LIPASE-MEDIATED ROUTE TO DIASTERO-PURE TRANEXAMIC ACID

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Diastereomerically pure tranexamic acid has been prepared via a diastereomeric separation of a trans/cis-mixture of 1,4-cyclohexanedicarboxylic acid using lipase PS (Pseudomonas sp. Amano).

KEY WORDS tranexamic acid; diastereomeric separation; lipase-mediated transesterification; lipase-mediated hydrolysis; antiplasmin activity

Tranexamic acid, trans-4-aminomethylcyclohexanecarboxylic acid (1), is a clinically important compound being used as a potent antiplasmin agent.1) Because its synthesis owes to hydrogenation of an aromatic precursor,2) it is always accompanied by the inseparable cis-diastereomer which has been found to be 50 times less effective.1a,b) We wish to report here with a diastereoselective route to pure tranexamic acid (1) from a trans/cis-mixture of 1,4-cyclohexanedicarboxylic acid (t/c-2) via a lipase-mediated diastereomeric separation.3)

\[ \text{tranexamic acid (1)} \quad \rightarrow \quad \text{t/c-2} \]

Chart 1

Recently, a base-induced equilibrium between trans- and cis-1,4-cyclohexanedicarboxylic acids (2) was reported to give a trans-enriched mixture.4) This led us to anticipate that the commercially available trans/cis-mixture (t/c-2) will be a vital precursor for pure tranexamic acid (1) if the mixture is readily separable after the equilibrium. Thus, we first examined its separation by application of the enzymatic transesterification procedure in an organic solvent.5) The mixture (t/c-2) (t/c=70:30), on reaction with vinyl acetate in tert-butyl methyl ether in the presence of lipase PS (Pseudomonas sp., Amano), furnished a readily separable mixture of the trans-enriched diacetate (t-3) (t/c=94:6) and the cis-enriched monoacetate (4) (t/c=17:83) in 74 and 10% yields, leaving the pure cis-diol (c-2) in 15% yield after 48 h.6) The pure trans-diacetate (t-3) could be obtained in 77% yield, accompanied by the monoacetate mixture (4) (t/c=63:37) (13%) and the diol mixture (2) (t/c=48:52) (5%), by a single repetition of the same enzymatic treatment on the trans-enriched diol (t-2) obtained from trans-enriched diacetate (t-3). Overall yield of the pure trans-diol (t-2) from the starting diastereomeric diol (t/c-2) after a twice-repeated

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enzymatic operation was 57%. The pure trans-acetate (t-3) thus obtained afforded pure trans-diol (t-2) in quantitative yield on methanolysis in the presence of potassium carbonate at room temperature.

\[
\begin{align*}
\text{t/c-2 (70:30)} & \xrightarrow{\text{lipase PS \, tert-BuOMe \, room temp. \, 48 h}} \text{AcO} & \text{t-3 (94:6) 74\%} & + \text{HO} & \text{c-4 (83:17) 10\%} & + \text{HO} & \text{c-2 (>99:1) 15\%}
\end{align*}
\]

Chart 2

We next examined the lipase-mediated hydrolysis of the diacetate mixture (t/c-3) in aqueous condition. It is well recognized that when the same lipase is used, both the transesterification and the hydrolysis occur at the same chiral center.\(^7\) The same is true for the diastereomeric mixture (t/c-3) (trans/cis=70:30), which afforded the pure trans-diol (t-2) in 66% yield accompanied by 31% yield of the cis-enriched monoacetate (c-4) (trans/cis=16:84) in the presence of lipase PS in a phosphate buffer solution,\(^8\) though the process took 7 days. Thus, it was concluded that hydrolytic conditions were more appropriate for the separation of pure trans-diol (t-2).

\[
\begin{align*}
\text{AcO} & \xrightarrow{\text{lipase PS \, phosphate buffer- \, acetone \, room temp. \, 7 days}} \text{t-2 (>99:1) 66\%} & + \text{HO} & \text{c-4 (84:16) 31\%}
\end{align*}
\]

Chart 3

The pure cis-diol (c-2) as well as the mixture diol (t/c-2) recovered from the mixture acetates (t/c-3) and (t/c-4) could be equilibrated to the trans-enriched mixture (t/c=4:1), which may be recycled. Thus, the pure cis-diol (c-2) was fused at 180 °C with sodium hydroxide (0.1 eq) for 15 min\(^4\) to furnish a mixture consisting of 82 parts of the trans-2 and 18 parts of the cis-2 in 92% yield after direct distillation (\~165 °C/5 Torr) from a reaction flask.

Having obtained pure trans-1,4-cyclohexanediolmethanol (t-2), we converted it to tranexamic acid (1). To minimize ditosylation, the trans-diol (t-2) was treated with 0.7 eq of p-toluenesulfonyl chloride to afford the monotosylate (5) in 47% yield (84% based on the consumed 2) with recovery of 44% of the unchanged 2, which was recycled. Treatment of the tosylate (5) with sodium azide gave the azide (6), in 93% yield, which on Jones oxidation afforded the carboxylic acid (7) in 87% yield. Finally, the azide
(7) was hydrogenated on 10% palladium-on-charcoal in 1% hydrochloric acid to give pure tranexamic acid (1) as hydrochloride\(^1\) in 70% yield after purification by recrystallization.

\[
\begin{align*}
\text{TsCl (0.7 eq.)} & \quad \text{Et}_{3}\text{N (1.4 eq.)} \\
\text{DMAP (cat.)} & \quad \text{CH}_{2}\text{Cl}_2 \\
\text{room temp.} & \quad 36 \text{ h} \\
\rightarrow & \quad \text{H} \\
\text{R} & \quad \text{H} \\
\text{OH} & \quad \text{H} \\
\text{Jones oxid.} & \quad \text{NaN}_3 (2 \text{ eq}) \\
\text{DMF, 60 °C} & \quad 7 \text{ h} \\
\rightarrow & \quad \text{CO}_2\text{H} \\
\text{R} & \quad \text{N}_3 \\
\text{H}_2 & \quad \text{10% Pd-C} \\
\text{1% HCl} & \quad \rightarrow 1 \cdot \text{HCl}
\end{align*}
\]

**Chart 4**

In conclusion, the present investigation has established a route to diastereomerically pure tranexamic acid (1) starting from a diastereomeric mixture of trans- and cis-1,4-cyclohexanediol (t/c-2) formally without loss of the cis-component, employing a lipase-mediated separation and a base-induced equilibration.

**REFERENCES AND NOTES**


3) A description of a lipase-mediated separation of a 1,4-cyclohexanediol mixture (t/c-2) by acylation in an organic solvent appeared while the present investigation was going on. However, neither lipase PS nor hydrolytic conditions were employed. See S. Gersh, E. Elbaz, R. Glaser, *Tetrahedron*, 49, 4939 (1993).


6) The reaction was carried out in tert-butyl methyl ether (10 ml/mmol of t/c-2) in the presence of lipase-PS-on-Celite (10 mg/mmol of t/c-2) at room temperature and separated by silica gel column chromatography. The trans/cis ratio was determined by \(^1\)H NMR analysis (300 MHz) of the diacetate (3): t-3 exhibits AcOCH\(_2\)- at \(\delta 3.46\), while the c-3 exhibits them at \(\delta 3.55\).


8) The reaction was carried out in a mixture of 0.2 M phosphate buffer and acetone (9:1) (10 ml/mmol of 3) in the presence of lipase PS-on-Celite (10 mg/mmol of 3) and separated by silica gel column chromatography.

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