Pergamon

Tetrahedron: Asymmetry Vol. 5, No. 8, pp. 1621-1626, 1994
Elsevier Science Ltd
Printed in Great Britain
0957-4166/94 $7.00+0.00

0957-4166(94)00229-0

A Four-step, Highly Enantioselective Synthesis and Enzymatic Resolution of 3,4-dichloro-phenylalanine.

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Abstract : *(S)*-3,4-Dichloro-phenylalanine hydrochloride **6** 98.3% *S* has been synthesized in 4 steps, 66% overall yield and with recovery of the chiral auxiliary **1** using our modified Yamada's method. Both enantiomers of the chiral auxiliary **1** being available, the *R* isomer of **6** with the same enantiomeric purity can also be prepared easily. Kinetic resolution with Subtilisin A of the racemic ethyl N-acetyl-3,4-dichloro-phenylalaninate **8** provided the same hydrochloride **6** of *S* configuration in 32% yield with about the same or slightly lower enantiomeric purity, the *R* isomer being available from the un-transformed **8**.

Mono-, di- and trichloro-phenylalanines have been shown to be specific precursors for the corresponding chlorophenylacetic or chlorobenzoic acids known as potent plant growth-regulating agents and/or powerful herbicides[1]. Destruction of the susceptible species could thus occur by a « lethal synthesis » of the active molecule *in vivo*. Racemic 3,4-dichloro-phenylalanine is usually synthesized in satisfactory yield (75%) using the acetamidomalonate route[2,3]. However, although this amino acid appeared to have interesting bioactivity in its racemic form[1] no studies have been devoted to its resolution or asymmetric synthesis.

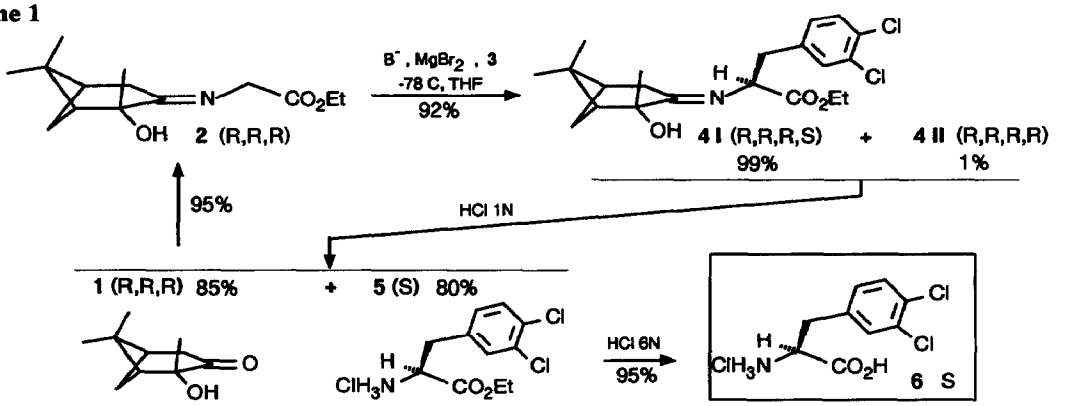
We report here the first short synthesis of ~98.3% enantiomerically pure (*S*)-3,4-dichloro-phenylalanine hydrochloride **6** and the first enzymatic resolution of ethyl N-acetyl-3,4-dichloro-phenylalaninate **8**.

Enantioselective Synthesis

During work on the synthesis of optically active unnatural D-amino acids[4] we had shown that addition of 0.5 to 1 equivalent of $MgBr_2$ to the lithium enolate of iminoglycinate **2** derived from 2-hydroxy pinanone **1** could increase the diastereoselectivity of alkylations up to 95-100/5-0. Because the 3,4-dichloro-phenyl benzyl bromide **3** was readily available from 3,4-dichloro-toluene we decided to apply our method to the synthesis of *(R)*- or *(S)*-3,4-dichloro-phenylalanine, scheme 1.

The *(R,R,R)*-iminoglycinate **2** was obtained in high yield (95%) from *(R,R,R)*-2-hydroxypinanone **1** and inexpensive ethyl glycinate using BF_3-etherate as catalyst[4,5]. Alkylation at -78°C with 3,4-dichloro-phenyl benzyl bromide **3** of the lithium enolate of iminoester **2** but after addition of freshly prepared $MgBr_2$ (1 equiv.) then afforded, as expected from our previous results[4] , 99% of one diastereomer **4I** (determined on the 200MHz ^1H NMR spectrum of crude product). After smooth hydrolysis (HCl 10% in THF at 25°C) the chiral

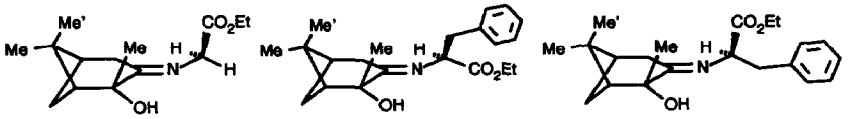
Scheme 1

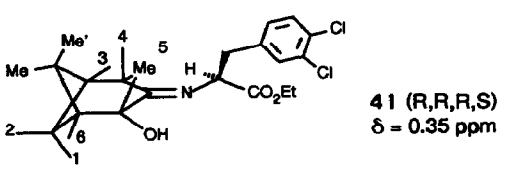


auxiliary **1** was recovered in high yield (>85%) and the aminoester hydrochloride **5** isolated as a pale yellow powder (80%). Subsequent hydrolysis of **5** (HCl 6N, 120°C) and recrystallization in ethanol-95% afforded the desired aminoacid hydrochloride **6** : $[\alpha]^{25}_D$= +8 (c = 2.08, MeOH).

The enantiomeric purity was determined to be 98.3/1.7 using ligand-exchange HPLC[6], consistent with the ^{1}H NMR data obtained on the crude product of the alkylation step, **4I/4II** = 99/1.

The *(S)*-configuration was assigned on the basis of our folded-dimeric model[4], scheme 2, which predicts formation of an *S* configuration at C-2 as already observed in the cases of known *(S)*-alanine and *(S)*-phenylalanine[5] and of the Me' singlet which is found at 0.35 ppm in **4I** as compared to 0.29 ppm in diastereomer **7I**-*(R,R,R,S)* , while in diastereomer **7II**-*(R,R,R,R)* and in the starting iminoester **2** the Me' singlets are found at 0.80 ppm and 0.84 ppm respectively.



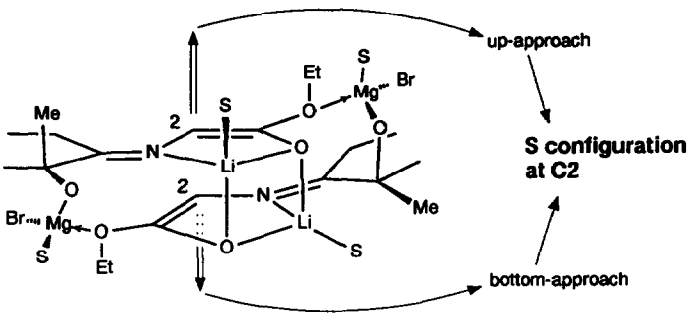


This is thus a short (4 steps) and high yield (overall 66%) synthesis of (S)-3,4-dichloro-phenylalanine hydrochloride **6** *with 98.3% (S) configuration.*

Resolution

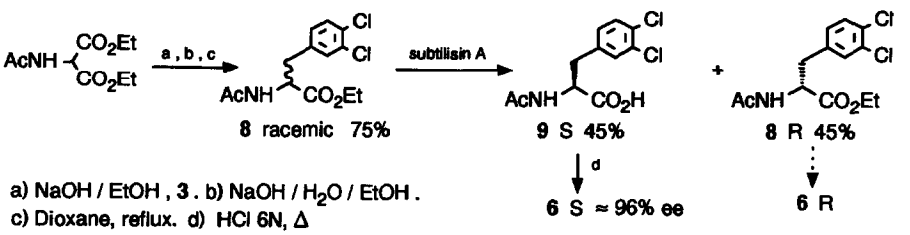
Selective hydrolysis of racemic ethyl N-acetyl-3,4-dichloro-phenylalaninate **8**, obtained in 75% yield through the acetamidomalonate method, Scheme 3, was carried out using subtilisin A (the major component of the serine-type endoproteinase isolated from *Bacillus licheniforms*)[8,9]. After incubation at 39°C f or 30 hours at

Scheme 2



pH=6.5-7 according to a literature procedure[9], (*S*)-N-acetyl-3,4-dichloro-phenylalanine **9** was isolated in 45% yield from the aqueous phase and (*R*)-ethyl N-acetyl-3,4-dichloro-phenylalaninate **8** was recovered from the organic phase. Subsequent hydrolysis of (*S*)-**9** (HCl 6N, reflux) afforded the (*S*)-3,4-dichlorophenylalamine hydrochloride **6** : $[\alpha]^{25}_{D} = +7$ (c = 0.74, MeOH).

Scheme 3



The specific rotations of the 3,4-dichloro-phenylalanine hydrochlorides **6** obtained through asymmetric synthesis or resolution might, because of the differences in the concentrations used (respectively 2.08 g/mL and 0.74 g/mL), be within the expected variations of the rotations Therefore the sample obtained from subtilisin kinetic resolution, if not lower, could well be of the same optical purity, but, because of the low yields and long incubation time, the enantiomeric purity of this sample has not been checked precisely.

Conclusion

(*S*)-3,4-Dichloro-phenylalanine hydrochloride **6** of 98.3% *S* enantiomeric purity was synthesized in 4 steps and 66% overall yield from (*R,R,R*)-hydroxypinanone **1**. The (*S,S,S*)-hydroxypinanone being also readily available, the (*R*)-enantiomer of compound **6** will, of course, be easily obtained in the same yield and the same enantiomeric purity. It is worth noting that, while resolution affords about 32% of the desired (*S*)-aminoacid hydrochloride, the asymmetric synthesis provides 66% of it and with recovery of the chiral auxiliary **1** (which can thus be used again). It is also of note that the synthesis of the chiral auxiliary **1** has been conducted without special equipment and without any difficulty from 400g of α-pinene and the aminoacid hydrochloride **6** from 50g of starting iminoester **2**.

Experimental Section

Infrared spectra were recorded on a Perkin-Elmer Model 257 spectrometer (ν in cm^{-1}). ^1H NMR (200 MHz) and ^{13}C NMR (50.3 MHz) spectra were recorded on a Bruker AC-200 (δ in ppm refered to TMS, $\Delta\nu$ and J in Hz, signs of the J_{AB} coupling constants not given). Rotations were measured on a Perkin-Elmer Model 241MC polarimeter. M.p (uncorrected) were determined on a Reichert Microscope. Flash-chromatography were performed using silicagel (70-230 mesh) purchased from Merck. Kieselgel $60F_{254}$ plates (from Merck) were used for TLC. Elemental analyses were performed by the « Service d'analyse du CNRS », Strasbourg, France. THF was distilled before use over Na/benzophenone. The diisopropylamine was dried by distillation over KOH and stored over molecular sieves. Ethyl glycinate hydrochloride, (-)-α-pinene 81% e.e. and 3,4-dichlorobenzyl bromide were purchased from Lancaster.

(+)-(1*R*,2*R*,5*R*)-2-hydroxypinan-3-one, 1 : was prepared by $KMnO_4$ oxydation of (-)-α-pinene ($[\alpha]_D$ = -42 (neat), 81% optical purity) following a known procedure.[10, 11] The distilled pinanone was recrystallized three times from pentane : yield : 35%. Colourless prisms, m.p. 36°C (lit.[12] : 34.5-35.5°C) ; $[\alpha]_D$ = +40 (c = 2.11, $CHCl_3$), optical purity about 98%.(lit.[12] : $[\alpha]_D$ = -41 , c = 0.04, $CHCl_3$). IR ($CHCl_3$) : ν_{OH}(free) = 3580 , ν_{OH}(bonded) = 3420 , $\nu_{C=O}$ = 1710. ^1H NMR ($CDCl_3$) : 0.89 (s, 3H, Me1) ; 1.36 (s, 3H, Me2) ; 1.37 (s, 3H, Me3) ; 1.67 (d, 1H, H1, J_{1-2}=10) ; 2.12 (m, 2H, H3 and H6) ; 2.47 (d.t.t, 1H, H2, J_{1-2}=10, J_{2-3}=J_{2-6}=6, J_{2-4}=J_{2-5}=1,5) ; 2.62 (broad m., 2H, H4 and H5). ^{13}C NMR ($CDCl_3$) : 22.9, 25.2 and 27.3 (CH_3) ; 28.4 (CH_2) ; 38.3 (CH) ; 39.3 (C) ; 43.0 (CH_2) ; 49.7 (CH) ; 77.1 (C) ; 214.2 (C=O). Anal. Calcd for $C_{10}H_{16}O_2$: C, 71.39 ; H, 9.59. Found : C, 71.41 ; H, 9.59.

(1*R*,2*R*,5*R*)-Ethyl 2-hydroxy-pinan-3-imino-acetate, 2. A mixture of ethyl glycinate (54 mmol, 2 eq.)[13]-,hydroxy-pinan-3-one (27 mmol), benzene (80 mL) and boron trifluoride etherate (0.1 g) was refluxed for 4-5 hours under argon using a Dean Stark apparatus. After evaporation of the benzene *in vacuo*, the yellow oil was purified by chromatography (Et_2O, silicagel pretreated with a 5% solution of Et_3N in Et_2O). Yield : 95%. Crystals have been obtained by crystallization in Et_2O/hexane (8/2) : m.p. 80-81°C. $[\alpha]_D$ = +6 (c = 2.54, $CHCl_3$). IR ($CHCl_3$) : ν_{OH}(free) = 3540 , ν_{OH} (bonded) = 3420 ; $\nu_{C=O}$=1720 ; $\nu_{C=N}$=1640. ^1H NMR ($CDCl_3$) : 0.88 (s, 3H, Me1) ; 1.30 (t., 3H, Me, J=7) ; 1.34 (s, 3H, Me2) : 1.53 (s, 3H, Me3) ; 1.57 (d, 1H, H1, J_{1-2}=10) ; 2.07 (m., 2H, H3 and H6) ; 2.36 (d.t.t., 1H, H2, J_{1-2}=10 , J_{2-3}=J_{26} = 6, J_{24}=J_{2-5}=1.5) ; 2.50 (broad m., 2H, H4 and H5) ; 2.61 (s, 1H, OH) ; 4.17 (s, 2H, NCH_2) ; 4.23 (q., 2H, OCH_2, J=7). ^{13}C NMR ($CDCl_3$) : 13.8, 22.4, 26.9 and 27.7 (CH_3) ; 27.6 and 33.3 (CH_2) ; 37.9 (CH) ; 38.1 (C) ; 50.1 (CH) ; 52.0 and 60.4 (CH_2) ; 75.9 (C) ; 169.8 (C=N) ; 179.5 (C=O). Anal. Calcd. for $C_{14}H_{23}NO_3$: C, 66.37 ; H, 9.15 ; N, 5.53. Found : C, 66.13 ; H, 9.32 ; N, 5.49.

Alkylation of the iminoester 2 with 3 . To a solution of diisopropylamine (1.6 mmol, 2 eq.) in anhydrous THF (4 mL) at -78°C, a 1.5 M solution of BuLi in hexanes (1.6 mmol, 1,1 mL) was added dropwise and the solution stirred for 30 min. at -78°. A solution of the iminoester **2** (0.8 mmol ,1 eq.) in anhydrous THF (2 mL) was then added at -78°C under argon, and the mixture stirred for 15 min. at -78°C. After addition of freshly

prepared $MgBr_2$ (0.6 equiv. in THF) the temperature was allowed to increase to -35°C for one hour. The solution was then cooled again to -78°C , 3,4-dichlorobenzylchloride **3** (2 eq.) was added dropwise and the mixture stirred at -78°C for 4 hours (the reaction was monitored TLC). The mixture was then poured into a cold saturated solution of NH_4Cl (15 mL). The aqueous phase was extracted with Et_2O (3 x 15 mL) ; the combined organic layers were dried over Na_2SO_4, filtered, concentrated *in vacuo* and the residue chromatographied on silicagel pretreated with a 5% solution of Et_3N in Et_2O/hexane (70/30) : 92% yield.

(*R,R,R,S*)-Ethyl 2-hydroxy-pinan-3-imino-(3,4-dichlorophenyl)alaninate, 4I. Pale yellow oil. $[\alpha]_D$ = -67 (c = 3.83, $CHCl_3$). Rf = 0.41 (Et_2O/hexane, 70/30). IR ($CHCl_3$) νco=1730. ^1H NMR ($CDCl_3$) : 0.35 (3H, s, Me1) ; 1.24 (3H, s, Me2) ; 1.27 (3H, t, J=7, Me) ; 1.41 (3H, s, Me3) ; 1.48 (1H, d, ^2J=10.5, H1) ; 1.85 (1H, qd, J_{32}=6, 3 J=3, H3) ; 2.0 (2H, m, H4 and H6) ; 2 25 (1H, dtd, J_{21}=10.5, J_{23}=J_{26}=6, J_{24}=2.5, H2) ; 2.49 (1H, dd, ^2J=18, J_{53}=3, H5) ; 3.18 (2H, AB part of an ABX system, ^2J=13.5, ^3J=4, ^3J=10, Δν=33) ; 4.19 (2H, q, CH_2O) ; 4.39 (1H, dd, X part of the ABX system, CH) ; 7.05 (1H, dd, ^3J=8, ^4J=2, H_{arom}) ; 7.25 (1H, d overlaped with $CHCl_3$, $H_{arom.}$) ; 7.30 (1H, d, ^3J=8, $H_{arom.}$). ^{13}C NMR ($CDCl_3$) : 14.1 (Me) ; 21.8 (Me1) ; 27.0 (Me2) ; 27.8 (CH_2) ; 28.3 (Me3) ; 33.3 (CH_2) ; 38.0 (C) ; 38.1 (CH) ; 38.2 (CH_2) ; 49.8 (CH) ; 61.3 (CH_2) 63.3 (CH) ; 76.5 (C) ; 128.9 and 131.6 ($CH_{arom.}$) ; 130.6, 132.0 and 138.4 (C_{arom}) ; 170.6 (C=N) ; 179.3 (C=O).

Hydrolysis of the imine bond . To a solution of the alkylated iminoester **4I** (1.47 mmol) in THF (50 mL) was added a 1.2N HCl solution (40 mL) and the mixture was stirred at 25°C for 3 days. After evaporation of the THF *in vacuo*, the aqueous phase was extracted with Et_2O (6 x 10 mL). the combined organic phases, after drying and concentration, afforded after chromatography hydroxypinanone **1** in 85% yield. The aqueous phase was evaporated to dryness (using four successive EtOH-additions/evaporation processes) the precipitate obtained was carefully crushed and rinsed with Et_2O affording a pale yellow solid (80%).

***(S)*-Ethyl 3,4-dichloro-phenylalaninate hydrochloride 5 .** M.p. 160-4° (dec.). $[\alpha]_D$ = +27 (c = 0.51, H_2O/EtOH, 2/1). ^1H NMR (Acetone d6) : 1.06 (3H, t, ^3J=7,CH_3) ; 3.22 (2H, AB part of an ABX system, ^2J=14, ^3J=8, ^3J=6, Δν=18, CH_2) ; 4.14 (2H, AB part of ABX_3 system, ^2J=11, 3 ^3J=7, CH_2O) ; 4.31 (1H, X part of the ABX system, CH) ; 7.21 (1H, dd, ^3J=8, ^4J=2, H_{arom}) ; 7.41 (2H, m, H_{arom}). ^{13}C NMR (CD_3OD) : 14.4 (Me) ; 36.5 (CH_2) ; 54.8 (CH) ; 63.9 (CH_2O) ; 130.6, 132.2 and 132.8 (CH_{arom}) ; 132.9, 134.5 and 134.5 ($C_{arom.}$) ; 168.8 (C=O). Anal. Calcd for $C_{11}H_{14}Cl_3NO_2$: C, 43.65 ; H, 4.66 ; N, 4.63. Found : C, 43.67 ; H, 4.61 ; N, 4.79.

Hydrolysis of the ester function. The above ester **5** (1.17 mmol) was dissolved in 6N HCl (100 mL) and the solution heated (120°C) under stirring for 3 hours. After evaporation to dryness the precipitate was recrystallized in EtOH 95% : white solid, 95% yield.

***(S)*-3,4-dichloro-phenylalanine hydrochloride 6 (from asymmetric synthesis) :** M.p. 254-57° (dec). $[\alpha]_D$ = +8 (c = 2.08, MeOH). IR (KBr) $\nu_{C=O}$ 1720. ^1H NMR (CD_3OD) : 3.25 (2H, AB part of an ABX system, ^2J=15, ^3J=6, ^3J=7, Δν=18, CH_2) ; 4.30 (1H, X part of the ABX system, CH) ; 7.27 (1H, dd, ^3J=8.5, ^4J=2, H_{arom}) ; 7.51

(2H, m, $H_{arom.}$). ^{13}C NMR (CD_3OD) : 36.8 (CH_2) ; 54.7 (CH) ; 130.5, 132.0 and 132.6 (CH_{arom}) ; 132.8, 133.6 and 136.5 (C_{arom}) ; 171.0 (C=O). Anal. Calcd for $C_9H_{10}Cl_3NO_2$: C, 39.96 ; H, 3.73. Found : C, 39.99 ; H, 3.58.

Racemic Ethyl N-acetyl-3,4-dichloro-phenylalaninate 8 was obtained in three steps and 75% yield from diethyl acetamidomalonate following a known procedure[9].

(*S*)-N-acetyl-3,4-dichloro-phenylalanine 9 was obtained, in 45 % yield, through resolution with subtilisin following a known procedure[9]. Starting from 27g of **8**, 250mg of Subtilisin were necessary and incubation lasted for 30 hours. White solid : m.p. 145-147°C. $[\alpha]_D$ = +41 (c = 1.15, MeOH). Anal. Calcd for $C_{11}H_{11}Cl_2NO_3$: C, 47.84 ; H, 4.01 ; N, 5.07. Found : C, 47.96 ; H, 3.88 ; N, 4.79.

(*S*)-3,4-dichloro-phenylalanine hydrochloride 6 (from resolution): was obtained, in 95 % yield, from the above compound **8** through usual acidic deprotection[9]. White solid : m.p. 253-57° (dec). $[\alpha]_D$ = +7 (c = 0.74, MeOH). IR (KBr), ^1H NMR (CD_3OD) and ^{13}C NMR (CD_3OD) were identical to those of the sample obtained from asymmetric-synthesis (*cf.* above).

References

1) Taylor D.C. ; Wightman F. ; *Phytochemistry*, **1987**, *26*, 1279-1288 and 2155-2166.
Taylor D.C. ; Wightman F. ; Kazakoff C. WW. ; *Phytochemistry*, **1988**, *27*, 51-71.

2) Burckhalter J.H. ; Stephens V.C. ; *J. Am. Chem. Soc.*, **1951**, *73*, 56 and 3502.

3) Taylor D.C. ; Wightman R.H. ; Wightman F., Wand A.J. ; *Bioorganic Chem.*, **1987**, *15*, 335-45.

4) Solladié-Cavallo A. ; Simon M.C. ; *Tetrahedron Lett.*, **1989**, *30*, 6011.
Solladié-Cavallo A. ; Simon-Wermeister M.C., Schwarz J.; *Organometallics*, **1993**, *21*, 3743.

5) Yamada S. ; Oguri T. ; Shioiri T. ; *J.C.S. Chem. Commun.*, **1976**, 136.
Oguri t. ; Kaway N. ; Yamada S. ; *Chem. Pharm. Bull. Jpn*, **1978**, *26*, 803.

6) The enantiomeric purity has been determined in Prof. R. Marchelli's group (University of Parma) using their method of Cu(II) ligand-exchange HPLC.

7) We have shown from X-ray diffraction of a single crystal of **2** that, in the solid state, the conformation is as shown , with the ester group up.

8) Chen S.T., Wang K.T., Wong C.H. ; *J.C.S. Chem. Commun.*, **1986**, 1514-16.

9) Rao P.N., Burdett J.E., Cessac J.W., Dinunno C.M., Peterson D.M., Kim H.K. *Int. J. Peptide Protein Res.*, **1987**, *29*, 118-125.

10) Carlson R.G. ; Pierce J.K. ; *J. org. Chem.*, **1971**, *36*, 2319.

11) Solladié-Cavallo A. ; Simon M.C. ; Fischer J. ; DeCian A. ; *Bull. Soc. Chim. Fr.*, **1989**, 544.

12) Delépine M. ; Horeau A. ; Grandperrin M. ; *Ann. Chim. (Paris)*, **1943**, *18*, 250.

13) The ethyl glycinate was obtained by bubbling gazeous ammonia into a suspension of glycinate chlorohydrate in benzene followed by filtration of the ammonium chloride formed.

Acknowledgment. *We are grateful for finantial support of this work from Neosystem SA and french MRT (CIFRE n° 90-T-0207 to JS) and for helpful discussions with Dr. S. Plaué (Neosystem SA).*

(*Received in UK* 13 *June* 1994)