Mechanism of the yeast mediated reduction of nitrostyrenes in light petroleum

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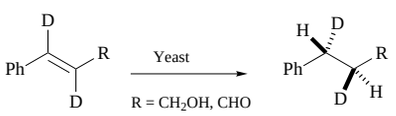
The yeast mediated reduction of a range of α- and β-deutero substituted nitrostyrenes has been conducted in light petroleum in the presence of a small amount of water or D_2O. NMR analysis of the products from these reactions has allowed the determination of the mechanism of this yeast reduction reaction. The results indicate that initially, a reversible non-stereoselective protonation occurs at the β-centre, followed by stereoselective addition of hydride at the α-position.

Introduction

The yeast mediated reduction of β-nitrostyrenes † has been studied using yeast in both an aqueous [1,2] and an organic solvent system.[3] In all cases reported, the reduction of nitrostyrenes with substituents in the β-position gave rise to the production of racemic products, whilst stereoselective reduction was observed for α-substituted nitrostyrenes. The lack of stereoselectivity in the aqueous systems had been attributed to racemisation of the product under the mildly basic reaction conditions,[4] however it was shown that this racemisation was not occurring in the organic solvent system.[3] In order to explain this unusual lack of stereoselectivity associated with a yeast reduction reaction, a clearer understanding of the reaction mechanism is required.

Even though yeast has been extensively used to effect chiral reductions in organic synthesis [5] very little work has been undertaken to elucidate the mode of action of this reduction reaction. Fuganti and co-workers have studied the mechanism of the yeast mediated reduction of cinnamyl alcohol and cinnamaldehyde (Scheme 1) [6] and a number of α,β-unsaturated



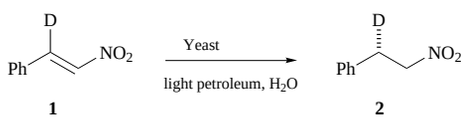
Scheme 1

lactones [7–9] using an aqueous reaction system. Through the use of deuterium labelling they have shown that yeast stereoselectivity delivers both the hydride and the proton in an *anti* fashion. The yeast reduction of nitrostyrenes must involve a somewhat different mechanism since a racemic product results.

Results and discussion

Reduction of α-deutero-β-nitrostyrene 1

α-Deutero-β-nitrostyrene **1** was prepared from nitromethane and α-deuterobenzaldehyde in good yield with 100% deuterium incorporation at the α-carbon. Reduction with bakers yeast using the previously reported reaction conditions (1 mmol substrate, 11 g yeast, 0.8 ml water per g yeast, 50 ml light petroleum) [3] gave 1-deutero-2-nitro-1-phenylethane **2** in 65% isolated yield and with no loss of deuterium from the α-carbon (Scheme 2).



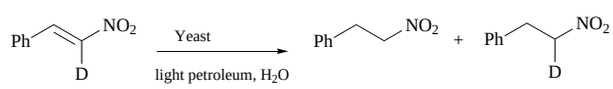
1 **2**

Scheme 2 ‡

The product obtained had a specific rotation of $+0.28\ 10^{-1}$ deg cm^2 g^{-1} indicating that some stereoselectivity had occurred at the α-carbon. To determine the enantiomeric excess of the product the nitro group was reduced to the amine using $LiAlH_4$ and the Mosher's amide formed. NMR analysis of the amide in the presence of the shift reagent $Eu(fod)_3$ indicated a 61% ee based upon integration of the proton at the α-centre. The finding that the reduction was partially stereoselective is not surprising since α-methyl-β-nitrostyrene is stereoselectively reduced by yeast in an aqueous reaction system;[2] it is at the β-centre that the lack of stereoselectivity has been observed.[1,3]

Reduction of β-deutero-β-nitrostyrene 3

β-Deutero-β-nitrostyrene **3** was prepared from [$^2H_3$]nitromethane and benzaldehyde in good yield and with 95% incorporation of deuterium at the β-carbon when the reaction was conducted in D_2O. Reduction of this compound with yeast in the organic solvent system gave a 59% yield of a mixture of 2-nitro-1-phenylethane **4** and 2-deutero-2-nitro-1-phenylethane **5** in a ratio of 4 : 1 (Scheme 3).



3 **4** **5**

Scheme 3

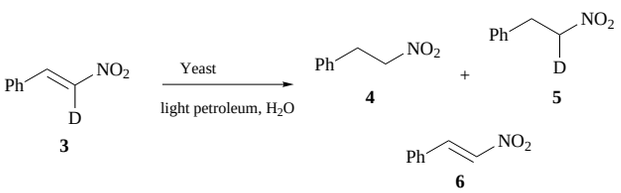
The loss of deuterium is a clear indication that exchange is occurring at some stage during the reaction. We have previously shown that deuterium is not incorporated at the β-centre when 2-nitro-1-phenylethane **4** is stirred in light petroleum, D_2O and yeast, indicating that exchange does not occur between the water present in the reaction and the reduced product **4**.[3] To show that exchange does not occur between protons bound to

† Throughout the text the compounds are referred to as β-nitrostyrenes with the substituents prefixed as α- or β- (*e.g.*: α-deutero-β-nitrostyrene) to assist in the description of where hydride and proton attack is occurring. IUPAC nomenclature, (*E*)-2-nitro-1-phenyl[$^{1\text{-}2}$H]propene, is used in the Experimental section.

‡ It is assumed that the major isomer produced is the (*S*)-enantiomer as this is the case for reduction of α-methyl-β-nitrostyrene under similar conditions.

the yeast and the reduced nitrostyrene, 2-deutero-2-nitro-1-phenylethane **5**, prepared by sodium borohydride reduction of β-deutero-β-nitrostyrene **3**, was stirred in light petroleum, D_2O and yeast. No loss of deuterium was observed. Taken together, these two observations confirm that the hydrogen atoms bound to the β-carbon in 2-nitro-1-phenylethane **4** are non-exchangeable under the reaction conditions; the exchange at the β-centre must therefore be occurring prior to product formation.

Reduction of β-deutero-β-nitrostyrene **3** using a small amount of yeast (7 g $mmol^{-1}$) resulted in incomplete reduction and the recovered starting material **6** did not contain deuterium (Scheme 4). The formation of reduced material, devoid of deuterium, therefore arises *via* reduction of non-deuterated nitrostyrene, rather than by loss of deuterium from the reduced product.

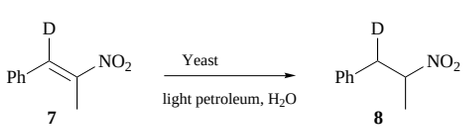


Scheme 4

The production of both deuterated and non-deuterated products and substrates in the reaction can best be explained if the initial step in the reaction is a reversible protonation of the nitrostyrene at the β-carbon followed by hydride attack at the α-carbon. The protonation is yeast catalysed, as loss of deuterium does not occur if β-deutero-β-nitrostyrene **3** is stirred in light petroleum and water in the absence of yeast. Previous reports on the mechanism of yeast reduction of carbon–carbon double bonds have not determined whether hydride attack or protonation occurs first, but have assumed that the mechanism is similar to metal hydride reduction where hydride attack is the first step. Clearly, in the case of nitrostyrenes, the first step in the reduction is a yeast catalysed protonation rather than a hydride attack.

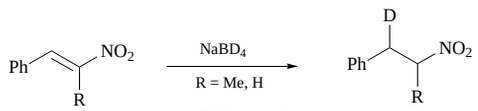
Reduction of α-deutero-β-methyl-β-nitrostyrene 7

Whilst the foregoing reactions have provided considerable evidence regarding the mechanism of the yeast mediated reduction of β-nitrostyrenes, of more interest was the mechanism of reduction of β-methyl-β-nitrostyrenes, since this reaction produces a potentially useful chiral centre, as previously mentioned however, all attempts to date have produced racemic material. α-Deutero-β-methyl-β-nitrostyrene **7** was prepared from nitroethane and α-deuterobenzaldehyde in 50% yield with 100% incorporation of deuterium at the α-carbon. The yeast mediated reduction of this compound gave 1-deutero-2-nitro-1-phenylpropane **8**, as an equimolar mixture of diastereomers, in 66% yield with no loss of deuterium from the α-position (Scheme 5). The retention of the deuterium could be clearly seen in a ^1H–^1H COSY NMR experiment which showed no crosspeak between the two diastereotopic protons at the α-position indicating there was no material present containing two protons at this carbon atom and therefore complete retention of the deuterium had occurred. This retention of deuterium supports the mechanism proposed for the reduction of β-nitrostyrenes, with reversible protonation occurring at the β-carbon and hydride attack at the α-position. It is of course not possible to directly observe the reversible nature of the protonation in this case due to the presence of the methyl group.



Scheme 5

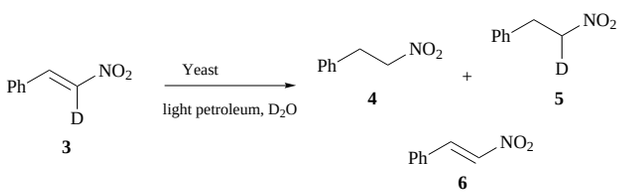
The fact that no deuterium loss occurs in the reduction of α-deutero-β-nitrostyrene **1** and α-deutero-β-methyl-β-nitrostyrene **7** indicates that the reversible protonation occurs exclusively at the β-carbon and hydride attack therefore occurs solely at the α-carbon. The electronic preference for hydride attack at the α-carbon was demonstrated by the reduction of these nitrostyrenes using $NaBD_4$, a reaction which delivers deuteride exclusively to the α-carbon (Scheme 6).



Scheme 6

Reactions using D_2O

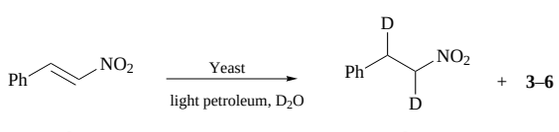
Other workers have concluded that in yeast mediated reduction reactions conducted in aqueous systems, the yeast delivers the hydride but the proton comes from the water.[7] If this mechanism is reflected in the present system, replacing the water with D_2O should result in little or no loss of deuterium from the β-centre. Reduction of β-deutero-β-nitrostyrene **3** with yeast (7 g $mmol^{-1}$) in light petroleum and using D_2O, in place of the small amount of water usually employed, resulted in the recovery of β-nitrostyrene **6** and 2-nitro-1-phenylethane **4** as well as the corresponding β-deuterated analogues, **3** and **5** (Scheme 7). The loss of deuterium in this system indicates that the protonation at the β-centre is not only yeast catalysed but also that the proton is delivered by the yeast rather than coming directly from the water present.



Scheme 7

Reduction of β-nitrostyrene **6** using the same D_2O system resulted in the production of the same four compounds **3**, **4**, **5** and **6**. The production of the deuterated compounds in this system indicates that (not surprisingly) ready exchange occurs between the yeast and the D_2O, with the result that the yeast can deliver both protons and deuterons.

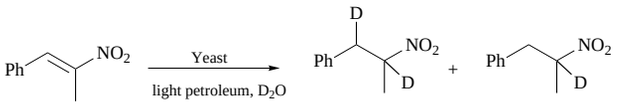
In both of the previous reactions a small amount (<5%) of the dideurated product 1,2-dideutero-1-phenyl-2-nitroethane **9** was detected (Scheme 8). The identity of this dideuterated product was confirmed by ^2H–^2H TOCSY NMR spectroscopy. A crosspeak was observed between the deuterium signals at the 1- and 2-position indicating that both signals came from a single molecule. This result strongly suggests that some exchange is occuring between the D_2O and the NADPH in the yeast, so that the yeast can deliver both a deuteride and a deuteron.



Scheme 8

Further evidence for this exchange came from the reduction of β-methyl-β-nitrostyrene **10**, using the D_2O reaction system,

where a significant amount of the dideurated product 1,2-dideutro-2-nitro-1-phenylpropane **11** was detected using ^2H–^2H TOCSY NMR spectroscopy (Scheme 9). Since protonation has



10 **11**

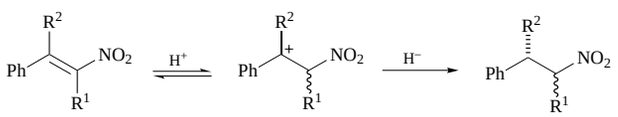
Scheme 9

been shown to occur exclusively at the β-carbon the incorporation of deuterium at the α-position can only come from exchange of deuterium between NADPH and the D_2O.

Guenther *et al*. have demonstrated *in vitro* that the NADH specific diaphorase, lipoamide dehydrogenase, catalyses the exchange of hydride between NADH and water.[10] Lipoamide dehydrogenase is a subunit of the enzyme pyruvate dehydrogenase, an enzyme that is abundant in yeast. It is proposed that the exchange between NADPH and D_2O is catalysed by an enzyme of this type present in the yeast.

Conclusion

The reduction of a number of deuterated nitrostyrenes in an organic solvent system incorporating a small amount of either water or D_2O has allowed us to determine that the yeast mediated reduction of these compounds proceeds *via* the following mechanism: a reversible non-stereoselective protonation at the β-carbon followed by a stereoselective addition of a hydride at the α-carbon (Scheme 10). It is unclear at this stage whether the



Scheme 10

reversible protonation is catalysed in a non-stereoselective manner or whether two (or more) enzymes with opposite enantioselectivities are involved. This mechanism accounts for the finding that stereoselective reduction occurs at the α-centre but not at the β-centre, in yeast mediated reductions of this type. This work complements other studies concerned with the mode of action of yeast mediated reduction reactions, however the use of an organic solvent system has enabled a more detailed determination of the mechanism to be achieved.

Experimental

General

NMR spectra were recorded using Bruker DPX300 and DRX500 spectrometers at 300 and 500 MHz (^1H) and 46 MHz (^2H). ^1H NMR and ^1H–^1H COSY NMR spectra were recorded as deuterochloroform solutions using tetramethylsilane ($\delta = 0.0$ ppm) as an internal reference. *J* Values are given in Hz. ^2H NMR and ^2H–^2H TOCSY NMR spectra were recorded in chloroform solutions using deuterochloroform ($\delta = 7.26$ ppm) as an internal reference. ^2H–^2H TOCSY NMR spectra were recorded using a similar method to that described by Chandrakumar and Ramamoorthy.[11] Bulb-to-bulb distillations were carried out using a Buchi-GKR 50 distillation unit. $[a]_D$ Values are given in units of 10^{-1} deg cm^2 g^{-1}.

[α-^2H]Benzaldehyde was purchased from ISOTEC Inc. [$^2H_3$]Nitromethane and D_2O were purchased from Cambridge Isotopic Laboratories. All other chemicals were purchased from the Aldrich Chemical Company. AR grade light petroleum (40–60 °C) was used without further purification. Mauripan Instant Dry Yeast was generously supplied by Mauri Foods Ltd, Australia.

Preparation of nitrostyrenes

(*E*)-2-Nitro-1-phenylethene 6. A mixture of methanol (2 ml), benzaldehyde (1.0 ml, 9.8 mmol) and nitromethane (0.63 ml, 11.6 mmol) was cooled to −10 °C. To this mixture, sodium hydroxide (464 mg, 11.6 mmol) in water (1 ml) was added with stirring at a rate such that the temperature of the mixture did not exceed 15 °C and the reaction mixture was allowed to stand for 15 min. Water (7 ml) was added to dissolve the resultant precipitate and the solution was added slowly to 4 M HCl (4.7 ml) with stirring. The yellow precipitate formed was filtered off and washed with cold water. The precipitate was placed in a beaker immersed in hot water whereby the yellow precipitate melted and two layers were formed. On cooling, crude product solidified and the remaining liquid was removed. The crude solid was then recrystallised from ethanol to give 2-nitro-1-phenylethene (0.799 g, 54%) mp 57–58 °C (lit.,[12] 57–58 °C); δ_H(300 MHz) 7.53 (5H, m, Ph), 7.61 (1H, d, *J* 13.5, 2-H), 8.03 (1H, d, *J* 13.5, 1-H).

(*E*)-2-Nitro-1-phenyl[1-^2H]ethene 1. The title compound was obtained using [α-^2H]benzaldehyde in place of benzaldehyde. Yield: 0.874 g, 60% (100% deuterium incorporation at the α-carbon), mp 57–58 °C; δ_H(300 MHz) 7.53 (5H, m, Ph), 7.61 (1H, t, *J* 1.8, 2-H); δ_D(46 MHz) 8.02 (1-D).

(*E*)-2-Nitro-1-phenyl[2-^2H]ethene 3. The title compound was obtained using [$^2H_3$]nitromethane in place of nitromethane and the NaOH solution was made using D_2O instead of water. Yield: 0.825 g, 57% (95% deuterium incorporation at the β-carbon), mp 58 °C (lit.,[12] 54–55 °C); δ_H(300 MHz) 7.52 (5H, m, Ph), 8.02 (1H, t, *J* 2.1, 1-H); δ_D(46 MHz) 7.61 (2-D).

(*E*)-2-Nitro-1-phenylprop-1-ene 10. Nitroethane (1.0 ml, 13.9 mmol) and cyclohexylamine (1.3 ml) were added to benzaldehyde (1.0 ml, 9.8 mmol) in glacial acetic acid (5.3 ml). The mixture was held at 100 °C for 6 h, cooled and diluted with water (1 ml). The reaction mixture was cooled overnight in a water bath. The crystals formed were filtered and air dried. The crude solid was recrystallised from ethanol to give (*E*)-2-nitro-1-phenylprop-1-ene (0.99 g, 62%), mp 64–65 °C (lit.,[13] 64–65 °C) δ_H(300 MHz) 2.48 (3H, s, CH_3), 7.47 (5H, m, Ph), 8.11 (1H, s, 1-H).

(*E*)-2-Nitro-1-phenyl[1-^2H]prop-1-ene 7. The title compound was obtained using [α-^2H]benzaldehyde instead of benzaldehyde. Yield: 0.780 g, 50% (100% deuterium incorporation at the α-carbon), mp 62–63 °C; δ_H(300 MHz) 2.48 (3H, s, CH_3), 7.47 (5H, m, Ph); δ_D(46 MHz) 8.11 (1-D).

Yeast reactions

Reduction of (*E*)-2-nitro-1-phenylethene 6. (*E*)-2-Nitro-1-phenylethene **6** (0.3 g, 2.0 mmol), yeast (22.13 g, 11 g mmol^{-1} of **6**), H_2O (17.7 ml) (0.8 ml per g of yeast) and light petroleum (200 ml) were stirred at room temperature for 24 h. The solvent was removed by filtration and the yeast was washed with dichloromethane (3 × 50 ml). The filtrates were combined and evaporated *in vacuo* to give a residue which was bulb-to-bulb distilled to give 2-nitro-1-phenylethane **4** (0.216 g, 72%), bp 150 °C/0.5 mmHg; δ_H(300 MHz) 3.34 (2H, t, *J* 7.5, 1-H), 4.63 (2H, t, *J* 7.5, 2-H), 7.3 (5H, m, Ph).

Reduction of (*E*)-2-nitro-1-phenyl[1-^2H]ethene 1. (*E*)-2-Nitro-1-phenyl[1-^2H]ethene **1** (0.3 g, 2.0 mmol) was stirred with yeast (21.98 g, 11 g mmol^{-1} of **1**), H_2O (17.5 ml) and light petroleum (150 ml) for 24 h to give 2-nitro-1-phenyl[1-^2H]ethane **2** (0.198 g, 65%) bp 150 °C/0.5 mmHg; $[a]_D$ +0.28 (*c* 5 in $CHCl_3$); δ_H(300 MHz) 3.33 (1H, tt, *J* 9.6, 2.1, 1-H), 4.63 (2H, dt, *J* 7.5, 0.9, 2-H), 7.3 (m, 5H, Ph); δ_D(46 MHz) 3.33 (1-D). A sample of this nitroalkane was reduced to the amine using $LiAlH_4$ in diethyl ether and then converted into the MTPA amide. An NMR spectrum was taken in the presence of 0.2 M Eu(fod)$_3$ shift reagent. Integration of the methine proton in the 1-position indicated a 61% ee.

Reduction of (*E*)-2-nitro-1-phenyl[2-^2H]ethene 3. (*E*)-2-Nitro-1-phenyl[2-^2H]ethene **3** (0.4 g, 2.7 mmol) was stirred with yeast

(29.3 g, 11 g mmol^{-1} of **3**), H_2O (23.4 ml) and light petroleum (200 ml) for 24 h to give a mixture of 2-nitro-1-phenylethane and 2-nitro-1-phenyl[2-^{2}H]ethane **5** (0.176 g), bp 150 °C/0.5 mmHg; δ_H(300 MHz) **4**: 3.34 (2H, t, *J* 7.5, 1-H), 4.63 (2H, t, *J* 7.5, 2-H), 7.3 (5H, m, Ph); **5**: 3.34 (2H, d, *J* 7.4, 1-H), 4.62 (1H, tt, *J* 7.4, 2.1, 2-H), 7.3 (5H, m, Ph); δ_D(46 MHz) 4.60 (2-D).

Reduction of (*E*)-2-nitro-1-phenyl[1-^{2}H]prop-1-ene 7. (*E*)-2-Nitro-1-phenyl[1-^{2}H]prop-1-ene **7** (0.3 g, 1.2 mmol) was stirred with yeast (20.10 g, 11 g mmol^{-1} of **7**), H_2O (16.1 ml) and light petroleum (200 ml) for 24 h, to give 2-nitro-1-phenyl[1-^{2}H]propane **8** as a mixture of diastereomers (0.198 g, 66%), bp 150 °C/0.5 mmHg; δ_H(300 MHz) 1.57 (3H, dd, *J* 6.9, 1.2, CH_3), 3.02 (1H, dt, *J* 6.9, 1.8, 1-H), 3.33 (1H, dt, *J* 6.9, 2.1, 1-H), 4.81 (1H, quintet, *J* 6.6, 2-H), 7.28 (m, 5H, Ph).

Reduction of (*E*)-2-nitro-1-phenylprop-1-ene 10. (*E*)-2-Nitro-1-phenylprop-1-ene **10** (0.3 g, 1.8 mmol) was stirred with yeast (12.8 g, 7 g mmol^{-1} of **10**), D_2O (10.2 ml) and light petroleum (150 ml) for 24 h to give a mixture of starting material and reduced products (0.201 g, 67%) bp 150 °C/0.5 mmHg; δ_D(46 MHz) 2.99 (1-D), 3.31 (1-D, 4.77 (2-D).

Sodium borodeuteride reductions

2-Nitro-1-phenyl[1-^{2}H]ethane. (*E*)-2-Nitro-1-phenylnitroethene (0.15 g, 1 mmol) was dissolved in a mixture of 12 ml chloroform (8 ml per g silica gel) and 2.3 ml isopropanol (1.5 ml per g silica gel) and stirred with 1 g silica gel. $NaBD_4$ (0.16 g, 4.1 mmol) was added in small amounts, until the yellow colour due to the nitrostyrene disappeared. The mixture was then filtered, washed with brine (15 ml), extracted with dichloromethane (20 ml), dried over $NaSO_4$ and the dichloromethane evaporated *in vacuo* to give the title compound (0.11 g, 73%); δ_H(300 MHz) 3.33 (1H, tt, *J* 9.6, 2.1, 1-H), 4.63 (2H, dt, *J* 7.5, 0.9, 2-H), 7.3 (m, 5H, Ph); δ_D(46 MHz) 3.33 (1-D).

2-Nitro-1-phenyl[1-^{2}H]propane 8. Using a similar procedure (*E*)-2-nitro-1-phenylprop-1-ene **7** gave the title compound **8** as a mixture of diastereomers (0.096 g, 57%); δ_H(300 MHz) 1.57 (3H, dd, *J* 6.9, 1.2, CH_3), 3.02 (1H, dt, *J* 6.9, 1.8, 1-H), 3.33 (1H, dt, *J* 6.9, 2.1, 1-H), 4.81 (1H, quintet, *J* 6.6, 2-H), 7.28 (m, 5H, Ph); δ_D(46 MHz) 3.03 (1-D), 3.36 (1-D).

References

1 M. Takeshita, S. Yoshida and Y. Kohno, *Heterocycles*, 1994, **37**, 553.
2 H. Ohta, N. Kobayashi and K. Ozaki, *J. Org. Chem.*, 1989, **54**, 1802.
3 R. A. Bak, A. F. McAnda, A. J. Smallridge and M. A. Trewhella, *Aust. J. Chem.*, 1996, **49**, 1257.
4 K. Sakai, A. Nakazawa, K. Kondo and H. Ohta, *Agric. Biol. Chem.*, 1985, **49**, 2331.
5 S. Servi, *Synthesis*, 1990, **50**, 1; R. Csuk and B. I. Glanzer, *Chem. Rev.*, 1991, **91**, 49.
6 C. Fuganti, D. Ghiringhelli and P. Grasselli, *J. Chem. Soc.*, *Chem. Commun.*, 1975, 846.
7 G. Fronza, C. Fuganti and P. Grasselli, *Tetrahedron Lett.*, 1992, **33**, 6375.
8 G. Fronza, C. Fuganti, P. Grasselli, A. Meles and A. Sarra, *Tetrahedron Lett.*, 1993, **34**, 6467.
9 G. Fronza, C. Fuganti, P. Grasselli, S. Lanati and R. Rallo, *J. Chem. Soc.*, *Perkin Trans. 1*, 1994, 2927.
10 H. Guenther, F. Biller, M. Kellner and H. Simon, *Angew. Chem.*, *Int. Ed. Engl.*, 1973, **12**, 146.
11 N. Chandrakumar and A. Ramamoorthy, *J. Am. Chem. Soc.*, 1992, **114**, 1123.
12 D. E. Worral, in *Org. Synth.*, 1941, **Coll. Vol. I**, 413.
13 B. C. Gairaud and G. R. Lappin, *J. Org. Chem.*, 1953, **18**, 1.

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