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Kinetic Resolution of Substituted 1,3-4*H*-5,6-Dihydrooxazines with Carboxylesterase NP: Synthesis of (3*S*,1'*R*)-3-(1'-Hydroxyethyl)-Azetidin-2-one

Claudio Fuganti, Simonetta Lanati and Stefano Servi*

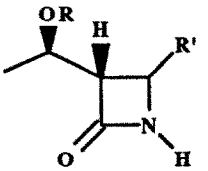
CNR, Centro per lo Studio delle Sostanze Organiche Naturali, Dipartimento di Chimica, Politecnico di Milano, Via Mancinelli 7, 20131 Milano, Italy

Abstract—The kinetic resolution of oxazinyl-ethyl carboxylates **4**, is reported employing carboxylesterase NP as biocatalyst. Starting from the *anti* racemic stereoisomers **4c–d**, the unreacted ester is obtained in 40 % yield and 93 % *e.e.* The enantiomeric acid is obtained in the open form. Cyclization of the latter and base equilibration allows recycling of the undesired stereoisomer. Compound **4d** has the absolute configuration required in the azetidinone **3d**, an intermediate in the synthesis of penems and carbapenems.

Introduction

β-Lactam antibiotics of synthetic origin are important targets for the pharmaceutical industry.[1] A number of penems[2,3] and carbapenems[4] are accessible through rather well established synthetic procedures starting from enantiomerically pure azetidinones of type **1**.[5,6]

In particular compound **2** has been commercially available for further elaboration. Access to this intermediate is given mainly by manipulation of synthons from the chiral pool like amino acids, β-hydroxy acids, or by degradation of penicillins or cephalosporins available by fermentation. Interest in this field is still active and we have recently studied an approach to **3d** via the selective reduction assisted by microorganisms of the appropriate α-alkyl-β-keto esters; the biotransformation was shown to occur with partial diastereoselection, giving as products mixtures of **3**.[7] The obtainment of **3d** was achieved by taking advantage of the observation that both chiral centers of any defined absolute configuration in **5** and not corresponding to the one required, can be transformed into the desired one, either by inversion of the secondary carbon in position 1' of **3**, or by inversion of the configuration at the other chiral centre through the thermodynamic equilibration of a cyclic intermediate (oxazine).[8] The latter opportunity has led us to devise a novel approach to chiral **3d** based on the observation of the equivalent triplicate structures indicated in each line of Figure 1. Compound **3d** bearing the required absolute configurations at the two chiral centres, has three other stereoisomers depicted in the Figure. For each of them is indicated the open α-benzoylamino methylene-β-hydroxy ester of equivalent chirality **5a–d** as well as the 1,3-oxazines **4a–d** from which the latter can be derived. Two of the stereoisomeric forms of the six membered ring compound **4** bear the two substituents in a pseudo-equatorial form (**4c** and **4d**) and are thermodynamically more stable than **4a** and **4b**. Since cyclization of compounds **5** to **4** occurs with inversion of configuration at carbon 3 of **5**, both **5b** and **5c** can be transformed into an oxazine (**4d** and **4a** respectively) with the *R* configuration at carbon 6 and hence at carbon 1' of **3d** to which it can be converted. Additionally in compounds **4a** and **4b** epimerization at carbon 5 is in principle possible through equilibration after base treatment. Overall the system is therefore rather flexible and allows recovery and/or reconversion of undesired stereoisomers.



1 R, R' = various groups
2 R = SitBuMe$_2$, R' = OCOR''
3 R, R' = H

Having applied these concepts to the reduction of β-ketoesters with suitable substitution patterns, we wish now to report on a preliminary result applied to the preparation of the same chiral azetidinone through the direct resolution of the racemic oxazine. In fact the presence of an ester function in **4** suggests the application of a hydrolytic enzyme in the kinetic resolution of the parent oxazines via diastereoselective hydrolysis. The operation can in principle be effected in three possible ways: a) separation of the two diastereoisomers and enantioselective hydrolysis of the appropiate racemic stereoisomer; b) application of the hydrolytic biocatalyst to the diastereoisomeric mixture with recycling of the undesired stereoisomer; c) equilibration of the diastereoisomeric oxazines to the more stable couple and resolution by enzyme catalysed hydrolysis of the couple obtained. The complementary approach with a transesterification procedure could in principle duplicate the above mentioned procedures. The three options are

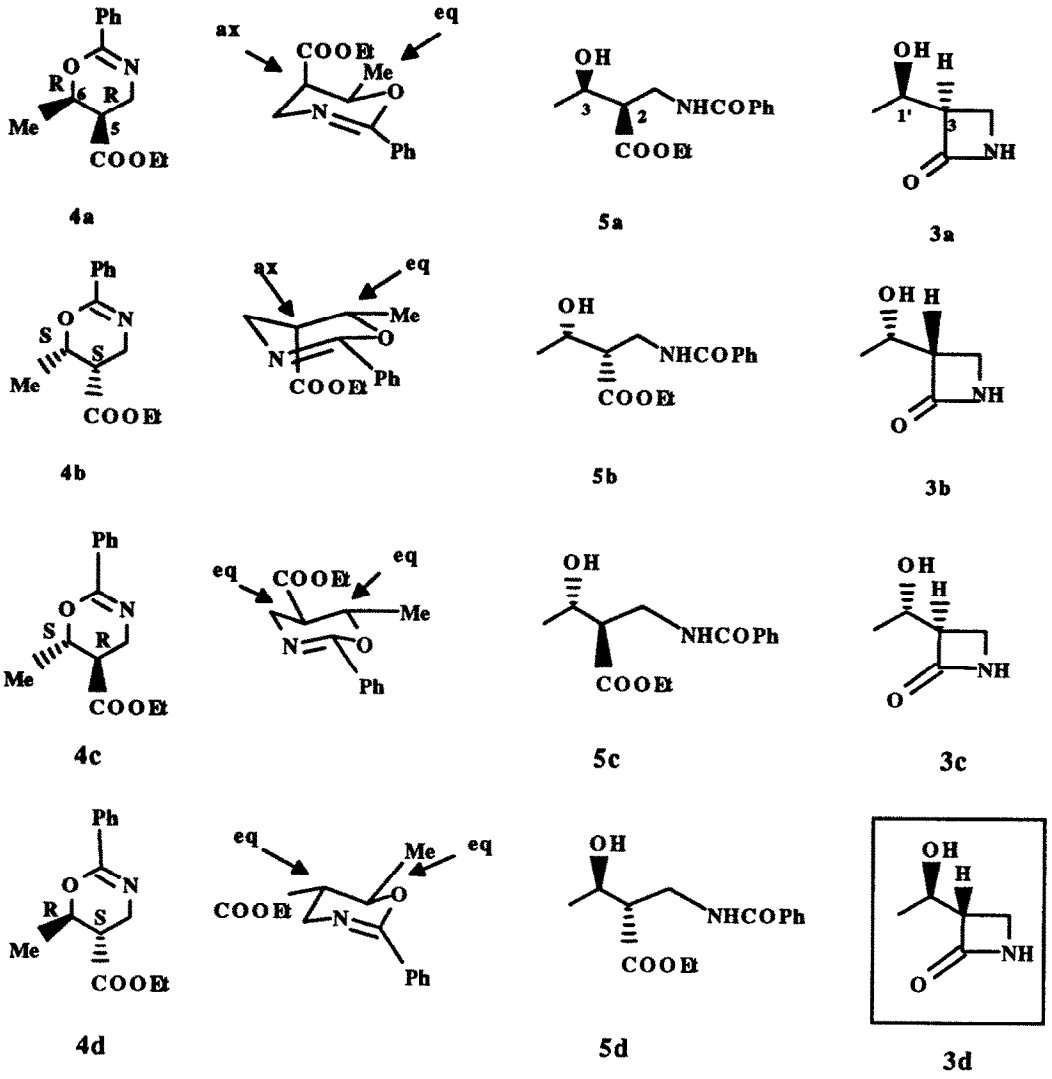
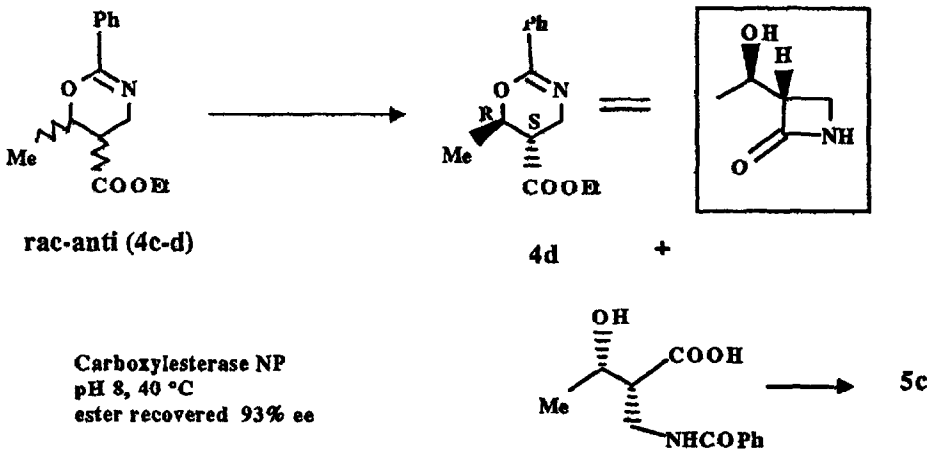


Figure 1.

equivalent if the efficiency of the resolution step is assumed to be equal for all the transformations. What is in fact crucial, is to find out which stereoisomer will preferentially (not) be hydrolysed and evaluate the efficiency of this recognition.

Results and Discussion

We initially attempted the resolution of the four stereoisomers with the aid of commercially available lipolytic and proteolytic enzymes starting with PLE, a natural candidate for an enantioselective hydrolysis of such compounds for the structural analogy with other racemic compounds which are efficiently resolved.[9] However this enzyme as well as other common lipases and esterases were not efficient in effecting the required enantioselective hydrolysis. We then considered carboxylesterase NP as a possible candidate, more for the known ability to hydrolyse carboxylic acid ethyl esters than for the intrinsic known specificity. Indeed this enzyme is reported to be very specific for propionates bearing an aromatic moiety in the α-position, deserving also the nickname of naproxen esterase.[10,11] However the behaviour of this enzyme with cyclic structures of type **4** has not previously been reported. The diastereoisomeric oxazines were in fact accepted as a substrate, although hydrolysed rather slowly. In a typical experiment the racemic diastereoisomeric mixture **4a–d** (10 mmol) was suspended in phosphate buffer pH 8 at 40 °C and hydrolysed in the presence of carboxyl esterase NP (440 ECU[12]) for 16–24 h. The course of the reaction is followed by HPLC on chiral stationary phase, allowing the direct determination of the complete course of the reaction. Indeed the relative amounts of the four stereoisomers were monitored in one single injection. The presence of the acid formed in the hydrolysis was, however, not detected with that analytical method. In this way we noticed that *anti* **4** was hydrolysed more rapidly than the corresponding *syn* stereoisomer giving rise to a mixture of enantiomerically enriched surviving esters corresponding to compounds **4d** and **4b** bearing the (5*S*,6*R*) and (5*S*,6*S*) absolute configuration respectively. HPLC analysis as well as optical rotation values allowed us to evaluate the enantiomeric excess of the two compounds as 93 and 95 % respectively. The hydrolysis thus occurs as is often the case with high enantioselectivity but with scarce diastereoselection. From a practical point of



Scheme I.

view separation of the two racemic *anti* and *syn* diastereoisomers via chromatography prior to enzymatic hydrolysis proved to be more convenient, allowing easier isolation and characterisation of the desired compound.

In this way starting from the racemic *anti* couple **4c–d**, (5*S*,6*R*) **4d** was obtained at 55 % conversion in about 40 % yield and 93% *e.e.* showing an $[\alpha]_D$ = + 73.1 ° (c = 0.3, dioxane, lit.[8] + 81.4 °). The acid of opposite absolute configuration was recovered in the open form and transformed into the ethyl ester of structure **5c** (Scheme I). The optical purity of the latter was surprisingly evaluated to be about 65 % *e.e.* (HPLC). From the racemic *syn* couple **4a–b**, (5*S*,6*S*) **4b** was obtained at 55 % conversion in about 30 % yield and 95 % *e.e.* showing an $[\alpha]_D$ = + 8.3 ° (c = 0.3, dioxane, lit.[8] –9.6 ° for the (5*R*,6*R*) enantiomer). The acid was also in this case isolated after acidic work-up in the open form of type **5**, with the absolute configuration corresponding to the one depicted in **5a**. Its optical purity was evaluated to be of 95 % *e.e.* by HPLC of the corresponding ethyl ester **5a**.

In conclusion the kinetic resolution of oxazines **4** offers a practical approach to enantiomerically pure **3d**. In particular it appears rather convenient if applied to the racemic *anti* oxazines **4c–d**: in this case, together with the unreacted ester **4d** bearing the absolute configuration required for transformation into **3d**, compound **5c** is obtained the transformation of which into **4d** should be possible through the double inversion following cyclization to **4a** and base equilibration to the more stable stereoisomer **4d**. However the low *e.e.* of the acid obtained in this case renders this approach impossible. We are currently investigating whether a partial racemization occurs during the work up procedure.

Experimental

6-Methyl-2-phenyl-5,6-dihydro-1,3,4H-oxazinyl-5-carboxylic acid ethyl ester 4a–b and 4c–d.

Thionyl chloride (4.1 mL, 56 mmol) is added to a solution of 2-benzamidomethylene-3-hydroxybutyric acid ethyl ester **5** (10 g, 37 mmol) in CH_2Cl_2 (40 mL) and stirred at room temperature for 3 h. The reaction solution is concentrated on a rotary evaporator, the residue is taken up in toluene and concentrated under vacuum. The residue is suspended in a small amount of ethyl acetate and the hydrochloride obtained in a crystalline form is filtered off, suspended in a small amount of ethyl acetate and stirred with a saturated $NaHCO_3$ solution. The organic phase is dried over anhydrous Na_2SO_4 and evaporated to give the free base as a colourless oil. The composition of the diastereoisomeric mixture is determined by HPLC to be a 2:1 *anti*:*syn* mixture (Chiralcel OD-DAICEL, *n*-hexane/2PrOH 95/5, 0.6 mL/min, UV = 254 nm. Retention times (min): (5*S*,6*R*) = 10.42, (5*R*,6*S*) = 11.08, (5*S*,6*S*) = 12.87, (5*R*,6*R*) = 25.08. The two diastereoisomeric oxazines are separated by column chromatography on silica gel with mixtures of hexane/ethyl acetate. TLC (silica gel, hexane/ethyl acetate 1:1). R_f:*anti* stereoisomer **4c–d** = 0.38, R_f:*syn* stereoisomer **4a–b** = 0.26. Assignment of the relative stereochemistry is done by comparison with the spectroscopic values reported in the literature.[8]

anti Stereoisomer **4c–d**, mp. 61–63 °C. ^{1}H NMR ($CDCl_3$): δ_H 1.3 (3H, CH_3, t), 1.45 (3H, CH_3, d), 2.6 (1H, CH, dt), 3.8 (2H, CH_2, m), 4.2 (2H, CH_2, q), 4.45 (1H, CH, m), 7–8 (5H, ArH).

syn Stereoisomer **4a–b**, oil. ^{1}H NMR ($CDCl_3$): δ_H 1.3 (3H, CH_3, t), 1.35 (3H, CH_3, d), 3.0 (1H, CH, m), 3.8 (2H, CH_2, m), 4.2 (2H, CH_2, q), 4.75 (1H, CH, m), 7–8 (5H, ArH).

General procedure for the enzymatic hydrolysis of 4

To a suspension of 10 mmol of substrate in 50 mL of Tritisol buffer pH = 8, 2 mL of carboxylesterase NP (440 ECU[12]) are added. The temperature is set at 40 °C and the pH is kept constant by automatic addition of 1 M NaOH. The solid substrate **4c–d** is added as a fine powder to the solution containing 1 % Tween 80. The oily substrate **4a–b** is dispersed as such and the stirring maintained at 300 rpm. At 55 % conversion the reaction mixture is extracted

with ethyl acetate. The organic layer containing the unreacted ester is dried and evaporated. The aqueous layer is acidified at pH 4 with 10 % HCl, extracted with ethyl acetate, dried and the solvent removed to give the corresponding α-benzoylaminomethylene-β-hydroxy acid which is then identified and characterised as the ethyl ester **5** in which it is converted by reaction with ethylbromide/K_2CO_3 in methyl-ethyl ketone as solvent at 60 °C. The analysis of the oxazines is made by HPLC as described previously. The enantiomeric excess of compounds **5** from hydrolysis, is determined by HPLC as described elsewhere.[7]

Oxazines **4c–d** give after hydrolysis unreacted **4d** at 55 % conversion (16 h) in about 40 % yield and 93% *e.e.* showing an $[\alpha]_D$ = + 73.1 ° (c = 0.3, dioxane, lit.[8] + 81.4 °). The acid of opposite absolute configuration is recovered in the open form and transformed into the ethyl ester of structure **5c** in 65% *e.e.*

Oxazines **4a–b** give after hydrolysis unreacted **4b** at 55 % conversion (24 h) in about 30 % yield and 95% *e.e.* showing an $[\alpha]_D$ = + 8.3 ° (c = 0.3, dioxane, lit.[8] –9.6 ° for the (5*R*,6*R*) enantiomer). The acid of opposite absolute configuration is recovered in the open form and transformed into the ethyl ester of structure **5a** in 95 % *e.e.*

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12. Enzyme activity is expressed in Esterase Cleavage Unit (ECU)/g using (*S*)-naproxen methyl ester as the substrate. One ECU is defined as the quantity of enzyme that will form 1 μmol of (*S*)-naproxen acid per minute at pH 7.5 and 32 °C.