Lipase-Catalysed Kinetic Resolution of Hydroxymethylchromanes

A. Czompa, T. Kovács and S. Antus*

Department of Organic Chemistry, University of Debrecen, P.O.Box 20, H-4010 Debrecen, Hungary

Received March 8, 2000

Dedicated to the Memory of Professor Raymond N. Castle

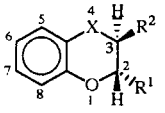
An effective kinetic resolution of hydroxymethylchromanes racemic **2a** and **3a** has been achieved by means of enantioselective transesterification with vinyl acetate in organic solvents. The alcohols (-)-*R*-**2a** and (-)-*S*-**3a** were obtained with high optical purities (94 and 98% ee) in 70% and 38% yields, respectively. The influence of the enzyme source and the character of the solvent on the enantioselectivity were studied.

J. Heterocyclic Chem., **37**, 991 (2000).

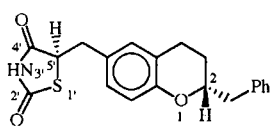
In the past decade the kinetic resolution of racemic alcohols catalyzed by enzymes has received considerable attention and has been utilized in the chemoenzymatic synthesis of optically active natural products and pharmaceuticals [1-5]. Among the enzymes, lipases are more extensively investigated as catalysts for both the enantioselective hydrolysis of racemic primary or secondary esters, and for the enantioselective acylation of racemic primary or secondary alcohols [6]. Recently, two of us reported [7] that (-)-2*S*-hydroxymethyl-1,4-benzodioxane (**1**) could be prepared from racemic **1** in the optically pure form by the *Pseudomonas fluorescens* (PsfL) Lipase-catalysed transesterification with vinyl acetate (VA) in dioxane at room temperature. A remarkable influence of the solvent on the enantioselectivity of the acylation has also been recognized.

In continuation of our investigations on the kinetic resolution of O-heterocycles we attempted to extend this simple procedure to the synthesis of the hydroxymethylchromane derivatives (-)-*R*-**2a** and (-)-*S*-**3a**, which served as suitable starting materials for the synthesis of a hypoglycemic agent (+)-2*R*, 5'*R*-**4** [8] and an anxiolytic substance (-)-3*S*-**5** [9,10] (Scheme 1).

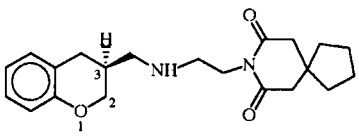
Scheme 1



	R^1	R^2	X
(-)-*S*-**1**	CH_2OH	H	O
(-)-*R*-**2a**	CH_2OH	H	CH_2
(-)-*S*-**3a**	H	CH_2OH	CH_2



englitazon [(+)-2*R*,5'*R*-**4**]



(-)-3*S*-**5**

Substrates racemic **2a** and -**3a** for the enzymatic reaction were prepared from 2-hydroxyacetophenone and salicylic aldehyde, according to the procedures described in the literature [11-13]. The enzyme catalyzed acylation of these compounds was carried out with vinyl acetate (VA) as an irreversible acyl donor in dry dichloromethane or dioxane at 24 °C, and the progress of the reaction was monitored by tlc. The reactions were terminated by filtration of the enzyme and the products containing the leavorotatory hydroxymethylchromane derivatives and their enantiomeric acetates were separated by means of preparative tlc. The conversion degrees were calculated from the yields of the recovered alcohols. The enantiomeric excess of alcohols (-)-**2a** and -**3a** was determined by measuring the optical rotation at the sodium-D line compared with that of the corresponding optically pure compound described in the literature [8,14]. Optical purities of the acetates [(+)-**2b**, -**3b**] were confirmed on the basis of the optical rotation of the corresponding alcohols prepared by a simple saponification with sodium methoxide in methanol at room temperature.

The results of the enzymatic transesterification of (±)-**2a** using different lipases are summerized in Scheme 2 and Table 1. Enzymatic transesterification of racemic

Scheme 2

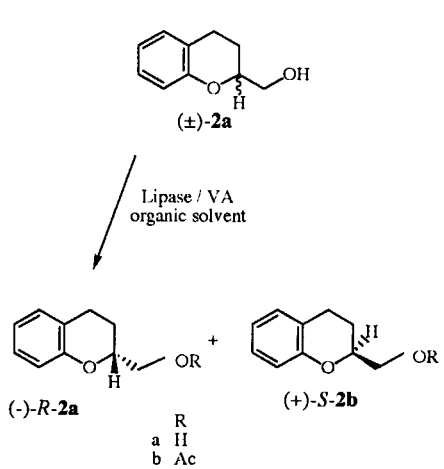


Table 1

Enzymatic Esterification of racemic **2a**

Entry	Lipase [a] (amount) [b]	Solvent [c]	Time h	Conv. % [d]	(-)-Alcohol [e] % ee, conf.		(+)-Acetate [e] % ee, conf.		E [f]
1	PsfL	dichloromethane	12.5	45	69	*R*	84	*S*	24,2
	(516)		22	51	80		80		18,5
	(525)		29	55	85		76		14,7
	(525)		38	59	92		77		14,3
2	PsfL	dioxane	40	65	94	*R*	58	*S*	9,9
	(525)								
3	PscL	dichloromethane	3.5	47	68	*R*	78	*S*	15,3
	(12193)		5.5	60	91		70		12,4

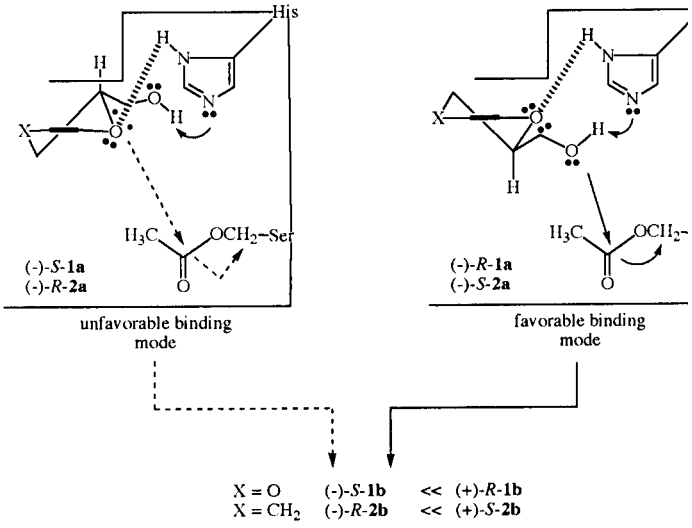
[a] PsfL denotes lipase from *Pseudomonas fluorescens* (Fluka), spec. act. (31.5 U/mg), PscL denotes lipase from *Pseudomonas cepacea* (Amano, PS-C), spec. act. (0.595 U/mg); [b] In unit of enzyme per mmol of racemic **2a**; [c] All the solvents were anhydrous and contained 36 mmol vinyl acetate/mmol racemic **2a**; [d] Conversion degrees were calculated from the yields of the recovered alcohols. The reactions were carried out at r.t.; [e] The enantiomeric excess was determined by measuring the optical rotation in methanol (c = 0.5-0.6) at the sodium D-line at r.t. $[\alpha]_D^{24}$ = -113.4 in methanol (c = 1.12) for optically pure *R*-**2a** [8]; [f] Calculated according to Ref. 22.

2a, performed under the same conditions used for the kinetic resolution of racemic **1** [7], took place significantly slower than in the case of racemic **1** both in dichloromethane (entry 1) and in dioxane (entry 2), to result in (-)-*R*-**2a** in 82% and 70% yields, respectively, (yields are calculated as the percentage of the theoretical 50% yield based on racemic **2a**) with excellent optical purity (92% and 94%). In addition, it should be noted that in contrast to our earlier result [7] neither the enantioselectivity nor the activity of the enzyme was influenced by the solvent. On the other hand, treatment of racemic **2** with the lipase from *Pseudomonas cepacea* (PscL) in dichloromethane for 5.5 hours resulted also in 60% conversion into the leavorotatory alcohol *R*-**2a** having 91% ee (entry 3).

Transesterification of racemic **3a** catalysed by PsfL was also attempted both in dichloromethane and in dioxane (Scheme 3). In general, its kinetic resolution has been found to be less effective in both solvents than in the case of racemic **2a** (Table 2). Thus, the lipase-mediated acylation of racemic **3a** resulted in a 57% conversion into (-)-*S*-**3a** of very modest optical purity (13% ee) in dichloromethane, which could be increased to 98% ee by a considerably increasing the rate of conversion (entry 1). It is remarkable that a significant decrease of the enantioselectivity was found in dioxane (entry 2). In agreement with our expectations, in runs using PsfL of higher activity (entry 3 and 4) acylation of racemic **3a** took place much faster, but the chiral recognition of this enzyme is drastically decreased. Interestingly, the resolution of racemic **3a** catalyzed by PscL in dichloromethane also gave (-)-**3a** of very poor optical purity (entry 5).

The different behaviour of racemic **2a** and -**3a** in the course of the enzyme-catalyzed acetylation can be explained by the conformation of their heteroring, and by the role of the heterocyclic oxygen atom in the binding to the active site of the lipases. Thus, according to our earlier result [7], binding of racemic **2a** takes place with the conformation of P-helicity defined by the torsion angle of C-5a, C-4, C-3, C-2 ($\omega > 0$) to lead to a preferable ES-complex. The tight fit of (+)-*S*-**2a** to the chiral binding site of the enzyme takes place due to a strong hydrogen bonding between the oxygen atom of the heteroring and the histidine residue in the active site of the enzyme (Figure 1 right-part). In this conformation the hydroxymethyl group adopts an equatorial position, and therefore its distance from the acyl-enzyme intermediate is optimum for an asymmetric acylation as depicted in Figure 1.

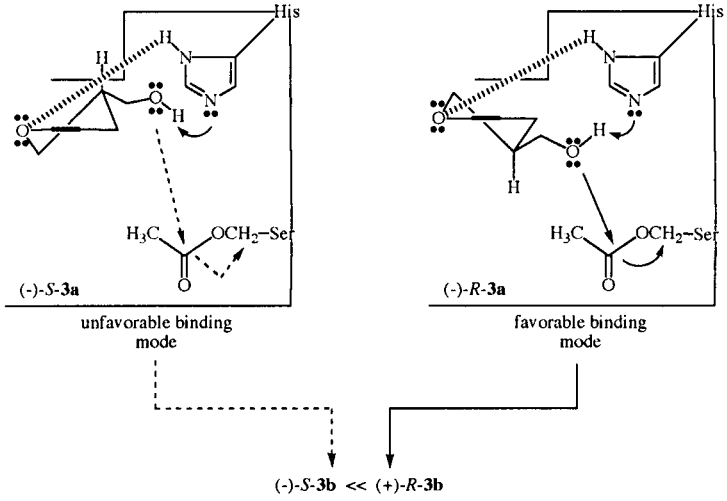
Figure 1



Taking into consideration this conformation of the fast-reacting enantiomer (+)-*S*-**2a** (Figure 1, left-part), the slow-reacting one [(-)-*R*-**2a**] must have a mirror image conformation to result in restricted binding to the active

site of the enzyme due to the unfavourable long distance between the hydroxymethyl group of the substrate and the acyl-lipase intermediate. Indeed, relying upon the positive sing of the ^1L_b-band cd, measured in dioxane at room temperature, an M-helicity of the heteroring of the slow-reacting enantiomer (-)-*R*-**2a**, carrying the hydroxymethyl group in equatorial position, was established by means of applying the helicity rule published by one of us [15].

Figure 2



As shown in Figure 2, on the basis of the above-mentioned stereochemical arrangement a less tight three-point interaction model of racemic **3a** at the chiral binding site of the enzyme can be predicted. Therefore, the differential degree of these spatial constraints should have to influence the migration of the acyl group from the acyl-lipase intermediate to the substrate, leading to dropped ee% values as compered to those obtained in the case of racemic **1** and **2**. As can be seen from Table 1 and 2 in fact, a valuable decrease of the enantioselectivity has been found for **3a**.

A final note must be made regarding the absolute stereochemical assignments. Our results are in full harmony with the literature data, that both PsfL [7,8,16,17] and PscL [18-20] possess high preference to selectively acylate the (*R*)-isomer of chiral primary alcohols. The stereochemical arrangement of the slow-reacting enantiomer of racemic **2a** [(-)--*R*-**2a**], depicted in Figure 1, clearly shows that this preference is actually also valid in this case, in spite that the absolute configuration of its chirality center is *S*. Thus, the change of the preference ($R \rightarrow S$) is due only to the Cahn-Ingold-Prelog priority rule [21], and not at all to the different fine structure of the enzyme-substrate complex.

We have demonstrated the utility of the *Pseudomonas fluorescens* (PsfL) and *cepacea* (PscL) lipase enzymes in the effective resolution of 2- and 3-hydroxymethylchromanes (racemic **2a**, **3a**). The enzymatic resolution of these chiral building blocks allows the more simple preparation of their enantiomers as described in the literature [8,14] and therefore this procedure may be useful for

Scheme 3

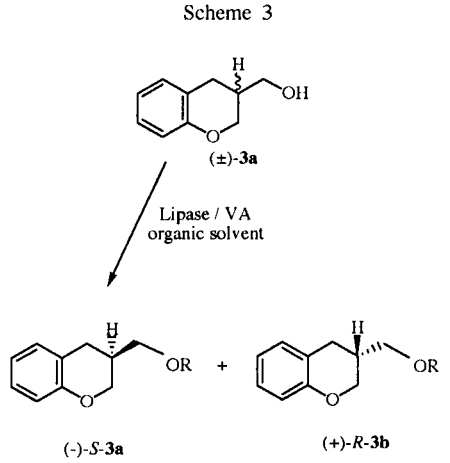


Table 2
Enzymatic Esterification of racemic **3a**

Entry	Lipase [a] (amount) [b]	Solvent [c]	Time h	Conv. % [d]	(-)-Alcohol [e] % ee, conf.		(+)-Acetate [e] % ee, conf.		E[f]
1	PsfL (748)	dichloromethane	24	57	13	*S*	15	*R*	1,4
			31	69	32		12		1,7
			41	81	98		8		5,7
2	PsfL (748)	dioxane	37	73	76	*S*	10	*R*	3,7
3	PsfL* (25402)	dichloromethane	3.5	59	19	*S*	14	*R*	1,6
4	PsfL* (25402)	dioxane	4.5	53	26	*S*	18	*R*	2,0
5	PscL (9765)	dichloromethane	7.5	11	11	*S*	30	*R*	19,2
			28	71	35		12		1,8

[a] PsfL denotes lipase from *Pseudomonas fluorescens* (Fluka), spec. act. (44.9 U/mg) and (3097 U/mg) marked by an asterick*; [b]-[d] see Table 1; [e] The enantiomeric excess was determined by measuring the optical rotation in methanol (c = 0.5-0.4) at the sodium D-line at r.t. $[\alpha]_D^{24} = -19$ in chloroform (c = 0.3) for optically pure *S*-**3a** [14]; [f] Calculated according to Ref. 22.

the synthesis of analogues of **4** and **5** for structure-activity studies. The observed stereochemical preference of PsfL as well as PscL during these kinetic resolutions has been also discussed.

EXPERIMENTAL

Optical rotations were determined on a Perkin-Elmer 241 polarimeter. Cd-spectra were recorded on a Jobin-Yvon Mark VI dichrograph at room temperature. ^{1}H nmr spectra were recorded with a Bruker AM 360 instrument in $CDCl_3$ with TMS as internal standard. Pre-coated silica gel plates (Kieselgel 60 F_{254}, 0.25 mm, Merck) were used for analytical and preparative tlc. Lipase from *Pseudomonas fluorescens* was purchased from Fluka. Lipase from *Pseudomonas cepacea* was gifted by Amano Pharmaceutical Co., Ltd.

General Procedure for the Lipase-Catalyzed Resolution of Racemic **2a** and **3a**.

A solution of racemic **2a** or **3a** (100 mg, 0.6 mmol) in dry dichloromethane or dioxane (4 ml) was treated with vinyl acetate (1 ml, 22 mmol) and the lipase from *Pseudomonas fluorescens* or *cepacea* (10 mg). The suspension was stirred at room temperature. The reaction was stopped by filtration of the enzyme, the filtrate was evaporated to dryness at reduced pressure and the residue was separated by means of preparative tlc (toluene:acetone or toluene:ethyl acetate) to give (-)-*R*-**2a** or (-)-*S*-**3a** and the corresponding acetates (+)-*S*-**2b** or (+)-*R*-**3b**, respectively.

(-)-*R*-**2a** colorless oil; $[\alpha]^{24}_D$ = -106.3 (c = 0.17, methanol), ee = 94 %, R_f = 0.27 (toluene:acetone = 10:1); ^{1}H nmr: δ = ppm 1.86-2.09 (m, 2H, 3-CH_2), 2.85 (dd, J = 4 and 11 Hz, 1H, 4-H_{eq}), 2.97 (dd, J = 6 and 12 Hz, 1H, 4-H_{ax}), 3.35 (s, 1H, OH), 3.88 and 3.93 (2xdd, J = 3.5, 6 and 12 Hz, 2H, CH_2OH), 4.15-4.25 (m, J = 3.5, 4.0, 6.2 and 9.5, 1H, 2-H_{ax}), 6.96 (dd, J = 8 and 16 Hz, 2H, 5-H, 6-H), 7.19 (dd, J = 9 and 18 Hz, 2H, 7-H, 8-H); ^{13}C nmr: δ = ppm 76.22 (2-C), 116.44, 120.14, 127.03 and 129.32 (5-C, 6-C, 7-C and 8-C), 23.47 and 24.23 (3-C and 4-C), 65.14 (-CH_2-OH), 121.73 (quaternary C), 154.24 (quaternary C attached to O); Cd (dioxane) λnm (Δε): 278 (0.09), 270 (0.06) 227 (-3.93); uv (dioxane) λnm (ε x 10^3): 283 (1.56), 275 (1.64) 220 (6.57); ms: (*m/z*) 164 (M^+, 42), 145 (69), 131 (100), 118 (15), 105 (33), 78 (42), 71 (65), 39 (82).

(+)-*S*-**2b** colourless oil; $[\alpha]^{24}_D$ = 56.2 (c = 0.38, methanol), ee = 58 %, R_f = 0.68 (toluene:acetone = 10:1); ^{1}H nmr: δ = ppm 1.71-1.82 (m, 2H, 3-CH_2), 2.06 (s, 3H, CH_3), 2.72 (dd, J = 4 and 11 Hz, 1H, 4-H_{eq}), 2.82 (dd, J = 7 and 12 Hz, 1H, 4-H_{ax}), 4.19 (dd, J = 4, 6 and 12 Hz, 2H, CH_2OH), 4.26-4.33 (m, 1H, 2-H), 6.78 (dd, J = 8 and 16 Hz, 2H, 5-H, 6-H), 7.01 (dd, J = 9 and 18 Hz, 2H, 7-H, 8-H); ^{13}C nmr: δ = ppm 20.88 (-CH_3), 73.51 (2-C), 116.86, 120.46, 127.36 and 129.48 (5-C, 6-C, 7-C and 8-C), 24.09 and 24.28 (3-C and 4-C), 66.31 (-CH_2-O-), 121.54 (quaternary C), 154.28 (quaternary C attached to O), 170.97 (carbonyl C).

(-)-*S*-**3a** mp 61-63 °C (n-hexane); $[\alpha]^{24}_D$ = -18.6 (c = 0.32, chloroform), ee = 98 %, R_f = 0.17 (toluene:ethyl acetate = 4:1); ^{1}H nmr: δ = ppm 1.32 (s, 1H, OH), 2.31-2.41 (m, 1H, 3-H), 2.68 (dd, J = 8 and 12 Hz, 1H, 4-H_{ax}), 2.97 (dd, J = 6 and 11 Hz, 1H, 4-H_{eq}), 3.73 and 3.81 (2xdd, J = 5, 8 and 12 Hz, 2H, CH_2OH), 4.08 (dd, J = 8 and 12 Hz, 1H, 2-H_{ax}), 4.38 (dd, J = 4 and 12 Hz, 1H, 2-H_{eq}), 6.91 (dd, J = 8 and 16 Hz, 2H, 5-H, 6-H), 7.15 (dd, J = 9 and 18 Hz, 2H, 7-H, 8-H); ^{13}C nmr: δ = ppm 77.65 (3-C), 120.45, 120.75, 127.55 and 127.88 (5-C, 6-C, 7-C and 8-C), 28.35 (4-C), 63.67 (-CH_2-OH), 71.14 (2-C), 113.87 (quaternary C), 155.24 (quaternary C attached to O). Cd (dioxane) λnm (Δε): 275 (-0.20); uv (dioxane) λnm (ε x 10^3): 282 (1.51), 275 (1.75); ms: (*m/z*) 164 (M^+, 40), 133 (100), 120 (10), 105 (58), 91 (105), 77 (40), 48 (28).

(+)-*R*-**3b** colorless oil; $[\alpha]^{24}_D$ = 1.9 (c = 0.93, chloroform), ee = 8%, R_f = 0.66 (toluene:ethyl acetate = 4:1); ^{1}H nmr: δ = ppm 2.02 (s, 3H, CH_3), 2.28--2.42 (m, 1H, 3-H), 2.55 (dd, J = 8 and 16 Hz, 1H, 4-H_{ax}), 2.82 (dd, J = 6 and 16 Hz, 1H, 4-H_{eq}), 3.89 (dd, J = 8 and 11 Hz, 1H, CH_2-H_{eq}), 3.99 (dd, J = 8 and 12 Hz, 1H, CH_2-H_{ax}), 4.09 (dd, J = 6 and 12 Hz, 1H, 2-H_{ax}), 4.19 (dd, J = 4 and 11 Hz, 1H, 2-H_{eq}), 6.77 (dd, J = 8 and 16 Hz, 2H, 5-H, 6-H), 7.01 (dd, J = 9 and 18 Hz, 2H, 7-H, 8-H); ^{13}C nmr: δ = ppm 31.93 (-CH_3), 93.02 (3-C), 116.66, 120.62, 127.45 and 129.94 (5-C, 6-C, 7-C and 8-C), 27.58 (4-C), 64.54 (2-C), 67.43 (-CH_2-O-), 113.64 (quaternary C), 156.10 (quaternary C attached to O), 170.06 (carbonyl C).

Aknowledgements.

The authors thank the National Science Foundation (OTKA T-23687) and Ministry of Education (Grant No. 460/1997) for financial support. We express our thanks to Amano Pharmaceutical Co. for generously providing a sample of lipase from *Pseudomonas cepacea*.

REFERENCES AND NOTES

[1] C. H.Wong and G. M. Whitesides, Enzymes in Synthetic Organic Chemistry, Pergamon, Oxford (1994).

[2] K. Faber, Biotransformations in Organic Chemistry, Springer-Verlag, Berlin (1995).

[3] A. M. P. Koskinen and A. M. Klibanov (Eds.), Enzymatic Reactions in Organic Media, Blackie Academic, Glasgow (1996).

[4] A. M. Klibanov, *Acc. Chem. Res.*, **23**, 114 (1990).

[5] S. Servi, *Synthesis*, 1 (1990).

[6] S. M. Roberts, K. Wiggins and K. Casy (Eds.), Preparative Biotransformations: Whole Cell and Isolated Enzymes in Organic Synthesis, John Wiley & Sons Ltd., New York (1993).

[7] S. Antus, Á. Gottsegen, J. Kajtár, T. Kovács, T. S. Tóth, and H. Wagner, *Tetrahedron Asymmetry*, 339 (1993).

[8] F. J. Urban, and B. S. Moore, *J. Heterocyclic Chem.*, **29**, 431 (1992).

[9] C. Melchiorre, A. Minarini, V. Tumiatti, E. Giraldo, G. B. Schiavi and M. Turconi, *Med. Chem. Res.*, **4**, 140 (1993).

[10] C. Marot, C. Comoy, M. C. Viaud, M. C. Rettori, B. Pfeiffer, L. Morin-Allory and G. Guillaumet, *Bioorg. Med. Chem. Lett.*, **6**, 1077 (1996).

[11] M. Payard, G. Mouysset, P. Tronche, P. Bastide, J. Bastide and M. J. Boas, *Eur. J. Med. Chem. Chim. Ther.*, **20**, 2, 117 (1985).

[12] R. P. Houghton, M. Voyle and R. Price, *J. Chem. Soc. Perkin Trans. 1.*, **5**, 925 (1984).

[13] G. Mouysset, M. Payard, G. Grassy, P. Tronche and H. Dabire, *Eur. J. Med. Chem. Chim. Ther.*, **22**, 539 (1987).

[14] G. Broggini, F. Folcio, N. Sardone, M. Sonzogni and G. Zecchi, *Tetrahedron Asymmetry*, **7**, 797 (1996).

[15] S. Antus, E. Baitz-Gács, J. Kajtár, G. Snatzke and A. L. Tökés, *Liebigs Ann. Chem.*, 497 (1994).

[16] B. Herradon and S. Valverde, *Tetrahedron Asymmetry*, **5**, 1479 (1994).

[17] H. Miyaoka, Y. Kajiwara, M. Hara, A. Suma and Y. Yamada, *Tetrahedron Asymmetry*, **10**, 3189 (1999).

[18] M. D. Ennis and D. W. Old, *Tetrahedron Lett.*, **33**, 6283 (1992).
[19] M. D. Ennis and N. B. Ghatal, *Tetrahedron Lett.*, **33**, 6287 (1992).
[20] D. Mauleon, C. Lobato and G. Carganico, *J. Heterocyclic Chem.*, **31**, 57 (1994).
[21] R. S. Cahn, C. Ingold and V. Prelog, *Angew. Chem.*, **78**, 413 (1966).
[22] C-S. Chen, Y. Fujimoto, G. Girdaukas and C. J. Sih, *J. Am. Chem. Soc.*, **104**, 7294 (1982).