Pergamon

Tetrahedron: Asymmetry Vol. 6, No. 6, pp. 1393-1398, 1995
Elsevier Science Ltd
Printed in Great Britain
0957-4166/95 $9.50+0.00

0957-4166(95)00172-7

Biocatalytic Resolution of a Tertiary Quinuclidinol Ester Using *Pig Liver Esterase*

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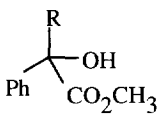
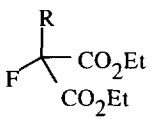
Abstract: The kinetic resolution of a tertiary acetylenic ester has been accomplished using *Pig liver esterase*. (R) and (S) isomers of 3-ethynyl-3-hydroxy quinuclidine have been isolated with high enantiomeric purity.

The enantiospecific kinetic hydrolysis of racemic esters using esterases and lipases is a well documented method for the preparation of alcohols and carboxylic acids in high enantiomeric purity.[1] Most publications have addressed the resolution of secondary alcohols or carboxylic acids from the corresponding acetates or α-mono substituted carboxylic esters. By comparison, few examples of the resolution of tertiary substituted systems are known. α,α-Disubstituted carboxylic esters[2], in particular **1**[3], and diesters[4], specifically **2**[5], have been hydrolysed and resolved with some success using a number of different lipases.



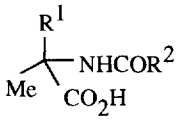
R = CF_3, Me

1



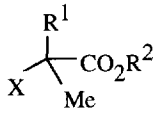
R = Me, Et

2



$R^1 = R^2 = CF_3$

3

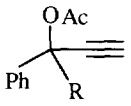


R = Aryl CH_2
X = $NHNH_2$,OH,
$NHCOCH_3$,
$NHNHCOCH_2Ph$

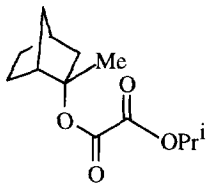
4

Using N-acylamino acids **3** as substrates, an acylase from hog kidney has been used in an enantioselective hydrolysis to yield the corresponding amino acids in high enantiomeric purity.[6] Indeed, a series of α-methyl carboxylates possessing a number of different hetero substituents **4** have been resolved with an esterase from *Candida lipolytica*[7], and recently, serine carboxypeptidases.[8] However, examples of the resolution of tertiary alcohol esters by biocatalysis are relatively few.

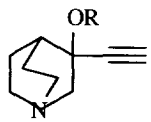
Recent publications have reported the hydrolytic resolution of tertiary acetylenic esters **5** with the lipase from *Candida cylindracea.*[9] When R = CF_3 an enantiomeric excess (ee) of 87% for the product alcohol and 75% for the residual acetate was obtained after 40% hydrolysis. The ee of the acetate was increased to >98% on 60% hydrolysis. Using an oxalate ester **6** as the substrate for the enzyme *Porcine pancreatic lipase*, a tertiary alcohol has been resolved in 42% yield and 90% ee.[10]



5



6



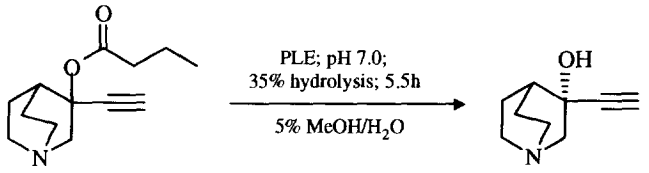
7 R = H
8 R = $COPr^n$

In the course of a research programme aimed at the synthesis of squalene synthase inhibitors,[11] we needed to prepare a series of enantiomerically pure analogues of a tertiary 3-substituted acetylenic quinuclidinol **7**. Biocatalytic resolution of 3-hydroxy quinuclidine has been reported.[12] We report here the preparation of (R) and (S) enantiomers of **7** by enantiospecific enzyme hydrolysis of the butyrate ester.

Results and discussion

Our initial approach to the resolution was to run a screen for enzyme activity on the acetate and butyrate esters of the alcohol. The esters were readily prepared from the racemic alcohol **7** on heating with the corresponding anhydride. The esters were treated in pH 7 buffer with a broad selection of lipases and esterases (*Candida cylindracea; Pig liver esterase(PLE);Porcine pancreatic lipase; Pseudomonas fluorescens; Rhizopus javanicus; Humicola lanuginosa; Chromobacterium viscosum; Butyryl cholinesterase).* Only *PLE* with the butyric ester, showed any significant hydrolysis.

The reaction was scaled up to 20 m.mole and allowed to proceed at pH 7.0 to 35% hydrolysis. From the resultant aqueous mixture we were able to isolate the (R) isomer of the quinuclidinol in 36% yield and 97% ee. When the racemic ester **8** was hydrolysed to 56%, the residual ester fraction was obtained in high yield and 99% ee. Hydrolysis of this product with KOH gave the required (S) enantiomer in 40% yield overall and 98% ee.[13,14]

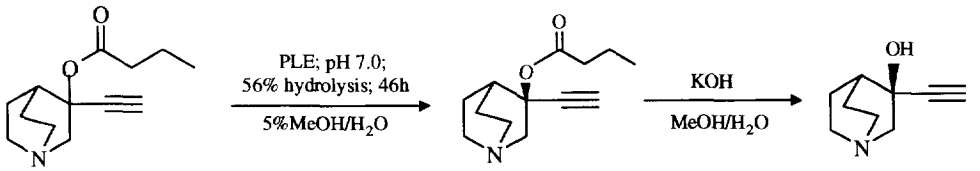


8 **9**

R-(+) 97% ee
36% yield

Enantiomeric excesses were determined by 400 MHz ^{1}H NMR. (R) and (S)-1,1-bi-2-naphthol have been

reported as useful agents for the determination of the enantiomeric purity of amines, alcohols and amino alcohols.[15] We therefore investigated the use of R-(+)-1,1-bi-2-naphthol(RBN) as a chiral solvating agent



8 **10** S-(-) 99% ee **11** S-(-) 99% ee 40% yield

(CSA) in this case. Some separation of signals occurred; in the presence of 5.0 mol. equivalents of RBN, a separation of 2.1Hz was observed for the alkyne proton and the protons attached to C2 in **7**. However, if a weak organic acid e.g. acetic, butyric or benzoic acid was added to the mixture, the signal separation of these protons in the resultant diastereomeric complex was greatly enhanced. On addition of butyric acid, these separations were increased dramatically. The optimum effect was obtained with 2.0 mol. equivalents of acid, when the separation observed for the alkyne protons of the enantiomers was increased ten-fold to 20.6Hz.[16] In the presence of an inorganic acid (e.g. HCl) no significant enhancement of signal separation occurred. Clearly, this is not simply a salt effect. A complex formed between the RBN, organic acid and the oxygen and nitrogen atoms of the quinuclidine nucleus will possess weaker acid-base properties and higher conformational rigidity than the quinuclidine alone; the shielding properties of the large aromatic rings will also play a significant role in the resulting chiral discrimination. Using this method a value for enantiomeric purity was calculated by comparison of the signals obtained for the acetylenic proton in the individual enantiomers.
The resolution is not successful when alkyl or aryl groups are substituted for the alkyne. This finding appears to support the view that the enzyme sees the small acetylene substituent as essentially the same as a hydrogen atom, thus enabling hydrolysis to occur.[9]

Conclusion

In the manner described the resolution of the tertiary alcohol (RS)-3-hydroxy-3-ethynyl quinuclidine is readily accomplished by enantiospecific enzymatic hydrolysis of the corresponding butyrate ester to yield both (R) and (S) isomers in high enantiomeric excess.

EXPERIMENTAL

Apparatus, materials and methods

^{1}H NMR spectra were recorded on a Brucker 400 MHz NMR spectrometer in $CDCl_3$. All chemical shifts are reported in ppm. Optical rotations were measured using a Perkin Elmer Model 241 polarimeter. Melting points were determined using a Buchi melting point apparatus and are uncorrected.

(R)-(+)-bi-2-naphthol was obtained from Aldrich Chemical Co. Enzymes from Sigma Chemical Co.(PLE, Butyryl cholinesterase) and Biocatalysts. (RS)-3-hydroxy-3-ethynyl quinuclidine was prepared by the method described in the literature.[17]

(RS)-(±)-3-ethynyl-3-oxbutyryl quinuclidine 8. Compound **7** (15.1g.; 0.1m) was stirred with butyric anhydride (60ml) at 120°C for 5h. After cooling, the mixture was added to saturated aqueous sodium carbonate solution(1lit.) and stirred for 3h. The product was extracted with diethyl ether (3x100ml.). The extracts were added dropwise to saturated aqueous Na_2CO_3 solution (1lit.) over 1h. and stirred for a further 2h. The organic phase was separated and the aqueous phase washed with diethyl ether (2x100ml.). The organic phases were combined, washed with sodium carbonate solution and dried over magnesium sulphate. Evaporation of the solvent gave 20.2g.(91%) of **8** as a brown oil. 400MHz ^{1}H NMR, **8** (3.3mg;1.0m.) in the presence of butyric acid (1.30µl; 0.95m) and (R)-(+)-1,1-bi-2-naphthol (21.76mg; 5.1m): 0.92-0.97,m,6H; 1.35-1.44,m,1H; 1.51-1.65,m,5H; 1.72-1.80,m,1H; 1.93-2.04,m,1H; 2.20-2.29,m,4H; 2.45-2.48,m,1H; 2.55,s,0.5H (S)-(-) isomer, 2.57,s,0.5H (R)-(+) isomer (alkyne H of enantiomers $\Delta\delta$ = 6.4Hz); 2.71-2.77,m,4H; 3.08-3.12,d,1H; 3.29-3.34,m,1H; 5.04,s,11H; 7.12-7.15,d,10H; 7.24-7.37,m,30H; 7.86-7.88,d,10H; 7.92-7.95,d,10H. MS (CI+): 222(MH)$^+$. Calc. for $C_{13}H_{19}NO_2$: C 70.7; H 8.65; N 6.33. Found: C 70.6; H 8.7; N 6.4.

(R)-(+)-3-ethynyl-3-hydroxy quinuclidine 9. A solution of (RS)-(±)-3-ethynyl-3-oxbutyryl quinuclidine **8** (4.42 g; 20 m.moles) in de-ionised water (700 ml) containing methanol (35 ml) was adjusted to pH 7.0 using an autotitrator. Pig liver esterase (8.0 ml; 9,200 units; 9 mg/ml) was added and the mixture stirred at room temperature with the pH maintained at a constant 7.0 using 0.1M sodium hydroxide dispensed by a pH stat/autotitrator. After 5.5 h, 71.3 ml sodium hydroxide had been consumed i.e. hydrolysis was 35% complete. pH of the reaction mixture was adjusted to 2.5 with 2M hydrochloric acid and stirred for 10 min. After addition of 2M sodium hydroxide to pH 7.05, the mixture was extracted with diethyl ether (3 x 200 ml; 12 x 150 ml). The remaining aqueous phase was freeze dried over 48 h. to an off-white solid. This solid was dissolved in de-ionised water (30 ml), filtered, and the filtrate was basified to pH 9 with 10.8M caustic liquor. Filtration of the solid precipitated gave the title compound **9** as an off-white solid (554 mg; 36%) mp 204-207°C; $[\alpha]^{20}_{589}$ = +54.5 (c 0.99, MeOH); 97.4% ee; 400 MHz ^{1}H NMR **9** (1.65mg; 1.0m) in the presence of (R)-(+)-1,1-bi-2-naphthol (16.68mg; 5.32m) and butyric acid (0.9µl; 2.0m): 0.91-0.94,t,6H; 1.30-1.33,m,1H; 1.54-1.65,m,5H; 1.88-1.92,m,1H; 2.00-2.03,m,1H; 2.19-2.23,t,4H; 2.41,s,0.013H (S)-(-) isomer; 2.46,s,0.987H (R)-(+) isomer; 2.72-2.83,m,4H; 2.98-3.02,d,1H; 3.14-3.18,m,1H; 5.03,s,12H; 7.12-7.14,d,10H; 7.26-7.37,m,30H; 7.86-7.88, d,10H; 7.92-7.94,d,10H. MS(CI+) 152(MH)$^+$; 134(152-H2O)$^+$. Calc. for $C_9H_{13}NO$: C 71.5; H 8.67; N 9.26. Found: C 71.8; H 8.8; N 9.2.

(S)-(-)-3-ethynyl-3-hydroxy quinuclidine 11. (RS)-(±)-3-ethynyl-3-oxbutyryl quinuclidine **8** (4.42 g.; 20 m.moles) was dissolved in de-ionised water (700 ml) containing methanol (35 ml) and the pH adjusted to 7.0 using an autotitrator. Pig liver esterase (3.0 ml; 3450 units; 9.0 mg/ml) was added, the mixture stirred at room temperature and maintained at pH 7.0 (by addition of 0.1M sodium hydroxide from a pH stat) for 46 h. 112.5 ml of sodium hydroxide were consumed over this period (i.e. 56% hydrolysis). The reaction mixture was adjusted to pH 2.52 with 2M hydrochloric acid and stirred for 20 min. After addition of 2M sodium hydroxide

to pH 7.01, the mixture was extracted with diethyl ether(12 x 150 ml). The organic extracts were combined, dried over magnesium sulphate and evaporated to give (-)-3-ethynyl-3-oxbutyryl quinuclidine **10** (2.43 g containing butyric acid) as a brown oil; ≥ 99% ee; 400 MHz ^{1}H NMR **10** (4.2mg; 1.0m) with (R)-(+)-1,1-bi-2-naphthol (27.69mg; 5.1m) and butyric acid (1.7µl; 1.0m): 0.90-0.96,m,6H; 1.33-1.41,m,1H; 1.46-1.56,m, 1H; 1.57-1.76,m,5H; 1.90-2.01,m,1H; 2.18-2.22,t,2H; 2.25-2.28,t,2H; 2.43-2.45,m,1h; 2.54,s,1H (alkyne H); 2.63-2.69,m,4H; 3.01-3.05,dd,1H; 3.23-3.27,dd,1H; 5.00,s,11H; 7.09-7.20,d,10H; 7.24-7.41,m,30H; 7.82-7.90,d,10H; 7.90-7.99,d,10H.

Compound **10** isolated as above was treated with a solution of potassium hydroxide flake (2.24 g) in methanol (50 ml). After stirring for 2h at room temperature, the solution was evaporated. The residue was treated with de-ionised water (2 ml); the solid obtained was filtered, washed with water (2 x 2 ml) and dried under vacuum over P_2O_5 to give the title compound **11** as a cream solid (611 mg; 40% from **8** mp 199-202°C; $[\alpha]^{20}_{589}$ = -56.1 (c 1.02, MeOH); 98.8% ee. 400 MHz ^{1}H NMR **11** (1.6mg) with (R)-(+)-1,1-bi-2-naphthol (16.0mg; 5.2m) and butyric acid (1.0µl; 1.0m): 0.88-0.97,t,3H; 1.30-1.40,m,1H; 1.50-1.67,m,3H; 1.84-1.95,m,1H; 1.94-2.07,m,1H; 2.18-2.26,t,2H; 2.43,s,0.994H (S)-(-) isomer; 2.48,s,0.006H (R)-(+) isomer; 2.69-2.86,m,4H; 2.97-3.04,dd,1H; 3.12-3.18,dd,1h; 5.00,s,12H; 7.09-7.20,d,10H; 7.23-7.42,m,30H; 7.81-7.90,d,10H; 7.90-7.99,d,10H. MS CI+ 152(MH)$^+$; 134(151-H_2O)$^+$. Calc. for $C_9H_{13}NO$: C 71.5; H 8.67; N 9.26. Found: C 71.7; H 8.6; N 9.1.

ACKNOWLEDGEMENTS

We are grateful to B. Wright and P. McNally for ^{1}H NMR data, E. Clayton and S. Taylor for Mass Spectra, Mrs. K. Barber for microanalyses and M. J. Taylor for samples of racemic quinuclidines.
Our thanks are also due to Prof. Mary McPartlin of the University of North London for X-ray data.

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(*Received in UK* 25 *April* 1995)