Highly enantioselective reductionof ethyl 4-chloro-3-oxobutanoate to L- and D- 3-hydroxyesters with baker's yeast

Arrigo Forni,* Emilia Caselli, Fabio Prati, Maria Bucciarelli, and Giovanni Torre

Dipartimento di Chimica, Università di Modena e Reggio Emilia,
via Campi 183, 41100 Modena, Italy
E-mail: forni.arrigo@unimo.it

This paper is dedicated to Professor D. Spinelli

(received 04 Oct 02; accepted 15 Nov 02; published on the web 23 Nov 02)

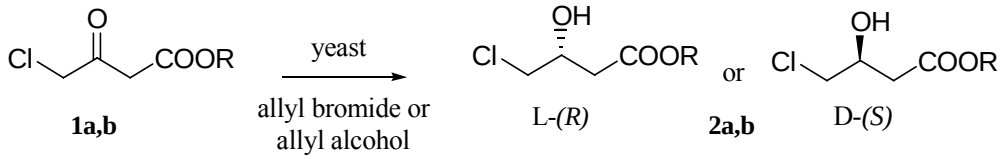
Abstract

Reduction with baker's yeast of ethyl or methyl 4-chloro-3-oxobutanoate in the presence of allyl bromide or allyl alcohol as additive afforded the corresponding L- and D-3-hydroxyesters. Several reduction conditions were tested, involving: different concentrations of additive; variations in the yeast/substrate ratio; the presence and absence of glucose; different pre-incubation periods; a range of temperatures. The best conditions gave a complete conversion of the substrate within 1–2 h and a very high enantioselectivity, 90–97% *e.e.*, for the two enantiomers.

Keywords: *(R)*- and *(S)*- Ethyl 4-chloro-3-hydroxybutanoate, yeast reduction, allyl alcohol, allyl bromide

Introduction

Optically active L- *(R)*- and D- *(S)*- ethyl or methyl 4-chloro-3-hydroxybutanoate (**2a,b**) are very useful chiral building blocks in the synthesis of pharmaceutical target compounds. For example, the L- enantiomer is an important chiral building block for the synthesis of (−)-macrolactin A,[1] L-carnitine[2] and *(R)*-γ-amino-β-hydroxybutyric acid (GABOB),[3] or can be converted into (+) negamicyn[4] or to a chiral 2,5-cyclohexadienone synthon.[5] On the other hand the D enantiomer is a key chiral intermediate in the enantioselective synthesis of slagenins B and C[6] and in the total synthesis of a class of HMG-CoA reductase inhibitors[7] or can be converted into a 1,4-dihydropyridine-type β-blocker.[8] Of the two enantiomers, the *(S)*- is the more common and more easily available, whereas *(R)*- is in greater demand yet less readily available.[9] The development of new, simple and economical methods of synthesizing the latter is therefore of overriding interest.



1a,b L-*(R)* **2a,b** D-*(S)*

a) R=Et: **b**) R=Me

Several synthetic routes have been developed to obtain both enantiomers, comprising asymmetric synthesis[2,10] and biocatalytic reduction,[8,11] as well as a lipase catalyzed kinetic resolution through ammonolysis of racemic 4-chloro-3-hydroxybutanoate.[12] Biocatalytic reduction appears to have attracted most attention, several enzymatic systems having been studied with very good results, particularly for the *(S)* enantiomer.[8,13] Of these, baker's yeast continues to play an important role in the attempts to synthesize chiral compounds by biocatalysis[14] because it is inexpensive, readily available, and very easy to handle, and also because, in recent years, some examples have been reported in which both enantiomers can be obtained from yeast reduction of the same substrate by adopting appropriate reduction conditions such as the use of organic solvents,[15] the use of additives[16] and the application of particular cell culture conditions.[17]

In this respect, we recently observed[18] that when allyl bromide[15] and allyl alcohol,[19] two commonly available chemicals, are added to baker's yeast reduction of 4-halogenated-3-oxo-butanoates, they interact with the enzymatic system of yeast by changing the relative activity of L- and D-enzymes, thereby affording one isomer or the enantiomer in good enantiomeric excess by reducing the substrate in suitable conditions with the additive. In particular, we reported that by reducing methyl 4-chloro-4,4-difluoro-3-oxobutanoate (the analogous 4,4 difluorurate of substrate **1b**) with baker's yeast in the presence of allyl bromide or allyl alcohol, we obtained efficient stereochemical control of both *(R)*- and *(S)*- hydroxy derivatives.[18b] Here we present our results obtained from the reduction of ethyl or methyl 4-chloro-3-oxobutanoate (**1a,b**) with baker's yeast and in the presence of the additive allyl bromide or allyl alcohol, which yielded both L-*(R)*- and D-*(S)*-4-chloro-3-hydroxybutanoates in nearly optically pure form and with total conversion of the substrate.

Results and Discussion

Reductions in the presence of allyl bromide. Yeast reduction, in water, of ethyl 4-chloro-3-oxobutanoate affords the corresponding hydroxy derivative in 14–55% *e.e.* for the D-*(S)*-enantiomer, depending upon the yeast/substrate ratio and in the presence of glucose.[15,20] It is common knowledge that the poor enantiomeric excess observed is because baker's yeast contains at least seven enzymes which are able to reduce the substrate following opposite stereochemical routes.[8] The predominant activity among these enzymes has been associated with a fatty acid synthetase complex isolated from yeast which reduces beta-ketoesters to D-*(S)*-

carbinols.[21] When allyl bromide is added to yeast reductions of beta-ketoesters,[16,18] it is seen to strongly suppress D-enzyme activity until carbinols with L-stereochemistry are obtained.

We started therefore by reducing **1** according to the reaction conditions previously reported and which gave best results of conversion and *e.e*. for L-*(R)* methyl 4-chloro-4,4-difluoro-3-hydroxybutanoate.[18b] In these reaction conditions, namely, 0.5 mmole substrate, 1.4 g of yeast, 3 g l^{-1} of allyl bromide and 0.75 g of glucose (Entry 1, Table 1), we obtained the interesting result of 80% substrate conversion with 64% *e.e.* for the L-*(R)*-enantiomer of the hydroxyester. Allyl bromide thus confirmed its capacity to strongly suppress the activity of D-enzymes that are involved in the reduction of 4-halogenated-3-oxobutanoates.

Since the conversion was not complete, we studied the effect of the yeast/substrate ratio (Entries 2 and 3, Table 1) in order to obtain a 100% conversion.[22] A 100% conversion together with 83% *e.e.* was obtained with 5.6 g of yeast for 0.5 mmol of substrate and glucose.

Table 1. Yeast reduction of ethyl 4-chloro-3-oxobutanoate in the presence of allyl bromide

Entry	Substrate mmol	Water ml	Glucose g	Allyl bromide g l^{-1}	BY g	Conv./*e.e* L, (*R*)-(+)
1	0.5	12.5	0.75	3	1.4	80/64
2	0.5	12.5	1.5	3	2.8	83/62
3	0.5	25	3	3	5.6	100/83
4	0.5	25	-	3	5.6	100/77
5	0.5	25	-	4	5.6	100/86
6	0.5	25	-	5	5.6	94/84
7	0.5	25	-	6	5.6	85/84
8	3	600	-	4	135	100/88[a](±4)
9	3[b]	600	-	4	135	98/97

(a) Mean value of four reductions. (b) Substrate added in five steps.

With this yeast/substrate ratio we carried out the reduction in the absence of glucose[22] and we also studied the effect of varying the concentration of allyl bromide from 3 to 6 g l^{-1} (Entries 4–7). Although, in the absence of glucose, total conversion and 77% *e.e.* for the L-isomer was observed with 3 g l^{-1} of additive, the best result for *e.e.* (86%) was achieved with 4 g l^{-1} of allyl bromide, while higher concentrations of the additive reduced the conversion without affecting the enantiomeric excess (Entries 6–7).

When reduction corresponding to Entry 5 was repeated on the scale of 3 mmol we did not obtain total conversion of the substrate at once; therefore, to achieve 100% conversion we increased the yeast/substrate ratio and also the amount of water (4x, Entry 8). We observed the total transformation of the substrate after 1 h together with 88% *e.e.* (mean value of four reductions). In view of the observation reported in ref. 17, namely, that the highest enantiomeric

excesses of ethyl L-*(R)*-4-chloro-3-hydroxybutanoate are obtained when the concentration of the oxo-ester is kept low by slow addition of the substrate, reduction Entry 8 was repeated, but adding the substrate in five steps over a period of one h. We obtained the best result for the *R*-enantiomer of 97% *e.e.* and 98% conversion after 2 h (Entry 9).

Reductions in the presence of allyl alcohol. The treatment of bakers' yeast cells with allyl alcohol in order to control the stereochemical course of yeast reduction was introduced by NaSkamura,[19] who investigated the baker's yeast reduction of beta-ketoesters in the presence of additives. In reducing 4-chloro-3-oxobutanoate with baker's yeast and in the presence of 2 g l^{-1} of allyl alcohol, he obtained the corresponding D-hydroxy-ester in enhanced enantiomeric excess: 85% *e.e.* together with 42% chemical yield, as opposed to 43% *e.e.* and 62% chemical yield obtained in the absence of the additive.[19a] More recently, we observed that the action of allyl alcohol was able to switch the usual L-stereochemistry observed in baker's yeast reduction of 4,4,4-trifluoro-3-hydroxybutanoate to the D-enantiomer.[18a] We therefore set out to investigate more thoroughly the capacity of this additive to obtain the D-enantiomer of ethyl 4-chloro-3-hydroxybutanoate on the basis of our previous results, although recent studies have been reported on the effects of allyl alcohol as additive in baker's yeast reduction of ethyl 4-chloro-3-oxobutanoate.[23]

The approach with this additive was the same as with allyl bromide. We started by applying the conditions of Entry 1 with allyl alcohol (3 g l^{-1}) as additive (Entry 10, Table 2). In this case, in line with the literature, only 30 min of pre-incubation time was allowed before adding glucose and substrate. The D-enantiomer was obtained in 75% *e.e.* and with 83% conversion.

Table 2. Yeast reduction of ethyl 4-chloro-3-oxobutanoate in the presence of allyl alcohol

Entry	Substrate Mmol	Water[a] ml	Glucose g	Allyl alcohol[b] g l^{-1}	BY g	Conv./*e.e.* D (*S*)-(–)
10	0.5	12.5	0.75	3	1.4	83/75
11	0.5	50	3	3	5.6	100/91
12	0.5	12.5	-	3	1.4	81/80
13	0.5	50	-	3	5.6	100/90
14	0.5	50	-	2	5.6	100/87
15	0.5	50	-	4.5	5.6	100/80
16	0.5	50	-	6	5.6	62/87
17	0.5	50	-	3[c]	5.6	41/84
18	0.5	50	-	2[c]	5.6	76/85

(a) ml of water refers to a constant ratio with yeast. (b) 30 min of pre-incubation at 30 °C before adding the substrate. (c) 60 min of pre-incubation.

Total substrate conversion and 91% *e.e.* were obtained by increasing the yeast from 1.4 to 5.6 g and glucose from 0.75 to 3 g (Entry 11). These two reductions were repeated without glucose and the results were almost the same (Entries 12, 13), showing that the contribution of glucose was not influential. In the conditions of Entries 14–16 the effect of allyl alcohol concentration, from 2 to 6 g l^{-1}, was studied. Since a higher concentration of allyl alcohol (Entries 15, 16) did not succeed in enhancing the enantiomeric excess, we investigated other aspects of the action of allyl alcohol by repeating the reductions of Entry 13 and 14 with a longer pre-incubation time (one h); however, this caused a decrease in the *e.e.* and a much greater decrease in conversion (Entries 17, 18). Furthermore, we studied the effects of temperature by raising it from 20 °C to 40 °C in steps of 5 degrees (Entries 19–23, Table 3).

Table 3. Yeast reduction of ethyl 4-chloro-3-oxobutanoate in the presence of allyl alcohol: effect of temperature

Entry	Substrate Mmol	Water ml	Temperature °C	Allyl alcohol[a] g l^{-1}	BY g	Conv./*e.e.* D (*S*)-(−)
19	0.5	50	20	2	5.6	100/81
20	0.5	50	25	2	5.6	100/85
21	0.5	50	30	2	5.6	100/91(±1)[b]
22	0.5	50	35	2	5.6	64/82
23	0.5	50	40	2	5.6	79/77

a) 30 min of pre-incubation at 30 °C before adding the substrate. b) Mean value of five reductions.

These results confirm an optimal working temperature for yeast of 30 °C, corresponding to 100% conversion and 91% *e.e.*

In conclusion, our findings relating to the effect of allyl alcohol in the yeast reduction of ethyl 4-chloro-3-oxobutanoate gave a maximum *e.e.* of 91% for the D-hydroxy-ester; this suggests that the additive is unlikely to be able to suppress the activity of all the L-enzymes. Nevertheless, the present result compares well with that already reported:[23a] the *e.e.* is somewhat better (91% *vs*., 87–90%), and it is achieved without glucose, thereby avoiding problems connected with fermentation, such as higher volumes and the presence of by-products; moreover, in these conditions total conversion was reached after two hours.

Reduction of methyl 4-chloro-3-oxobutanoate. In order to assess the effect of additive in relation to the entity of the ester function, the optimal reduction conditions, without glucose, were also checked in the reduction of methyl 4-chloro-3-oxobutanoate without additive and together with allyl alcohol or allyl bromide. It has been reported that, in the absence of additive, the D-enantiomer is the one preferentially formed, corresponding to a decrease in size of the

ester grouping of 4-chloro-3-oxobutanoates.[20] Our results, reported in Table 4, show that the expected trend was not observed: the D- enantiomer, obtained in the presence of allyl alcohol, was recovered in only 72% *e.e.* whereas, in the presence of allyl bromide, the same interesting result was obtained as on the ethyl derivative, *i.e.,* the L- enantiomer was obtained in 96% *e.e.* with 100% conversion.

Table 4. Yeast reduction of methyl 4-chloro-3-oxobutanoate, effects of the additives

Entry	Substrate Mmol	Water ml	BY g	Additive g l^{-1}	Conv./*e.e.*/config.
24	0.5	25	5.6	-	100/12/D
25	0.5	100	22.7	AllylBr (4)	100/96/L
26	0.5	25	5.6	AllylOH (2)	100/72/D

Conclusions

We studied baker's yeast reduction of ethyl- and methyl 4-chloro-3-oxobutanoate in the presence of the additives allyl bromide and allyl alcohol. A very good stereochemical control was obtained in the reduction that gave in turn L- or D-enantiomer with total conversion of the substrate and with 96–97% and 72–91% *e.e.*, respectively. The 100% conversion was obtained without adding glucose and in a very short time (1–2 h). It is worth pointing out that very high *e.e.* for the *(R)*-L-enantiomer in reducing 4-chloroacetoacetate with bakers' yeast has previously been obtained only with purified L-enzymes [21a] or with yeast in particular growing conditions.[17] Moreover, yeast reduction to the *(R)*-L-enantiomer was carried out with the recovery of the purified product which was obtained in 75% chemical yield.

Experimental Section

General Procedures. Fresh baker's yeast from FALA, Strasbourg, was purchased locally. Ethyl and methyl 4-chloro-3-oxobutanoate were purchased from Fluka; allyl bromide and allyl alcohol were purchased from Merck; commercially available glucose was used.

Yeast reductions were performed in an ISCO incubator. Optical rotations were measured on a Perkin-Elmer 241 polarimeter at 20 °C. GLC analyses were performed on a Hewlett-Packard 5890 A gas chromatograph; the conversions were evaluated on a DB 1 column (30 m x 0.53 mm i.d. and 5 μm film phase) from J&W Scientific, while the enantiomeric excesses were evaluated on a chiral alpha-dex 120 column (30 m x 0.5 mm i.d. and 0.25□m film phase) from Supelco. Temperature programming for enantiomeric excess determinations for ethyl ester: 100 °C constant; first eluted D- enantiomer R_{t1} 32.93 min, R_{t2} 33.96 min, helium flow rate 15 psi;

temperature programming for methyl ester, 60 °C (0 min) – 1 °C/min – 120 °C; first eluted D-enantiomer R_{t1} 36.18 min, R_{t2} 36.45 min, helium flow rate 25 psi; accuracy was within ± 2%. The ^{1}H-NMR spectrum was recorded in $CDCl_3$ on a Bruker Advance DPX 200 MHz spectrometer; chemical shifts are reported in δ values from TMS as internal standard (s singlet, d doublet, dd double doublet, t triplet, q quartet, m multiplet). Mass spectra were determined on a Hewlett-Packard 5970 mass-selective detector.

Reduction conditions. The reductions were carried out on 0.5 mmol of substrate following the simple procedure described here.[18b] Baker's yeast was suspended in distilled water in a 4x volume Erlenmeyer flask and additive was added. The mixture was kept at 30 °C in an incubator and shaken with a magnetic stirrer for two h with the allyl bromide additive, and for 0.5 or 1 h with the allyl alcohol additive (the flask being capped with cotton-flock). Thereafter, the substrate **1a** and glucose, if included, were added and the suspension was stirred again at 30°C. The conversion was checked directly by GLC on the water suspension of a centrifuged sample every 15 min and we observed that maximum conversion is reached within one or two h; longer reaction times led to the formation, in small quantities, of several by-products. The reaction mixture was then centrifuged, the yeast washed with water and the total water phase extracted with ethyl acetate; finally, it was dried over $MgSO_4$, concentrated *in vacuo* and examined for final conversion and enantiomeric excess.

When the reduction was repeated on 3 mmol of substrate with allyl bromide as additive, we observed a drop in conversion. So we started with an increased yeast/substrate ratio (x4) in a correspondingly increased volume of water in order to maintain a constant yeast/additive ratio. After two h pre-incubation the suspension was centrifuged, the recovered yeast suspended in a ¼ volume of supernatant and the substrate added. Within one or two h, at 100% conversion, the reaction mixture was worked up as described above. Moreover, in the reduction of Entry 9, Table 1, the substrate (3 mmole) was added in five steps at 10 min intervals, and, after total conversion, the product was recovered as described above. The organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo.* The crude extract was chromatographed on silica gel, diethyl ether/methylene chloride 1:4 as eluent, the product being recovered in 75% yield. After distillation (boiling point 47–50 °C at 0.3 mmHg), the recovered hydroxyester **2a** displayed 97% enantiomeric excess by GLC and $[\alpha]_D = +19.7$ (c = 1.62, $CHCl_3$).

Compound **2a** gave the following GLC-mass and ^{1}H-NMR spectroscopic data:
MS (m/z): M^+ not found, 123, 121, 117, 89, 85, 79, 75, 71, 60, 49, 45, 44. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ (ppm) 1.31 (3H, t, *CH*$_3$, J=7.3), 2.6 (1H, b, *OH*), 2.56–2.74 (2H, m, *CH*$_2$COO), 3.5–3.71 (2H, m, Cl*CH*$_2$), 4.21 (2H q, COO*CH*$_2$), 4.23–4.35 (1H, m, C<u>H</u>OH).

Acknowledgements

This work was supported by Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR), Rome.

References

* Author to whom correspondence should be addressed, E-mail: forni.arrigo@unimo.it

1. Marino, J.P.; McClure, M.S.; Holub, D.P.; Comasseto, J.V.; Tucci, F.C. *J. Am. Chem. Soc.* **2002**, *124*, 1664.
2. (a) Song, C.E.; Lee, J.K.; Lee, S.H.; Lee, S. *Tetrahedron: Asymmetry* **1995**, *5*, 1063. (b) Kitamura, M.; Ohkuma, T.; Takaya, H.; Noyori, R. *Tetrahedron Letters* **1988**, *29*, 1555.
3. Kolb, H.C.; Bennani, Y.L.; Sharpless, K.B. *Tetrahedron: Asymmetry* **1993**, *4*, 133.
4. Davies, S.G.; Ichihara, O. *Tetrahedron: Asymmetry* **1996**, *7*, 1919.
5. Hikichi, S.; Hareau, G. P.-J.; Sato, F. *Tetrahedron Letters* **1997**, *38*, 8299.
6. Jiang, B.; Liu, J-F.; Zao, S.-Y. *Organic Letters* **2001**, *3*, 25, 4011.
7. Karanewsky, D. S.; Badia, M.C.; Ciosek Jr., C. P.; Robl, J. F.; Sofia, M. J.; Simpkins, L. M.; DeLange, B.; Harrity, T.W.; S Biller, A.; Gordon, E. M. *J. Med. Chem*. **1990**, *33*, 2952.
8. Kita, K.; Kataoka, M.; Shimizu, S. J. *Bioscience and Bioengineering* **1999**, *88*, 591.
9. Wang, G.; Hollingsworth, R. I. *Tetrahedron: Asymmetry* **1999**, *10*, 1895.
10. Zhou, Y.-G.; Tang, W.; Wang, W-B.; Li, W.; Zhang, X. *J. Am. Chem. Soc*. **2002**,*124*, 4952.
11. (a) Wada, M.; Kawabata, H.; Yoshizumi, A.; Kataoka, M.; Nakamori S.; Yasohara, Y.; Kizaki, N.; Hasegawa, J.; Shimizu, S. J. *Bioscience and Bioengineering* **1999**, *87*, 144. (b) Kataoka, M.; Yamamoto, K.; Kawabata, H.; Wada, M.; Kita, K.; Yanase, H.; Shimizu, S. *Appl. Microbiol. Biotechnol.* **1999**, *51*, 486. (c) Yasohara, Y.; Kizaki, N.; Hasegawa, J.; Takahashi, S.; Wada, M.; Kataoka, M.; Shimizu, S. *Appl. Microbiol. Biotechnol*. **1999**, *51*, 847. (d) Molinari, F.; Gandolfi, R.; ;R. Occhiato, E.G. *Tetrahedron: Asymmetry* **1999**, *10*, 3515.
12. Garcia-Urdiales, E.; Rebolledo, F.; Gotor, V. *Tetrahedron: Asymmetry* **1999**, *10*, 721.
13. (a) Yadav, J. S.; Nanda, S.; Thirupathi Reddy, P.; Bhaskar Rao, A. *J. Org. Chem*. **2002**, *67*, 3900. (b) Yasohara, Y.; Kizaki, N.; Hasegawa, J.; Wada, M.; Kataoka, M.; Shimizu, S. *Tetrahedron: Asymmetry* **2001**, *12*, 1713. (c) Patel, R. N.; McNamee, C. G.; Banerjee, A.; Howell, J. H.; Robinson, R. S.; Szarka, L. J. *Enzyme Microb. Technol*. **1992**, *14*, 731.
14. (a) Ohno, A. *Reviews on Heteroatom Chemistry* **1999**, *20*, 29. (b) D'Arrigo, P.; Pedrocchi-Fantoni, G.; Servi, S. *Advances in Applied Microbiology* **1997**, *44*, 81.
15. Rotthaus, O.; Krüger, D.; Demuth, M.; Schaffner, K. *Tetrahedron* **1997**, *53*, 935.
16. Ushio, K.; Hada, J.; Tanaka, Y.; Ebara, K. *Enzyme Microb. Technol*. **1993**, *15*, 222.
17. Dahl, A.C.; Madsen, J. *Tetrahedron: Asymmetry* **1998**, *9*, 4395.

18. Davoli, P.; Forni, A.; Moretti, I.; Prati, F.; Torre, G. *Enzyme Microb. Technol*. **1999**, *25*, 149. (b) Antolini, L.; Forni, A.; Moretti, I;. Prati, F.; Torre, G. *Gazz. Chim. Ital.* **1995**, *125*, 549.
19. (a) Nakamura, K.; Inoue, K.; Ushio, K.; Oka, S.; Ohno, A. *Chemistry Letters* **1987**, 679. (b) Nakamura, K.; Kawai, Y.; Oka, S.; Ohno, A. *Bull. Chem. Soc. Jpn*. **1989**, *62*, 875.
20. Zhou, B.; Gopalan, A.S.; Van Middlesworth, F.; Shieh, W.R.; Sih, C.J. *J. Am. Chem. Soc*. **1983**, *105*, 5925.
21. (a) Shieh, W. R.; Gopalan, A. S.; Sih, C. J. *J. Am. Chem. Soc*. **1985**, *107*, 2993. (b) Ward, O. P.; Young, C. S. *Enzyme Microb. Technol*. **1990**, *12*, 482.
22. Bucciarelli, M.; Forni, A.; Moretti, I.; Torre, G. *Synthesis* **1983**, *11*, 897.
23. (a) Dahl, A. C.; Fjeldberg, M.; Madsen, J. *Tetrahedron: Asymmetry* **1999**, *10*, 551. (b) Nielsen, J.K.; Madsen, J. *Tetrahedron: Asymmetry* **1994**, *5*, 403. (c) Naoshima, Y.; Meda, J.; Munakata, Y. *J. Chem. Soc. Perkin Trans*. **1992**, *1*, 659.

