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TETRAHEDRON: *ASYMMETRY*

A facile synthesis of (*R*)-(−)-2-azido-1-arylethanols from 2-azido-1-arylketones using baker's yeast

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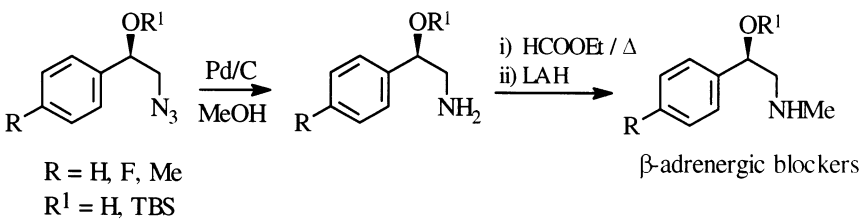
Abstract—A novel and efficient stereoselective reduction of 2-azido-1-aryl ketones using baker's yeast is described.

1. Introduction

In recent years the use of biocatalysts i.e. enzymes or whole microbial cells in synthesis of chiral products is well recognized. Biocatalysts display greater substrate specificity, regio- and stereoselective transformations of organic compounds under extremely mild reaction conditions.[1] Currently much emphasis has been given to obtain optically active compounds in good yields with high enantiomeric purity under environmentally friendly conditions.[2] Many drugs contain stereogenic centers and the enantiomers of such molecules often have different biological activities.[3] The β-adrenergic drugs have historically been made as racemates; however, there is an increasing demand to market them as single enantiomers because the biological activity resides mainly in the (*R*)-enantiomer of the drug.[4] Preparation of optically active β-adrenergic blockers generally involves chemical resolutions or rather lengthy chemical syntheses from chiral precursors.[5]

Enzymes are known to catalyze the reduction of carbonyl groups which have found important applications in enantioselective synthesis.[6] Among the biocatalysts used for the asymmetric reductions of prochiral carbonyl groups, the *Saccharomyces cerevisiae* (baker's yeast) mediated transformation has attracted new interest as the fungus shows a wide range of substrate specificity and good enantioselectivity in organic transformations.[7] The stereospecific synthesis of azidoalcohols, which are key intermediates for adrenergic drugs,[8] are of great importance (Scheme 1).

Because we are interested in the preparation of (*R*)- and (*S*)-2-azido-1-phenyl ethanols[9] herein, we wish to report a novel, efficient and environmentally friendly stereospecific reduction of 2-azido-1-arylketones to give (*R*)-2-azido-1-arylethanols using fermented baker's yeast in aqueous medium (Scheme 2).



Scheme 1.

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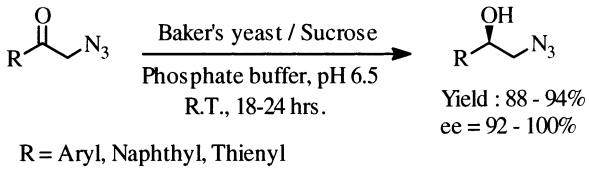
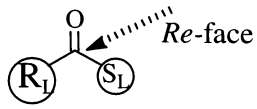


Scheme 2.

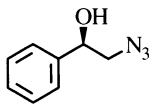
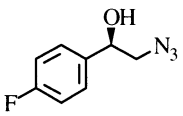
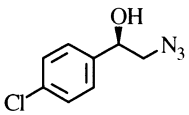
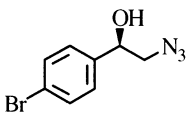
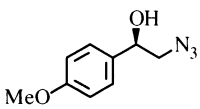
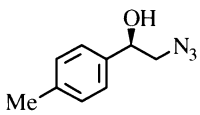
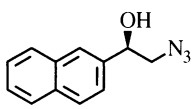
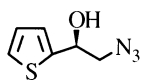
2. Results and discussion

It was observed that fermentation yeast reduced a variety of *para*-substituted α-azidoacetophenones to corresponding alcohols in higher yields and with good enantiomeric excess (e.e.). These 2-azido-1-arylalcohols were found to have (*R*)-configuration by comparing their specific rotations with those of authentic samples.[10] In this reduction the observed stereochemistry could be explained in accordance with Prelog's rule.[11] The hydride is transferred to the *re*-face of the α-azidoarylketone with respect to the methyl azido group which is effectively smaller than an aryl group.



From Table 1, it was found that ketones with an electron-withdrawing group adjacent to the ketone moi-

Table 1. Reduction of α-azido acetophenones by baker's yeast with sucrose in aqueous medium

Entry	Product[a]	Time (h.)	Yield[b] (%)	e.e. (%)[c]	Configuration[d]
1		18	92	100	*R*
2		18	90	98	*R*
3		20	93	100	*R*
4		22	91	100	*R*
5		20	94	97	*R*
6		22	91	96	*R*
7		24	92	96	*R*
8		22	88	92	*S*[e]

a : All products were characterised by ^{1}H, ^{13}C NMR, IR, Mass spectral data.

b : Isolated yields after the column chromatography

c : E.e. determined by chiral HPLC analysis using chiral OD coloumn with iPrOH : *n*-hexane system (1.5 : 8.5)

d : The absolute configuration assigned by sign of rotation.[9]

e : The absolute configuration of entry 8, assigned by analogy,[16]

ety were good substrates for the reduction. The stereospecificity of the reaction is said to depend upon the physiological state of fermenting yeast, the substrate concentration in the medium, the stage of growth of the cells and the reaction pH and temperature.[12] In this case however, it was observed that there was no significant change in the specificity of reaction in variation of the above mentioned parameters, except in the case of variation of substrate concentration, where the enantiomeric excess of the product dropped significantly when the substrate concentration was increased above >50 mg/mL in the given conditions.

One major complication in using intact cells for the catalytic reduction of ketones is the reduction of compounds by oxidoreductase iso-enzymes where both faces of the carbonyl group are equally favored.[13] In our studies, the fermented yeast cells were treated with allyl alcohol to inhibit the opposite (*S*)-oxidoreductase enzyme; thus, we obtained exclusively (*R*)-isomers of azido alcohols in all the reactions studied except thienyl azidoalcohol. This is further confirmed by comparing apparent kinetic parameters V_{max} and K_m of the enzyme reaction (Table 2). The rate constant K_m of the enzyme greatly affects the rate of the enzyme catalyzed reaction. The substrate constant (K_m) in the treated yeast cells was significantly higher than untreated cells, thus the study confirms the inhibition of (*S*)-isomer reductase to improve the e.e. of the (*R*)-2-azido-1-aryl alcohol product.

The reduction of heterocyclic aromatic azido ketones like 2-thienyl azidoketone (Table 1, entry 8) with baker's yeast gave the 2-azido-1-(2-thienyl)ethanol with good yield and e.e. In order to determine its absolute configuration the azido alcohol was reduced to 2-amino-1-(2-thienyl)ethanol **9** and the specific rotation measured. Interestingly, **9** showed the *levo*-rotation, indicating an original (*S*)-configuration as reported by Verbit et al.[16] Thus, unlike phenyl and naphthyl azidoketones reduction with baker's yeast provided the (*R*)-series of azido alcohols, whereas the reduction of thiophenyl azidoketone with baker's yeast gave the (*S*)-azido alcohol.

3. Conclusion

In conclusion, we have described a simple and cost-effective method for the synthesis of (*R*)-(−)-2-azido-1-arylethanols using baker's yeast. The reaction uses mild environmentally friendly conditions and the products are formed with excellent enantioselectivity. Further work is currently being directed into widening the application of this methodology.

4. Experimental

4.1. General

Melting points were recorded on Buchi R-535 apparatus. IR spectra were recorded on Nicolet-740 FT-IR spectrophotometer. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ solution on Varian Gemini-200 MHz spectrometer and chemical shifts are reported in ppm. Mass spectra were recorded on VG micromass-7070H (70 eV). CHN analysis was performed on a Vario EL analyzer. The optical rotations were recorded on Jasco Dip 360 digital polarimeter. HPLC analysis was performed on a Schimadzu liquid chromatography LC-6A, equipped with a SCE-6A, system controller, SPD-6A, fixed wavelength UV monitor as detector and chromatopac C-R4A data processor as integrater. The column was 4.6×250 mm, chiral cell OD column (Dai cell). The eluents were hexane–*iso*-propanol (HPLC grade, 85:15) at 0.5 mL per min flow rate and monitored at a wavelength of 254 nm. Baker's yeast type-1 was obtained from SIGMA Chemicals Co. All the starting materials were prepared according to the literature procedure. All the products were purified by chromatography using 60–120 Å silica gel, hexane–EtOAc (analytical grade, 90:10).

4.2. General procedure for the reduction of 2-azido-1-aryl ketones

Dry baker's yeast (1 g) was mixed with 0.1 M phosphate buffer pH 6.5 (20 mL) containing sucrose (2 g) and $MgCl_2$ (0.1 g) and the mixture incubated at 30–35°C for 12 h. The fermented cells were treated with allyl alcohol (20 mL) and stirred for 3 h. The solvent was decanted. To the treated yeast cells was added the azidoketone (0.5 g). The reaction mixture was incubated in an orbital shaker (150 rpm) for a specific time. The progress of the reaction was monitored by TLC and HPLC. After completion of the reaction, the fermentation medium was saturated with NaCl and the products extracted into ether and filtered through celite, the filtrate was dried over Na_2SO_4, concentrated and

Table 2. Apparent kinetic constants[a]

Compound	V_{max} (μmol/min/mg protein)	K_m (mmol)	V_{max}/K_m (μmol/min/mg)
Entry 1			
Baker's yeast	15+1	25+3	480
Treated baker's yeast	98+2	400+20	125
Entry 7			
Baker's yeast	8+1	13+5	540
Treated baker's yeast	78+5	200+20	165

[a] The apparent enzyme kinetic constants K_m and V_{max} were calculated by linear regression methods.[14] Protein content in the fermentation medium was estimated using the Bradford method.[15]

the residue was chromatographed (60–120 mesh silica gel, *n*-hexane–EtOAc, 90:10) to give product in good yield. The structure of each compound was confirmed by spectral analysis. The e.e.'s were determined by chiral HPLC.

4.2.1. (*R*)-(−)-2-Azido-1-phenylethanol 1. Light yellow oil: $[\alpha]_D^{25} = -80.1$ ($c = 0.78$, $CHCl_3$). ^1H NMR (200 MHz, $CDCl_3$): δ 2.28 (d, 1H, $J = 2.2$), 3.48 (m, 2H), 4.88 (m, 1H), 7.30 (m, 5H). ^{13}C NMR (200 MHz, $CDCl_3$): δ 58.2, 73.5, 125.9, 128.5, 128.9, 140.8. MS (CI): m/z 163 (M^+). IR ($CHCl_3$, cm^{-1}): 3410, 2100. Anal. calcd for $C_8H_9N_3O$: C, 58.89; H, 5.56; N, 25.75. Found: C, 58.85; H, 5.52; N, 25.73%.

4.2.2. (*R*)-(−)-2-Azido-1-(4-fluorophenyl)ethanol 2. Oil: $[\alpha]_D^{25} = -14.7$ ($c = 2.0$, $CHCl_3$). ^1H NMR (200 MHz, $CDCl_3$): δ 2.30 (brs, 1H), 3.60 (dt, 2H, $J = 6.8$, 2.6), 4.90 (t, 1H, $J = 5.8$), 7.15 (t, 2H, $J = 8.6$), 7.40 (m, 2H). ^{13}C NMR (200 MHz, $CDCl_3$): δ 58.2, 72.8, 115.5, 115.9, 127.3, 127.7, 136.4, 160.1, 165.3. MS (CI): m/z 181 (M^+). IR ($CHCl_3$, cm^{-1}): 3410, 2085. Anal. calcd for $C_8H_8FN_3O$: C, 53.04; H, 4.45; N, 23.19. Found: C, 53.01; H, 4.43; N, 23.14%.

4.2.3. (*R*)-(−)-2-Azido-1-(4-chlorophenyl)ethanol 3. Colorless needles: $[\alpha]_D^{25} = -79.1$ ($c = 1.25$, $CHCl_3$). Mp 47.5–48°C, ^1H NMR (200 MHz, $CDCl_3$): δ 2.35 (d, 1H, $J = 2.6$), 3.42 (d, 2H, $J = 6.8$), 4.85 (m, 1H), 7.35 (m, 4H). ^{13}C NMR (200 MHz, $CDCl_3$): δ 58.2, 72.8, 127.5, 128.7, 134.3, 140.1. MS (CI): m/z 197 (M^+). IR ($CHCl_3$, cm^{-1}): 3395, 2085. Anal. calcd for $C_8H_8ClN_3O$: C, 48.62; H, 4.08; N, 21.26. Found: C, 48.58; H, 4.01; N, 21.22%.

4.2.4. (*R*)-(−)-2-Azido-1-(4-bromophenyl)ethanol 4. White crystalline solid: mp 66–67.5°C. $[\alpha]_D^{25} = -36.4$ ($c = 0.95$, $CHCl_3$). ^1H NMR (200 MHz, $CDCl_3$): δ 2.55 (d, 1H, $J = 2.6$ Hz), 3.55 (m, 2H), 4.86 (m, 1H), 7.26 (d, 2H, $J = 6.8$), 7.50 (d, 2H, $J = 6.8$). ^{13}C NMR (200 MHz, $CDCl_3$) δ 39.8, 73.3, 122.2, 127.8, 131.6, 139.3. MS (CI): m/z 242 (M^+). Anal. calcd for $C_8H_8BrN_3O$: C, 39.69; H, 3.33; N, 17.36. Found: C, 39.66; H, 3.26; N, 17.28%.

4.2.5. (*R*)-(−)-2-Azido-1-(4-methoxyphenyl)ethanol 5. Colorless liquid: $[\alpha]_D^{25} = -39.0$ ($c = 1.0$, $CHCl_3$). ^1H NMR (200 MHz, $CDCl_3$): δ 2.20 (brs, 1H), 3.42 (m, 2H), 3.79 (s, 3H), 4.80 (dd, 1H, $J = 7.8$, 4.0), 6.85 (d, 2H, $J = 6.7$), 7.26 (d, 2H, $J = 6.7$). ^{13}C NMR (200 MHz, $CDCl_3$): δ 55.5, 58.3, 73.2, 114.3, 127.4, 132.5, 159.8. MS (CI): m/z 193 (M^+). IR ($CHCl_3$, cm^{-1}): 3428, 2099. Anal. calcd for $C_9H_{11}N_3O_2$: C, 55.95; H, 5.74; N, 21.75. Found: C, 55.97; H, 5.71; N, 21.72%.

4.2.6. (*R*)-(−)-2-Azido-1-(4-methylphenyl)ethanol 6. Colorless liquid: $[\alpha]_D^{25} = -28.2$ ($c = 1.2$, $CHCl_3$). ^1H NMR (200 MHz, $CDCl_3$): δ 2.20 (brs, 1H), 2.30 (s, 3H), 3.35 (m, 2H), 4.80 (m, 1H), 7.12 (d, 2H, $J = 8.4$), 7.22 (d, 2H, $J = 8.4$). ^{13}C NMR (200 MHz, $CDCl_3$): 21.2, 57.6, 73.3, 125.5, 129.4, 137.8, 138.0. MS (CI): m/z 177 (M^+). IR ($CHCl_3$, cm^{-1}): 3428, 2095. Anal. calcd for $C_9H_{11}N_3O$: C, 61.00; H, 6.26; N, 23.71. Found: C, 60.95; H, 6.22; N, 23.69%.

4.2.7. (*R*)-(−)-2-Azido-1-(2-naphthyl)ethanol 7. White solid: mp 80–81.5°C. $[\alpha]_D^{25} = -80.1$ ($c = 0.55$, $CHCl_3$). ^1H NMR (200 MHz, $CDCl_3$): δ 2.42 (d, 1H, $J = 2.4$), 3.50 (m, 2H), 5.08 (m, 1H), 7.50 (m, 3H), 7.85 (m, 4H). ^{13}C NMR (200 MHz, $CDCl_3$): δ 58.2, 73.0, 124.0, 125.1, 126.8, 127.2, 128.1, 128.4, 129.2, 133.8, 137.4. MS (CI): m/z 213 (M^+). IR ($CHCl_3$, cm^{-1}): 3398, 2095. Anal. calcd for $C_{12}H_{11}N_3O$: C, 67.59; H, 5.20; N, 19.71. Found: C, 67.57; H, 5.15; N, 19.66%.

4.2.8. (*S*)-(−)-2-Azido-1-(2-thiophenyl)ethanol 8. Oily liquid: $[\alpha]_D^{25} = -34.2$ ($c = 1.2$, $CHCl_3$). ^1H NMR (200 MHz, $CDCl_3$): δ 2.50 (brs, 1H), 3.50 (m, 2H), 5.05 (dd, 1H, $J = 7.3$, 2.2), 6.95 (m, 2H), 7.25 (d, 1H, $J = 5.6$). ^{13}C NMR (200 MHz, $CDCl_3$): δ 57.9, 69.8, 124.6, 125.4, 126.9, 144.1. MS (EI): m/z 169 (M^+). IR ($CHCl_3$, cm^{-1}): 3408, 2104. Anal. calcd for $C_6H_7N_3OS$: C, 42.59; H, 4.17; N, 24.83. Found: C, 42.52; H, 4.08; N, 24.75%.

4.3. Reduction of 2-azido-1-(2-thienyl)ethanol 8 to (*S*)-(−)-2-amino-1-(2-thienyl)ethanol 9

To a suspension of $LiAlH_4$ (190 mg, 5 mmol) in dry THF (5 mL) under nitrogen at 0°C was added a solution of **8** (338 mg, 2 mmol) in THF (2 mL) over a period of 5 min. The reaction mixture was stirred for 15 min at 0°C and then for 2 h at room temperature. The reaction mixture was quenched with water (0.5 mL) and filtered. The filtrate was dried over Na_2SO_4 and concentrated in vacuo. The residue was recrystallized from hexane–EtOAc (9:1) to afford the pure **9** (271 mg, 95% yield). The e.e. was determined by chiral HPLC as 94%.

White solid: mp 60–61.5°C. $[\alpha]_D^{25} = -39.1$ ($c = 2.0$, $CHCl_3$). ^1H NMR (200 MHz, $CDCl_3$): δ 2.25 (brs, 3H), 2.90–3.15 (m, 2H), 4.80 (m, 1H), 7.00 (m, 2H), 7.25 (m, 1H). ^{13}C NMR (200 MHz, $CDCl_3$): δ 49.1, 70.4, 123.5, 124.3, 126.7, 146.7. MS (EI): m/z 143 (M^+). IR (KBr, cm^{-1}): 3325 (NH), 3471 (OH). Anal. calcd for C_6H_9NOS: C, 50.32; H, 6.33; N, 9.78. Found: C, 50.08; H, 6.18; N, 9.33%.

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