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SUBSTRATE SPECIFICITY AND ENANTIOSELECTIVITY OF PENICILLINACYLASE CATALYZED HYDROLYSIS OF PHENACETYL ESTERS OF SYNTHETICALLY USEFUL CARBINOLS

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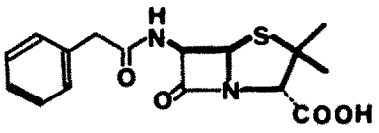
Abstract: Penicillinacylase from E.coli, immoblized on Eupergit C beads catalyzes the hydrolysis in water/CH_3CN 10:1, at pH 7.5 and 23 °C, of a set of O-phenylacetate esters of primary carbinols. The highest enantioselectivity is observed in the case of the 2,2-dimethyl-1,3-dioxolane-4-methanols structurally related to the penicillin (1) framework. Minor modifications of this basic structure are not altering the acceptability by the enzyme, but significantly decrease the enantioselectivity of the hydrolysis, as does the use of benzene as solvent and Sepharose-bound enzyme.

There is a current interest[1] in preparative organic chemistry for new synthetic applications of enzymes, due to reaction specificity, enantioselectivity, and rate enhancement these unique catalysts can achieve in comparison with non-biologically based systems. Organic chemists are now being facilitated in this exploratory work from the circumstance that an increasing number of enzymes is becoming commercially available, some of which in stable, immobilized form, suitable for long term, continuous or batchwise operations.

In this contest, for some time now we have been studying the behavior towards unnatural [2,3] substrates of penicillinacylase (EC 3.5.1.11) from E.coli, using a commercial preparation immobilized on Eupergit C beads. This enzyme selectively transfers the phenacetyl moiety of penicillin G (1) to water giving 6-aminopenicillanic acid (2) and phenylacetic acid[4] and it is used industrially[5] in the production of (2), which is a key intermediate in the preparation of semisynthetic penicillins[6].

Recently we observed the aptitude of immobilized penicillinacylase to hydrolyze at pH 7.5 O-phenacetyl esters to the corresponding alcohols and phenylacetic acid and briefly[7] referred on the enantioselectivity of the hydrolysis of the phenacetyl esters of a series of 2,2-dimethyl-1,3-dioxolane-4-methanols. The relevant enantioselectivity observed in the hydrolysis of some members of the above set of materials induced us to further extend our studies on substrate specificity and enantioselectivity of this synthetically useful enzymic reaction and we now report on the results obtained.



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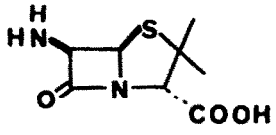

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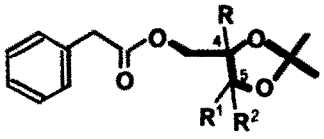


(3) R= R^1= R^2= H
(4) R= Me: R^1= R^2= H
(5) R= R^1=H; R^2= Me (4,5-anti)
(6) R= R^1= H; R^2= CH_2OH (4,5-anti)
(7) R= R^1= Me; R^2= H (4,5-syn)
(8) R= H; R^1= R^2= Me
(9) R= R^1=H; R^2= Me (4,5-syn)
(10)R=R^1= H; R^2= Et (4,5-syn)
(11)R= Me; R^1= H; R^2= Et (4,5-syn)

RESULTS AND DISCUSSION

In the present study the ability of immobilized penicillinacylase to hydrolyze the O-phenylacetate esters of a series of relatively small, highly functionalized, structurally related primary and secondary carbinols was considered, together with the enantioselectivity of the reaction. The compounds examined are accessible in optically active form either by modification of chiral natural products or by asymmetric synthesis and are being currently used as starting material in the synthesis of optically active forms of natural products and drugs. It was thus of interest to verify in the context of the present work designed to establish the structural limits of acceptability as substrates by penicillinacylase of phenylacetate esters, if this hydrolytic method could give a direct access to a set of synthetically useful chiral synthons.

In the first instance we chose as substrates for the hydrolysis reaction the phenylacetate esters (3)-(11), holding structural similarities with penicillin G (1). Thus, comparison of structural formulas (3)-(11) and (1) indicates that part of the framework of (1) is matching the 1,3-dioxolane moiety of (3)-(11) (see heavy bonds), being the gem-dimethyl groups at homologous positions in (1) and (3)-(11) separated from the bonds to be broken in the hydrolysis by the same number of atoms.

The esters (3)-(11) are indeed hydrolyzed to the corresponding carbinols with t 1/2 of 3-4 h for all the materials. However, the absolute configuration of the products obtained in the hydrolysis and the enantiomeric eccess (ee) (table 1), strongly depends upon the nature of the 5-substituent(s) and the 4,5 stereochemistry. The highest ee values (ca. 0.9) are obtained for the 4-methyl substituted dioxolane methanols derived from (4) and (11), respectively, being the absolute configuration inverted upon substituting an hydrogen at position 5 for an ethyl group in syn relationship with the 4-CH_2O group.

The significance for the enantioselectivity of the hydrolysis of the presence of the gem-dimethyl moiety at position 2 of the 1,3- dioxolane system is supported from the fact that (12) and (13),

the gem-diethyl analogs of (3) and (10), afforded, at 50% hydrolysis, (4S) and (4R, 5S) carbinols, showing 0.3 and 0.4 ee, respectively, being these values remarkably lower than 0.6 and 0.8 observed with (3) and (10). Moreover, when the sp^3carbon bearing the two methyl groups in position 2 of the dioxolane framework is substituited for an sp^2 carbonyl carbon, as in the cyclic carbonates (14) and (15), a rapid hydrolysis occurs (t 1/2 ca. 1 h), but the carbinols obtained at 50% hydrolysis resulted devoid of optical activity in the case of (14) and (15). Subsequently, the framework of the dioxolane methanols (3), (5) and (9) was substituted in α-position with a vinyl group, thus mimicking in some way , in terms of number of atoms and electron density, the carbonyl group of the β-lactam ring of (1). However, the resulting secondary allylic esters (16)-(18) (as a mixture of diasteroisomers) were not hydrolyzed, even when CH_3CN in the reaction medium was substituited for 10% glycerol or 10% dimethylformamide. On the other side, penicillinacylase does

TABLE I : Absolute configuration and enantomeric excess values for the products obtained by 50% penicillinacylase hydrolysis of the corresponding phenylacetate esters.

parent ester	abs. configuration of the alcohol	ee	Methods used for determining ee.
(3)	(4S)	0.6	a,c
(4)	(4S)	0.9	a,b,d
(5)	(4S,5S)	0.5	a,c
(6)	(4S,5S)	0.52	a,c
(7)	(4S,5R)	0.65	d
(8)	(4R)	0.33	a,c
(9)	(4R,5S)	0.46	a,c
(10)	(4R,5S)	0.8	a,c
(11)	(4R,5S)	0.9	a,c
(12)	(4S)	0.3	
(13)	(4R,5S)	0.4	
(14)	optically inactive		
(15)	optically inactive		
(16)	(4R,5S)	0.3	
(20)	(2S)	0.85	a,c
(23)	(2S)	0.07	a,c
(25)	(4R,5S)	0.7	a,c
(26)	(S)	0.28	a,c

a) Optical rotation measurements. b) NMR studies on (-) camphanyl esters. c) NMR studies on the acetyl derivative in the presence of tris- 3-(heptafluoropropylhydroxymethylene)camphoratoeuropium(III). d) NMR studies on the (-)-methoxy(trifluoromethyl)phenylacetyl esters.

catalyze the hydrolysis of the β-phenyl substituted secondary allylic phenylacetate (20), yielding with t 1/2 of 2 h a (2R) carbinol of 0.8-0.85 ee.[8]

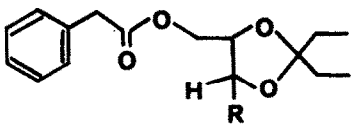
Furthermore, product (20), the phenylacetate of glycidol (2,3-epoxypropan-1-ol), in which the three adjacent oxygen functions present in (3) are mantained, but the pentaatomic dioxolane system is substituted by the oxyrane system, is rapidly hydrolyzed, whereas the ester which survived to 50% hydrolysis resulted optically inactive. Similarly, the glycerol derivative (21) behaves towards penicillinacylase as (20). The hydrolysis proceeds with t 1/2 of 1 h, but the survived ester is devoid of optical activity. The same is true for the 2-phenylacetate of 1-phenyl-1,2-ethanediol (22) which yields with t 1/2 of 1 h a (2S)-diol with $[\alpha]^{20}_D$ +2.4° (c 3,acetone) (lit.[9] +39°). When the secondary alcohol of (22) was esterified with phenylpropionic acid, the resulting diester (23) was not hydrolyzed.

However, the phenylacetate ester (24), in which the 1,3-dioxolane system bears at position 5 a bulky substituent, is hydrolyzed (t 1/2 ca. 2h), affording, at 50% conversion, a (4R,5S) carbinol with 0.7 ee, readly converted upon controlled acidic hydrolysis, into 2-deoxy-D-ribose[10] . Furthermore, when penicillinacylase is acting onto (25), the phenylacetate ester of 3,4-isopropylidenedioxy butan-1-ol, an (S) carbinol was obtained with 0.28 ee. Comparison of this result

with that obtained with the lower homologue (3) indicates a decrease of optical purity on introduction of an additional CH_2 group between the bond to be broken and the asymmetric carbon atom. Finally an hydrolysis experiment was carried on using Sepharose bound penicillinacylase in benzene saturated with water. In this circumstance, the extent of the hydrolysis was followed by HPLC analysis. However, the carbinol obtained at ca. 50% hydrolysis from the phenylacetate (9) was devoid of optical activity.

CONCLUSIONS

In the present work an attempt has been made to estabilish some structural rules for acceptabi-



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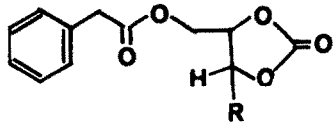
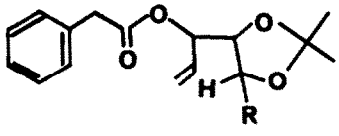
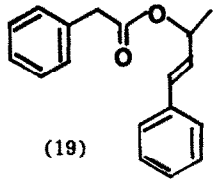
(13) R= Et (4,5-syn)



(14) R= H
(15) R= Me (4,5-anti)



(16) R= H
(17) R= Me (4,5-anti)
(18) R= Me (4,5-syn)



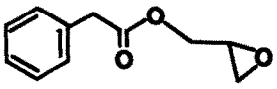
(19)

lity by penicillinacylase, a synthetically important, readly accessible enzyme, of non conventional substrates.

The results otained with the above sets of O-phenacetyl esters of structurally related alcohols, bearing, in most instances, two oxygen functions in α and β positions, indicate the facile hydrolysis of the derivatives of primary carbinols, being, however, the enantioselectivity strongly depending upon some structural features. In particular, amongst the compounds matching in part the molecule of penicillin G (1), the natural substrate of the enzyme, those hydrolyzed with higher enantioselectivity are the 2,2-dimethyl-1,3-dioxolane derivatives (3)-(11). Minor modifications of this framework, such as the substitution of the 2,2 dimethyl moiety for the 2,2-diethyl or the 2-carbonyl as in (12)-(15), determines a significant decrease of the ee values.

Furthermore, the allylic, secondary derivatives (16)-(18) are not hydrolyzed, altough the less hindered allylic ester (19) affords a carbinol of high ee value. The primary carbinols bearing oxygen functions in α and β position(s) are obtained from the corresponding esters but without noticeable enantioselectivity. The relevance of the solvent in determining the steric course of the enzymic reactions[12] is demonstrated by the experiment with Sepharose bounded penicillinacylase acting in benzene upon the ester (9), which afforded a carbinol devoid of optical activity, whereas in water, with the Eupergit C bounded enzyme a material with 0.46 ee. was obtained. The above experiments, seen together, illustrate the synthetic utility of penicillinacylase in the preparation of chiral carbinols by means of the hydrolysis of the corresponding phenylacetate

esters and the empiricism still informing the predictability of the substrate specificity and stereospecificity of enzymatic transformations of non conventional substrates.



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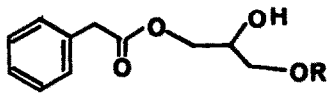
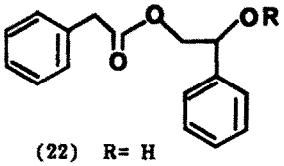
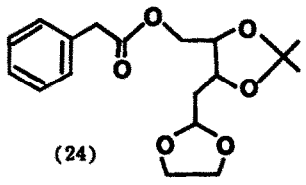


(21) R= $SO_2C_6H_4CH_3(p)$



(22) R= H

(23) R= $COCH_2CH_2Ph$



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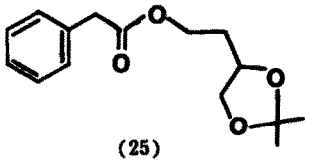

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EXPERIMENTAL

Materials and Methods

The enzyme penicillinacylase from E.coli,strain ATCC 9637, was a commercial preparation devoid of β-lactamase, provided by SCLAVO , Divisione Biochimica, De.Bi.. The enzyme had been covalently coupled on Eupergit C (activated oxirane acrilic beads) obtained from ROHM Pharma Weiterstadt FRG., following the instruction of the manufacturer.

The supported enzyme was stored in aqueous solutions containing 80% glycerol at the temperature of 4° C. In these conditions it mantained constant activity for almost one year.

In one of the experiments the penicillinacylase used was a preparation coupled to Sepharose CL 4B Pharmacia, following the procedure of Aksen[11].

Before use, the beads were filtered and washed several times with distilled water in order to remove glycerol traces. The beads were air dried on the water pump: in this way about a 60% content of water was retained. The activity of enzyme preparations was determined with penicillin G as substrate (4% w/V solution, in 0.002 M phosphate buffer, pH 7.8 at 28 C). 50% hydrolysis of penicillin G required 20 min..

The activity of Eupergit C supported penicillinacylase was 190 IU/g wet weight; the activity of the sepharose bound one was 153 IU/g wet weight.

Kinetics of enzymatic hydrolysis were followed by alkaline titration (1M NaOH) using a Methrom pH-stat equipped with pH-meter E362, Impulsomat E-473, Dosimat E-415.

Preparation of Substrates

The alcohols used in the present experiments were either commercial samples or prepared by synthesis according to literature procedures. The O-phenacetyl esters were obtained according to the following general procedure:. the alcohol (0.1 mole) in 100 ml of dry CH_2Cl_2, in the presence of 0.2 moles of pyridine, was treated dropwise within 15 min. at 0° C with phenacetyl chloride (0.12 mole). After standing overnight at room temperature, the reaction mixture was sequentially washed with water, 1N HCl, 5% $NaHCO_3$ solution, to give, after evaporation of the dried (Na_2SO_4) solution, the phenacetyl ester in 70-90% yield. The material was purified either by distillation or by SiO_2 column cromathography using hexane-ethyl acetate as eluents. The structural assignement is based on elemental analysis, ^1H NMR and IR spectra performed, when possible, on samples obtained by bulb to bulb vacuum distillation.

The following analytical data were obtained:

Product (3)	Anal. Calcd. for $C_{14}H_{18}O_4$	: C, 71.77; H, 7.74.	Found: C, 71.63; H, 7.61;
(4)	for $C_{15}H_{20}O_4$	: C, 68.16; H, 7.63.	Found: C, 68.22; H, 7.66;
(5)	for $C_{15}H_{20}O_4$	: C, 68.16; H, 7.63.	Found: C, 68.19; H, 7.63;
(6)	for $C_{15}H_{20}O_5$	: C, 64.27; H, 7.19.	Found: C, 64.16; H, 7.21;
(7)	for $C_{15}H_{20}O_4$	: C, 68.16; H, 7.63.	Found: C, 68.23; H, 7.56;
(8)	for $C_{16}H_{22}O_4$	: C, 69.04; H, 7.97.	Found: C, 69.08; H, 7.81;
(9)	for $C_{15}H_{20}O_4$	: C, 68.16; H, 7.63.	Found: C, 68.11; H, 7.58;
(10)	for $C_{16}H_{22}O_4$	: C, 69.04; H, 7.97.	Found: C, 69.11; H, 7.91;
(11)	for $C_{17}H_{24}O_4$	: C, 69.83; H, 8.27.	Found: C, 69.76; H, 8.22;
(12)	for $C_{16}H_{22}O_4$	: C, 69.04; H, 7.97.	Found: C, 69.01; H, 7.89;
(13)	for $C_{18}H_{26}O_4$	: C, 70.56; H, 8.55.	Found: C, 70.59; H, 8.47;
(14)	for $C_{12}H_{12}O_5$	: C, 61.01; H, 5.12.	Found: C, 61.03; H, 5.14;
(15)	for $C_{13}H_{14}O_5$	: C, 62.39; H, 5.64.	Found: C, 62.32; H, 5.68;
(16)	for $C_{16}H_{20}O_4$	: C, 69.54; H, 7.30.	Found: C, 69.58; H, 7.32;
(17)	for $C_{17}H_{22}O_4$	: C, 70.32; H, 7.64.	Found: C, 70.28; H, 7.60;
(18)	for $C_{17}H_{22}O_4$	: C, 70.32; H, 7.64.	Found: C, 70.29; H, 7.62;
(19)	for $C_{18}H_{18}O_2$	: C, 81.17; H, 6.81.	Found: C, 81.09; H, 6.83;
(20)	for $C_{11}H_{12}O_3$	: C, 68.73; H, 6.29.	Found: C, 68.77; H, 6.22;
(21)	for $C_{18}H_{18}O_6S$	: C, 59.66; H, 5.01.	Found: C, 59.71; H, 5.06;
(22)	for $C_{16}H_{16}O_3$	: C, 74.98; H, 6.29.	Found: C, 74.93; H, 6.22;
(23)	for $C_{25}H_{24}O_4$	: C, 77.30; H, 6.23.	Found: C, 77.26; H, 6.21;
(24)	for $C_{18}H_{24}O_6$	: C, 64.27; H, 7.19.	Found: C, 64.21; H, 7.21;
(25)	for $C_{15}H_{20}O_4$	: C, 68.16; H, 7.63.	Found: C, 68.11; H, 7.61.

Hydrolysis Experiments

The following general procedure was applied to all ester hydrolysis. The substrates used in a concentration range of 0.1-1 mMolar were suspended in 10% CH_3CN aqueous solutions 0.002 M phosphate buffer, pH 7.5. The reaction vessel was maintained at room temperature and was mechanically stirred. The pH-stat was set to keep the pH at 7.5.

Penicillinacylase beads were added in the amount of about 50 IU/mmole of ester. The 50% of hydrolysis was extrapolated by the equivalents of NaOH added.

No aspecific hydrolysis was detected in the absence of the enzyme. The presence of 5-10% of CH_3CN, THF or CH_3OH was accepted by the enzyme beads without significative modifications on the activity and substrate tolerance.

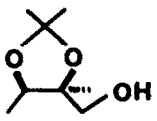
The enzyme activity after a long permanence in the reaction vessel in the presence of substrate and 10% CH_3CN, was tested and presented no significative reduction. Even if the reaction mixtures were often non-omogeneous and hence far from the Michaelis-Menten conditions, a good reproducibility of the results (i.e. ee and hydrolysis rates) was observed.

Isolation of the Reaction Products and Determination of their Optical Purity

The mixture resulting from the enzymic hydrolysis, once separated from the beads by filtration, was extracted 3-5 times with CH_2Cl_2. The residue obtained upon concentration of the dried (Na_2SO_4) solution, was chromatographed on SiO_2 column with hexane-ethyl acetate, obtaining the survived ester in the first eluate, and subsequently, upon increasing the amount of ethyl acetate, the carbinol produced in the hydrolysis. The materials, when possible, were subsequently distilled under vacuum in a bulb-to-bulb apparate. The absolute configuration of the isolated carbinols was assigned on the basis of the optical rotation and comparison with authentic samples. When required (see below) a chemical correlation with known materials was made.

Thus, the chiral forms of the carbinols obtained from the esters (3),(6) and (22) are known and commercially available, whereas those from (5),(9),(11) and (24), already prepared by us,[13,14,15] were directly used for comparison. Finally, the optically active forms of the carbinols arising from the esters (4), (8), (14) and (25) are reported in the literature.[16, 17, 8, 18].

The absolute configuration of the carbinol (26), obtained in the hydrolysis of the ester (7), was determined by conversion, in two steps, into known[19] α,β-unsaturated ester (27).



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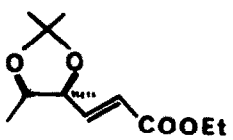
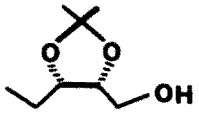


(27)

Moreover, the alcohol (28) arising from (10) was assigned the (4R, 5S) configuration because of its conversion, on acid hydrolysis, into 1,2-dideoxy-L-erythro-pentitol (29).[20] The same is holding for the diethyl analog of (28) formed in the hydrolysis of the ester (13).



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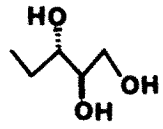
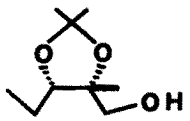


(29)

The carbinol obtained in the hydrolysis of the ester (11) was assigned the (4R, 5S) configuration depicted in (30) because of its conversion into the known [14] aldehyde (31).



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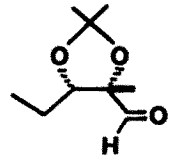
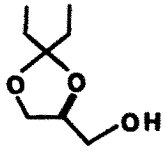
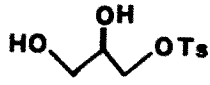


(31)

Finally the material obtained in the hydrolysis of the 2,2-diethyl derivative (12) was assigned the (4S) configuration (32) because it gave, once converted into the 4-toluenesulfonyl derivative, on acid hydrolysis, (R) 3-tosyloxy-1,2-propanediol (33).[21]



(32)



(33)

The optical purity of the products obtained in the enzymic reactions, was determined by optical rotation measurements and comparison with authentic samples, ^{1}H NMR studies on the esters prepared from the alcohols with either (-)-α-methoxy-α-trifluoromethylphenylacetic acid[22] or with (-)-camphanic acid [23], via reaction of the acid chloride with the carbinol in CH_2Cl_2 in the presence of dry pyridine (1:1:2 molar ratio). Alternative or complementary to the above measurements were ^{1}H NMR studies on the survived esters and on the acetate esters of the alcohols obtained in the hydrolysis in the presence of tris 3-(heptafluoropropylhydroxymetylene)-d-camphorato europium (III), integrating the base-line separated peaks due to the two enantiomers.

(4S, 5R) 4-(2-carbethoxyethenyl)-2,2,4,5-tetramethyl-1,3-dioxolane (27) from (4S, 5R)-2,2,4,5-tetramethyl-1,3-dioxolane-4-methanol (26).

The carbinol (26) used in this experiment was obtained at 30% conversion, and showed $[\alpha]_D^{20}$ -19.5° (c 2, $CHCl_3$). To a stirred solution of oxalyl chloride (0.96 mL, 11 mmol, 1.1 equiv) in CH_2Cl_2 (40 mL) at -78 °C, Me_2SO was added (1.56 mL, 22 mmol, 2.2 equiv) over 5-min period. After the suspension was stirred for 20 min, product (26) (1.6 g, 10 mmol, 1 equiv) in CH_2Cl_2 (10 mL) was added at -78 °C. After stirring 1h at -78 °C, Et_3N (7 mL, 50 mmol, 5 equiv) was added and the cooling bath was removed. The suspension, after stirring 5h at room temoerature was diluted with Et_2O (200 mL), washed with water, dried (Na_2SO_4), filtered, evaporated, and carefully distilled in a bulb-to-bulb apparate. The clear oil so obtained (ca. 0.7 g) in 50 mL of dry benzene, was refluxed 2 days with carbethoxymethylene-triphenylphosphorane (3,6 g, 10 mmol) in the presence of 40 g of benzoic acid. The solvent was evaporated and the residue purified on silica gel. Elution with hexane-ethyl acetate (9:1) gave product (27) (0.69 g, 30% yield from (26)), $[\alpha]_D^{20}$ +4.8° (c 4, $CHCl_3$), (lit.[19] -4.6° for the (4R, 5S) enantiomer); ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.23 (3H, d), 1.32 (3H, t), 1.42 (3H, s), 1.45 (3H, s), 1.54 (3H, s), 4.07 (1H, q), 4.22 (2H, q), 6.07 (1H, d), 6.90 (1H, d). Anal. Calcd for $C_{12}H_{20}O_4$: C, 63.13; H, 8.38. Found: C, 63.15; H, 8.92.

Conversion of (4R, 5S) 2,2-dimethyl-5-ethyl-1,3-dioxolane-4-methanol (28) into 1,2-dideoxy-L-erythro-pentitol (29).

The carbinol (29) used in this experiment was obtained at 30% hydrolysis and showed $[\alpha]_D^{20}$ +31.2° (c 2, $CHCl_3$). A solution in 20% aqueous trifluoroacetic acid of product (29) (0.8g, 5 mmol) was heated for 5h on a steam bath, untill tlc indicated that the reaction was complete. The reaction mixture was taken to dryness under vacuum at 50-60°C and the residue was distilled (90-100 °C/ 0.1 mm Hg) to give (29) (0.4 g, 67%), oil which solidified on standing, $[\alpha]_D^{20}$ +6.35° (c 1, H_2O) (lit.[20] +6.2°). ^1H NMR (90 MHz, CD_3OD) δ 0.97 (3H, t, J 7Hz), 1.50 (s H, m), 3.2-3.8 (4H, m). Anal. Calcd for $C_5H_{12}O_3$: C, 49.98; H, 10.07. Found: C, 50.02; H, 9.95.

(4R, 5S) 2,2,4-trimethyl-5-ethyl-1,3-dioxolane-4-carboxaldehyde (31) from (4R, 5S) 2,2,4-trimethyl-5-ethyl-1,3-dioxolane-4-methanol (30).

The carbinol (30) used in this experiment was obtained at 30% hydrolysis and showed $[\alpha]_D^{20}$ +7.8° (c 2, $CHCl_3$). The oxidation of (30) to (31) was performed as reported above, starting from (26), and afforded the aldehyde (31), $[\alpha]_D^{20}$ +10.7° (c 1, EtOH) (lit[21] +11.9°) in 45% yield. ^1H NMR (90 MHz, $CDCl_3$) δ 1.02 (3H, t, J 7.5Hz), 1.38 (3H, s), 1.45-1.60 (2H, m), 1.50 (3H, s), 1.58 (3H, s), 3.78 (1H. dd, J 8.5 and 3.7 Hz), 9.63 (1H, s). Anal. Calcd for $C_9H_{16}O_3$: C, 62.76; H, 9.36. Found: C, 62.71; H, 9.25.

(R) 3-Tosyloxy-1,2-propanediol (33) from (4S) 2,2-diethyl-1,3-dioxolane-4-methanol (32).

The carbinol (32) obtained at ca. 50% hydrolysis showed $[\alpha]_D^{20}$ +2° (c 2, $CHCl_3$). A solution of carbinol (32) (0.8 g, 5 mmol) in 25 mL of dry pyridine was treated at 0 °C for 2 days with 4-toluene sulfonyl chloride (2.75 g, 15 mmol). The reaction mixture was poured into ice/water and extracted (2x150 mL) with Et_2O. The organic phase was washed with cold 1% HCl (3x50 mL), 3% $NaHCO_3$ (2x50 mL), water and dried (Na_2SO_4). The thick oil obtained upon evaporation of the solvent was taken up in acetone (25 mL) and 1N HCl (25 mL) and heated on a steam bath for 1h. The resulting solution was concentrated to dryness and the residue was dissolved in CH_2Cl_2 (80 mL). After drying (Na_2SO_4) and concentration, the resulting oil solidified upon standing. Residual solvents were removed at 40 °C and 0.5 mm Hg over 16h to give (R) 3-tosyloxy-1,2-propanediol (33) (0.95 g, 75%), $[\alpha]_D^{20}$ -2.8° (c 5, MeOH) (lit.[22] -9.3°). ^1H NMR ($CDCl_3$)δ 2.4 (3h, s), 3.3-4.3 (7H, m), 7.35 and 7.8 (4H, 2d, J 8Hz).

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