Pergamon

Tetrahedron: Asymmetry, Vol. 8, No. 17, pp. 2913–2920, 1997
© 1997 Elsevier Science Ltd. All rights reserved.
Printed in Great Britain
PII: S0957-4166(97)00349-2
0957-4166/97 $17.00 + 0.00

An efficient access to the enantiomers of α-methyl-4-carboxyphenylglycine via a hydantoin route using a practical variant of preferential crystallization AS3PC (Auto Seeded Programmed Polythermic Preferential Crystallization)[11]

E. Ndzié,[a] P. Cardinael,[a] A.-R. Schoofs[b] and G. Coquerel[a,*]

[a] Laboratoire de Modélisation Moléculaire, CRUS–IRCOF, IFRMP no 23, Université de Rouen, 76821 Mont-Saint-Aignan Cedex, France

[b] Centre Européen de Bioprospective, 24 rue J. Boutrolle, 76131 Mont-Saint-Aignan Cedex, France

Abstract: The enantiomers are obtained in preparative amounts without a resolving agent via the following sequence: hydantoin synthesis, resolution by entrainment, and ring cleavage by means of hydrolysis in basic conditions. The new variant of preferential crystallization (AS3PC), implemented without using seeds, shows good reproducibility and yield. © 1997 Elsevier Science Ltd

Introduction

Non-proteinogenic α,α-disubstituted glycines play an important role when incorporated into small- to medium-size peptides[1,2] due to their ability to stabilize some secondary structures. Peptides containing these amino acids as building blocks exhibit an increased stability towards biological and chemical degradation. Among them the α-methylphenylglycine series has been the object of intensive work as putative tools for investigating the role of the so-called metabotropic glutamate receptors in the neuronal synaptic function.

S-(+)-Enantiomer of α-methyl-4-carboxyphenylglycine **4a** (M4CPG) is a potent and selective antagonist of presynaptically mediated depressant responses and cyclic AMP-coupled metabotropic glutamate receptor subtypes mGluR1 and mGluR2.[3,4] In a recent paper, Coudert *et al.*[5] described the successful preparation of the two M4CPG enantiomers (+)-**4a** and (−)-**4b** by using an ion-exchange chromatography separation of the corresponding (S)-Leu diastereomeric dipeptides. This process needs non racemizing conditions during the coupling, the separation and the hydrolysis, and requires the setting of appropriate chromatographic parameters that will not hamper these steps.

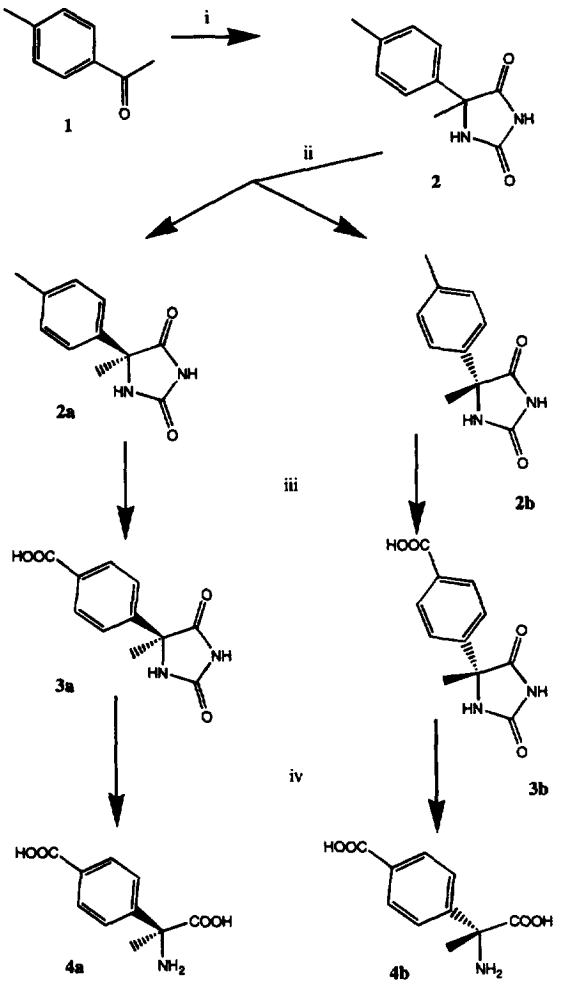
The aim of our study is a convenient 4-step method for the preparation of M4CPG enantiomers (+)-**4a** and (−)-**4b** without using a resolving agent, such a method would allow these amino acids to be obtained with a good overall yield and a high enantiomeric excess (ee>98%).

Method

According to Scheme 1, the two enantiomers of M4CPG are obtained by synthesis of the hydantoin derivative **2** in a 'one pot' reaction using the Bücherer–Bergs method on the corresponding ketone **1**.[6–8] The 5-methyl-5-(4-methylphenyl)-hydantoin **2** obtained crystallizes as a conglomerate and can thus be resolved by preferential crystallization. The obtained enantiomers **2a** and **2b** of absolute configuration[14] (S)-(+) and (R)-(−) respectively are first submitted to oxidation of the para methyl substituent on the phenyl group to obtain (S)-(+)-**3a** and (R)-(−)-**3b**. These acids are subsequently opened under basic conditions to yield the enantiomerically pure forms of (S)-(+)-M4CPG **4a** and

* Corresponding author. Email: Gerard.Coquerel@univ-rouen.fr

(R)-(−)-M4CPG **4b** without racemization. In this series of reactions, the retention of configuration is observed from the hydantoin derivative **2a**, **2b** to the afforded amino acids[13] **4a** and **4b**.



Scheme 1. i) 0.9 eq. (71.7 g) NH_4HCO_3, 0.3 eq. (19.5 g) KCN, 0.25 eq. (33.5 g) ketone, 100 mL of ethanol, 75 mL of NH_4OH, 60°C, 3–4 h, 25 mL H_2O; ii) AS3PC[11]; iii) 0.015 eq. (3.06 g) of **2**, **2a**, **2b**, 0.046 eq. (7.27 g) $KMnO_4$, 0.3 eq. (12.0 g) NaOH, 180 mL H_2O, 90–95°C, 2–4 h; iv) 1 eq. **4a**, **4b**, **4** eq. NaOH, 20 mL H_2O, 4 h, 140°C.

Preferential crystallization is a well-known resolution method[9,10] which consists in alternate crystallizations of each antipode from a supersaturated mother liquor containing a slight excess of one enantiomer, by seeding with fine crystals of the enantiomer that is in excess. This method can be applied provided no stable (or metastable) racemate appears and it leads quantitatively by continuous recycling of the mother liquor to the two enantiomers without the use of any resolving agent.

We apply here a new variant of preferential crystallization named AS3PC (Auto Seeded Programmed Polythermic Preferential Crystallization),[11] which is based on a different approach from the conventional method. The differences between the AS3PC process and the usual method can be highlighted by comparison of the manipulations in the two processes. Below are listed the sequences of operations starting from the mother liquor obtained at the end of the previous run which is taken to have yielded M grams (for the conventional process) and M′ grams (for AS3PC) of crude enantiomer *e.g.* (+).

Conventional process[9,10]

- Addition of M grams of (±)-mixture equal to the mass of the previous crop.
- Homogenization by heating to a temperature higher than the temperature (T_{HOMO}) of complete dissolution of the total amount of solute.
- Cooling to T_F (the temperature of filtration at the end of the crystallization).
- Seeding with the same enantiomer *i.e.* (−) that is in excess in the supersaturated solution.
- Crystallization induced by the seeds; this step includes secondary nucleation and growth.
- Filtration to yield M grams of the (−)-enantiomer.

AS3PC process[11]

- Addition of M′ grams of (±)-mixture; the crystal size distribution of this powder is well defined.
- Heating to T_B defined as $1/2(T_{HOMO}+T_L)$; T_L stands for the temperature of dissolution of the total amount of racemic mixture only in the same quantity of solvent (above T_L the solution has an optical purity different from 0). T_{HOMO} stands for the temperature of homogenization *i.e.* the lowest temperature that allows complete dissolution of the solute: racemic mixture plus the enantiomer in excess.
- Annealing at T_B, during a period of time t_A to ensure the complete dissolution of the undersaturated enantiomer and relaxation of crystals of the enantiomer in excess. Thus, a suspension consisting of crystals of pure enantiomer in excess and its saturated solution is obtained in thermodynamic equilibrium.
- Cooling from T_B to T_F according to well defined kinetics with an adapted rate of stirring so that T_B represents the beginning of crystallization. Actually from T_B to T_L only the enantiomer already present as crystals can grow and from T_L to T_F, the real entrainment effect occurs.
- Filtration of M′ grams of (−)-enantiomer at temperature T_F prior to the spontaneous nucleation of the counter enantiomer.

The main advantages of the AS3PC method (tested up to now on more than 25 different molecules — covalent, of ionic character or solvate in a great variety of solvents) are:

- suppression of the seeding constraint (the crystallization is initiated without inoculation of seeds of pure enantiomer),
- improvement of the yield (M′>M) and of the enantiomeric purity of the crops,
- improvement of the reproducibility of the process,
- shortened duration of each cycle,
- adjustable crystal size distribution and ease of scaling up,
- ease of filtration.

Because the AS3PC method does not require inoculation of any solid to initiate the crystallization and because the temperature is programmed, the crystallization can be monitored and automated more easily.

The (±)-mixture added at the beginning of each run must sometimes first undergo one of the following pre-treatments:

- grinding and sieving,
- precipitation in a separate operation. When the unsolvated racemic mixture is actually a racemate this precipitation must be carried out in the appropriate solvent in which the conglomerate of solvate is stable,
- a fusion and then rapid cooling prior to grinding and sieving.

These operations are designed to ensure a reproducible fine crystal size distribution so that a large surface of growth is created and the equilibrium at T_B is reached more quickly.

- At T_B, a biphasic system is obtained so that about 25% of the initial enantiomeric excess is present as fine crystals in equilibrium with their saturated solution, and these crystals represent a large number of seeds ready to grow as soon as the temperature is decreased according to the cooling program (auto seeded solution).

When a high enantiomeric excess of the mother liquor is obtained from the previous run and when, in an appropriate solvent, $Z = \left[\frac{d(T_{HOMO})}{d(e.e)}\right]_{T_L \text{ constant}} = \left[\frac{d(T_{HOMO})}{d(e.e)}\right]_{(\pm)\text{concentration constant}}$ ratio is high enough (about 1°C/1% e.e); the AS3PC method is particularly efficient and easy to perform as shown in Table 2. If one of two properties is not fulfilled, the AS3PC method can still be carried out if an accurate T_B temperature control is achieved.

- Moreover, when the Z ratio is favorable, T_B may be adjusted closer to T_L so that up to 40% of the total crop is present in equilibrium at the initial point of crystallization (i.e. the starting point of the cooling) and will induce the crystallization of only 60% of the total harvest. Thus, the exothermic heat transfer phenomenon resulting from the crystallization is much weaker compared to the classical seeded process and it lasts over the entire duration of the cooling. Moreover, this exothermic phenomenon is integrated in the cooling program.
- The temperature versus time function is adapted so that the crystal growth is favored instead of nucleation. The stirring mode and rate are also adapted so that the mother liquor is renewed around the crystal and kept below the secondary nucleation induction threshold. The imposed cooling program provides full control of the selective crystallization.
- As $T_B < T_{HOMO}$, fewer by-products are generated. This can be a decisive advantage because of the continuous recycling of the mother liquor to which the racemic mixture is added at the end of each run.
- Filtration is preferably carried out in isothermal conditions (at T_F); because the crystals are larger with smooth faces, the retention of mother liquor is drastically decreased.

In conclusion, we have illustrated with the preparation of both enantiomers of M4CPG a new variant for preferential crystallization that may offer dramatic advantages over the classical process. Moreover, in order to show the wide range applicability of this process several other examples are given at the end of this paper (Table 6). These examples include some comparative results with the conventional procedure. The additional data highlight the determining roles of the Z factor together with the e.e. of the mother liquor reached at the end of each run, in the required accuracy of the temperature control.

Experimental

General methods

Solvents are used without further purification. Melting points are determined on Differential Scanning Calorimeter DSC 101 Setaram apparatus and are corrected by using two standards (benzoic acid and Indium). ^{1}H NMR spectra are recorded on a 200 MHz Bruker Spectrometer using tetramethylsilane as internal reference. Structural assignments for all new compounds are consistent with their spectra.

Multiplicities (δ, ppm) are reported as s (singlet), d (doublet), t (triplet), q (quadruplet) and m (multiplet). Specific rotation determinations are performed on a Perkin Elmer 241C polarimeter with a 1 dm cell length. The enantiomeric excess of the compounds is checked by HPLC with a Teicoplanin Chirobiotic T column (Astec, USA). The hydantoin hydrolysis is performed in a high pressure Vinci Technologies steel reactor (France).

Synthesis of racemic 5-(4-methylphenyl)-5-methylhydantoin

0.9 eq. (71.1 g) of ammoniumhydrogenocarbonate, 0.3 eq. (19.5 g) of potassium cyanide, 0.25 eq. (33.5 g) of 4′-methylacetophenone, 100 mL of ethanol and 75 mL ammonium hydroxide are respectively introduced in a 500 mL flask provided with a reflux condenser and a mechanical stirrer. The mixture is maintained at 60°C for 3 to 4 h **under hood**. 25 mL of water is added at the end of the reaction to induce the crystallization of the hydantoin. The (±) hydantoin is filtered off, dried in an oven and recrystallized twice in 200 mL of a 60/40 mixture of ethanol/water. 45.9 g of racemic hydantoin derivative are obtained (yield: 90%). Melting point: 205°C. ^{1}H NMR (DMSO-d_6) (δ ppm): 1.65 (s, 3H, C*H*$_3$); 2.3 (s, 3H, C*H*$_3$); 7.3–7.5 (m, 4H, Ar*H*); 8.6 (s, 1H, N$_1$-*H*); 10.75 (s, 1H, N$_3$-

H). HPLC Teicoplanin column (15×4.6 cm). Mobile phase hexane/ethanol (v/v: 50/50). Flow: 1 ml min^{-1}. Detector UV λ=225 nm: 6.5 min; 15.8 min.

Resolution of 5-(4-methylphenyl)-5-methylhydantoin

Resolution by diastereomeric salt formation

The two enantiomers are obtained by diastereomeric salt formation using a chiral base (R)-(+) or S-(−)-1-phenylethylamine[12] or by preferential crystallization[11] detailed in the following section. $[\alpha]_D^{20}$=+110.1(±)0.5 and −110.0(±)0.5 (c 1, DMSO).

Auto Seeded Programmed Polythermic Preferential Crystallization

In order to apply the AS3PC method, accurate solubility-versus-temperature data of the racemic mixture in an appropriate solvent (2-methoxyethanol; purity 99%) are needed.

Solubility of racemic mixture in 2-methoxyethanol versus temperature

Temperature (°C)	15	25	39	45	50
Solubility s (mass %)	12.9	14.4	17.0	19.5	20.1

Table 1

Solubility of R-(−)-enantiomer: 7.5% mass at 25°C, α=s (±)/s (−)=2.26

T_{HOMO} as a function of the enantiomeric excess with a racemic concentration (17.0% and T_L constant) remained constant. These data give the limits of the biphasic domain (enantiomer in excess plus its saturated solution in equilibrium) which are used in the auto seeding at T_B. The latter temperature is the starting point of the cooling program.

Enantiomeric excess (%)	0	2	4	6	8
T_{HOMO} (°C)	T_L = 39.0	T_B = 41.3	43.7	46.0	48.3

Table 2

Cooling program

T_B=41.3°C; T_F=14°C. T=f(t) temperature versus time law.

Temperature (°C)	T_B = 41.3	31	31	21	21	T_F = 14
Time (minutes)	0	15	30	45	50	60

Table 3

Initial conditions

T_L=39°C, L point coordinates: 17.0% mass percent at 39°C. Enantiomeric excess: 7.4%.

mass of solvent (g)	mass of racemic mixture (g)	mass of enantiomer (g)
27.68	5.667	0.458

Table 4

Additional parameters of the crystallization

Duration of the supersaturation of the L homogeneous solution undergoing the above cooling program: 70 minutes with 150 rpm stirring rate. Duration of the crystallization: 60 minutes. Duration of the annealing at T_B: 30 minutes. Stirring rate: 150 rpm at the beginning and 200 rpm at the end of the crystallization.

Results

According to the description of the AS3PC process above, the results of twelve consecutive runs are listed below.

Run number	1	2	3	4	5	6	7	8	9	10	11	12
Mass of pure enantiomer (g)	0.882	0.914	0.860	0.887	0.905	0.929	0.882	1.002	1.064	1.164	1.165	1.071
enantiomeric excess (%)	(+) 93	(-) 91	(+) 93	(-) 95	(+) 96	(-) 95	(+) 93	(-) 99	(+) 93	(-) 94	(+) 90	(-) 92

Table 5

Mean mass of pure enantiomer: 0.977 g. Average enantiomeric excess at the beginning of the next run: 7.9%. Mean enantiomeric excess of the crude crystals: 93.6% **without any recrystallization or washing.** Z=1.16.

Theoretically, this value should be 100%, the lower value obtained is due to the fact that the crystals are partially impregnated by the mother liquor containing the remaining racemic mixture thus slightly decreasing the optical purity. The crude enantiomers of the different batches are collected and recrystallized in pure ethanol before characterization (e.e.>99.7%). ^{1}H NMR (DMSO-d_6) (δ ppm): 1.65 (s, 3H, CH_3); 2.3 (s, 3H, CH_3); 7.3–7.5 (m, 4H, ArH); 8.6 (s, 1H, N_1-H); 10.75 (s, 1H, N_3-H). HPLC Teicoplanin column (15×4.6 cm). Mobile phase hexane/ethanol (v/v: 50/50). Flow: 1 ml min^{-1}. Detector UV λ=225 nm: 6.5 min; 15.8 min. Melting point: 250°C (enantiomer). $[\alpha]_D^{20}$=+110.0(±)0.5 and −109.8(±)0.5 (c 1, DMSO).

Synthesis of (−) or (+) enantiomeric forms of 5-(4-carboxyphenyl)-5-methylhydantoin

0.015 eq. (3.06 g) of the pure enantiomeric form of 5-(4-methylphenyl)-5-methylhydantoin is introduced into a solution of 0.046 eq. (7.27 g) of $KMnO_4$ and 0.3 eq. (12.0 g) of NaOH in 180 mL of distilled water. The mixture is slightly refluxed at 95°C for 3 h. 25 mL of ethanol is added during the cooling to react with the excess of $KMnO_4$. The precipitated MnO_2 is filtered off twice to ensure the complete removal of this brown solid. If a green color remains in the filtrate, ethanol is added in order to eliminate the excess of potassium permanganate. The filtrate is then acidified by a mineral acid solution (N HCl).

The filtrations of these carboxylic acids are difficult due to their flocculent character. The suspension is preferably left for one night at room temperature to obtain well crystallized particles. This waiting time is designed to ease the filtration. The (−)- or (+)-carboxylic acids are recrystallized twice, whether by using acidobasic reactions or by using a (50/50) water/ethanol mixture, 2.28 g of white crystals are obtained. Yield: 65%. ^{1}H NMR (DMSO-*d6*) (ppm): 1.67 (s, 3H, CH_3); 7.60–7.95 (d,d, 4H, ArH); 8.68 (s, 1H, N_1-H); 10.84 (s, 1H, N_3-H); 13.03 (s, 1H, COOH). $[\alpha]_D^{20}$=+100.4(±)0.5 and −100.0(±)0.5 (c 1, DMSO).

The melting points of **3a** and **3b** are too high to allow an easy analysis by means of Gas Chromatography coupled with Mass Spectrometry, so the analytical methods have been performed on the methyl ester derivative.

Esterification of the carboxylic hydantoin derivative

4.10^{-3} mol (0.93 g) of the carboxylic derivative and 5 mL of concentrated H_2SO_4 are dissolved in an excess of methanol (25 mL). The mixture is refluxed for 2 h after which cold water is added and a homogeneous solution is obtained. The organic phase is extracted with CH_2Cl_2, and 0.89 g of white crystals are obtained after evaporation of this solvent. The solid is purified and recrystallized in the same solvent. Yield: 90%. Melting point: 186°C. ^{1}H NMR (DMSO-d_6): 1.66 (s, 3H, CH_3); 3.9 (s, 3H, OCH_3); 7.6–8.0 (dd, 4H, ArH); 8.7 (s, 1H, N_1-H), 10.8 (s, 1H, N_3-H). HPLC Teicoplanin column (15×4.6 cm). Mobile phase ethanol. Flow: 1 ml min^{-1}. Detector UV λ=225 nm: 3.33 min; 5.83 min.

compound	solvent	concentration mass of (±) (%)	T_{HOMO} (°C)	T_B (°C)	T_L (°C)	T_F (°C)	final e.e(%)	Z	Enantiomeric excess %	cooling rate °C/min	At T_B (min) (b)	T_B to T_F (min) (c)
1*	water	49.0	51.1	44.4	42.8	30.0	10.3	0.92	95.0	0.26	30	55
1**[15]	water	51.7	56.0	-	-	32.0	8.6	-	72.6	0.08-0.13	-	150
2*	toluene	34.6	33.6	29.5	27.9	19.5	9.0	0.63	91.0	0.25	60	42
2**[16]	toluene	34.0	60.0	-	-	20.0	5.9	-	83.0	0.66	-	60
3*	water	23.1	68.5	58.0	54.5	31.0	8.5	1.75	96.5	0.45	40	60
3**[17] (a)	water	23.1	80.0	-	-	20.0	8.0	-	84.2	1.0	-	60
4[18]	2-methoxyethanol	10.0	60.0	56	53	34.0	15.8	0.92	94.5	0.73	40	65
5	ethanol	12.5	29.8	27.5	27.0	19.4	6.7	0.35	94.2	0.4	20	60
6	2-methoxyethanol	21.5	45.5	40.0	37.0	20.0	6.2	1.41	91.0	0.5	30	60
7	water	46.0	18.3	17.3	16.3	12.0	5.3	0.40	95.5	0.2	40	20

Table 6

* AS3PC process ** conventional process

(a) no seeds; (b) Duration of annealing at T_B. (c) Duration of the crystallization

The enantiomeric excess of the crops is expressed without washing nor recrystallization: 1) *Glutamic acid hydrochloride*; 2) *1-phenylethanol-3,5-dinitrobenzoate*; 3) Threonin; 4) 5-(4'-methoxyphenyl)-5-methylhydantoin; 5) Threitol; 6) 5-methyl-5-phenylhydantoin; 7) Sodium ammonium tartrate tetrahydrate.

Hydrolysis of hydantoin derivatives[13,15–18]

0.013 eq. (3.04 g) of hydantoin and 0.052 eq. (2.08 g) of NaOH are dissolved in distilled water, and heated up under pressure to 140°C for 4 h. The mixture is cooled down to room temperature and concentrated HCl is then added. At neutral pH, the resulting white solid is submitted to filtration.

The afforded aminoacid is recrystallized twice in a (50/50) water/ethanol mixture and 2.43 g of white crystals are obtained (yield=90%). ^{1}H NMR (D_2O) (δ ppm): 1.66 (s, 3H, CH_3); 7.28 (m, 4H, Ar*H*). $[\alpha]_D^{20}$=+89.6 (±)0.5 and −89.6 (±)0.5 (c 1, DMSO). Melting point: 280°C (dec.)

References

1. (a) D. Obrecht, U. Bohdal, J. Daly, C. Lehmann, P. Schönholzer and K. Müller *Tetrahedron* **1995**, 51, 40, 10883–10900. (b) D. Obrecht, U. Bodhal, C. Broger, D. Bur, C. Lehmann, R. Ruffieux, P. Schönholzer, C. Spiegler and K. Müller *ibid.* **1995**, 78, 563–580.
2. (a) D. Obrecht, C. Spiegler, P. Schönholzer, K. Müller, H. Heimgartner and F. Stierli *Helv. Chim. Acta* **1992**, 75, 1666–1696. (b) D. Obrecht, H. Karajiannis, C. Lehmann, P. Schönholzer, C. Spiegler and K. Müller *ibid* **1995**, 78, 703–714.
3. M. Kemp, P. Roberts, P. Pook, D. Jane, A. Jones, P. Jones, D. Sunter, P. Udvarhelyi and J. Watkins *Eur. J. Pharm. Mol. Pharm. Section* **1994**, 266, 187–192.
4. (a) S. R. Glaum, D. C. Sunter, P. M. Udvarhelyi, J. C. Watkins and R. J. Miller *Neuropharmacology* **1993**, 32, 1419–1425. (b) J. Watkins and G. Collingridge *Trends. Pharmacol. Sci.* **1994**, 15, 333–342.
5. E. Coudert, F. Acher and R. Azerad *Tetrahedron: Asymmetry* **1996**, 7, 2963–2970.
6. H. R. Henze and A. Furman Isbell *J. Am. Chem.* Soc. **1954**, 76, 4152–4156.
7. H. R. Henze and Robert J. Speer *J. Am. Chem. Soc.* **1942**, 64, 522–523.
8. (a) H. T. Bücherer and V. A. Lieb *J. Prakt. Chem.* **1934**, 141, 5 (b) L. H. Goodson, I. L. Honigberg, J. J. Lehman and W. H. Burton *J. Org. Chem.* **1960**, 25, 1920–1924.
9. J. Jacques, A. Collet and S. H. Wilen *Enantiomers, Racemates and Resolutions,* Krieger Publishing Company, Malabar Florida, **1994**.
10. E. Eliel, S. H. Wilen and L. N. Mander *Stereochemistry of Organic Compounds,* Wiley-Interscience, New York, **1994**, 153–214, 297–322.
11. G. Coquerel, M.-N. Petit and R. Bouaziz. *PCT Patent* WO 95/08522.
12. G. Coquerel, M.-N. Petit, R. Bouaziz and D. Depernet *Chirality* **1992**, 4, 400–403.
13. E. Ware *Chem. Rev.* **1950**, 46, 403–470.
14. G. Coquerel and S. Petit *J. Cryst. Growth.* **1993**, 130, 173–180.
15. Noguchi Institute *English Patent* n° 1 197 809.
16. M. J. Brienne, A. Collet and J. Jacques *Synthesis* **1983**, 9, 704–705.
17. G. Amiard *Bull. Soc. Chim. Fr.* **1956**, 447.
18. E. Ndzié, G. Coquerel *Unpublished results.*

(Received in UK 16 June 1997; accepted 8 August 1997)