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Homochiral Amine Synthesis by Baker's Yeast Resolution of a β-Keto Amide: 1-Phenylethylamine

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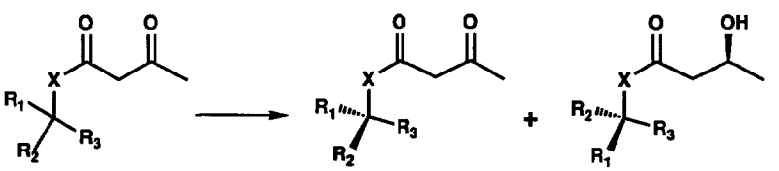
Abstract: Preliminary investigation of the resolution potential of baker's yeast reduction of β-keto amides has been carried out with 1-phenylethylamine. Enantiomeric excess was determined by direct comparison of resolved enantiomers with derivatives prepared from commercially available (R)-(+)-1-phenylethylamine and was found to be 56-58.6% for the S enantiomer and 76-84.6% for the R enantiomer.

The enzymatic reduction of 1,3-dicarbonyl compounds by Baker's yeast and other strains is well established as a reliable procedure for the generation of oxygenated homochiral centers.[2-6] Recently, this type of reduction was applied for the first time to β-keto esters of several prochiral alcohols as means of their resolution, providing both enantiomers of the parent alcohols with enantiomeric excesses of 0-77%.[7] The slower reacting enantiomers **2a** remained unreduced and were easily separated from the β-hydroxy esters **3a**, Figure 1.The rationale for this application was based on the assumption that the secondary resolution site, the alkoxy carbon of the ester moiety, maintained identical distance from the primary reaction site at the ketone carbonyl. The results of these prochiral resolutions confirmed this assumption and provided practical means for resolution of prochiral alcohols.



1a X=O
1b X=NH

2a
2b

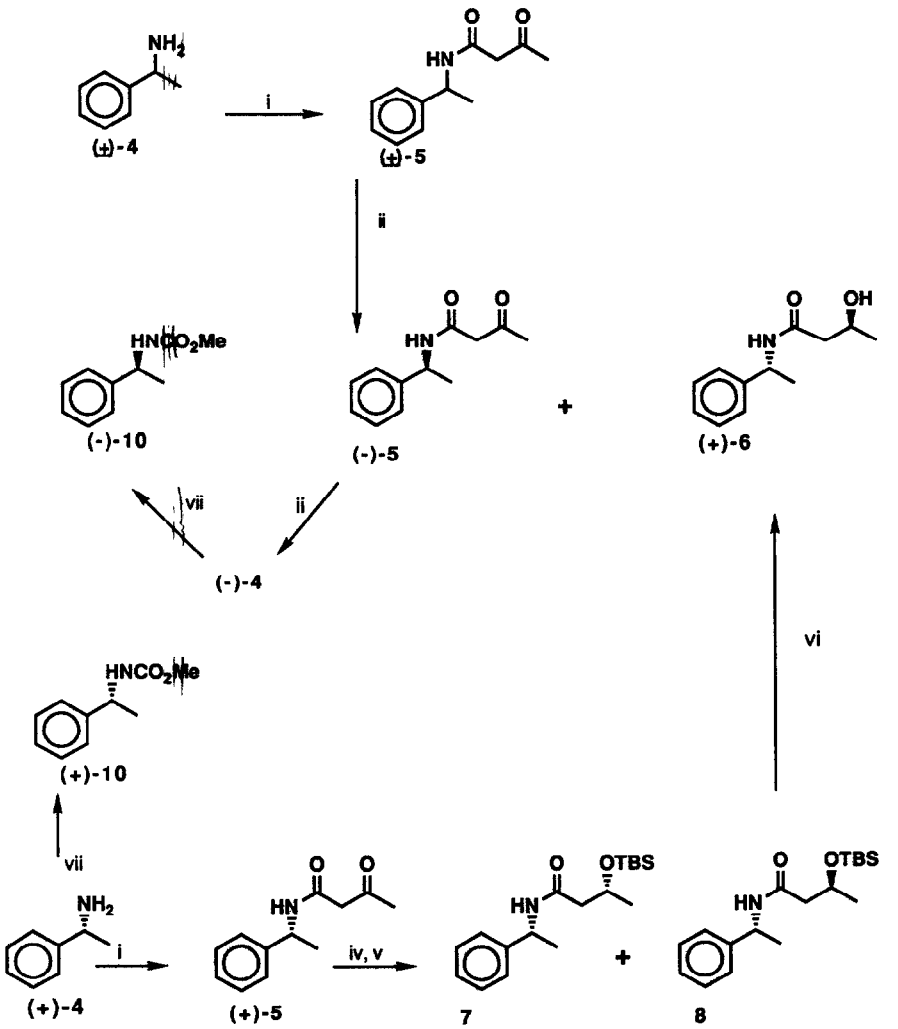
3a
3b

Figure 1. Yeast reduction of β-keto esters and β-keto amides

It is well known that the extent of secondary resolution at auxiliary chiral centers falls off with the increasing distance of the second center from the site of primary enzymatic recognition. We chose to study this process with β-keto amides in order to extend its applicability to the potential resolution of amines. Homochiral amines are available by a number of methods ranging from traditional resolution,[8] preparation from aminio acids[9] or sugars,[10] or amination of chiral substrates.[11] Recently, a report appeared describing the enzymatic reduction of oximes and esters[12] or oxime ethers[13] to amines with ca. 24-44 % ee for the former and 79-92 % ee for the latter. Herein we report the results of resolution of 1-phenylethylamine.

Racemic 1-phenylethylamine (Aldrich) was acylated with diketene in the presence of triethylamine to provide keto amide **5** in 91% yield, Scheme 1. The keto amide **5** was dissolved in DMSO and added to a solution of α-D-glucose, yeast extract, and active dry yeast (Fleischmann's Yeast Inc., Oakland, CA, 94603).

The mixture was incubated at 30–35 °C while air was bubbled through the medium. The reaction was continued for a period of 48 hours to 1.5 weeks to determine the profiles of the resolution. The reaction was then centrifuged and extracted with ethyl acetate. Reproducible and optimized conditions were established for a reduction time of one week. The unreacted starting β-keto amide was easily distilled from the crude reaction mixture (Kugelrohr 170°C, .001 mm Hg), and the β-hydroxy amide **6** was purified by flash chromatography on silica deactivated with 10 % water. The enantiomeric excess at the benzylic center was determined by several methods.



Scheme 1.

Reagents: (i) DMAP, triethylamine, diketene; (ii) α-D-glucose, yeast extract, H_2O, Fleischman's yeast; (iii) 50 % aqueous KOH; (iv) $NaBH_4$, MeOH; (v) TBSCl, DIPEA, DMF; (vi) THF, TBAF; (vii) $ClCO_2Me$, Na_2CO_3.

Optical rotation comparison with standards. Hydrolysis of separated amides afforded the free bases whose enantiomeric excess was determined by direct comparison of $[\alpha]_D$ with that of enantiomers prepared from commercially available standards. (See Experimental Section.) The percent ee of (-)-**4** from hydrolysis of (-)-**5** was initially determined to be 25–30%. (+)-**6** did not undergo hydrolysis after 12 h at 130 °C in 50 % aqueous KOH. To ensure that free amine was not susceptible to air oxidation, which would complicate analysis by optical rotation, methylcarbamates were prepared from both the commercial samples and hydrolysis products. The ee for **(-)-10** determined by this method was 58.6% , a more reliable value.

Conversion to derivatives. To determine the absolute stereochemistry of the reduction and to provide additional means of analysis for the level of ee, pure (+)-1-phenylethylamine was converted to its keto amide (+)-**5** and compared with (-)-**5**. This comparison showed an ee of 56 % in agreement with the value obtained for methylcarbamate (-)-**10**. Compound (+)-**5** was reduced with $NaBH_4$. The diastereomeric alcohols were silylated with *tert*-butyldimethylsilyl chloride to furnish **7** and **8** and allow for their separation, Scheme 1. Deprotection of amide **8** gave material identical in all respects with **6**. Conversely, **6** protected with *tert*-butyldimethylsilyl chloride provided **8**, Scheme 1. This experiment established that the (-) enantiomer of **5** was relatively unreactive toward the reduction, whereas the (+) enantiomer was reduced at a faster rate, which allowed for kinetic resolution. These results indicate that the resolution parameters inherent for the reduction of β-keto esters operate also at a remote chiral center through both ester and amide linkages. Whichever enantiomer is subject to faster enzymatic reduction yields a compound in which the extent of resolution at the primary reaction site has been communicated to the secondary one. Further applications of this process to the preparation of other homochiral amides through the use of other more efficient microorganisms[14] are in progress and will be reported in due course.

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Experimental Section:

All nonhydrolytic reactions were carried out in a nitrogen or argon atmosphere, with standard techniques for the exclusion of air and moisture. Glassware used for moisture-sensitive reactions was flame-dried with an internal inert gas sweep. Dichloromethane was distilled from calcium hydride. Analytical TLC was performed on silica gel 60-F_{254} plates. Flash chomatography was performed on Kieselgel 60 (230-400 mesh). Mass spectra were recorded on DuPont 20-491 or a Varian MAT-112 instrument (low resolution). Infared spectra were recorded as neat samples (NaCl plates) on a Perkin-Elmer 1600 Series FT spectrometer. Proton and ^{13}C NMR spectra were obtained on a Bruker WP-270 instrument. Proton shifts are reported in parts per million (ppm) relative to chloroform (7.24 ppm). Carbon chemical shifts are reported in parts per million relative to the center line of the $CDCl_3$ triplet (77.0 ppm). The multiplicity as indicated by CH_3, CH_2, CH, or C was determined by INEPT experiments.

(±)-*N*-(1-phenylethyl)-3-oxobutyramide (5). 4-Dimethylaminopyridine (30 mg, 3.66 mmol), triethylamine (1.26 mL, 9.0 mmol), and (±)-1-phenylethylamine **4** (1.06 mL, 8.25 mmol) were dissolved in CH_2Cl_2 (10 mL), at 0 °C under an argon atmosphere. The reaction mixture was stirred for 10 min, then diketene (1.24 mL, 16.5 mmol) was added dropwise. Stirring was continued for 3.5 h. The reaction was

quenched with 5% aqueous KOH and extracted with CH_2Cl_2 (20 x 2 mL). The combined extracts were dried (Na_2SO_4) and evaporated to give 1.96 g of a reddish oil. The crude product was distilled (Kugelrohr; 170 °C/0.001 torr) yielding 1.54 g (7.50 mmol, 91%). An analytical sample was obtained by recrystallization from cyclohexane. **R_f**= 0.27 (silica gel; hexane/EtOAc, 3:7); **mp** = 52 °C; **IR** (KBr) cm^{-1} 3256, 3079, 1725, 1665, 1648 cm^{-1}; **^{1}H NMR** ($CDCl_3$) δ, 7.22 - 7.38 (m, 5H), 5.13 (q, J =7.0 Hz, 1H), 3.42 (s, 2H), 2.26 (s, 3H), 1.50 (d,J= 6.9 Hz, 3H); **^{13}C NMR** ($CDCl_3$) δ 204.7 (C), 164.4 (C), 143.1 (C), 128.7 (CH), 127.3 (CH), 126.0 (CH), 49.6(CH2), 49.0(CH), 31.0 (CH_3), 22.1 (CH_3); **MS** (EI) *m/z* (rel. intensity) 205 (M+, 0.54), 162 (0.14), 120 (1.0), 106 (0.86), 91 (0.13), 77 (0.25); **Anal. calcd for** $C_{12}H_{15}O_2N$: C 70.22; H 7.37; **Found**: C 70.04; H 7.38.

(+)-N-(1(R)-phenylethyl)-3(S)-hydroxybutyramide (6) and (-)-N-(1(R)-phenylethyl)-3-oxobutyramide (5). α-D-Glucose (5 g, 0.028 mol) and yeast extract (0.2 g) were dissolved in water (100 mL) at 25–35 °C and stirred. Active dry yeast (4 g, Fleischmann's Yeast Inc., Oakland, CA, 94603) was added and the mixture stirred open to the air for 30 min. The keto amine **5** (800 mg, 3.89 mmol), dissolved in DMSO, was slowly added and the mixture stirred at 35-40°C with air bubbling through for 23 h. α-D-Glucose (2.5 g, 0.014 mol), yeast extract (0.1 g) and active dry yeast (2 g) were added and stirring continued under the same conditions for another 25 h. The reaction mixture was centrifuged (10 min., 5000 rpm) and the solution decanted from the cells. The centrifugate was suspended in CH_2Cl_2 and centrifuged (10 min., 5000 rpm), this process was repeated once. The decanted solutions were combined, saturated with NaCl, and extracted with CH_2Cl_2 (3x25mL). The organic layers were combined and dried ($NaSO_4$). Solvents were evaporated under reduced pressure yielding 700 mg of a mixture of two products, purified by flash chromatography (silica gel deactivated with 10 % H_2O, hexane/ethyl acetate, 1:1). Hydroxy amide **6** was obtained as white crystals (95 mg, 0.461 mmol, 12%); this yield reflected isolation techniques and could be improved by distillation of unreacted keto amide from the crude reaction mixture prior to chromatography): **(+)-6: R_f**=0.185 (EtOAc); **mp** = 79-81 °C; **$[\alpha]_D^{25}$**= +95.65 (c=0.32, MeOH); **IR** (film) cm^{-1} 3275 3060, 2971, 1634 cm^{-1}; **^{1}H NMR** ($CDCl_3$) δ 7.37-7.22 (m, 5H), 6.26 (br. d,J= 5.67 Hz, 1H), 5.11 (q,J= 7 Hz, 1H), 4.20-4.09 (m, 1H), 3.80 (br. s, 1H), 2.36-2.21 (m, 2H), 1.47 (d,J= 6.93 Hz, 3H), 1.18 (d,J= 6.32 Hz, 3H); **^{13}C NMR** ($CDCl_3$) δ 171.3 (C), 143.1 (C), 128.7 (CH), 127.4 (CH), 126.1 (CH), 64.9 (CH), 48.8 (CH), 44.1 (CH_2), 22.8 (CH_3), 21.8 (CH_3); **MS** (EI) *m/z* (rel. intensity) 207 (M+, 55), 174 (14), 120 (66), 106 (100), *91* (8), 77 (24). The unreacted enantiomer **5** was isolated as a yellowish oil (494 mg, 2.4 mmol, 61%), which crystallized on standing. An analytical sample was obtained by recrystallization from cyclohexene, producing white crystals. **(-)-5: Rf**= 0.27 (silica gel, hexane/ethylacetate, 3:7); **mp** = 47-49 °C; **$[\alpha]_D^{25}$** -65.05(c = 3.34, MeOH); **IR** (KBr) u 3292, 3063, 1719, 1645 cm^{-1}; **^{1}H NMR** ($CDCl_3$) δ 7.17 - 7.44 (m, 5H), 5.13 (q, 1H, J= 7.1 Hz, 1H), 3.37 (s, 2H), 2.24 (s, 3H), 1.47 (d, 3H, J=4.6 Hz).

(+)-N-(1-phenylethyl)-3-oxobutyramide (5). Prepared as above from (R)-(+)-1-phenylethylamine (Aldrich). **$[\alpha]_D^{25}$**=+113.65(c=0.32, MeOH).

(+)-N-(1(R)-phenylethyl)-3(R)-*tert*-butyldimethylsilyloxybutyramide (7) and (+)-N-(1(R)-phenylethyl)-3(S)-*tert*-butyldimethylsilyloxybutyramide (8). Keto amide (+)-5 (prepared as above from (R)-(+)-1-phenylethylamine) (61.0 mg, 0.297 mmol) was dissolved in 0.7 ml MeOH at RT. To this

solution was added $NaBH_4$ (11.4 mg, 0.29 mmol) dissolved in 0.3 ml of MeOH. The reaction was complete in 30 min, and the solvent was removed under reduced pressure. The residue was dissolved in 1 ml of CH_2Cl_2 and filtered through celite. The solvent was removed under reduced pressure to yield 60 mg of crude material which was filtered through flash chromography (silica, hexane/ethylacetate 1:1) to yield white crystals (50 mg, 0.243 mmol 82 %) of **6** (two diastereomers). To a solution of *tert*-butyldimethylchlorosilane (590 mg, 3.93 mmol) in dry DMF (5 mL), diisopropylethylamine (0.79 ml, 4.59 mmols) was slowly added and HCl gas was swept away by a stream of argon. When all of the HCl gas was removed the crude mixture of diastereomeric hydroxyamides **6** (270 mg, 1.31 mmol, obtained from several reductions as described above) was dissolved in dry DMF (3 mL) and added dropwise. The reaction was stirred for 10 h and monitored by TLC (hexane/ethylacetate, 8:2) revealing a mixture of two products. Brine (5 ml) and ethylacetate (5 ml) were added and the mixture stirred for 10 minutes. The mixture was extracted with ethylacetate (3 x 5 ml) and, the organic layers washed with brine (3 x 5 ml). The organic layer was dried with Na_2SO_4 and the solvents were removed under reduced pressure. The two products were purified by flash chromatography (silica gel, hexane/ethylacetate, 8:2).

The less polar **8** (117.2 mg, 0.364 mmol, 47%) was obtained as a colorless oil . R_f= 0.45 (hexane/ethylacetate, 8:2); $[\alpha]_D^{25}$= + 91.22 (c=28.8, MeOH); **IR** (film) cm^{-1} 3283, 3067, 2956, 1639, 1544 cm^{-1}; **^{1}H NMR** ($CDCl_3$) δ 7.26-7.16 (m, 5H), 6.66 (br. d, J=6.9 Hz, 1H), 5.06 (m, 1H), 4.18-4.12 (m, 1H), 2.39 (dd, J=10.5, 4.1 Hz, 1H), 2.17 (dd, J=9.3, 5.4 Hz, 1H), 1.40 (d, J=6.9 Hz, 3H), 1.08 (d, J=6.2 Hz, 3H), 0.84 (m, 9H); **^{13}C NMR** ($CDCl_3$) δ 170.0 (C), 143.5 (C), 128.5 (CH),, 127.1 (CH), 126.2 (CH), 66.1 (CH), 48.4 (CH) 45.9(CH_2), 25.7 (CH_3), 22.8 (CH_3), 22.0 (CH_3),17.9 (CH_3), -4.6 (CH_3), -5.0 (CH_3); **MS** (EI) *m/z* (rel. intensity) 321 (M^+, 7), 264 (40), 160 (80), 105 (100); **Anal. calcd for** $C_{18}H_{31}O_2SiN$. : C 67.24; H 9.72; **Found**: C 67.30; H 9.75.

The more polar **7** (83.0 mg, 0.258 mmol, 33%) was isolated as a white solid. R_f= 0.39 (hexane/ethylacetate, 8:2); **mp**=97-98°C; $[\alpha]_D^{25}$= + 34.4 (c=15.0, MeOH); **IR** (film) cm^{-1} 3279, 3074, 2962, 1640, 1552 cm^{-1}; **^{1}H NMR** ($CDCl_3$) δ 7.32-7.26 (m, 5H), 6.75 (br. d, J=6.87 Hz, 1H), 5.13 (q, J= 6.98 Hz, 1H), 4.25-4.19 (m, 1H), 3.66 (s, 1H), 2.46 (dd, J=14.9, 4.2 Hz, 1H), 2.26 (dd, J=14.9, 5.1 Hz, 1H), 1.49 (d, J=6.8 Hz, 3H), 1.25 (d, J=6.2 Hz, 3H), 0.7 (s, 9H), 0.0 (d, J = 15.98 Hz, 6 H); **^{13}C NMR** ($CDCl_3$) δ 169.9 (C), 143.2 (C), 128.6 (CH), 127.2 (CH_2), 126.3 (CH), 65. 9 (CH), 48.6 (CH), 45.8 (CH2), 25.6 (CH3), 22.7 (CH3), 21.9 (CH3), 17.8 (CH3), -4.654 (CH_3); **MS** (EI) *m/z* (rel. intensity) 334 (M^+, 3), 321 (40), 264 (35), 160 (80), 105 (100); **Anal. calcd for** $C_{18}H_{31}O_2SiN$: C 67.24; H 9.72; **Found**: C 67.01; H 9.74.

Methyl (1-phenylethyl)carbamate (+)-(10). Amine (+)-**4** (0.5 g, 2.43 mmol) was dissolved in acetone (7 ml). To this was added Na_2CO_3 (1.50 g, 14.5 mmol) and then methyl chloroformate (0.918 g, 9.72 mmol) was added over 5 min. The reaction was stirred for two hours. Then the reaction mixture was filtered though celite, and the solvent was removed under reduced pressure to yield crude material (0.72 g) that was purified by flash chromography (silica, hexane/ethylacetate 2:1), which yielded white crystals (0.701 g, 3.93 mmol, 94 %), $[\alpha]_D$ ^{25}= +88.58 (c=1.13, MeOH). The free amine isolated from the hydrolysis of **5** was converted to this carbamate for evaluation of enantiomeric excess: **(-)-(10)** from hydrolysis of **(-)-(5)**: $[\alpha]_D$ ^{25}= -53.0 (c=0.32, MeOH), 58.6% ee.

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