Pergamon

Tetrahedron: Asymmetry, Vol. 7, No. 5, pp. 1237-1240, 1996

0957-4166/96 $15.00 + 0.00

S0957-4166(96)00131-0

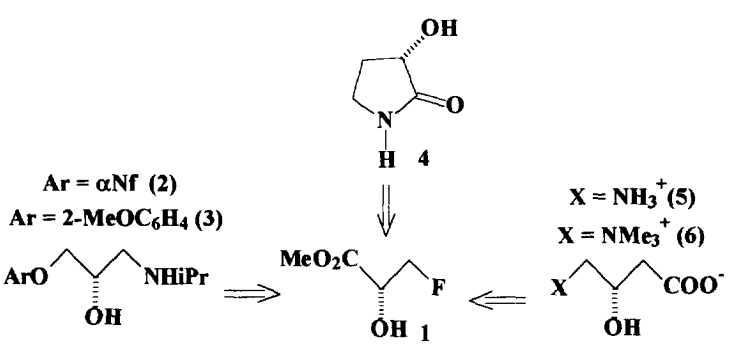
Enzymatic Laboratory Scale Production of Homochiral (*R*)-3-Fluorolactic Acid Methyl Ester via Enantiospecific Reduction of Sodium Fluoropyruvate Catalyzed by Rabbit Muscle L-Lactate Dehydrogenase (L-LDH).

L. P. B. Gonçalves; O. A. C. Antunes;* G. F. Pinto; E. G. Oestreicher.*
Instituto de Química, UFRJ, Cidade Universitária CT Bloco A, Rio de Janeiro, RJ 21945-970, Brazil.

Abstract: In the present work a simple laboratory procedure for the synthesis of (*R*)-3-Fluorolactic Methyl Ester(**1**) is described. A coupled enzymatic system formed by rabbit muscle L-lactate dehydrogenase (L-LDH), horse liver alcohol dehydrogenase (HLADH), fluoropyruvic acid sodium salt, NAD^+ and cis-1,2-bis(hydroxymethyl)cyclohexane were found to be very effective for production of (**1**) in 80% overall yield and ee > 99%. Copyright © 1996 Elsevier Science Ltd

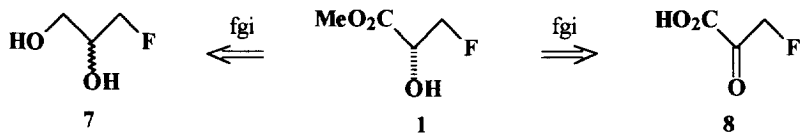
The enzymatic approach to chiral building blocks is a very important synthetic methodology due to the peculiar characteristics of the biological catalysts used for these purposes,[1] especially their enantio-, regio- and quimiospecificities. The enzymatic systems also show great efficiency under mild reaction conditions and are considered to be safe in terms of solvent disposal. The objective of the present work was the construction of a chiral synthetic equivalent[2] of glycerol. Three carbon chiral blocks shows great chemical versatility, as has been shown with other three carbon equivalents (*i. e.* (*R*)- and (*S*)-glyceraldehyde).[3]

The choice of (*R*)-3-fluorolactic methyl ester(**1**) as the target product of our process was based on its potential use as a precursor[4a,b] for (S)-propranolol(**2**),[4c-e] (S)-moprolol(**3**), (S)-3-hydroxypirrolidin-2-one(**4**), (S)-GABOB(**5**), and (S)-carnitine(**6**). Synthesis of these compounds is presently the goal of several groups.[4f-i] Since the use of L-LDH for production of **4** was recently reported,[5] and because we believe that transformation of **1** to **4** could be easily accomplished we have decided to disclose our results.



Although fluoroketones are believed to act as enzyme inhibitors,[6] several examples of enzymatic reactions of fluorocompounds have been described in the literature. [7a] As a very closely related example, the enzymatic production of **1** using fluorocompounds as starting material can be mentioned. [7b] The kinetic resolution of diol **7** by horse liver alcohol dehydrogenase (HLADH), followed by oxidation of the resulting hydroxyaldehyde catalyzed by aldehyde dehydrogenase (AldDH), with the use of glutamate dehydrogenase (GluDH) as a NADH recycling enzyme yielded the desired *(R)*-3-fluorlactic acid. Since this process

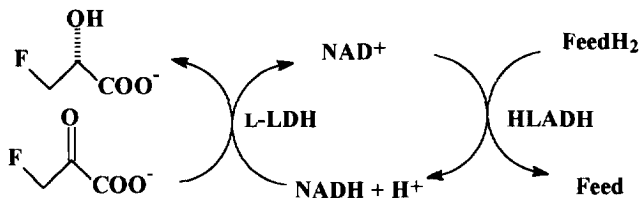
presents *a priori* the inconvenience that the theoretical yield can only be 50%, rabbit muscle L-lactate dehydrogenase (L-LDH, EC 1.1.1.27) was chosen as an enzyme able to produce **1** from **8**, in 100% yield.



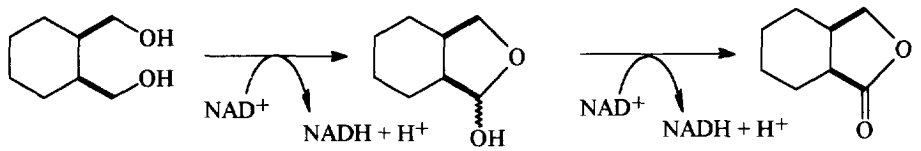
7 1 8

Kim and Whitesides[8] showed that **8** is a fairly good substrate for this enzyme (k_{cat}/K_m is about 22% of that determined for the natural substrate, pyruvate). These authors with their classical screening work determined the kinetic parameters (k_{cat} and K_m) of L-LDH from different sources for several α-ketoacids and showed that this enzyme is very useful in preparative scale enantiospecific reductions of some pyruvic acid derivatives. Although in this work, 3-halopyruvic acids were only used as substrates for estimation of the kinetic parameters, Hirschbein and Whitesides[9] showed earlier the use of rabbit muscle L-LDH in the multigram scale synthesis of (*R*)-3-chlorolactic acid with ee > 97%.

NADH is a very expensive reagent. To be used in stoichiometric concentrations with the prochiral substrate of the dehydrogenase, this coenzyme must be used in catalytic amounts to permit the economical viability of the enzymatic process. To achieve a good reaction condition, a convenient method to recycle the coenzyme is of fundamental importance. Therefore, several methods to recycle the coenzyme are described in the literature.[10a-f] For the enantioselective reduction of pyruvic acid derivatives catalyzed by L-LDH, Kim and Whitesides[8] and Hirschbein and Whitesides,[9] used a formate dehydrogenase(FDH)/ formate system to accomplish this task. Since FDH is an expensive enzyme, and also because HLADH very efficiently catalyzes the oxidation of several monocyclic mesodiols in the presence of NAD^+ leading to the production of the respective chiral lactones,[10b] we decided to test this system to recycle NADH. We then compared the mesodiol/ HLADH system with the ethanol/ HLADH system due to the simplicity and lower cost of the latter.



The first system studied involved reduction of pyruvic acid sodium salt coupled to the oxidation of ethanol to acetaldehyde,[11-14] in closed and open vessels, obtaining 93% and 100% conversion, respectively, after 8h of reaction. The better performance observed in open vessels was attributed to the partial evaporation of acetaldehyde, a well known mixed-type inhibitor of HLADH with respect to ethanol.[10c] When *cis*-1,2-bis(hydroxymethyl)cyclohexane (BHMC) (**9**) was used, 100% conversion was achieved in 3h of reaction under the same experimental conditions.[14a,b] This was not an unexpected result since it has been shown that the transformation of **9** into **11** presents better thermodynamic ($\Delta G^{\circ\prime}$) and kinetic (K_m / K_i) properties than the ethanol/ acetaldehyde reaction.[15a,b] Furthermore, this system allows the recycling of two equivalents of NAD^+ for each mol of BHMC (**9**) oxidized, thereby providing additional driving force to the desired reaction.



9 BHMC **10** **11**

Once defined as an efficient system to recycle NADH using sodium pyruvate as a model substrate, the enzymatic reduction of 3-fluoropyruvic acid sodium salt (3.25 mmols) catalyzed by L-LDH was undertaken, and after 9 h of reaction 100 % conversion was attained. Convenient work up of the reaction medium,[16] followed by CH_2N_2 methylation furnished **1** in 80% yield and ee > 99%.[16] Since the reduction of pyruvic acid derivatives catalyzed by L-LDH occurs enantiospecifically, the absolute configuration of the α-C must be the same for all the products.[8,9] Based on this fact, the configuration of **1** was considered to be (*R*).

This report shows that we have developed a very efficient enzymatic system to produce (*R*)-3-fluorolactic acid methyl ester, which constitutes a very useful chiral building block with several applications in organic synthesis. The process disclosed here can be followed by any synthetic laboratory, increasing the number of suitable chiral blocks available to synthetic chemists.

Acknowledgments: Financial support (grant no. 523227/94-9RE) from the Brazilian National Research Council (CNPq) as well as from Brazilian Ministry of Science and Technology (PADCT/ CNPq) is acknowledged. A fellowship from the Brazilian Ministry of Education (CAPES) to LPBG is also acknowledged. We also wish to express our gratitude to "Núcleo de Pesquisas de Produtos Naturais- UFRJ" , "Fundação Instituto Oswaldo Cruz" , and "Instituto de Química- UFF" for runing NMR analyses, as well as to Professor Maria da Conceição K. V. Ramos and LADETEC/IQ/UFRJ for runing GC and GC/MS analyses.

References and Notes:

1. a) Jones, J. B. *Tetrahedron* **1986**, *42*, 3351-3403; b) Hummel, W. ; Kula, M.-R. *Eur. J. Biochem.* **1989**, *184*, 1-13; c) Santaniello, E. ; Ferraboschi, P. ; Grisenti, P. ; Manzocchi, A. *Chem. Rev.* **1992**, *92*, 1071-1140; d) Faber, K. "Biotransformations in Organic Chemistry", Springer, Berlin, 1992. e) Kvittingen, L. *Tetrahedron* **1994**, *50*, 8253-8274; f) Wong, C.-H. ; Whitesides, G. W. "Enzymes in Synthetic Organic Chemistry", Elsevier, Oxford, 1994. g)Azerad, R. *Bull. Soc. Chim. Fr.* **1995**, *132*, 17-51. h) Fang, J.-M. ; Lin, C.-H. ; Bradshaw, C. W. ; Wong, C.-H. *J. Chem. Soc. Perkin Trans.1* **1995**, 967-978.

2. Corey, E. J. ; Cheng, X.-M. "The Logic of Chemical Synthesis"; John Wiley and Sons; New York; 1989.

3. Hanessian, S. "Total Synthesis of Natural Products The 'Chiron' Approach"; Pergamon Press; Oxford; 1983.

4. a)Harris, H.D. *J. Org. Chem.* **1963**, *28*, 745; b) Danilewicz, J.C.; Kemp, J.E.G. *J. Med. Chem.* **1973**, *16*,168; c) Fuganti, C.; Grasselli, P.; Seneci, P.F.; Servi, S.; Casati, P. *Tetrahedron Lett.* **1986**, *27*, 2061; d) Kan, K.; Miyama, A.; Hamaguchi, S.; Oshashi, T; Watanabe, K. *Agric.Biol.Chem.* **1985**, *49*, 207; e) Sasai, H.; Itoh, N.; Suzuki, T.; Shibasaki, M. *Tetrahedron Lett.* **1993**, *34*, 855. f) Ader, U.; Schneider, M.P. *Tetrahedron: Asymmetry* **1992**, *3*, 201; g) Ader, U.; Schneider, M.P. *Tetrahedron: Asymmetry* **1992**, *3*, 205; h) Ader, U.; Schneider, M.P. *Tetrahedron: Asymmetry* **1992**, *3*, 521; i) Klunder, J.M.; Ko, S.Y.; Sharpless, K.B. *J.Org.Chem.* **1986**, *51*, 3710.

5. Bentley, J. M. ; Wadsworth, H. J. ; Willis, C. L. *J. Chem. Soc. Chem. Commun.* **1995**, 231-232.

6. a) Bernardi, R. ; Bravo, P. ; Cardillo, R. ; Ghiringhelli, D. ; Resnati, G. *J. Chem. Soc. Perkin Trans.1* **1988**, 2831; b) Bernardi, R. ; Bravo, P. ; Cardillo, R. ; Ghiringhelli, D. ; Resnati, G. *J. Chem. Soc. Perkin Trans.1* **1990**, 579.

7. a) Bravo, P. ; Resnati, G. *Tetrahedron:Asymmetry* 1990, *1*, 661-692. b) Wong, C.-H. ; Matos, J. R. *J. Org. Chem.* **1985**, *50*, 1992.

8. Kim, M.-J. ; Whitesides, G. M. *J. Am. Chem. Soc.* **1988**, *110*, 2959-2964.
9. Hirschbein, B. L. ; Whitesides, G. M. *J. Am. Chem. Soc.* **1982**, *104*, 4458-4460.
10. a) Shaked, Z. ; Whitesides, G. M. *J. Am. Chem. Soc.* **1980**, *102*, 7104; b) Jakovak, I. J. ; Goodbrand, H. B. ; Lok, K. P. ; Jones, J. B. *J Am. Chem. Soc.* **1982**, *104*, 4659-4665; c) Lee, L. ; Whitesides, G. M. *J. Am. Chem. Soc.* **1985**, *107*, 6999-7008; d) Wong, C.-H. *Science* **1989**, *244*, 1145-1152; e) Itozawa, T. ; Kise, H. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 3304; f) Pereira, D. A. ; Pinto, G. F. , Oestreicher, E. G. *J. Biotechnol.* **1994**, *34*, 43-50.
11. Paiva, L. M. C. ; Pinto, G. F. , Oestreicher, E. G. *Biom. Biochim. Acta* **1991**, *50*, 25-29.
12. L-LDH assays were performed in phosphate buffer, pH 7.2,[13]. HLADH assays were performed essentially as described in reference 11.
13. Zewe, V.; Fromm, H. J. *J. Biol. Chem.* **1962**, *237*, 1668-1675.
14. a) Alcohol dehydrogenase from horse liver (EC 1.1.1.1), L-lactate dehydrogenase from rabbit muscle (EC 1.1.1.27), crystalline suspended in 2.1 M $(NH_4)_2SO_4$ solution at pH 6.1, NAD^+ (grade III-C) and bovine serum albumin (BSA) (Fraction V) were purchased from Sigma Chemical Company. BHMC, Diazald® kit and reagent and ethyl alcohol (spectrophotometric UV grade) were products from Aldrich Chemical Company. All other reagents used were of analytical grade obtained from E. Merck, Darmstadt, Germany. b) Reaction mixtures contained in a total volume of 123 mL: 0.1 M phosphate buffer, pH 7.2, 20 mM pyruvic (or 3-fluoropyruvic) acid sodium salt, 29 mM ethanol (or 15 mM BHMC), 700 International Units (IU) of L-LDH, 37 IU of HLADH and 0.1 % (w/v) BSA. Reaction mixtures were maintained under magnetic stirring and the temperature was kept at 25°C by circulating water with a thermocirculating bath. Reactions were started by adding 0.1 mM NAD^+. The extent of the reaction was measured by removing at different times aliquots (10 - 50 μL) of the reaction mixtures, that were immediately diluted with 1 mL of 0.1 M phosphate buffer, pH 7.2 and heated at 100°C for 5 min in order to inactivate the enzymes. The concentration of pyruvate (or 3-fluoropyruvate) present in these samples was determined by "end point"assays in the presence of 0.25 mM NADH and 14 IU of L-LDH in a total volume of 2.5 mL at 25°C. The decrease of NADH absorption was followed at 340 nm before and after the addition of the enzyme in silica cuvettes with a 1 cm light path using a Beckman DU 70 recording spectrophotometer until total consumption of the substrate. The concentration of pyruvate (or 3-fluoropyruvate) was calculated from the difference of NADH absorption (before and after the addition of L-LDH) by using a molar absorption coefficient for NADH of 6220 $M^{-1}cm^{-1}$.
15. a) $\Delta G^{0'}$ for the oxidation reaction of **9** to **11** was estimated to be 1787 cal/ mol, based on the values of: I) oxidation of ethanol to acetaldehyde, $\Delta G^{0'}$= 6930 cal/ mol (Bergmayer, H. U.; Grabe, M. ; Walter, H. E. "Biomedical Reagent for General Use" *In* "Methods of Enzymatic Analysis", vol. 2, pp 139-140, Bergmayer, H. U. (Ed.), VCH Verlagsgesellschaft, Weinhein, 1983) ; II) cyclization of D-glucose to D-glucopyranose, $\Delta G^{0'}$= -5043 cal/ mol (Conn, E. E. ; Stumpf, P. K. "Outlines of Biochemistry", 2nd. ed. , John Wiley & Sons, New York, p38, 1967) ; III) oxidation of D-glucose to D-gluconolactone, $\Delta G^{0'}$= -100 cal/ mol (Mahler, H. R. ; Cordes, E. H. "Biological Chemistry", 2nd. ed. ,p532, Harper Int. Ed. , New York, 1971. b) K_m/K_i= 0.3 for the system BHMC (**9**)/ lactone (**11**) and, K_m/K_i = 3 for the ethanol/acetaldehyde system.[10c] K_m is the Michaelis constant for the substrate (BHMC (**9**) or ethanol) and K_i is the inhibition constant of **11** or acetaldehyde, as defined by Lee and Whitesides.[10c]
16. Work up was conducted by extraction of BHMC (**9**) and corresponding lactone (**11**) from the aqueous phase with $CHCl_3$, after thermal treatment of reaction medium (100°C, 5 min), followed by HCl (1 M) addition until pH 1.5, and continuous extraction with Et_2O (72 h), and esterification with CH_2N_2. An overall yield of 80% and an ee greater than 99% was obtained. ^1H NMR spectrum showed the following signals: δ= 3,85 (3H, s, CH_3); 4,40 (1H , dt , J_{CHCF}=30 Hz , J_{CHCH2} = 3 Hz); 4,70 (2H , ddd , J_{CH2F} = 47 Hz , $J_{CH2(gem)}$ = 0,5 Hz , J_{CH2CH} = 3 Hz), which agreed with literature.[7b] Chiral high resolution gas chromatographic analysis was performed on a capillary column (30 m) coated with 2,3,6-tri-*O*-metil-β-ciclodextrin (PMCD/OV1701-OH), isothermally at 70°C. A sample of the corresponding racemate obtained by $NaBH_4$ reduction was used to test the column, and an almost base line resolution was obtained, thus showing two peaks of almost the same area. The chiral sample showed no trace of the first peak (ee > 99%) and GC co-elution, and GC-MS analysis confirmed these results. ^1H NMR analysis of the chiral sample in the presence of a chiral shift reagent showed no trace of the other enantiomer. $[\alpha]^{25}_D$= -5 (c=1, EtOH).

(*Received in USA* 15 *February* 1996; *accepted* 20 *March* 1996)