

0957-4166(95)00224-3

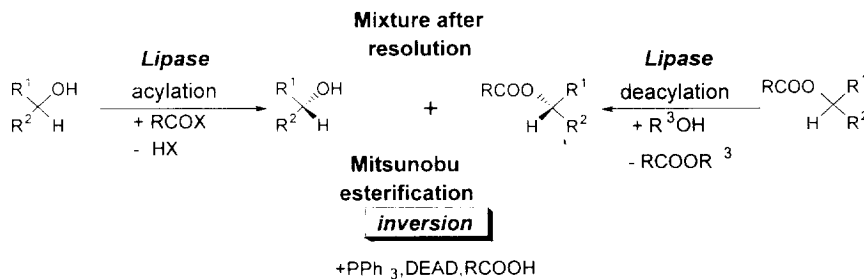
## Combination of the Lipase-Catalysed Resolution with the Mitsunobu Esterification in One Pot

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**Abstract:** A chemo-enzymatic method for the preparation of homochiral esters of 14 secondary alcohols with 100% theoretical yields is described in one pot through two steps: the lipase-catalysed kinetic resolution followed by the Mitsunobu esterification of the free alcohol enantiomer *in situ* in the resolution mixture. Mathematical equations which link the enzymatic and chemical steps were derived, resulting in an enantioconvergent synthetic tool for the preparation of chiral intermediates.

Stereoselectivity of different kinds of enzymes is widely exploited for the production of optically active compounds.<sup>1</sup> Especially lipase-catalysed kinetic resolutions of racemic alcohols and carboxylic acid derivatives through acylation or deacylation in organic solvents are of great importance (Scheme 1).



Scheme 1

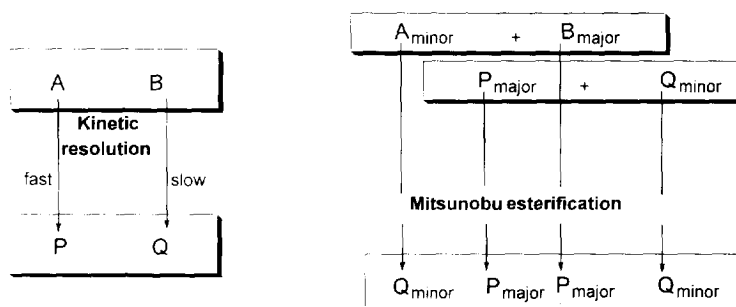
When the value of the enantiomeric ratio ( $E$ ; the ratio of the specificity constants of the enantiomers)<sup>2</sup> for such a reaction is high enough one of the enantiomers is obtained as a free alcohol and the other as a less reactive (or unreactive) carboxylic acid derivative simultaneously with high enantiomeric purities. The separation of these resolution products often means tedious chromatographic procedures. As a more serious drawback of conventional resolutions (chemical or enzymatic), half of the starting material has the wrong absolute configuration for certain purposes.

Our aim has been to introduce an one-pot method which can be used to avoid the above-mentioned disadvantages of lipase-catalysed resolution and by thus doing to obtain the desired enantiomer with 100%

theoretical yield when calculated according to the racemic starting material. One way to do this has been the recycling of an undesired enantiomer through racemization *in situ* during the reaction or through a repeated resolution after the separation of that enantiomer from the resolved mixture.<sup>3-5</sup> Another possibility for the creation of the correct stereochemistry has been an inversion at the stereogenic centre.<sup>6-14</sup> We resorted to this procedure and used resolution followed by the Mitsunobu esterification<sup>15</sup> of a free alcohol enantiomer by means of diethyl azodicarboxylate (DEAD)-triphenylphosphine (PPh<sub>3</sub>) and a carboxylic acid as a logical choice for the enantioconvergent S<sub>N</sub>2 step in the two-stage one-pot synthesis (Scheme 1). When the acid RCOOH of the S<sub>N</sub>2 step corresponds to the acid component of the ester in the resolved mixture the chemically produced and original optically active esters become configurationally and chemically identical, the chemical yield of the ester enantiomer then being increased to 100% in theory. The choice between enzymatic acylation and deacylation together with the selectivity of a lipase, on the other hand, determines the absolute configuration of the ester produced. To our knowledge, similar kinds of an one-pot procedure connected to lipase-catalysed resolution have been used in four cases before.<sup>16-19</sup> However, these procedures have been unambitious as to the prediction of the usability of the method for practically enantiospecific resolutions. In this paper, mathematical equations are proposed in order to introduce theoretical plots for synthetic usability. The one-pot resolution-inversion method has been tested for the lipase-catalysed resolutions of 14 different secondary alcohols.

## Results and Discussion

In the present one-pot method, the final product consists of the chemically produced inverted ester and of the enzymatically produced ester (Schemes 1 and 2).



Scheme 2

Expecting that the enantiomer A is the faster reacting enantiomer in the racemic mixture of A and B and that the resolution proceeds enantiospecifically the enantiomeric purity of the final product ( $ee_{\text{final}}$ ) should equal with the enantiomeric purities of the new product ( $ee_{\text{P}}$ ) and the less reactive starting material ( $ee_{\text{B}}$ ), *i.e.*,  $ee_{\text{final}} =$

$ee_p = ee_B = 1.0$  at the termination conversion ( $c_{\text{termination}} = 0.5$ ), but outside this  $ee_{\text{final}}$  is not intuitively predictable. A general equation relating the value of  $ee_{\text{final}}$  to the  $ee_p$  and  $ee_B$  values is clearly needed. For synthetic purposes, the most important equations are those relating the maximal enantiomeric excess of the produced ester [ $ee_{\text{final}}(\text{max})$ ], the corresponding optimal termination conversion of an enzymatic reaction [ $c_{\text{termination}}(\text{max})$ ] and  $E$ .

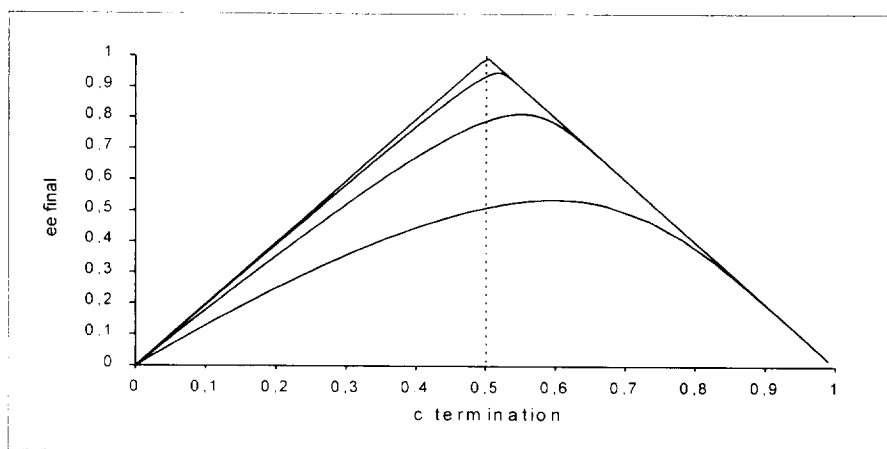
**Derivation of Equations** - When the racemic mixture of alcohols A and B (Scheme 2) is stereoselectively acylated in the presence of a lipase  $ee_B$  and  $ee_p$  can be expressed by equations (1) and (2) and the conversion at these  $ee$  values by equation (3). In the equations, A and B refer to the concentrations of the substrate enantiomers and P and Q to those of the product enantiomers. According to Scheme 2, the enantiomeric purity of the resulting total ester in the resolution-inversion sequence is obtained by equation (4) which can be further expressed in the terms of  $ee_B$  and  $ee_p$  by equation (5).<sup>20</sup>

$$(1) ee_B = \frac{B-A}{B+A} \quad (2) ee_p = \frac{P-Q}{P+Q} \quad (3) c = \frac{ee_B}{ee_B + ee_p}$$

$$(4) ee_{\text{final}} = \frac{(B+P)-(Q+A)}{(B+P+Q+A)} \quad (5) ee_{\text{final}} = \frac{2ee_B ee_p}{ee_B + ee_p}$$

In order to link an inversion step to a particular resolution, the enantiomeric ratio of the resolution was included leading to equations (6) and (7).<sup>21</sup> The plots of  $ee_{\text{final}}$  vs.  $c_{\text{termination}}$  at fixed  $E$  values can now be obtained (Figure 1).

$$(6) ee_{\text{final}} = 2B + 1 - 2B_0 - \frac{(2 - 2B_0)}{B_0^E} B^E \quad (7) c_{\text{termination}} = -\frac{(1 - B_0)}{B_0^E} B^E - B + 1$$



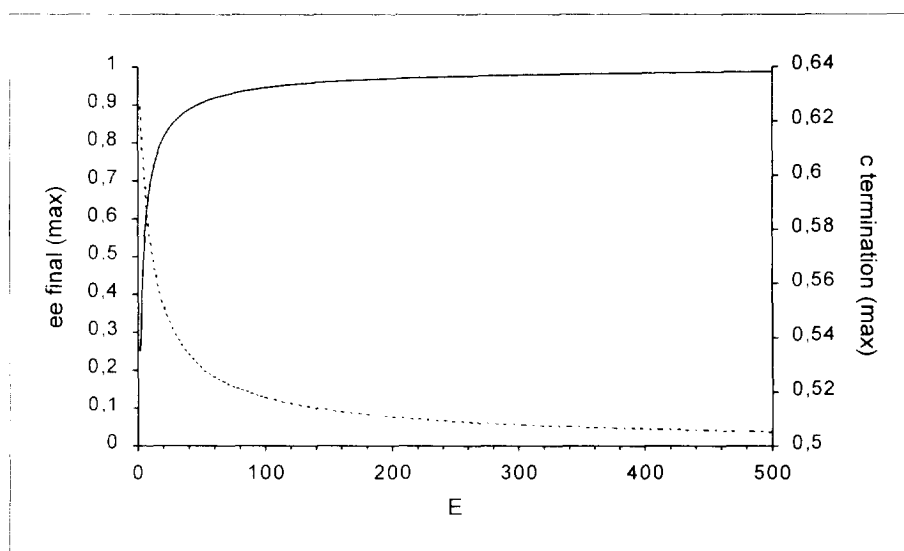
**Figure 1.**  $ee_{\text{final}}$  vs.  $c_{\text{termination}}$  at fixed values of  $E$ .  $E$  from top to bottom 1000, 100, 20, 5.

According to the plots, there is always a maximum for  $ee_{\text{final}}$  which lies above  $c_{\text{termination}}=0.5$ . It is also obvious that with moderate  $E$  values the one-pot method results in a low enantiomeric purity for the final ester product.

Finally we end in the two relations expressed in equations (8) and (9), giving maximal  $ee_{\text{final}}(\text{max})$  and the corresponding  $c_{\text{termination}}(\text{max})$  values as a function of  $E$  in the cases where racemic starting mixture is used, *i.e.*, when  $B_0=0.5$ . Equations (8) and (9) are obtained by substituting the zero point of the derivatized equation (6) into equations (6) and (7), respectively.

$$(8) \quad ee_{\text{final}}(\text{max}) = 2 \left( \frac{2^{1-E}}{E} \right)^{\frac{1}{E-1}} - 2^E \left( \frac{2^{1-E}}{E} \right)^{\frac{E}{E-1}}$$

$$(9) \quad c_{\text{termination}}(\text{max}) = 1 + 2^{E-1} \left( \frac{2^{1-E}}{E} \right)^{\frac{E}{E-1}} - \left( \frac{2^{1-E}}{E} \right)^{\frac{1}{E-1}}$$



**Figure 2.** The theoretical plots of  $ee_{\text{final}}(\text{max})$  [solid line] and  $c_{\text{termination}}(\text{max})$  [broken line] as a function of  $E$  according to equations (8) and (9)

The plots of  $ee_{\text{final}}(\text{max})$  and  $c_{\text{termination}}(\text{max})$  vs.  $E$  are shown in Figure 2. The  $E$  value of a resolution is often reliably obtained by modern analytical methods such as chiral chromatography at the early stage of the reaction. Once this is done, Figure 2 enables useful predictions of the methodology. Thus, *e.g.*, as is seen from the Figure or more accurately by iteration of equations (8) and (9), the resolution should have the  $E$  value higher than 110 and the reaction should be stopped at 0.52 conversion in order to have  $ee_{\text{final}}(\text{max}) > 0.95$ .

The formulation of the above equations was based on lipase-catalysed resolution by an acylation. The functions are, of course, equally valid in the case of enzymatic deacylation followed by the inversion at the configuration of the produced alcohol.<sup>22</sup>

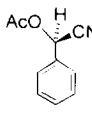
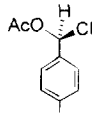
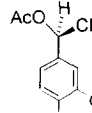
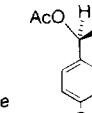
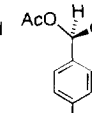
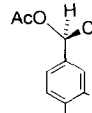
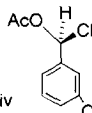
**Table 1** Final products of the lipase-catalysed resolution followed by the Mitsunobu esterification *in situ*

Compound	1	2	3	4	5	6	7
<b>RESOLUTION</b>							
Enzyme <sup>a</sup> /Selectivity	PPL/R	CCl/R	PPL/R	APS/R	APS/S	AK/S	APS/S
Resolution type <sup>b</sup>	A	D	A	A	A	A	D
Solvent	<i>i</i> -Pr <sub>2</sub> O	<i>i</i> -Pr <sub>2</sub> O	<i>i</i> -Pr <sub>2</sub> O	<i>i</i> -Pr <sub>2</sub> O	Et <sub>2</sub> O	<i>i</i> -Pr <sub>2</sub> O	PhMe
Cosubstrate <sup>c</sup>	T	P	VB	VA	VA	VA	P
ee <sub>B</sub> /%	98 <sup>d</sup>	94 <sup>c</sup>	95 <sup>d</sup>	>99 <sup>f</sup>	99 <sup>g</sup>	98 <sup>g</sup>	97 <sup>h</sup>
ee <sub>P</sub> /%	90 <sup>c</sup>	65 <sup>d</sup>	81 <sup>c</sup>	>99 <sup>c</sup>	98 <sup>i</sup>	86 <sup>i</sup>	98 <sup>i</sup>
Conversion <sup>k</sup> /%	52	59	54	50	50	53	50
<i>E</i>	85	16	35	>1000	>500	60	420
<b>MITSUNOBU</b>							
Isolated yield/%	100	86	86	97	80	88	100
ee <sub>final</sub> /%	94 <sup>c</sup>	63 <sup>c</sup>	88 <sup>c</sup>	97 <sup>c</sup>	90 <sup>l</sup>	91 <sup>l</sup>	97 <sup>h</sup>
Calculated <sup>l</sup> ee <sub>final</sub> /%	93	77	88	>99	98	92	98
Configuration	R	S	R	R	S	S	R

<sup>a</sup> PPL=porcine pancreatic lipase, CCl=*Candida rugosa* lipase, APS=*Pseudomonas cepacia* lipase, APK=*Pseudomonas sp.* lipase. <sup>b</sup> A=acylation, D=deacylation. <sup>c</sup> T=2,2,2-trifluoroethyl butyrate, P=1-propanol, VB=vinyl butyrate, VA=vinyl acetate. <sup>d</sup> ee by chiral GLC on permethyl-β-cyclodextrin column as an acetate. <sup>e</sup> ee by chiral GLC on permethyl-β-cyclodextrin column. <sup>f</sup> ee by chiral GLC on permethyl-β-cyclodextrin column as propionate. <sup>g</sup> ee by chiral HPLC on Chiralcel OD column as propionate. <sup>h</sup> ee by chiral GLC on Chiralcel-L-Val column. <sup>i</sup> ee by chiral HPLC on Chiralcel OD column. <sup>j</sup> ee by chiral GLC on Chiralcel-L-Val column as propionate. <sup>k</sup> calculated according to equation (3). <sup>l</sup> calculated according to equation (5).

**Resolution-Inversion Method in Practice.** - In order to test the validity of the above theoretical consideration, several lipase-catalysed resolutions of secondary alcohols by acylation or deacylation in organic solvents were performed following the procedures described in the literature.<sup>4,23-28</sup> The results are shown in Tables 1 and 2. Compounds **6** and **12-14** were not resolved before. First the *E* values were determined in order to be able to stop an enzymatic reaction by filtering off the enzyme at the conversion reasonable according to Figure 2.<sup>29</sup> Depending on the acyl component of the ester product in the resolution mixture, the Mitsunobu esterification with acetic or butyric acid was performed<sup>30</sup> and the values of ee<sub>final</sub> were determined using chiral

**Table 2** Aromatic cyanohydrins prepared by the lipase-catalysed resolution followed by the Mitsunobu esterification *in situ*

							
Compound	8	9	10	11	12 <sup>a</sup>	13 <sup>a</sup>	14 <sup>a</sup>
<b>RESOLUTION</b>							
Enzyme <sup>b</sup> /Selectivity	APS/S	APS/S	APS/S	APS/S	APS/S	APS/S	APS/S
Resolution type <sup>c</sup>	D	D	D	D	D	D	D
Solvent	i-Pr <sub>2</sub> O	i-Pr <sub>2</sub> O	Et <sub>2</sub> O	i-Pr <sub>2</sub> O	i-Pr <sub>2</sub> O	i-Pr <sub>2</sub> O	i-Pr <sub>2</sub> O
Cosubstrate <sup>d</sup>	P	P	P	P	P	P	P
ee <sub>B</sub> <sup>e</sup> /%	97 <sup>c</sup>	95 <sup>c</sup>	79 <sup>f</sup>	93 <sup>c</sup>	>99 <sup>f</sup>	91 <sup>f</sup>	96 <sup>f</sup>
ee <sub>P</sub> <sup>e</sup> /%	95 <sup>g</sup>	91 <sup>g</sup>	>99 <sup>h</sup>	95 <sup>g</sup>	96 <sup>h</sup>	>99 <sup>h</sup>	-
Conversion <sup>g</sup> /%	51	51	44	50	51	48	52 <sup>j</sup>
E	160	80	>400	130	>260	>600	65
<b>MITSUBU</b>							
Isolated yield/%	79	92	83	60	81	81	68
ee <sub>final</sub> <sup>e</sup> /%	93 <sup>c</sup>	63 <sup>c</sup>	61 <sup>f</sup>	78 <sup>c</sup>	97 <sup>f</sup>	81 <sup>f</sup>	63 <sup>f</sup>
Calculated ee <sub>final</sub> <sup>e</sup> /%	96	93	>88	94	>98	>95	92 <sup>l</sup>
Configuration	R	R	R	R	R	R	R

<sup>a</sup> Piv=Trimethylacetyl. <sup>b</sup> APS=*Pseudomonas cepacia* lipase. <sup>c</sup> D=deacylation. <sup>d</sup> P=1-propanol. <sup>e</sup> ee by chiral GLC on permethyl- $\beta$ -cyclodextrin column. <sup>f</sup> ee by chiral HPLC on Chiralcel OD column. <sup>g</sup> ee by chiral GLC on permethyl- $\beta$ -cyclodextrin column as propionate. <sup>h</sup> ee by chiral HPLC on Chiralcel OD column as propionate. <sup>i</sup> calculated according to equation (3). <sup>j</sup> determined by HPLC on RP18 column. <sup>k</sup> calculated according to equation (5). <sup>l</sup> calculated according to  $ee_{\text{final}}=2ee_{\text{B}}(1-c)$ .

chromatography by the methods described in the Tables as well as calculating according to equation (5). A difference between the observed and calculated values can reveal racemization or retention of configuration during the Mitsunobu esterification. The results of Table 1 show almost complete inversion of configuration. In the case of aliphatic cyanohydrin **2** (Table 1) the deviation is due to the racemization of (*R*)-2-hydroxyoctanenitrile during the esterification as was shown by the racemic acetate obtained when the cyanohydrin (ee 65%) was esterified with acetic acid instead of butyric acid (the original acyl component of the substrate in the deacylation) under the Mitsunobu conditions. Contrary to this result, the Mitsunobu esterification of (*R*)-2-hydroxypentanenitrile with phenylacetic acids was previously reported to proceed with complete retention of configuration.<sup>6</sup> It is worth mentioning that only the free alcohol in the resolution mixture reacts under the essentially neutral Mitsunobu conditions at ambient temperatures.

Optically active aromatic cyanohydrins are potential synthons in organic synthesis, the resolution of which by lipase catalysis was found to proceed with excellent enantioselectivity.<sup>4</sup> The separation of the cyanohydrin enantiomer from the corresponding ester isomer in the resolution mixture may, however, lead to considerable decomposition and racemization of the first. This is due to the lability of aromatic cyanohydrins especially in the presence of bases. In order to avoid the separation problem the resolution-inversion method was applied for the preparation of structurally different aromatic cyanohydrin acetates. In many cases, however, the results of Table 2 show considerable drops in  $ee_{\text{final}}$ , the degree of the drop depending on the structure of the cyanohydrin subjected to the Mitsunobu esterification. Extensive racemization in the case of compounds **9-11** can be explained by electron-donating substituents which favour unimolecular nucleophilic substitution. On the other hand, electron-withdrawing substituents at the benzene ring should favour esterification with the  $S_N2$  mechanism. This is seen as a successful inversion-esterification in the case of compound **12**. For acetates **13** and **14**, however, the values of  $ee$  are considerably lower than calculated. As in the case of 2-hydroxyoctanenitrile cyanohydrin **14** esterified using propionic acid instead of acetic acid produced racemic cyanohydrin propionate. As a possible explanation, the *meta*-substituent exposes the cyanohydrin to racemization by acetate ion or its steric crowding affect the reaction.

As a conclusion, the present one-pot two-step resolution-esterification procedure for the preparation of optically active esters of secondary alcohols is an easy and convenient method in avoiding typical problems of separation and low chemical yields involved in conventional resolution. The method is excellently valid when  $E$  is of the order of 100 or higher (Figure 2). In the case of moderate to good enantioselectivity, the present method results in an optically enriched ester which can be further subjected to enzymatic deacylation. Also in this case the chemical yield is higher than in the conventional double resolution without the Mitsunobu esterification. As the main limitation of the method, the structural features of the esterified alcohol must favour acylation with the bimolecular nucleophilic substitution mechanism.

**Acknowledgements.** We thank the Technology Development Centre (TEKES) for financial support.

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20.  $(B+A+P+Q)=1$ ;  $B=(1-c)(0.5+0.5ee_B)$ ;  $A=(1-c)(0.5-0.5ee_B)$ ;  $P=c(0.5+0.5ee_P)$  and  $Q=c(0.5-0.5ee_P)$  is substituted into equation (4). For a nonracemic substrate the value of  $ee_{final} = ((2ee_B ee_P + ee_0(ee_P - ee_B)) / (ee_P + ee_B))$  obtained by using  $c = (ee_0 + ee_B) / (ee_B + ee_P)$  in the place of equation (3),  $ee_0$  being the excess of the faster reacting enantiomer at the beginning.
21.  $E = \ln(A/A_0) / \ln(B/B_0)$  is transformed into the form a)  $A=f(B)$ . According to stoichiometry b)  $P=A_0-A$ ; c)  $Q=B_0-B$ ; d)  $A_0=1-B_0$ . Substituting a) and d) into b) and replacing A, P and Q in equation (4) with the resultant expressions gives  $ee_{final}$  as a function of B. Equation (7) is the result of substitution of a)  $A=f(B)$  and d)  $A_0=1-B_0$  into:  $c=1-((A-B)/(A_0-B_0))$ .
22. In the case of deacylation followed by the Mitsunobu esterification  $ee_{final} = ((Q+A)-(B+P)) / (B+P+Q+A)$ . If E is the same for acylation and deacylation  $Q+A$  of deacylation =  $B+P$  of acylation, and  $B+P$  of deacylation =  $Q+A$  of acylation. i.e., the equations for the two cases become identical.
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29. Calculated according to  $E = \ln((1-ee_B)/(1+(ee_B/ee_P))) / \ln((1+ee_B)/(1+(ee_B/ee_P)))$
30. The resolutions were run in 20 mmol scale in organic solvents. At a suitable conversion the enzyme was filtered off and the solution evaporated. The evaporated resolution mixture and 1.2 equivalents of triphenylphosphine and a carboxylic acid were dissolved in 30 ml of diethyl ether. At 0 °C, under vigorous stirring 1.2 equivalents of diethyl azodicarboxylate was added during 10-15 minutes. The mixture was allowed to stand at room temperature for 1 h and evaporated together with sufficient amount of chromatography grade silica to make the residue almost free flowing powder. This was transferred to a flash chromatography column and the product was isolated by normal chromatographic practice.

(Received in UK 1 June 1995)