activated Amberlist-15 was added, and the mixtures were shaken at 800 rpm for 5 h. The resulting clear solutions were filtered, and the solids were washed with MeOH (400 μL). The combined organic solutions were concentrated under reduced pressure to afford the required libraries.

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Supporting Information Available. HRMS and percent yeast growth for selected library compounds (purity by ELS ≥ 80%), NMR data for model compounds **1**{*14*}, **1**{*16*}, **1**{*19*}, **1**{*21*}, **5**{*14*}, **5**{*16*}, **5**{*19*}, **5**{*21*}, **9**{*14*}, **9**{*16*}, **9**{*19*}, **9**{*21*}, **13**{*14*}, **13**{*16*}, **13**{*19*}, and **13**{**21**}, and a graphical representation of the percent of yeast growth

inhibition for all library members. This material is available free of charge via the Internet at http://pubs.acs.org.

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(20) Discrimination between C1 and C4 adducts was not possible in the analytical conditions used for libraries **4**, **5**, **12**, and **13**.

(21) Differences found between enantiomeric anhydrides **4** and **5** are surprising. These could be the result of unpredictable partial acetate hydrolysis of starting anhydride **4**.

(22) Libraries **9**−**13** were synthesized from an independent set of libraries **1**−**5**. Thus, the observed differences in average purities could be caused y the inherent variability among each set of libraries.

(23) Unpublished results from our laboratory.

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