Sequential biocatalytic resolution of (±)-*trans*-cyclohexane-1,2-diamine. Chemoenzymatic synthesis of an optically active polyamine

Ignacio Alfonso, Covadonga Astorga, Francisca Rebolledo and Vicente Gotor*

Departamento de Química Orgánica e Inorgánica, Universidad de Oviedo, 33071 Oviedo, Spain

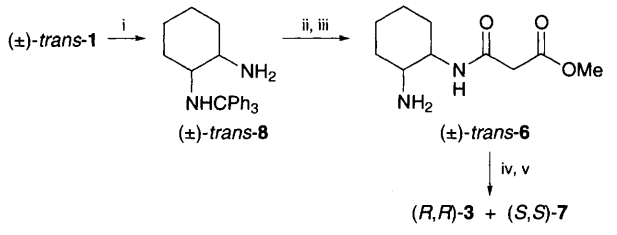
***Candida antarctica* lipase-catalysed double monoaminolysis of dimethyl malonate by (±)-*trans*-cyclohexane-1,2-diamine allows the sequential resolution of the latter compound, affording an enantiopure bis(amidoester), (*R*,*R*)-3, which is subsequently transformed into an optically active polyamine, (*R*,*R*)-9.**

Hydrolytic enzymes such as lipases have been widely used in simple, single-step, kinetic resolutions of racemates.[1] However, if the substrate structure allows the coupling of two sequential kinetic resolutions, the second step can improve the enantiomeric purity of the product. This strategy has been successfully used for the preparation of optically active binaphthols[2] and pentane-2,4-diols.[3] Racemic diamines are other interesting bifunctional substrates which can undergo a sequential biocatalytic resolution. With a suitable acyl donor, an appropriate enzyme can catalyse the enantioselective monoacylation of the diamine and the subsequent enantioselective acylation of the resulting optically enriched monoamide, thus affording a diamide of a higher enantiomeric purity. Here we report the resolution of (±)-*trans*-cyclohexane-1,2-diamine [(±)-*trans*-**1**] by means of two sequential aminolysis reactions, using dimethyl malonate **2** as the acyl donor and *Candida antarctica* lipase (CAL) as the catalyst. Our interest in enantiopure *trans*-cyclohexane-1,2-diamine stems from its usefulness as chiral auxiliary in asymmetric synthesis,[4] as well as from its biological and medicinal importance.[5] The selection of the acyl donor is related to the synthetic utility of the resulting products for the preparation of optically active polyamines. CAL is the catalyst of choice owing to its well-documented activity in aminolysis processes,[6] and particularly in the preparation of optically active diamides from propane-1,2-diamine.[7]

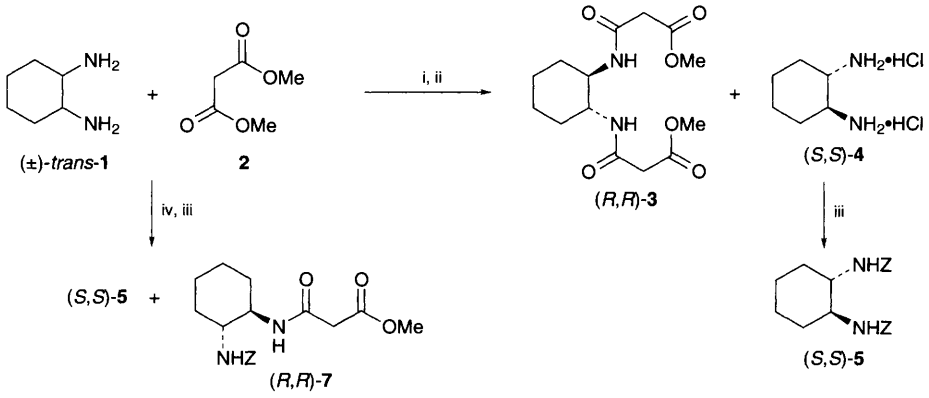
Exposure of an equimolecular mixture of (±)-*trans*-**1** and **2** to CAL in 1,4-dioxane,† until disappearance of the diester (7 h), affords the enantiopure bis(amidoester) (*R*,*R*)-**3**‡ (Scheme 1). The unreacted, air-sensitive (*S*,*S*)-**1** is isolated as its ammonium salt, (*S*,*S*)-**4**, formed by treatment of the reaction mixture with dry HCl. To determine the optical purity of (*S*,*S*)-**1**, its salt (*S*,*S*)-**4** is converted into the *N*,*N*′-bis(benzyloxycarbonyl) derivative (*S*,*S*)-**5**, whose chiral HPLC analysis shows an 83% ee.§

The formation of (*R*,*R*)-**3** involves two biocatalytic steps [diamine to mono(amidoester) to bis(amidoester)] and, therefore, the above mentioned enantiopurity of (*R*,*R*)-**3** (for a conversion of *ca.* 50%)¶ has to be clearly determined by the enantioselectivities of both steps. In order to investigate the enantioselectivity exhibited by the CAL in each step, we have calculated E_1 and E_2 values. Since the disappearance of the diamine is influenced only by the first aminolysis, E_1 could be calculated from the ee of the remaining (*S*,*S*)-**1** and the percentage of overall conversion (*c*).[8] However, the easy oxidation of **1**, as well as the final persistence of trace amounts of mono(amidoester) (detected in the derivatization process of **4**), make the accurate determination of *c* difficult. For these reasons, E_1 is determined when a 2 : 1 molar ratio of diamine : acyl donor is used and the reaction is stopped at the mono(amidoester) stage (Scheme 1). In this process, substrate **1** and product **6** are actually isolated as their carbamates (*S*,*S*)-**5** and (*R*,*R*)-**7**, with 33% and 94% ee, respectively.‖ Conversion (c = 26%) and enantioselectivity (E_1 = 45) are determined from these values.[8]

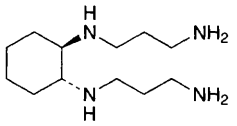
Enantioselectivity of the second step (E_2) is also determined from the single-step aminolysis reaction between racemic mono(amidoester) **6** and dimethyl malonate **2** (Scheme 2).



Scheme 2 *Reagents and conditions*: i, Ph_3CCl, Et_3N, CH_2Cl_2; ii, $ClC(O)CH_2CO_2Me$, Et_3N, CH_2Cl_2; iii, HCO_2H, Pd-black, MeOH; iv, **2** [0.5 mol mol^{-1} of (±)-*trans*-**6**], CAL, 1,4-dioxane; v, ZCl, Na_2CO_3



Scheme 1 *Reagents and conditions*: i, 1 : 1 molar ratio of (±)-*trans*-**1** : **2**, CAL, 1,4-dioxane; ii, HCl (g); iii, ZCl, Na_2CO_3, H_2O; iv, 2 : 1 molar ratio of (±)-*trans*-**1** : **2**, CAL, 1,4-dioxane, 1 h (Z = $PhCH_2OCO$)



(*R,R*)-**9**

Compound (±)-*trans*-**6** is prepared by standard reactions: monotritylation of (±)-*trans*-**1**, treatment with malonyl chloride monomethyl ester and, finally, removal of the trityl group. Enzymatic aminolysis of (±)-*trans*-**6** and **2** (molar ratio 2 : 1) yields a mixture of (*S,S*)-**6**, isolated as its carbamate, (*S,S*)-**7**, and the bis(amidoester) (*R,R*)-**3**, with 33 and 96% ee, respectively. Conversion (c = 26%) and enantioselectivity (E_2 = 68) are determined from these values.[8]

It should be pointed out that CAL shows the same stereochemical preference towards the *R,R* enantiomer of the substrate in both steps. This fact, besides the strategy of the sequential resolution, are the keys to the successful preparation of enantiopure (*R,R*)-**3**. As it can be deduced of the above results, enantiopure products cannot be obtained with single-step biocatalytic processes with E values of 45 or 68.

On the other hand, since E_1 value is high, diamine (*S,S*)-**1** can be obtained with very high ee if the reaction is carried out with an excess of **2** (molar ratio 1 : 1.2) and it is allowed to react beyond 50% of conversion. In fact, enantiopure (*S,S*)-**1** (for a conversion of 57%)** and (*R,R*)-**3** with 97% ee are obtained after 9 hours of reaction. A small amount of mono(amidoester) is also detected but it cannot be isolated.

Finally, the enantiopure (*R,R*)-**3** is easily transformed into the polyamine (*R,R*)-**9**. Polyamines are interesting compounds which play major roles in cellular differentiative and proliferative processes.[9] Treatment of (*R,R*)-**3** with ammonia in methanol and subsequent reduction of the resulting tetraamide with BH_3·THF afford polyamine (*R,R*)-**9** with 82% yield,†† calculated from the starting bis(amidoester) (*R,R*)-**3**.

We are grateful to the Comisión Interministerial de Ciencia y Tecnología (Project BIO 95-0687) for financial support. I. A. thanks to the Ministerio de Educación y Ciencia for a predoctoral scholarship. We also thank Novo Nordisk Co. for the generous gift of the CAL.

Footnotes

† All the enzymatic reactions are carried out with 60 mg of immobilised CAL (NOVOZYM 435)® and 4 ml of 1,4-dioxane for each mmol of nitrogen substrate (typical scale 5 mmol); the amount of acyl donor is specified in each case.

‡ Enantiomeric excess for compound **3** is determined by chiral HPLC analysis using a CHIRALCEL-OD column (4.6 × 250 mm), a mixture hexane–ethanol 85 : 15 as eluent, and a flow rate of 0.8 cm^3 min^{-1}. For (±)-*trans*-**3** two well resolved peaks (t_R 4.2 and 5.6 min) are detected. For enantiopure (*R,R*)-**3** {$[\alpha]_D^{20}$ + 57.0 (c 0.50, $CHCl_3$)} only one peak (t_R 5.6 min) is observed. (*R,R*)-Configuration is assigned by comparison of the sign of its optical rotation with that of a sample obtained from the commercially available (*R,R*)-cyclohexane-1,2-diamine.

§ Determined with a CHIRALCEL-OD column, hexane–ethanol 90 : 10 as eluent, flow rate of 0.8 cm^3 min^{-1}, t_R 4.6 min. For (±)-*trans*-**5** two well resolved peaks (t_R 4.6 and 5.6 min) are observed. Specific rotations: (*S,S*)-**5**, $[\alpha]_D^{25}$ −6.6 (c 0.59, $CHCl_3$), 83% ee; (*S,S*)-**4**, $[\alpha]_D^{25}$ + 13.3 (c 0.46, H_2O).

¶ This conversion is estimated from the complete disappearance of the diester **2**, and taking into account that only a trace of mono(amidoester) is detected (see below in the text).

‖ The enantiomeric excess for compound {(*R,R*)-**7** ($[\alpha]_D^{25}$ + 8.0 (c 0.45, $CHCl_3$), 94% ee} is also determined by chiral HPLC analysis (CHIRALCEL-OD column, hexane–ethanol 95 : 5 as eluent, flow rate of 0.8 cm^3 min^{-1}, t_R 20.8 min). For (±)-*trans*-**7** two well resolved peaks (t_R 18.2 and 20.8 min) are detected.

** Calculated from E_1 value and the ee of (*S,S*)-**1** (see ref. 8).

†† Polyamine (*R,R*)-**9** is stored as its tetrahydrochloride. ^{13}C NMR (75 MHz, D_2O) for (*R,R*)-**9**·4HCl δ 22.2 (CH_2), 24.5 (CH_2), 26.2 (CH_2), 37.2 (CH_2), 43.4 (CH_2), 58.2 (CH). MS (70 eV) *m/z* for (*R,R*)-**9**: 228 (M^+, < 1), 154 (100).

References

1 K. Faber, *Biotransformations in Organic Chemistry*, 2nd edn., Springer, New York, 1995; F. Theil, *Chem. Rev.*, 1995, **95**, 2203.
2 R. J. Kazlauskas, *J. Am. Chem. Soc.*, 1989, **111**, 4953.
3 Z.-W. Guo, S.-H. Wu, C.-S. Chen, G. Girdaukas and C. J. Sih, *J. Am. Chem. Soc.*, 1990, **112**, 4942.
4 For some recent applications see: B. M. Trost and R. C. Bunt, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 99; Y. Belokon, N. Ikonnikov, M. Moscalenko, M. North, S. Orlova, V. Tararov and L. Yashkina, *Tetrahedron: Asymmetry*, 1996, **7**, 851; J. L. Leighton and E. N. Jacobsen, *J. Org. Chem.*, 1996, **61**, 389; Y. Nishibayashi, Y. Arikawa, K. Ohe and S. Uemura, *J. Org. Chem.*, 1996, **61**, 1172.
5 S. S. Yoon and W. C. Still, *J. Am. Chem. Soc.*, 1993, **115**, 823; J. Reedijk, *Chem. Commun.*, 1996, 801.
6 For the resolution of esters see: M. J. García, F. Rebolledo and V. Gotor, *Tetrahedron: Asymmetry*, 1993, **4**, 2199; M. C. Zoete, A. C. Kock-van Dalen, F. Rantwijk and R. A. Sheldon, *J. Chem. Soc., Chem. Commun.*, 1993, 1831; M. C. Zoete, A. A. Ouwehand, F. Rantwijk and R. A. Sheldon, *Recl. Trav. Chim. Pays-Bas*, 1995, **114**, 171. For the resolution of amines see: M. J. García, F. Rebolledo and V. Gotor, *Tetrahedron*, 1994, **50**, 6935; M. T. Reetz and C. Dreisbach, *Chimia*, 1994, **48**, 570; C. Chamorro, R. González-Muñiz and S. Conde, *Tetrahedron: Asymmetry*, 1995, **6**, 2343.
7 C. Astorga, F. Rebolledo and V. Gotor, *J. Chem. Soc., Perkin Trans. 1*, 1994, 829.
8 C.-S. Chen, Y. Fujimoto, G. Girdaukas and C. J. Sih, *J. Am. Chem. Soc.*, 1982, **104**, 7294; C.-S. Chen and C. J. Sih, *Angew. Chem., Int. Ed. Engl.*, 1989, **28**, 695.
9 R. A. Campbell, D. R. Morris, D. Bartos, G. D. Daves Jr., and F. Bartos, in *Advances in Polyamine Research*, Raven Press, New York, 1978, vols. 1 and 2; S. H. Goldenburg and J. D. Algranati, *The Biology and Chemistry of Polyamines*, JCSU Press, Oxford, 1990.

Received, 30th July 1996; Com. 6/05322J