Chemo-enzymatic synthesis of chiral 3-hydroxy-azetidin-2-ones

S S Bari*, S Madan & M K Sethi
Department of Chemistry, Panjab University, Chandigarh 160014, India

Received 7 April/998; accepted (revised) 4 December 1998

n-Tributyltin hydride reduction of 3-acetoxy-4,4-bis(alkylthio)-azetidin-2-ones 4a and 4b produces 4-unsubstituted azetidinone 5 and 4-alkylthio-azetidin-2-one 9 respectively. Azetidin-2,3-diones 8,11,13 which can be conveniently prepared by basic hydrolysis of 3-acetoxy-azetidin-2-ones 5,9,4c followed by oxidation, on biocatalytic reduction with fermenting and immobilised Baker's Yeast in aqueous and organic media, respectively affords chiral 3-hydroxy-azetidin-2-ones 6,10,12,14 with high level of enantioselectivity.

Stereocontrolled synthesis of nonfused monocyclic \(\beta\)-lactams continues to be a fascinating area of crucial importance in the field of \(\beta\)-lactam antibiotics. Periodical discoveries of new \(\beta\)-lactam antibiotics in nature has sustained the interest of the synthetic organic chemists. Besides, these strained heterocycles are increasingly becoming important synthetic building blocks for a variety of other compounds.\(^1\)

Chiral 3-hydroxy-azetidin-2-ones 1 are useful synths for different antibiotics\(^4\) as well as \(\alpha\)-hydroxy-\(\beta\)-amino acids\(^5\) derivatives which occur in many biologically active compounds such as taxol\(^6\) and taxotere, the promising anticancer drugs as well as in bestatin\(^7\), a low molecular weight peptide enzyme inhibitor with antimicrobial, anticancer and immunomodifier properties. A number of synthetic approaches involving chemical\(^8\)\(^9\) as well as biocatalytic transformations\(^10\) have been developed for their synthesis.

We wish to report here a stereoselective synthesis of 3-hydroxy-azetidin-2-ones of type 1 mediated by microbial reduction of azetidin-2,3-diones of type 2 using fermenting and immobilised Baker's yeast (\textit{Saccharomyces cerevisiae} Type-I) in aq. and organic media, respectively.

It was envisaged that in order to assess the potential of microbial reduction of azetidin-2,3-diones 2, three different types of \(\beta\)-lactam substrates namely, C-4 unsaturated \(\beta\)-lactam, C-4 monosubstituted \(\beta\)-lactam and \(\beta\)-lactam having two equivalent substituents at C-4 be used in this biotransformation study. These azetidin-2,3-diones are easily prepared from 3-acetoxy-4,4-bis(alkylthio)-azetidin-2-ones 4a-c which in turn can be synthesised\(^16\)\(^17\) by reacting di-thiocarbonimidates 3a-c\(^15\) with acetoxyacetyl chloride in presence of triethyl amine as shown in Scheme I.

![Scheme I](image)

Reagents: i, (C\(_2\)H\(_5\))\(_3\)N, CH\(_2\)Cl\(_2\), 0°C.

4-Unsubstituted Azetidin-2,3-dione. 1-(4'-Methoxy-phenyl)-3-acetoxy-4,4-bis(methylthio)azetidin-2-one 4a was submitted to desulphurization reaction using n-butylltin hydride (2 molar) under N\(_2\) in presence of radical generator AIBN in refluxing toluene (\textit{Scheme II}). TLC profile of the products showed formation of three products. Solvent evaporation followed by column chromatographic separation afforded 4-unsubstituted azetidin-2-one 5 along with two other azetidin-2-ones 6 and 7 in 58%, 10% and 32% proportions, respectively. The structures of

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\(\dagger\) Dedicated to Prof. S.V. Kessar, Senior Scientist, INSA, Department of Chemistry, Panjab University, Chandigarh
these azetidin-2-ones were confirmed on the basis of spectroscopic data (IR, PMR).

Mild basic hydrolysis of 1-(4'-methoxyphenyl)-3-acetoxy-azetidin-2-one 5 with 1% sodium hydroxide in aqueous methanol at 0°C produced 3-hydroxy-azetidin-2-one 6 in quantitative yield whose structure was established on the basis of its spectral data. This 3-hydroxy-azetidin-2-one was oxidized\(^1\) to azetidin-2,3-dione 8 using \((\text{CH}_3)_2\text{SBr/Et}_3\text{N}\) (Corey’s reagent) in methylene chloride which was purified by column chromatography. The structure was confirmed on the basis of its spectral data (UV, IR, PMR).

4-Monoalkythio-azetidin-2,3-dione. Controlled reduction of 4,4-bis(ethythio)-azetidin-2-one 4b using n-tributyltin hydride \((1.02 \text{ molar})\) in presence of AIBN in refluxing toluene afforded cis-3-acetoxy-4-ethylthio-azetidin-2-one 9 along with two other compounds, cis-3-hydroxy-4-ethylthio-azetidin-2-one 10 and 3-hydroxy-azetidin-2-one 6 in 82%, 6% and 12% proportion, respectively (Scheme II). The exclusive cis-\(\beta\)-lactam formation\(^2\) takes place as expected since the hydrogen atom approaches the C-4 planar radical from the less hindered side of C-3 proton. The column chromatographic separation of the mixture afforded all the components in pure form whose structures were established on the basis of spectral data.

CH\(_3\)COO)=t1;l SRI HO
\(=\)I .
\(\text{SR } \rightarrow \text{SR } \)
\(\text{R } \rightarrow \text{R } \)
\(\text{R } \rightarrow \text{R } \)
\(4 \text{ (a-c) } 12 \text{ (a-c) } 13 \text{ (a-c) } 4,12,13 \text{ R } \)

Reagents: i, \(\text{n-BuSnH, AIBN, toluene, reflux} \); ii, 1% NaOH, \(\text{aq. methanol, 0°C} \); iii, \((\text{CH}_3)_2\text{S, Br, (C}_2\text{H}_5)_2\text{N, CH}_2\text{Cl}_2, 0°C\).

Scheme II

cis-3-Acetoxy-azetidin-2-one 9 on hydrolysis with 1% NaOH in \(\text{aq. methanol at 0°C} \) produced cis-3-hydroxy-azetidin-2-one 10 which on oxidation with Corey’s reagent in methylene chloride at 0°C gave 4-ethylthio-azetidin-2,3-dione 11 as a yellow crystalline solid. The structure was confirmed on the basis of spectroscopic data.

4,4-Bis(alkythio)-azetidin-2,3-diones. 3-Acetoxy-4,4-bis(methylthio)-azetidin-2-one 4a on hydrolysis with 1% NaOH in \(\text{aq. methanol gave 3-hydroxy-azetidin-2-one 6a in quantitative yield (Scheme III)} \). However, this azetidinone was found to be unstable at room temperature and slowly changed to a new compound with higher R\(_f\) than the initially hydrolysed product. Thus 3-hydroxy-azetidin-2-one after work up was immediately submitted to Corey’s oxidation in methylene chloride at 0°C which cleanly afforded 4,4-bis(methylthio)-azetidin-2,3-dione 13a as a yellow crystalline material. Azetidin-2,3-diones 13b,c were also prepared following the same procedure from 3-acetoxy-4,4-bis(alkythio)-azetidin-2-ones 4b, c.

CH\(_3\)COO)=t1;l SRI HO
\(=\)I .
\(\text{SR } \rightarrow \text{SR } \)
\(\text{R } \rightarrow \text{R } \)
\(\text{R } \rightarrow \text{R } \)
\(4,12,13 \text{ R } \)

Reagents: i, 1% NaOH, \(\text{CH}_2\text{OH} \); ii, \((\text{CH}_3)_2\text{S, Br, (C}_2\text{H}_5)_2\text{N, CH}_2\text{Cl}_2, 0°C\).

Scheme III

The structures of these azetidin-2,3-diones were established on the basis of their spectral data. Since 3-hydroxy-azetidin-2-ones 12a, b were found to be unstable at room temperature, the corresponding azetidin-2,3-diones 13a, b were considered unsuitable for biotransformation. However, it is pertinent to mention here that 3-hydroxy-4,4-bis(n-propythio)-azetidin-2-one 12c was found to be stable at room temperature and thus the corresponding azetidin-2,3-dione 13c was considered suitable for bioreduction.

Chiral 3-hydroxy-azetidin-2-ones. Various azetidin-2,3-diones prepared were used as substrates for microbial reduction (Scheme IV). Thus 1-(4'-methoxyphenyl)-azetidin-2,3-dione 8 was submitted to biocatalytic reduction using Baker’s Yeast (Saccharomyces cerevisiae Type-I, Sigma Chemicals, USA) in \(\text{aq. media at 28°C} \) containing small amount of methanol to dissolve the substrate as well as to serve as energy source for the microorganism. The stirring was continued for 24 hr at the same tempera-
The crude product formed in 72% yield after chromatographic purification afforded optically active 1-(4'-methoxyphenyl)-3-hydroxy-azetidin-2-one 6 in 57% isolated yield. The structure was confirmed on the basis of its spectral data (IR, PMR) which was identical to that produced by basic hydrolysis of 3-acetoxy-azetidin-2-one 5.

The biocatalytically produced 3-hydroxy-azetidin-2-one 6 on treatment with acetic anhydride and pyridine in methylene chloride furnished optically active 3-acetoxy-azetidin-2-one 5 which was purified by column chromatography and showed specific rotation as $[\alpha]_D^{20} +20.72$ (c 2.17, CHCl$_3$). The structure was corroborated by its spectral data (IR, PMR).

Similarly azetidin-2,3-dione 8 was submitted to microbial reduction$^{23}$ using immobilised Baker’s Yeast (Saccharomyces cerevisiae, type-I) in hexane at 37±1°C. The substrate was dissolved in small amount of methanol which also served as a nutrient for Baker’s Yeast. The reduction was found to be slow and was complete in 72 hr. The crude product formed in 36% yield on acylation with Ac$_2$O/Py in methylene chloride at 0°C followed by purification by column chromatography afforded pure optically active 3-acetoxy-azetidin-2-one 5 in 31% isolated yield. The structure was confirmed through its spectral data (IR, PMR) and it showed specific rotation as $[\alpha]_D^{20} + 20.97$ (c 0.429, CHCl$_3$).

The other azetidin-2,3-diones 11 and 13c were also reduced with Baker’s Yeast in aq. medium following the procedure as mentioned for azetidin-2,3-dione 8. The corresponding 3-hydroxy-azetidin-2-ones 10, 12c and 14 after acylation with Ac$_2$O/Py were purified by column chromatography and their structures established through their spectral data (IR, PMR).

**Enantiomeric / Diastereomeric Excess Determination.** The enantiomeric excess (ee) or diastereomeric excess (de) of optically active 3-acetoxy-azetidin-2-ones obtained by microbial reduction was determined by NMR spectroscopy using chiral shift reagents such as tris(3-heptafluoropropylhydroxymethylene)-(+) -camphorato)-praseodymium (III), Pr(hfc)$_3$. However to check the suitability of this technique for correct enantiomeric excess (ee) determination, studies regarding base line separation of enantiotopic C$_1$-H resonance and enantiotopic acetate methyl resonance in the PMR spectrum were conducted using Pr(hfc)$_3$, and racemic 3-acetoxy-azetidin-2-one 5 as well as racemic 3-hydroxy-azetidin-2-one 6. No base line separation was observed for 3-hydroxy-azetidin-2-one 6 where as enantiotopic C$_1$-H resonance at $\delta$ 5.73 and acetate methyl resonance at $\delta$ 2.15 showed complete base line separation with Pr(hfc)$_3$ shift reagent. The enantiomeric excess (ee) of various optically active 3-acetoxy-azetidin-2-ones were determined by using Pr(hfc)$_3$ as shown in Table I.

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**Table I—Enantiomeric Excess (ee) of chiral 3-acetoxy-azetidin-2-ones**

<table>
<thead>
<tr>
<th>Substrate No</th>
<th>Baker’s Yeast (Medium)</th>
<th>Product No. Yield (%)</th>
<th>Enantiomeric excess (ee) $[\alpha]_D^{20}$ (c in g/100mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Fermenting Baker’s Yeast (FBY) (aqueous)</td>
<td>6 (57)</td>
<td>66</td>
</tr>
<tr>
<td>8</td>
<td>Immobilised Baker’s Yeast (IBY) (Hexanes)</td>
<td>6 (31)</td>
<td>19</td>
</tr>
<tr>
<td>11</td>
<td>FBY</td>
<td>10 (50)</td>
<td>18</td>
</tr>
<tr>
<td>12c</td>
<td>FBY</td>
<td>14 (9)</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12c (51)</td>
<td>-98</td>
</tr>
</tbody>
</table>

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**Scheme IV**

Reagents: i. Fermenting Baker’s Yeast (Type I), 28°C or Immobilised Baker’s Yeast, (Type I), hexanes, 37°C; ii. Ac$_2$O, Py; CH$_2$Cl$_2$, 0°C.
Experimental Section

M.Ps. are uncorrected and reported in °C. IR spectra were recorded on a Perkin Elmer Model 1430 spectrometer (\(\nu_{\text{max}}\) in cm\(^{-1}\)) and PMR spectra on a Varian EM 390, 90 MHz spectrometer or Bruker AC 300F, 300 MHz multinuclear spectrometer. Enantiomeric excess (ee) and CMR spectra were obtained on Bruker AC 300F, 300 MHz spectrometer in CDCl\(_3\) with TMS as internal standard (chemical shifts in \(\delta\) ppm). Mass spectra (EI, CIMS) were obtained at 70 eV on VG Analytical 11-250-J 70S spectrometer.

Optical rotations were measured on Bruker A C 300F, 300 MHz spectrometer using sodium vapour lamp as a source of light. Elemental analyses were carried out on a Perkin Elmer 2400 (CHN) Elemental Analyser. Unless otherwise stated all organic extracts were dried over anhyd. sodium sulphate.

Dialkyl-1-(4'-methoxyphenyl)-dithiocarbonimidates 3a-c. These dithiocarbonimidates were prepared following the reported procedure.\(^{15}\)

1-(4'-Methoxyphenyl)-3-acetoxy-4, 4-bis(methylthio) azetidin-2-one 4a. A solution of acetoxycetil chloride (0.75g, 5.5 mmole) in methylene chloride (20 mL) was added dropwise to a stirred solution of dithiocarbonimidate 3a (1.14g, 5 mmol) and trimethylamine (2.1 mL, 15 mmole) in methylene chloride (50 mL) at 5°C. The progress of the reaction was checked by TLC. Thereafter the reaction mixture was washed with 5% aq. sodium bicarbonate solution (2×20 mL), water (30 mL), brine (30 mL) and organic layer dried. The residue after solvent evaporation on chromatography using silica gel and ethyl acetate-hexanes (1:9) furnished the azetidin-2-one 4a as a thick oil which was crystallized from methylene chloride-hexanes in 72% yield (1.17 g) m.p. 69-70°; IR (KBr): 1755, 1710, 120-150, PMR: 2.15 (s, 3H, OCOCH\(_3\)), 2.30 (s, 6H, 2×SCH\(_2\)), 3.80 (s, 3H, OCH\(_3\)), 6.05 (s, 1H, C\(_4\)-H), 7.30 (dd, 4H, aromatic protons); CMR: 13.01, 13.70, 20.01, 55.02, 79.91, 81.91, 113.80, 120.00, 157.92, 160.00, 166.81; CIMS: (NH, as reagent gas): m/z 345 (M+18\(^+\)). Anal. Found: C, 61.20; H, 5.15; N, 4.19. Calc. for C\(_{14}\)H\(_{17}\)NO\(_2\)S\(_2\): C, 51.35; H, 5.23; N, 4.28%.

1-(4'-Methoxyphenyl)-3-acetoxy-4, 4-bis(ethylthio) azetidin-2-one 4b. This was prepared from dithiocarbonimidate 3b following the procedure for 4a in 76% yield as a thick oil which showed the following spectral data; IR (neat): 1770, 1715; PMR: 1.11 (t, 3H, J = 7.0 Hz, SCH\(_2\)CH\(_3\)), 1.21 (t, 3H, J = 7.0 Hz, SCH\(_2\)CH\(_3\)), 2.30 (s, 3H, OCOCH\(_3\)), 2.71 (m, 4H, 2×SCH\(_2\)CH\(_3\)), 3.82 (s, 3H, OCH\(_3\)), 6.05 (s, 1H, C\(_4\)-H), 6.90-7.85 (dd, 4H, aromatic protons); CMR: 13.43, 13.96, 20.44, 24.75, 25.39, 55.37, 80.26, 114.28, 120.55, 128.71, 157.34, 159.90, 168.67. Anal. Found: C, 53.92; H, 5.79; N, 3.85. Calc. for C\(_{19}\)H\(_{21}\)NO\(_2\)S\(_2\): C, 54.06; H, 5.95; N, 3.94%.

1-(4'-Methoxyphenyl)-3-acetoxy-4, 4-bis(n-propylothio) azetidin-2-one 4c. This was prepared from dithiocarbonimidate 3c following the same procedure in 61% yield as an oil; IR (neat): 1770, 1720; PMR: 0.94 (m, 6H, 2×SCH\(_2\)CH\(_2\)CH\(_3\)), 1.56 (m, 4H, 2×SCH\(_2\)CH\(_2\)CH\(_3\)), 2.25 (s, 3H, OCOCH\(_3\)), 2.74 (m, 4H, 2×SCH\(_2\)), 3.82 (s, 3H, OCH\(_3\)), 6.05 (s, 1H, C\(_4\)-H), 6.50-7.35 (dd, 4H, aromatic protons); CMR: 13.40, 13.53, 20.50, 22.00, 22.40, 32.57, 55.38, 80.31, 82.58, 114.29, 120.53, 128.75, 157.35, 160.10, 168.75. Anal. Found: C, 56.21; H, 6.62; N, 3.59. Calc. for C\(_{24}\)H\(_{27}\)NO\(_2\)S\(_2\): C, 56.37; H, 6.57; N, 3.65%.

1-(4'-Methoxyphenyl)-3-acetoxy-azetidin-2-one 5. n-Tributyltin hydride (1.19g, 1.1 mL, 4.08 mmole) was added dropwise via syringe to a stirred solution of azetidin-2-one 4a (0.65g, 2 mmol) in dry toluene (50 mL) containing catalytic amount of azobisisobutyronitrile (AIBN) under nitrogen. The reaction mixture was refluxed and the progress of the reaction was checked by TLC. Thereafter the toluene was evaporated in vacuo and the residue was redissolved in methylene chloride (50 mL) and washed with water (4×20 mL), brine (30 mL) and dried. Solvent evaporation gave the residue (0.94 g) consisting of three products (TLC profile) which on chromatographic separation (silica gel, ethyl acetate-hexanes, 2:8) gave azetidin-ones 5,6,7 in 58%, 10% and 32% proportion, respectively. The major component 5 (R, 0.51), m.p. 97-98° was characterised as 1-(4'-methoxyphenyl)-3-acetoxy-azetidin-2-one and showed the following spectral data; IR (KBr): 1750, 1710; PMR: 2.15 (s, 3H, OCOCH\(_3\)), 3.59 (dt, 1H, C\(_4\)-H), 3.80 (s, 3H, OCH\(_3\)), 4.00 (dt, 1H, C\(_5\)-H), 5.74 (m, 1H, C\(_6\)-H), 7.12 (dd, 4H); CMR: 20.40, 47.65, 55.44, 73.78, 114.45, 117.99, 131.05, 156.67, 160.73, 169.80; CMR (DEPT 135); 20.41(+), 47.65(-), 55.44(+), 73.78(+), 114.45(+), 117.99(+). Anal. Found: C, 61.39; H, 5.65; N, 6.01. Calc. for C\(_{19}\)H\(_{17}\)NO\(_2\): C, 61.28; H, 5.53; N, 5.95%.

The fast moving component (RF 0.65, 32%) was identified as cis-1-(4'-methoxyphenyl)-3-acetoxy-4-methylthio-azetidin-2-one 7 from its spectral data, m.p.
125 °, IR (nujol): 1775, 1760, 1510; PMR: 2.05 (s, 3H, SCH₂), 2.25 (s, 3H, OOCCH₃), 3.80 (s, 3H, OCH₃), 5.25 (d, 1H, J = 4.55 Hz, C₂-H), 5.95 (d, 1H, J = 5.45 Hz, C₃-H), 6.90-7.60 (dd, 4H); CMR: 11.44, 20.40, 55.37, 63.37, 76.45, 114.47, 118.94, 129.61, 156.96, 160.66, 169.20.

The third component (Rₜ 0.23, 10%) was assigned the structure as 1-(4'-methoxyphenyl)-3-hydroxy-azetidin-2-one 6. on the basis of the spectral data, IR (neat): 3400, 1740; PMR: 3.51 (m, 1H, C₃-H), 3.79 (s, 3H, OCH₃), 3.85 (t, 1H, C₁-H), 5.00 (m, 1H, C₄-H), 6.01 (d, 1H, J = 7.50 Hz, exchangeable with D₂O), 7.10 (dd, 4H); CMR: 49.41, 55.10, 74.22, 114.10, 117.51, 131.51, 155.90, 165.92; CMR (DEPT 135): 49.41(-), 55.10(+), 74.19(+), 114.09(+), 117.51(+).

1-(4'-Methoxyphenyl)-3-hydroxy-azetidin-2-one 6. A solution of sodium hydroxide (6.01 g, 76%) in methanol (20 mL) was added dropwise to a solution of 3-acetoxy-azetidin-2-one 5 (1.17 g, 5 mmole) in methanol (100 mL) at 0 °C. The progress of the reaction was monitored by TLC and pH of reaction mixture was made neutral. Residue after solvent evaporation at room temperature was redissolved in methylene chloride (50 mL) and washed with water (3 x 20 mL), brine (20 mL) and dried. Solvent evaporation followed by chromatography of the residue (silica gel, ethyl acetate-hexanes, 2:3) afforded 1-(4'-methoxyphenyl)-3-hydroxy-azetidin-2-one 6 (0.92 g, 96%) whose structure was established through its spectral data.

1-(4'-Methoxyphenyl)-azetidin-2,3-dione 8. A solution of bromine (0.45 mL, 4.6 mmole) in methylene chloride (10 mL) was added to a stirred solution of dimethyl sulphide (0.45 mL, 6 mmole) in methylene chloride (20 mL) at 0 °C. Light yellow precipitates were obtained and mixture was cooled to -25 °C. Then a solution of 3-hydroxy-azetidin-2-one 6 (0.58 g, 3 mmol) in methylene chloride (20 mL) was added slowly and the stirring continued for 1 hr. Thereafter, triethylamine (1.25 mL, 9 mmole) in methylene chloride (10 mL) was added dropwise. The contents were allowed to warm to room temperature and washed with water (3 x 25 mL), brine (30 mL) and dried. The residue after solvent evaporation followed by chromatographic purification (silica gel, ethyl acetate-hexanes, 1:9) afforded 1-(4'-methoxyphenyl)-azetidin-2,3-dione 8 (0.44 g, 76%) as a pale yellow crystalline solid, m.p. 148-49°C; IR (KBr): 1780, 1740; PMR: 3.84 (s, 3H, OCH₃), 4.30 (s, 2H, C₄-H₅), 7.25 (dd, 4H); CMR: 55.56, 59.57, 114.81, 118.98, 131.00, 158.21, 159.80, 190.41; CI-MS (CH₄ as reagent gas): m/z 192 (M+H⁺). Anal. Found: C, 62.98; H, 4.81; N, 7.31. Calc. for C₁₂H₁₇NO₃: C, 62.82; H, 4.74; N, 7.33%.

cis-1-(4'-Methoxyphenyl)-3-acetoxy-4-ethylthio-azetidin-2-one 9. This was prepared from 3-acetoxy-4,4-bis(ethylthio)azetidin-2-one 4b (0.71 g, 2 mmol) and n-butyltin hydride (0.59 g, 2.04 mmole) in presence of catalytic amount of AIBN in refluxing toluene following earlier procedure which afforded a mixture of three components (0.58 g). The major component which was obtained as an oil (68%, Rₜ 0.54) was characterised as cis-1-(4'-methoxyphenyl)-3-acetoxy-4-ethylthio-azetidin-2-one 9 on the basis of its spectral data; IR (neat): 3400, 1740, 1710; PMR: 1.20 (t, 3H, SCH₂CH₃), 2.65 (m, 2H, SCH₂CH₃), 3.80 (s, 3H, OCH₃), 5.30 (d, 1H, J = 4.4 Hz, C₂-H), 5.95 (d, 1H, J = 4.4 Hz, C₃-H), 7.25 (dd, 4H, Ar); CMR: 14.41, 20.50, 23.72, 55.41, 64.02, 76.38, 114.51, 119.22, 129.61, 157.01, 160.73, 169.30. Anal. Found: C, 57.08; H, 5.86; N, 4.68. Calc. for C₁₂H₁₇NO₃: C, 56.93; H, 5.80; N, 4.74%.

The minor component (17%, Rₜ 0.28) was identified from spectral data as cis-1-(4'-methoxyphenyl)-3-hydroxy-4-ethylthio-azetidin-2-one 10, IR (neat): 3400, 1740; PMR: 1.31 (t, 3H, SCH₂CH₃), 2.7 (m, 2H, SCH₂CH₃), 3.55 (d, 1H, J = 9.00 Hz, OH), 3.8 (s, 3H, OCH₃), 5.05 (dd, 1H, J = 4.4 Hz, C₂-H), 5.25 (d, 1H, J = 4.4 Hz, C₃-H), 7.3 (dd, 4H, Ar); CMR: 14.88, 24.41, 55.52, 65.31, 76.52, 114.40, 119.21, 130.04, 157.81, 163.83.

The third component slow moving (Rₜ 0.23, 15%) was assigned structure as 1-(4'-methoxyphenyl)-3-hydroxy-azetidin-2-one 6 on the basis of its spectral data.

cis-1-(4'-Methoxyphenyl)-3-acetoxy-4-ethylthio-azetidin-2-one 10. This was prepared by hydrolysing 3-acetoxy-azetidin-2-one 9 (1.47 g, 5 mmole) with 1% sodium hydroxide (0.2 g, 5 mmole) in aq. methanol (50 mL) at 0 °C in 90% yield as an oil (1.17 g) whose structure was confirmed on the basis of its spectral data as reported earlier.

1-(4'-Methoxyphenyl)-4-ethylthio-azetidin-2,3-dione 11. This was prepared from 3-hydroxy-4-ethylthio-azetidin-2-one 10 (0.75 g, 3 mmole) and Corey's reagent prepared from dimethyl sulphide (0.57 g, 4.6 mmole) following the procedure already...
reported for 8 in 66% yield (0.5 g) as a yellow crystalline solid whose structure was confirmed on the basis of its spectral data, m.p. 93-94°C, IR (nujol): 1780, 1740; PMR: 1.21 (t, 3H, SCH₂CH₃), 2.55 (q, 2H, SCH₂CH₃), 3.8 (s, 3H, OCH₃), 5.65 (s, 1H, C₆-H), 7.22 (dd, 4H, Ar); CMR: 13.37, 22.60, 32.74, 55.50, 87.72, 114.43, 120.90, 128.37, 157.90, 158.70, 191.87. Anal. Found: C, 56.48; H, 6.13; N, 4.23. Calc. for C₁₆H₁₁N₀₃S₂: C, 56.61; H, 6.23; N, 4.13%.

1-(4'-Methoxyphenyl)-3-hydroxy-4, 4-bis(methylthio) azetidin-2-one 12a. This was synthesised from azetidin-2-one 4a (1.63 g, 5 mmol) by hydrolysing with 1% aq. NaOH in methanol (50 mL) following the procedure reported for 6 in 83% yield (0.98 g), IR (neat): 3400, 1745; PMR: 2.15 (s, 3H, SCH₃), 2.25 (s, 3H, C₆H₅), 3.81 (s, 3H, OCH₃), 4.75 (bs, 1H, OH, D₂O exchangeable). 5.15 (s, 1H, C₆-H), 7.3 (dd, 4H, Ar); CMR: 12.71, 14.02, 55.43, 81.20, 83.21, 114.31, 121.12, 128.42, 157.41, 164.30.

1-(4'-Methoxyphenyl)-3-hydroxy-4, 4-bis(methylthio)azetidin-2,3-dione 13a. This was prepared from 3-hydroxy-azetidin-2-one 12a (0.86 g, 3 mmol) by oxidation with Corey’s reagent prepared from diacetyl-2,3-dione 13a. This was prepared from 3-acetoxy-azetidin-2-one 4b (1.76 g, 5 mmol) when hydrolysed with 1% aq. NaOH in methanol (50 mL) giving 3-hydroxy-azetidin-2-one 12b (0.97 g, 61%) as a thick oil, IR (neat): 3400, 1750; PMR: 0.85 (m, 6H, 2xSCH₂CH₃), 2.70 (m, 4H, 2xSCH₂CH₃), 3.7 (s, 3H, OCH₃), 5.05 (s, 1H, C₆-H), 6.8-7.8 (dd, 4H, Ar).

1-(4'-Methoxyphenyl)-3-hydroxy-4, 4-bis(propylthio)azetidin-2-one 12c. This was prepared from 3-acetoxy-azetidin-2-one 4c using the procedure reported for 6 in 83% yield as oil, IR (nujol): 3390, 1750; PMR: 0.85 (m, 6H, 2xSCH₂CH₃), 1.50 (m, 4H, 2xSCH₂CH₃), 2.70 (m, 4H, 2xSCH₂CH₃), 3.7 (s, 3H, OCH₃), 5.2 (s, 1H, C₆-H), 6.20 (bs, 1H, OH), 6.85-7.80 (dd, 4H, Ar).

1-(4'-Methoxyphenyl)-3-hydroxy-4, 4-bis(n-propylthio) azetidin-2,3-dione 13c. This was prepared by oxidation of 3-hydroxy-azetidin-2-one 12c by following the procedure reported for 8 as a crystalline solid (57%), m.p. 84-85°C, IR (KBr): 1775, 1740; PMR: 0.91 (t, 6H, 2xSCH₂CH₃), 1.51 (m, 4H, 2xSCH₂CH₃), 2.60 (m, 4H, 2xSCH₂CH₃), 3.81 (s, 3H, OCH₃), 7.52 (dd, 4H, Ar); CMR: 13.37, 22.60, 32.74, 55.50, 87.72, 114.43, 120.90, 128.37, 157.90, 158.70, 191.87.

Optically active 1-(4'-methoxyphenyl)-3-hydroxy-azetidin-2-one 6

a) By fermenting Baker’s Yeast

Dry Baker’s Yeast cells (10 g, Type-I, 90% active, Sigma Chemical Co., USA) were slurried in warm distilled water (100 mL) and stirred for 5 min. at ambient temperature. Azetidin-2,3-dione 8 (0.25 g, 1.3 mmole) dissolved in methanol (3 mL) was added dropwise to it and the mixture incubated on a rotary shaker at 28±1°C. Progress of the reaction was checked by TLC and shaking was continued for 4 hr. Thereafter, the reaction mixture was centrifuged (5000 r.p.m. for 10 min.) and the supernatant was collected, saturated with sodium chloride and extracted with methylene chloride (8×25 mL). The combined extract was washed with water (2×30 mL), brine (30 mL) and dried. The residue, after solvent evaporation, on chromatography (silica gel, ethyl acetate-hexanes, 3:7) gave optically active 3-hydroxy-azetidin-2-one 6 (0.14 g, 57%). The structure was confirmed on the basis of spectroscopic data (IR, PMR, CMR) which was in exact agreement with that reported for racemic 6.

Optically active 1-(4'-methoxyphenyl)-3-acetoxy-azetidin-2-one 5. Chiral 3-hydroxy-azetidin-2-one 6 (0.13 g, 0.7 mmol) was acylated by treating with acetic anhydride (0.08 g, 0.78 mmol) in dry methylene chloride containing a drop of pyridine. After evaporating the solvent in vacuo, the residue was redissolved in methylene chloride (30 mL) and washed with water (2×10 mL), brine (10 mL) and dried. Chromatographic purification of residue gave optically active 3-acetoxy-azetidin-2-one 5 (0.15 g, 91%) whose structure was confirmed on the basis of its spectral data which was in close agreement with that of (±)-3-acetoxy-azetidin-2-one 5. The enantiomeric excess (ee) was determined to be 66% by using chiral shift reagent, Pr(hfc).

b) By immobilised Baker’s Yeast

A solution of 1-(4'-methoxyphenyl)-azetidin-2,3-dione 8 (0.38 g, 2 mmole) in methanol (3 mL) was
added dropwise to stirred immobilised Baker’s Yeast beads (2-3 mm) in hexane (100 mL) prepared from active Baker’s Yeast cells (5 g) and sodium alginate. The contents were incubated at 37±1° on a rotary shaker for 72 hr, thereafter the reaction mixture was filtered and beads washed with hexanes (2x30 mL), brine (20 mL) and dried. The residue after solvent evaporation on chromatography afforded the optically active 3-hydroxy-azetidin-2-one 6 (0.12 g, 31%). Structure was confirmed on the basis of its spectral data (IR, PMR).

It was further converted to optically active 3-acetoxy-azetidin-2-one 5 using acetic anhydride and pyridine. The structure was confirmed on the basis of its spectral data. The enantiomeric excess (ee) was determined to be 19.02% and optical rotation as [α]D20+20.97 (c 2.17, CHCl₃).

Optically active cis and trans 1-(4’-methoxyphenyl)-4-ethylthio-3-hydroxy-azetidin-2-one 10 and 14. Reduction of azetidin-2,3-dione 11 (0.37 g, 1.5 mmole) in methanol (3 mL) with fermenting Baker’s Yeast Type-I slurred in distilled water at 28±1° following the procedure adopted for optically active 6 gave a mixture of optically active 1-(4’-methoxyphenyl)-4-ethylthio-3-hydroxy-azetidin-2-ones 10 and 14, respectively (0.22 g, 58%) as evident from the PMR spectra. This mixture on treatment with acetic anhydride (90 mg, 0.87 mmole) in presence of pyridine at 0°C afforded cis and trans 3-acetoxy-4-ethylthio-azetidin-2-ones 9 and 15, respectively in the ratio 6:1 as inferred from PMR spectra. The major cis 84% (0.19 g) and minor trans 14% (0.03 g) were separated by column chromatography and characterised by their spectral data. The optical rotation and enantiomeric excess (ee) were determined for these azetidin-2-ones as reported in Table 1.

Optically active 1-(4’-methoxyphenyl)-3-hydroxy-4,4-bis(n-propylthio)azetidin-2-one 12c. Reduction of azetidin-2,3-dione 13c (0.5 g, 1.5 mmole) with fermenting Baker’s Yeast (10 g, Type-I) following the procedure adopted for 8 gave optically active 3-hydroxy-4,4-bis(n-propylthio)-azetidin-2-one 12c (0.26 g, 51%). The structure was confirmed on the basis of its spectral data.

Optically active 1-(4’-methoxyphenyl)-3-acetoxy-4,4-bis(n-propylthio)-azetidin-2-one 4c. 

| Hydroxy-azetidin-2-one 12c (0.17 g, 5mmole) on treatment with acetic anhydride (0.06 g, 0.6 mmole) and pyridine following the earlier procedure adopted for optically active 5 afforded optically active 4c (0.15 g, 88%). The structure was confirmed on the basis of spectral data. The enantiomeric excess (ee) was determined to be more than 98% and optical rotation measured as [α]D20 + 10.14 (c 0.493, CHCl₃). |

Acknowledgement
The authors gratefully acknowledge the financial support of this work from Council of Scientific and Industrial Research, New Delhi and Department of Science and Technology, New Delhi (Project No. SP/SI/G-29/94). We also thank the Regional Sophisticated Instrumentation Centre (RSIC), Panjab University, Chandigarh for spectral facilities.

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