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Preliminary investigation of the yeast-mediated reduction of β-keto amides derived from cyclic amines as potential resolution methodology

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Abstract—2-Methylpiperidine and 2-cyclohexylpiperidine were converted to their respective β-keto amides by treatment with diketene. Several strains of yeast were used to reduce the racemic β-keto amides. The unreacted enantiomers were separated from the β-hydroxy amides, the amides cleaved, and the extent of resolution was determined for the cyclic amines. Detailed experimental and spectral data are provided for all compounds.

1. Introduction

The reduction of β-keto esters and other dicarbonyl compounds by enzymes from natural yeast strains is well established.[1] Further advances in this field provided recombinant strains that are either (*R*)- or (*S*)-specific for the configuration of the resulting β-hydroxy carbonyl compounds.[2] These biotransformations yield valuable chiral building blocks and, in those instances where additional unsaturation is present in the substrates, are superior to other methods of reduction such as Noyori hydrogenation.[3]

The next logical step was to investigate asymmetric induction at remote stereogenic centers in the keto ester portion,[4] as indicated in Figure 1. The results of these experiments showed that the enantiomeric excess at remote centers decreases with the increasing distance (d_2) of such centers from the reaction site.[5] Surprisingly, there have been no investigations reported on the extent of asymmetric induction at a prochiral center contained within the alkoxide part of the molecule. The distance of this center (d_1) from the site of the enzymatic reduction remains constant.

In 1991, we reported the first example of the resolution of racemic alcohols by the yeast-mediated reduction of their keto esters,[6] as shown in Figure 2. We found that reasonable levels of enantiomeric enrichment were produced for those alcohols containing larger groups.[7] An application of this methodology in the field of pyrrolizidine alkaloid synthesis led to the concise preparation of several natural products.[8,9] Keto ester **9** was reduced to β-hydroxy ester **10**, as shown in Figure 3.

At longer reaction times, the unreduced enantiomer **11** was racemized, via the enolized dienic ester **12a**, possibly formed by an intramolecular abstraction of the proton from the enolized β-keto ester **12b**. Thus, all the mass of

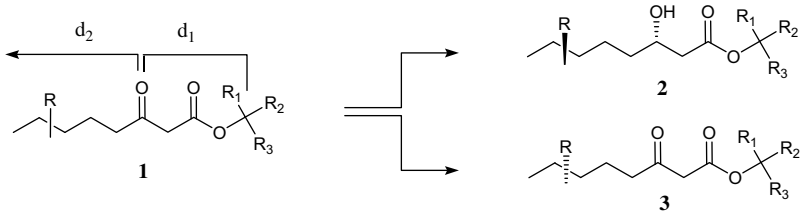

Figure 1. Enantiomeric enrichment at prochiral centers as a function of distance from the reduction site; $\lim_{d_2 \to \infty} (ee) = 0$.

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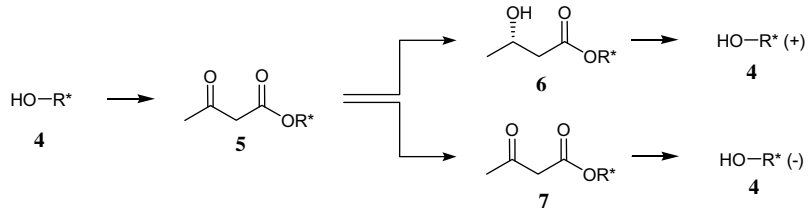


Figure 2. Yeast-mediated resolution of alcohols via the reduction of β-keto esters [(+) and (−) arbitrarily assigned].

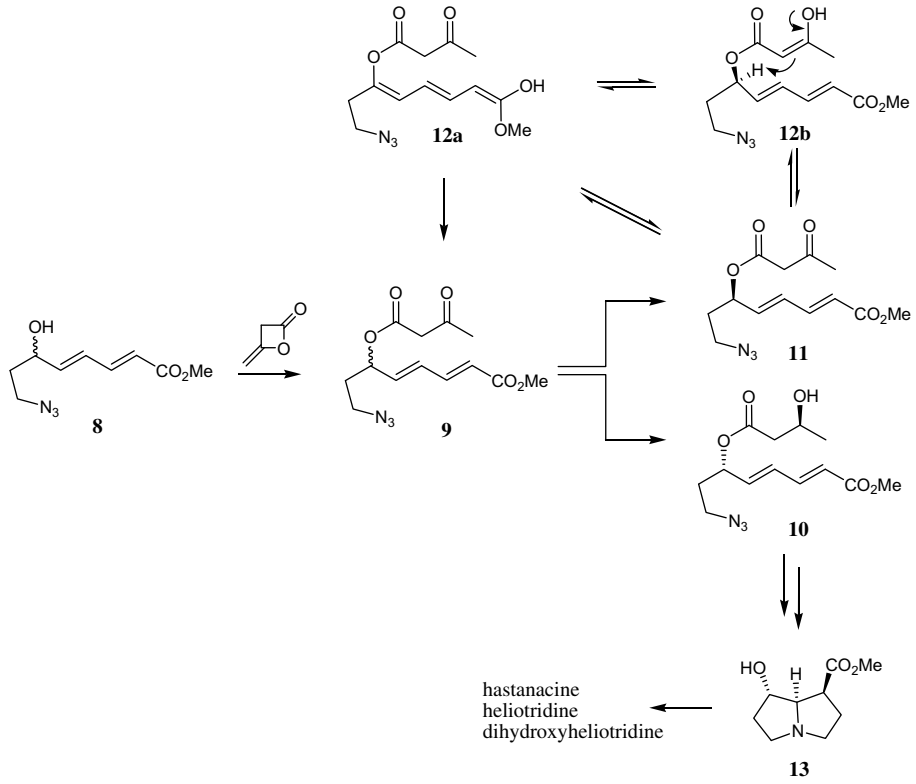


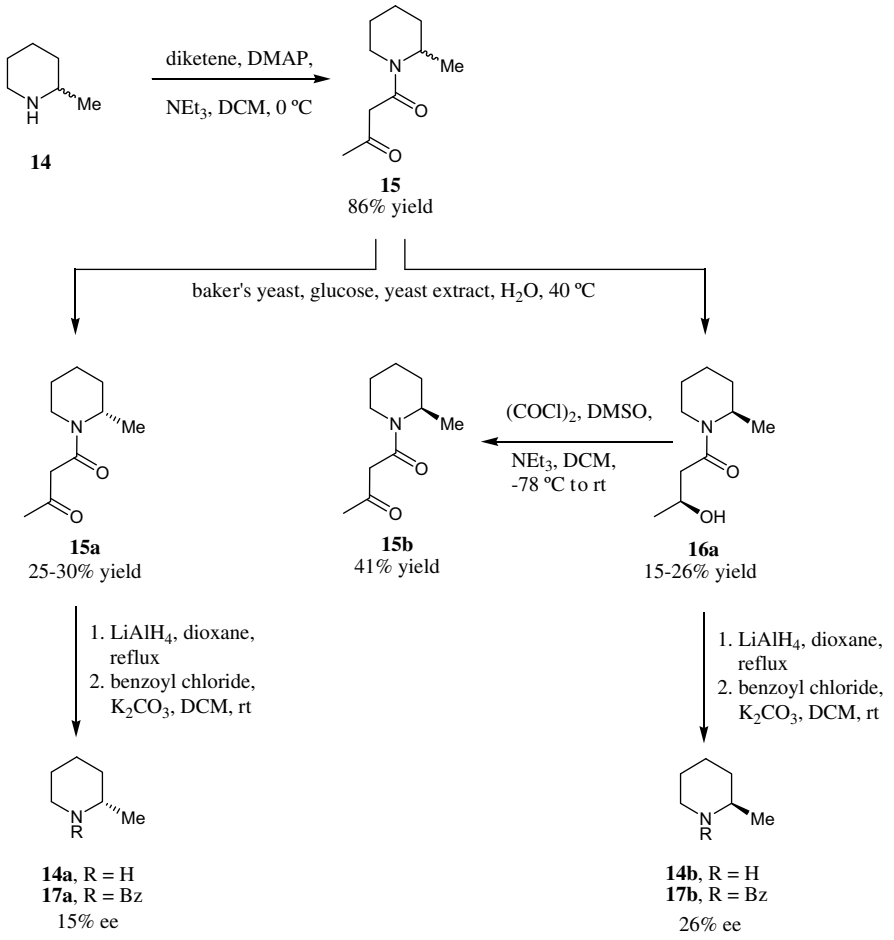
Figure 3. Asymmetric synthesis of pyrrolizidine alkaloids by [4+1] pyrroline annulation and yeast-mediated resolution of β-keto esters.

racemic **9** was converted to hydroxyl ester **10**, whose further reactions provided the pyrrolizidine ester **13**, a precursor to several naturally occurring alkaloids.

In an extension of this method to similar reductions of β-keto amides,[10] we found that the extent of resolution of conformationally flexible amides derived from acyclic amines was lower than that found for β-keto esters of racemic alcohols. Herein, we report the preliminary results of the reductions of β-keto amides derived from cyclic amines as a means of their resolution. As cyclic amines containing alkyl groups at C-2 are commonly used as organocatalysts,[11] yeast-mediated reduction could potentially lead to both enantiomers of such amines, not always available from naturally derived substances.

2. Results and discussion

For our initial study, we chose two cyclic amines containing substituents of varying size α to the nitrogen atom and converted them to the corresponding β-keto amides by reaction with diketene, as shown in Scheme 1. The progress of the yeast-mediated reduction of **15** was followed by GC/MS, and was stopped after 50% conversion of the starting material. The reaction provided β-keto amide **15a** and β-hydroxy amide **16a**, which were separated by flash column chromatography and converted to the free amines by treating either β-keto amide **15a** or β-hydroxy amide **16a** with an excess of $LiAlH_4$ in refluxing dioxane. Conversion of the amines to their Mosher amides did not allow the estimation of the enantiomeric excess by ^{19}F NMR, ^{1}H NMR, GC/MS, or HPLC, as no separation or distinction of the diastereomeric compounds was achieved by these methods. ^{19}F NMR spectra of the diastereomeric mixture showed only one signal for both compounds. No suitable conditions were found for the separation of the diastereomeric compounds by GC/MS or HPLC. The formation of benzamide derivatives **17a** and **17b**, prepared by the acylation of the free amines, led to a scalemic mixture, which was separated by chiral HPLC. An enantiomerically pure sample of **17a** for HPLC analysis was easily obtained



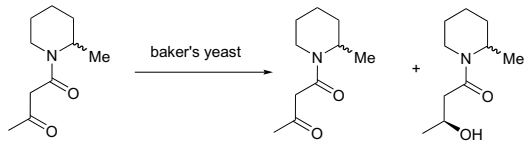
Scheme 1. Yeast reduction of 2-methylpiperidine β-keto amide **15**.

by the acylation of commercially available (*S*)-2-methylpiperidine with benzoyl chloride, allowing the assignment of the absolute configuration of the resolved amines.

The first cycle of the yeast reduction produced about 15% ee in β-keto amide **15a** and 26% ee in β-hydroxy amide **16a**, as determined at the stage of benzamides **17a** and **17b**. The β-hydroxy amide **16a** was oxidized in moderate yield by means of standard Swern conditions[12] to β-keto amide **15b**. Both keto amides, enriched as noted above, were then resubjected to the second cycle of yeast reduction.

As expected, the second bioreduction of β-keto amide **15a** provided enrichment to 25% ee, as determined after amide cleavage and acylation to the benzamide derivative. However, the results for the reduction of β-keto amide **15b**, obtained by the oxidation of β-hydroxy amide **16a**, were quite unexpected and revealed that the second yeast reduction did not enrich further the enantiomeric purity of the (*R*)-2-methylpiperidine. The results for both experiments are shown in Table 1.

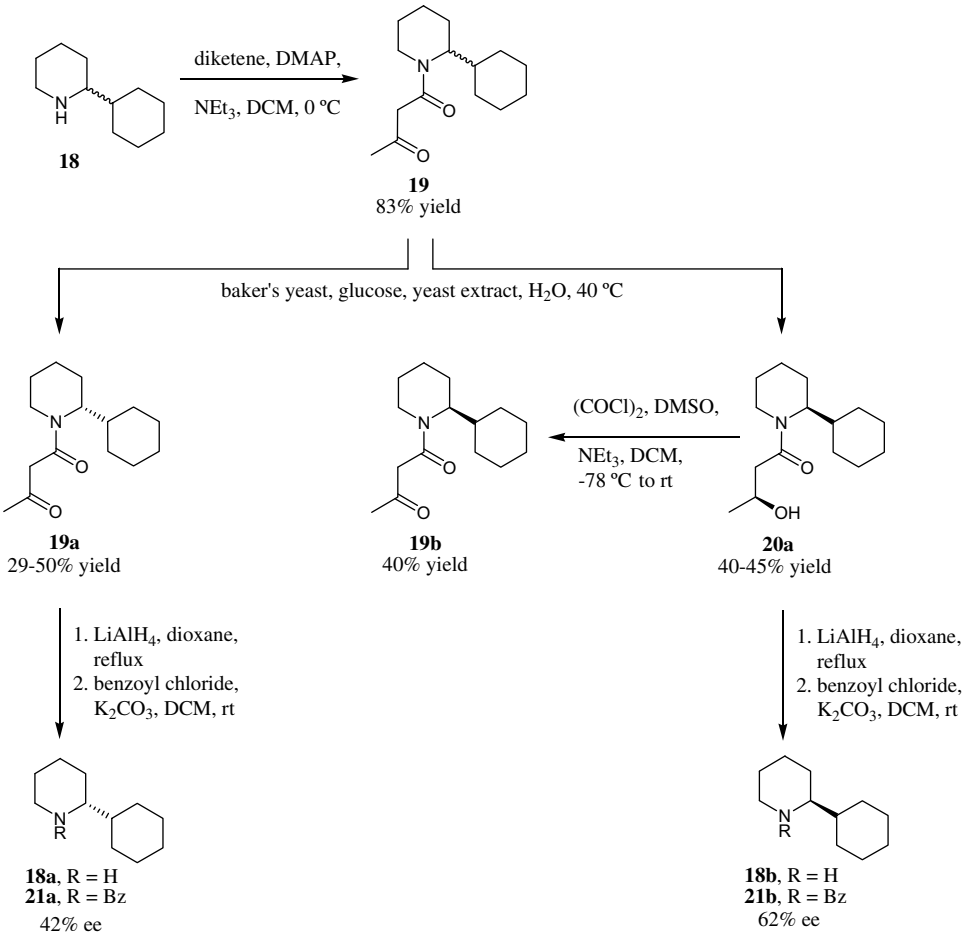
Table 1. Second yeast-reduction cycle of optically enriched **15a** and **15b**



	2nd Yeast-reduction cycle	
	β-Keto amide[a]	β-Hydroxy amide[a]
β-Keto amide **15a** (+)-15% ee	35–43% yield (+)-25% ee	26–35% yield (−)-12% ee
β-Keto amide **15b** (−)-26% ee	28–35% yield (−)-2% ee	39% yield (−)-2.5% ee

[a] Enantiomeric excess was determined by chiral HPLC, after the cleavage of the amide and acylation with benzoylchloride to **17a** and **17b**, respectively.

After the unexpected outcome of the second yeast-reduction cycle, we examined the resolution of 2-cyclohexylpiperidine **18** by the reduction of β-keto amide **19**, as shown in Scheme 2. The reduction of **19** produced higher enrichments in the first cycle: 42% enantiomeric excess in **19a** and 62% enantiomeric excess in β-hydroxy amide **20a**. The enantiomeric excess was determined after the conversion of **19a** and **20a** to the corresponding benzamide derivatives **21a** and **21b**, respectively. The second yeast-reduction cycles of compounds **19a** and **19b** (**19b** was obtained upon the oxidation



Scheme 2. Yeast reduction of 2-cyclohexylpiperidine β-keto amide **19**.

of β-hydroxy amide **20a**) showed a similar trend to the results obtained for the second yeast reduction cycle of the 2-methylpiperidine derivatives. The enantiomeric excess of β-keto amide **19a**, determined after amide cleavage and acylation with benzoyl chloride, was enriched further to 72%. The reduction of β-keto amide **19b** gave a slight decrease in the enantiomeric excess for both the β-keto amide and β-hydroxy amide derivative, as shown in Table 2.

The absolute stereochemistry of the 2-cyclohexylpiperidine derivatives was determined by the synthesis of an enriched standard **21a**, via the hydrogenation of (*R*)-2-phenylpiperidine **26**, which was obtained by a literature procedure,[13] as shown in Scheme 3. Cyclocondensation of (*R*)-phenylglycinol **22** with 5-phenyl-5-oxopentanoic acid **23**, followed by the reductive opening of cyclic amide **24** with 9-borabicyclo[3.3.1](9-BBN) yielded a mixture of two diastereomers in a ratio of approximately six to four. Removal of the benzylethanol group by hydrogenolysis afforded (*R*)-2-phenylpiperidine **26** {$[\alpha]_D^{23} = +3.6$ (*c* 0.6, $CHCl_3$), lit.[13] $[\alpha]_D^{22} = +63.8$ (*c* 0.5, $CHCl_3$)}, which was further hydrogenated and reacted with benzoyl chloride to give the enriched standard **21a** in 6% ee,[14] sufficient for assigning absolute stereochemistry to **21a** by comparison. The absolute stereochemistry of the alcohol portion was not determined, but was assumed to be (*S*) as expected from the baker's yeast reduction of β-keto esters or β-keto amides.[6,10]

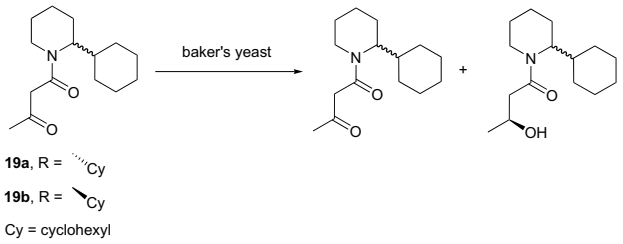
A third β-keto amide derivative, derived from 2-phenyl-3,4-dihydro-2*H*-quinoline, was also subjected to bioreduction. The resulting β-keto amide **27a** and the β-hydroxy amide **28** were both isolated in racemic form.[15] These results indicate that the yeast-mediated reduction of quinoline derivative **27** showed no kinetic resolution and that the reductase or reductases did not distinguish between the (*R*)- and (*S*)-enantiomer.

After the promising results in the resolution of 2-cyclohexylpiperidine with baker's yeast, we investigated the bioreduction and resolution with various yeast strains available from wine cultures. The results are shown in Table 3.

The K1 V1116 and EC 1118 strains achieved only 33% and 30% conversions, respectively, and no further conversion was observed even after an incubation of two weeks. In both cases the enantiomeric excess was lower than for bioreduction with baker's yeast from the local supermarket.

Biotransformations with CY 3079 and BA 11 strains furnished the β-keto amides with slightly higher enantiomeric excess compared to that obtained with baker's yeast (49% for CY 3079 and 41% for BA 11). In contrast, the enantiomeric excess of the β-hydroxy amides was significantly lower in both the cases.

Table 2. Second yeast-reduction cycle of enantiomerically enriched **19a** and **19b**



	2nd Yeast-reduction cycle	
	β-Keto amide[a]	β-Hydroxy amide[a]
β-Keto amide **19a** (+)-42% ee	40–48% yield (+)-72% ee	28–36% yield (−)-18% ee
β-Keto amide **19b** (−)-62% ee	28–36% yield (−)-45% ee	48% yield (−)-59% ee

[a] Enantiomeric excess was determined by chiral HPLC, after cleavage of amide and acylation with benzoylchloride to **21a** and **21b**, respectively.

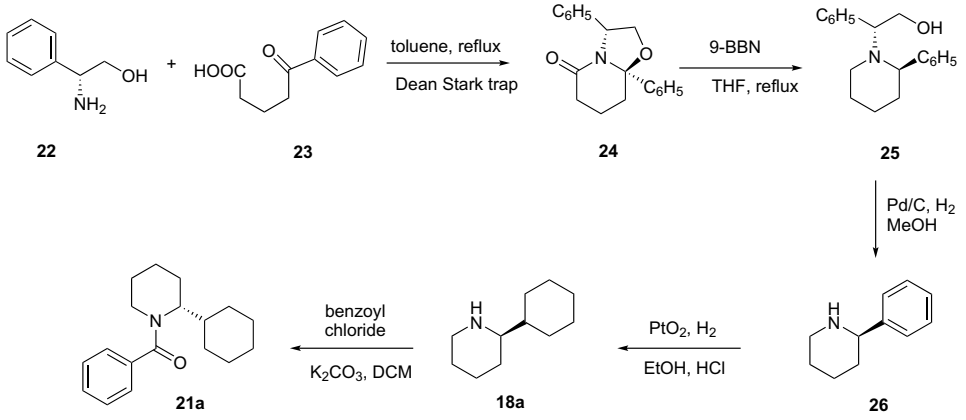
To compare the results with more traditional methods, we investigated the reduction of β-keto amide derivatives of 2-methylcyclopiperidine and 2-cyclohexylpiperidine with known asymmetric reducing agents or catalysts. In the case of Noyori's catalyst,[17] at a hydrogen pressure of up to 1400 psi, or borane reduction with Corey's catalyst,[18] only the starting material could be recovered. In the case of β-chlorodiisopinocampheylborane (DIP-Cl), the starting material was degraded.

3. Conclusion

The yeast-mediated reduction of keto amides derived from the two piperidines appears to follow principles similar to those in the reduction of β-keto esters or acyclic β-keto amides; that is, the extent of the resolution of the adjacent stereogenic center is a function of the steric bulk at the C-2 of the cyclic amine. At this time, we cannot explain the unexpected decrease in the enantiomeric excess during the second cycle of the yeast reduction of the keto amides obtained by the reoxidation of enriched β-hydroxy amides. As the mass balance of the reductions has not been completely accounted for, the lack of enrichment may be due to either the loss of material during purification or possibly due to unrelated metabolic processes operating during the fermentation. The results reported here are only preliminary and are based on the resolution of two compounds containing the substituents of differing *A*-values. A detailed investigation of 5- and 6-membered cyclic amines containing substituents of different steric bulk in the 2-position will be conducted to assess the practicality and potential of using this methodology for providing resolved cyclic amines for the use as catalysts. Further improvements will also be considered by using recombinant yeast strains[2] with known (*R*)- or (*S*)-selectivity.

4. Experimental

All non-aqueous reactions were carried out in an argon atmosphere using standard Schlenk techniques for the exclusion of moisture and air. Methylene chloride was distilled from calcium hydride. THF, benzene, and toluene were dried over potassium/benzophenone. Analytical thin layer chromatography was performed on Silicycle 60 Å 250 μm TLC plates with F-254 indicator. Flash column chromatography was performed using Natland 200–400 mesh silica gel. The two centrifuges used are the Beckman Model TJ-6 centrifuge using 50 mL conical tubes and the Eppendorf Centrifuge 5414 using 1.5 mL M.C. tubes. Melting points were recorded on a Hoover Unimelt apparatus



Scheme 3. Preparation of enantiomerically enriched standard **21a**.

Table 3. Results of the bioreduction of 2-cyclohexylpiperidine β-keto amide with various yeast strains

Entry	Yeast strain	Ketone ee (%)	Alcohol ee (%)	Conversion (%)
1	baker's yeast	42	62	50
2	K1 V1116 (*Saccharomyces cerevisiae cerevisiae*)[16]	32	46	33
3	EC 1118 (*Saccharomyces cerevisiae bayanus*)[16]	23	—	30
4	CY 3079 (*Saccharomyces cerevisiae*)[16]	49	26	50
5	BA 11 (*Saccharomyces cerevisiae cerevisiae*)[16]	41	30	50

and are uncorrected. IR spectra were obtained on a Perkin–Elmer One FT-IR spectrometer. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. The GC/MS data were obtained on a Perkin-Elmer Clarus 500 Gas Chromatograph and Mass Spectrometer using a Perkin Elmer Elite-5MS column, 10 m, 0.25 mm ID, 2 mL/min helium flow. For monitoring the conversion of the yeast reduction the following temperature program were used: for 2-methylpiperidine, 50 °C, 15 °C/min to 330 °C (2 min); for 2-cyclohexylpiperidine and 1,2,3,4-tetrahydro-2-phenylquinoline, 50 °C (2 min), 10 °C/min to 300 °C (3 min). Chiral HPLC analyses were performed on Agilent 1100 Series HPLC with a Daicel Chiralpak AS-H column, 1 mL/min flow, hexanes/isopropanol 9:1 at room temperature, courtesy of Dr. C. Metallinos of Brock University. Retention times for the (2-methylpiperidin-1-yl)-phenyl-methanone are 17.0 and 20.5 min, (2-cyclohexyl-piperidin-1-yl)phenyl-methanone are 18.5 and 20.9 min, and (3,4-dihydro-2-phenylquinolin-1(2*H*)-yl)-phenyl-methanone are 19.9 and 22.6 min. ^{1}H and ^{13}C NMR spectra were recorded on a Brucker (300 MHz or 600MHz) spectrometer. All chemical shifts are referenced to TMS or residual undeuterated solvent. Mass spectra were performed by Tim Jones, at Brock University. Combustion analyses were carried out by Atlantic Microlabs, Norcross, GA.

4.1. General procedure for the formation of β-keto amides

The amine (10 mmol), DMAP (4.4 mmol), and triethylamine (10 mmol) were dissolved in methylene chloride (15 mL) and cooled to 0 °C. Diketene (30 mmol) was added dropwise to the reaction mixture. After the addition was complete, the reaction mixture was allowed to warm to room temperature and stirred until complete conversion of the starting material. The reaction was quenched by the addition of 15% NaOH (15 mL). The mixture was extracted three times with methylene chloride. The organic layers were combined and washed four times with 1 M HCl and once with brine, then dried over anhydrous $MgSO_4$, and filtered. The solvent was evaporated under reduced pressure.

4.1.1. 1-(2-Methyl-piperidin-1-yl)-butane-1,3-dione 15. The crude product was purified by Kugelrohr distillation affording the title compound (86%) as a clear and colourless oil. Bp 140–150 °C (0.4 mmHg); R_f 0.5 (methylene chloride/methanol 96:4); IR ν 3483, 2939, 2865, 1721, 1634, 1582, 1441, 1359, 1272, 1181, 1152, 1008; ^{1}H NMR (300 MHz, $CDCl_3$) (two rotamers) δ 15.0 (s, 0.3H), 5.08 (s, 0.3H), 4.78–4.92 (m, 1H), 4.43 (d, $J = 11.5$ Hz, 1H), 3.90–4.01 (m, 1H), 3.35–3.56 (m, 5H), 3.09 (dt, $J = 13.0$, 2.3 Hz, 1H), 2.64 (t, $J = 13.0$ Hz, 1H), 2.21 (d, $J = 6.8$ Hz, 6H), 1.89 (s, 1H), 1.44–1.71 (m, 11H), 1.25–1.43 (m, 2H), 1.18 (d, $J = 6.9$ Hz, 3H), 1.11 (d, $J = 7.0$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.8, 202.7, 164.9, 164.7, 50.7, 50.2, 49.3, 44.0, 41.7, 36.5, 30.6, 30.1, 30.0, 29.7, 26.1, 25.4, 22.1, 18.7, 18.5, 16.6, 15.5; MS (EI) m/z (%): 183 (19), 168 (19), 140 (20), 98 (22), 97 (18), 85 (14), 84 (100), 56 (17), 55 (13), 43 (38), 42 (14), 41 (13); HRMS calcd for $C_{10}H_{17}NO_2$ 183.1259, found 183.1254. Anal. Calcd for $C_{10}H_{17}NO_2 \cdot 1/6H_2O$: C, 64.49; H, 9.38. Found: C, 64.69; H, 9.38.

4.1.2. 1-(2-Cyclohexyl-piperidin-1-yl)-butane-1,3-dione 19. The crude product was purified by flash column chromatography (hexanes/ethyl acetate 3:1→1:2) affording the title compound (83%) as a clear, slightly yellow oil; R_f 0.4 (methylene chloride/hexanes/diethyl ether 8:1:4); IR ν 3425, 3252, 2928, 2852, 2663, 1721, 1631, 1579, 1484, 1446, 1390, 1358, 1315, 1259, 1214, 1190, 1176, 1159, 1144, 1107, 1086, 1071, 1025, 1006; ^{1}H NMR (300 MHz, $CDCl_3$) (two rotamers) δ 15.0 (s, 0.3H), 5.13 (s, 0.3H), 4.54 (d, $J = 12.3$ Hz, 1H), 4.41 (d, $J = 6.6$ Hz, 1H), 3.33–3.58 (m, 4H), 3.05 (t, $J = 13.2$ Hz, 1H), 2.54 (t, $J = 12.6$ Hz, 1H), 2.26 (s, 4H), 1.25–2.04 (m, 24H), 1.10–1.26 (m, 6H), 0.92–0.98 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.8, 202.7, 165.4, 164.9, 59.6, 53.5, 50.8, 50.3, 42.5, 37.3, 35.4. 34.8, 30.4, 30.1, 30.0, 29.9, 29.6, 28.8, 26.4, 26.1, 26.1; MS (EI) m/z (%): 169 (13), 168 (74), 85 (16), 84 (100), 43 (15); HRMS calcd for $C_{15}H_{25}NO_2$ 251.1885, found 251.1882. Anal. Calcd for $C_{15}H_{25}NO_2 \cdot 1/4\ H_2O$: C, 70.41; H, 10.05. Found: C, 70.68; H, 10.04.

4.1.3. 1-(2-Phenyl-3,4-dihydro-2*H*-quinolin-1-yl)-butane-1,3-dione 27. The crude product was purified by flash column chromatography (hexanes/ethyl acetate 5:1) affording the title compound (57%) as a yellow oil. R_f 0.7 (hexanes/ethyl acetate 1:1); IR ν 3425, 3062, 3028, 2951, 1721, 1635, 1603, 1581, 1491, 1454, 1411. 1380, 1358, 1316, 1251, 1208, 1179, 1157, 1115, 1074, 1030; ^{1}H NMR (300 MHz, $CDCl_3$) δ 14.2 (s, 0.3H), 7.18–7.43 (m, 9H), 5.75 (s, 0.4H), 5.64 (t, $J = 8.1$ Hz, 0.4H), 3.60–3.74 (m, 1H), 2.63–2.73 (m, 3H), 2.06 (s, 2H), 1.89 (s, 1H), 1.73–1.79 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.6, 174.8, 171.6, 166.6, 143.4, 137.6, 128.4, 127.4, 126.7, 126.6, 126.0, 126.0, 125.8, 125.7, 125.4, 125.2, 124.8, 89.1, 88.8, 60.2, 56.6, 50.0, 34.3, 34.2, 30.4, 30.3, 26.6, 26.5, 26.5, 23.8, 21.7, 14.0; MS (EI) m/z (%): 217 (27), 209 (10), 134 (10), 133 (100), 132 (69), 131 (15), 130 (13), 118 (14), 117 (10), 85 (15), 84 (11), 77 (12), 69 (13), 43 (57); HRMS calcd for $C_{19}H_{19}NO_2$ 293.425, found 293.415.

4.2. General procedure for the chemical reduction of β-keto amides to racemic β-hydroxy amides

To a solution of the corresponding β-keto amide (1.09 mmol) in MeOH (5 mL) was added $NaBH_4$ (1.20 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature over 30 min followed by the addition of 1 M NaOH (2 mL).

The solvent was evaporated and the residue diluted with H_2O (5 mL). The aqueous layer was extracted three times with methylene chloride. The organic layers were combined and dried over anhydrous $MgSO_4$, filtered, and the solvent was evaporated under reduced pressure.

4.2.1. 3-Hydroxy-1-(2-methyl-piperidin-1-yl)-butan-1-one 16. The title compound obtained from the reaction did not require further purification; it was obtained in 96% yield as a clear and colourless oil. R_f 0.32 (hexanes/ethyl acetate 1:1); IR ν 3423, 2970, 2937, 2866, 1719, 1617,

1442, 1372, 1256, 1181, 1153, 1038; ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.98 (s, 1H), 4.46 (d, $J = 12.9$ Hz, 2H), 4.03–4.25 (m, 3H), 3.56 (d, $J = 13.1$ Hz, 1H), 3.07 (t, $J = 12.2$ Hz, 1H), 2.63 (t, $J = 14.2$ Hz, 1H), 2.11–2.55 (m, 4H), 1.48–1.75 (m, 10H), 1.29–1.45 (m, 2H), 1.15–1.26 (m, 9H), 1.07–1.15 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.9, 170.8, 64.3, 64.2, 64.1, 48.0, 47.9, 43.6, 43.5, 41.1, 40.6, 40.4, 36.0, 30.5, 29.7, 26.0, 25.4, 22.2, 22.1, 18.6, 16.5, 15.6, 15.5; MS (EI) *m*/*z* (%): 185 (21), 170 (12), 140 (10), 126 (12), 98 (11), 84 (100) 56 (13), 55 (12), 45 (15), 43 (19), 41 (13); HRMS calcd for $C_{10}H_{19}NO_2$ 185.1416, found 185.1421. Anal. Calcd for $C_{10}H_{19}NO_2$: C, 64.38; H, 10.34. Found: C, 64.49; H, 10.16.

4.2.2. 1-(2-Cyclohexyl-piperidin-1-yl)-3-hydroxy-butan-1-one 20. The residue was purified by flash column chromatography (hexanes/ethyl acetate 2:1) affording the title compound (85%) as a colourless oil. R_f 0.2 (methylene chloride/hexanes/diethyl ether, 8:1:4); IR *v* 3401, 2928, 2852, 1614, 1448, 1371, 1248, 1189, 1121, 1012; ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.52 (m, 2H), 4.38 (d, $J = 6.3$ Hz, 1H). 4.13–4.17 (m, 2H), 3.55 (d, $J = 13.8$ Hz, 1H), 2.95 (t, $J = 4.5$ Hz, 1H), 2.41–2.49 (m, 2H), 2.21–2.29 (m, 2H), 1.57–1.89 (m, 18H), 1.42 (m, 5H), 1.06–1.19 (m, 12H), 0.78–0.94 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 171.3, 171.1, 171.0, 64.3, 64.2, 64.1, 58.3, 58.2, 53.1, 41.2, 40.8, 36.8, 36.7. 35.4, 35.3, 34.8, 30.4, 30.2, 29.6, 29.0, 29.8, 26.4; MS (EI) *m*/*z* (%): 170 (48), 85 (16), 84 (100), 41 (12); HRMS calcd for $C_{15}H_{27}NO_2$ 253.2042, found 253.2042, Anal. Calcd for $C_{15}H_{27}NO_2 \cdot 1/4H_2O$: C, 69.86; H, 10.75. Found: C, 69.51; H, 10.58.

4.2.3. 3-Hydroxy-1-(2-phenyl-3,4-dihydro-2*H*-quinolin-1-yl)-butan-1-one 28. The residue was purified by flash column chromatography (hexanes/ethyl acetate 5:1) affording the title compound (88%) as a white solid. Mp 118–126 °C (methylene chloride); R_f 0.5 (hexanes/ethyl acetate 1:1); IR *v* 3683, 3401, 3019, 2400, 1630, 1583, 1521, 1491, 1400, 1215, 1047; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.17–7.31 (m, 9H), 5.70 (s, 1H), 4.21–4.23 (m, 1H), 2.61–2.88 (m, 7H), 2.25–2.33 (m, 1H), 1.98–2.02 (m, 1H), 1.12–1.24 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.0, 172.5, 128.5, 127.5, 127.4, 126.9, 126.7, 126.7, 126.0, 125.9, 125.8, 64.5, 42.4, 42.3, 42.2, 34.5, 34.4, 26.6, 26.5, 22.3, 22.0; MS (EI) *m*/*z* (%): 277 (10), 209 (45), 208 (17), 201 (13), 133 (100), 132 (65), 131 (14), 130 (28), 118 (11), 91 (11), 77 (16), 69 (38), 43 (19), 41 (17); HRMS calcd for $C_{19}H_{21}NO$ 295.1572, found 295.1574; Anal. Calcd for $C_{19}H_{21}NO_2 \cdot 1/4H_2O$: C, 76.10; H, 7.23. Found: C, 75.92; H, 7.25.

4.3. General procedure for the reduction of β-keto amides with baker's yeast

A mixture of glucose (12.00 g) and yeast extract (0.50 g) in distilled water (400 mL) was warmed to 40 °C in a 1000-mL Erlenmeyer flask. Fleischmann's Traditional baker's yeast (10.00 g) was added to the mixture and allowed to hydrate for 20 min before the addition of the substrate (400 mg) in ethanol (2 mL). The mixture was shaken at 180 rpm at 40 °C on an orbital shaker. At 24 h intervals, a suspension of yeast (2.00 g), glucose (3.40 g), and yeast extract (0.12 g) in distilled water (80 mL), hydrated at 35 °C for 20 min, was added to the reaction mixture to ensure further reduction of the starting material.

The conversion of the starting material was followed by GC/MS. For GC/MS analysis, an aliquot of 2 mL was taken from the reaction mixture and centrifuged at 13,000 rpm for 2 min. The supernatant was extracted 2× with chloroform (1 mL) and dried over anhydrous $MgSO_4$. The organic layer was filtered through a 9 in. pipette containing glass wool and was used directly for GC/MS analysis.

After 50% conversion had been achieved, the reaction mixture was centrifuged at 4000 rpm for 13 min. The supernatant was decanted into a separatory funnel. The centrifugate was suspended in chloroform, and the organic solvent was decanted and used for the extraction of the aqueous solution. The supernatant was either extracted five times with chloroform/methylene chloride 1:4 or continuously extracted with methylene chloride. The organic layers were combined, dried over anhydrous $MgSO_4$, filtered, and the solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography using methylene chloride/hexanes/diethyl ether 8:1:4 as an eluent for the separation of 1-(2-methyl-piperidin-1-yl)-butane-1,3-dione and 3-hydroxy-1-(2-methyl-piperidin-1-yl)-butan-1-one; 1-(2-cyclohexyl-piperidin-1-yl)-butane-1,3-dione and 3-hydroxy-1-(2-cyclohexyl-piperidin-1-yl)-butan-1-one; and hexanes/ethyl acetate 9:1→2:1 for 1-(2-phenyl-3,4-dihydro-2*H*-quinolin-1-yl)-butane-1,3-dione and 3-hydroxy-1-(2-phenyl-3,4-dihydro-2*H*-quinolin-1-yl)-butan-1-one.

In all the cases the analytical data of the isolated products were found to be identical to compounds **15** and **16**. In addition, the following specific rotation values were obtained: **15a**, $[\alpha]_D^{22} = +7.65$ (*c* 0.65, $CHCl_3$); **15a** (β-keto amide recovered from second yeast cycle of **15a**), $[\alpha]_D^{22} = +16.35$ (*c* 2.00, $CHCl_3$); **15b** (β-keto amide recovered from second yeast cycle of **15b**), $[\alpha]_D^{23} = -0.6$ (*c* 0.75, $CHCl_3$); **16a**, $[\alpha]_D^{22} = +53.6$ (*c* 1.10, $CHCl_3$); **16a** (β-hydroxy amide recovered from second yeast cycle of **15a**), $[\alpha]_D^{22} = +27.6$ (*c* 1.25, $CHCl_3$); **16a**(β-hydroxy amide recovered from second yeast cycle of **15b**), $[\alpha]_D^{23} = +44.3$ (*c* 1.10, $CHCl_3$); **19a**, $[\alpha]_D^{22} = +15.1$ (*c* 0.45, $CHCl_3$); **19a** (β-keto amide recovered from second yeast cycle of **19a**), $[\alpha]_D^{22} = +29.7$ (*c* 1.10, $CHCl_3$); **19b**(β-keto amide recovered from second yeast cycle of **19b**), $[\alpha]_D^{23} = -17.1$ (*c* 0.55, $CHCl_3$); **20a**, $[\alpha]_D^{24} = +7.2$ (*c* 0.30, $CHCl_3$); **20a** (β-hydroxy amide recovered from second yeast cycle of **19a**), $[\alpha]_D^{22} = +2.6$ (*c* 0.85, $CHCl_3$); **20a** (β-hydroxy amide recovered from second yeast cycle of **19b**), $[\alpha]_D^{23} = +6.8$ (*c* 1.00, $CHCl_3$).

4.4. General procedure for the reduction of β-keto amides with selected yeast strains

Yeast (0.5 g) was rehydrated in autoclaved distilled water (3 mL) for 15 min. A starter culture was prepared by the addition of the hydrated yeast solution (150 μL) to Yeast Peptone Dextrose (YPD) broth (3 mL) (10 g/L yeast extract, 20 g/L peptone, and 20 g/L glucose) and the culture

was incubated at 40 °C at 180 rpm in an orbital shaker overnight.

YPD broth (200 mL) was inoculated with the starter culture. The mixture was shaken at 40 °C at 180 rpm for 20 min, before the addition of the substrate (200 mg) dissolved in ethanol (2 mL). Shaking was continued at 40 °C with monitoring of the consumption of starting material by GC/MS. At 24 h intervals, a suspension of yeast starter culture (0.6 mL) in YPD broth (40 mL) was added to the reaction mixture to ensure further reduction of the starting material.

After 50% conversion of the starting material, as detected by GC/MS, the reaction mixture was worked up as described in the general procedure for the reduction of β-keto amides with baker's yeast.

4.5. General procedure for the oxidation of β-hydroxy amides

Oxalyl chloride (1.08 mmol) in methylene chloride (8.0 mL) was cooled to −65 °C, then DMSO (2.16 mmol) in methylene chloride (2 mL) was added. The reaction mixture was stirred at −65 °C for 10 min, before the β-hydroxy amide (0.54 mmol) in methylene chloride (1.0 mL) was added. After 15 min, triethylamine (5.4 mmol) was added dropwise. The reaction was warmed to 0 °C over 1 h and allowed to stir at room temperature until no more starting material was present by TLC. The reaction was quenched by the addition of H_2O (5 mL) and extracted four times with methylene chloride. The organic layers were combined and washed with 1 M HCl and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure.

4.5.1. (*R*)-1-(2-Methyl-piperidin-1-yl)-butane-1,3-dione 15b. Compound **15b** was obtained in 41% yield, following the general procedure for the oxidation of β-hydroxy amides after purification by flash column chromatography (methylene chloride/hexanes/diethyl ether 8:1:4). The analytical data were identical to compound **15**; $[\alpha]_D^{23} = -16.7$ (*c* 1.20, $CHCl_3$).

4.5.2. (*S*)-1-(2-Cyclohexyl-piperidin-1-yl)-butane-1,3-dione 19b. Compound **19b** was obtained in 40% yield following the general procedure for the oxidation of β-hydroxy amides after purification by flash column chromatography (methylene chloride/hexanes/diethyl ether, 8:1:4). The analytical data were identical to compound **19**; $[\alpha]_D^{22} = -26.3$ (*c* 0.75, $CHCl_3$).

4.6. General procedure for the reductive cleavage of amides

To a solution of the corresponding amide (0.45 mmol) in dioxane (2 mL) was added $LiAlH_4$ (2.30 mmol) and the reaction mixture was kept at reflux overnight until no more starting material was present by TLC. The reaction was cooled to room temperature and quenched by the addition of H_2O (0.1 mL), 15% NaOH (0.2 mL) and H_2O (0.3 mL). The reaction mixture was filtered and the filtrate was extracted three times with methylene chloride. The organic layers were combined, dried over anhydrous Na_2SO_4, and filtered. The solvent was carefully reduced and the crude reaction mixture (50 mg in 1 mL of DCM) was carried onto the next step without further purification.

4.7. General procedure for the formation of benzamides

Potassium carbonate (484 mg, 3.5 mmol) was added in one portion to the crude amine (0.7 mmol) in methylene chloride at 0 °C. Benzoyl chloride (147 mg, 1.05 mmol) was added dropwise and the reaction mixture was allowed to warm to room temperature. After complete consumption of the starting material (TLC), the reaction mixture was quenched by the addition of water. The aqueous layer was extracted three times with methylene chloride. The organic layers were combined, and washed with 1 M NaOH and 1 M HCl. The organic layer was dried over anhydrous $MgSO_4$, filtered, and the solvent was evaporated under reduced pressure.

4.7.1. (*S*)-(2-Methyl-piperidin-1-yl)-phenyl-methanone 17a. The crude product, using (*S*)-2-methylpiperidine (75 mg, 0.76 mmol) as the starting material, was purified by flash column chromatography (hexanes/ethyl acetate 6:1→4:1) affording 125 mg (96%) of the title compound as a white solid. ee = 96% (HPLC); $[\alpha]_D^{21} = +37.2$ (*c* 0.95, $CHCl_3$). The analytical data were identical to the reported data in the literature.[19]

The following compounds were prepared by the general procedure for the formation of benzamides.

Compound **17a**, ee = 15% (HPLC); $[\alpha]_D^{22} = +6.05$ (*c* 1.40, $CHCl_3$); **17b**, ee = 26% (HPLC); $[\alpha]_D^{21} = -8.7$ (*c* 1.95, $CHCl_3$); **17a** (benzamide derived from β-keto amide of second yeast cycle of **15a**), ee = 25% (HPLC); $[\alpha]_D^{23} = +8.1$ (*c* 0.45, $CHCl_3$); **17b** (benzamide derived from β-hydroxy amide of second yeast cycle of **15a**), ee = 12% (HPLC); $[\alpha]_D^{22} = -4.6$ (*c* 0.25, $CHCl_3$); **17b** (benzamide derived from β-keto amide of second yeast cycle of **15b**), ee = 2% (HPLC); $[\alpha]_D^{22} = -0.8$ (*c* 0.50, $CHCl_3$); **17b** (benzamide derived from β-hydroxy amide of second yeast cycle of **15b**), ee = 2.5% (HPLC); $[\alpha]_D^{22} = -0.8$ (*c* 0.40, $CHCl_3$).

4.7.2. (2-Cyclohexyl-piperidin-1-yl)-phenyl-methanone 21. The crude product was purified by flash column chromatography (hexanes/ethyl acetate 6:1→4:1) affording 183 mg (88%) of the title compound as a white solid. Mp 86–90 °C (ethyl acetate); R_f 0.5 (hexanes/ethyl acetate 2:1); IR *v* 3676, 3466, 3245, 2994, 2927, 2874, 2662, 2467, 2158, 1958, 1886, 1810, 1734, 1724, 1624, 1577, 1494, 1443, 1452, 1372, 1345, 1304, 1276, 1263, 1242, 1215, 1119, 1073, 1028, 1000; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (s, 5H), 4.56 (m, 1H), 3.33–3.52 (dd, $J = 12.9$, 8.1 Hz, 1H), 2.70–3.03 (dt, $J = 11.7$, 12.6 Hz, 1H), 1.51–1.80 (m, 11H), 1.41 (m, 1H), 0.98–1.31 (m, 4H), 0.57 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.3, 170.5, 137.3, 129.4, 128.9, 128.8, 128.5, 128.3, 126.6, 126.4, 60.3, 60.0, 53.3, 43.6, 37.5, 35.6, 35.0, 30.2, 29.5, 28.9, 26.5, 26.1, 25.5, 19.2, 14.2; MS (EI) *m*/*z* (%): 189 (17), 188 (79), 105 (100), 77 (24); HRMS calcd for $C_{18}H_{25}NO$ 271.1936, found

271.1935; Anal. Calcd for $C_{18}H_{25}NO$: C, 79.66; H 9.28. Found: C, 79.27; H 9.35.

The following compounds were prepared by the general procedure for the formation of benzamides. The analytical data were identical to compound **21**.

Compound **21a**, ee = 42% (HPLC); $[\alpha]_D^{23} = +21.7$ (*c* 0.30, $CHCl_3$); **21b**, ee = 62% (HPLC); $[\alpha]_D^{22} = -41.9$ (*c* 0.35, $CHCl_3$); **21a** (benzamide derived from β-keto amide of second yeast cycle of **19a**), ee = 72% (HPLC); $[\alpha]_D^{23} = +47.4$ (*c* 0.25, $CHCl_3$); **21b** (benzamide derived from β-hydroxy amide of second yeast cycle of **19a**), ee = 18% (HPLC); $[\alpha]_D^{23} = -10.1$ (*c* 0.60, $CHCl_3$); **21b** (benzamide derived from β-keto amide of second yeast cycle of **19b**), ee = 45% (HPLC); $[\alpha]_D^{23} = -29.7$ (*c* 0.55, $CHCl_3$); **21b** (benzamide derived from β-hydroxy amide of second yeast cycle of **19b**), ee = 59% (HPLC); $[\alpha]_D^{22} = -32.4$ (*c* 0.40, $CHCl_3$).

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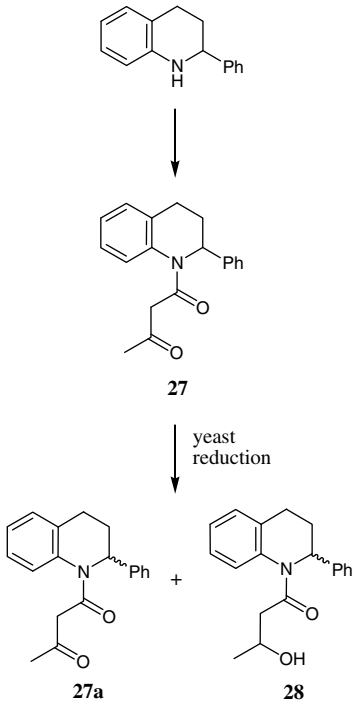

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