Lipase-Mediated Kinetic Separation of a Diastereomeric Mixture of 4-*tert*-Butylcyclohexanemethanol

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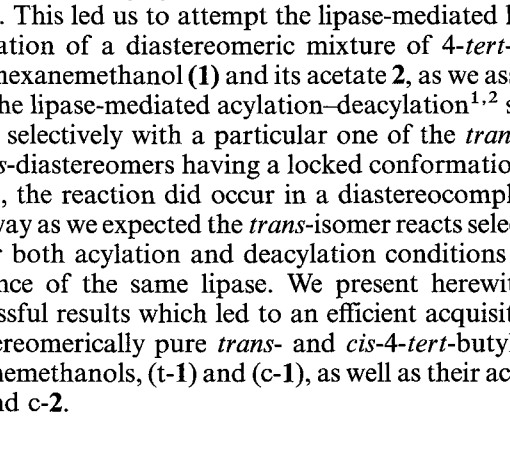
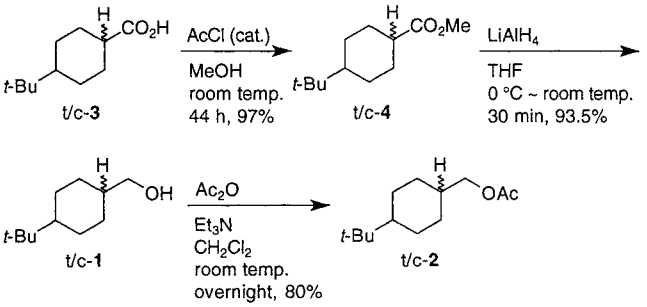
Diastereomerically pure *trans*- and *cis*-4-*tert*-butylcyclohexanemethanols have been obtained by kinetic acylation of the diastereomeric alcohol in an organic medium and by kinetic deacylation of the diastereomeric acetate in an aqueous medium both in the presence of the same lipase (lipase PS, *Pseudomonas* sp., Amano). The reactions take place preferentially with the *trans*-isomers both in organic and aqueous media to give the *trans*-acetate with recovery of the *cis*-alcohol on acylation in an organic medium, and the *trans*-alcohol with recovery of the *cis*-acetate on deacylation in an aqueous medium.

It is well recognized that a *tert*-butyl group on substituted cyclohexanes holds the cyclohexane ring in a single conformation keeping the *tert*-butyl equatorial as represented by 4-*tert*-butylcyclohexanemethanol (**1**) and its acetate **2**. This led us to attempt the lipase-mediated kinetic separation of a diastereomeric mixture of 4-*tert*-butylcyclohexanemethanol (**1**) and its acetate **2**, as we assumed that the lipase-mediated acylation–deacylation[1,2] should occur selectively with a particular one of the *trans*- and the *cis*-diastereomers having a locked conformation. Actually, the reaction did occur in a diastereocomplementary way as we expected the *trans*-isomer reacts selectively under both acylation and deacylation conditions in the presence of the same lipase. We present herewith our successful results which led to an efficient acquisition of diastereomerically pure *trans*- and *cis*-4-*tert*-butylcyclohexanemethanols, (t-**1**) and (c-**1**), as well as their acetates, t-**2** and c-**2**.

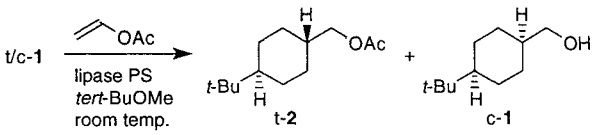


The substrate alcohol t/c-**1** was obtained in two steps in 91% overall yield as a diastereomeric mixture (t/c = 57:43) from a commercially available diastereomeric mixture (t/c = 57:43) of 4-*tert*-butylcyclohexanecarboxylic acid[3] (t/c-**3**) via methyl 4-tert-butylcyclohexanecarboxylate[4] (t/c-**4**). The alcohol mixture t/c-**1** was then transformed into the acetate substrate t/c-**2** (t/c = 57:43) in 80% yield on acetylation with acetic anhydride in the presence of triethylamine (Scheme 1). These two substrates, as well as both the acid **3** and the ester **4** mixtures could not be separated chromatographically, even on a TLC plate, and their diastereomeric ratio could be only determined by ^{1}H NMR spectra[5] (300 MHz) of the alcohol **1** and the acetate **2** in which the side-chain methylene protons appear as doublets at δ = 3.44 for t-**1**, 3.64 for c-**1**, 3.87 for t-**2**, and 4.08 for c-**2**, respectively.



Scheme 1

We first treated the alcohol mixture t/c-**1** with 25 molar excess of vinyl acetate in *tert*-butyl methyl ether[6] in the presence of lipase PS (*Pseudomonas* sp., Amano) at room temperature. The reaction was terminated after 38 hours to give the acetate **2** and the unreacted alcohol **1** in yields of 60 and 35% after separation by silica gel column chromatography (Table 1, Entry 1). ^{1}H NMR analysis revealed the acetate to be a *trans*-**2** major mixture containing 89% of the *trans*-**2** (78% de) and the alcohol being virtually the *cis*-**1** (> 99% de). The same reaction with a lesser amount of vinyl acetate (7.5 mol equiv) took a longer reaction time (100 h) to give almost the same result leaving *trans*-**2** (80% de) and *cis*-**1** (> 99% de) in comparable yields of 58 and 34% (Table 1, Entry 2). Thus, although *trans*-**2** could not be obtained in a diastereomerically pure form, a repeated treatment of the diastereomerically enriched alcohol t-**1** (91% de), obtained quantitatively from the diastereomerically enriched acetate t-**2** by base-catalyzed methanolysis, under the same treatment with five molar excess of vinyl acetate furnished the pure *trans*-acetate (t-**2**) (> 99% de) in 80% yield with the alcohol mixture c/t-**1** (53:47) in 15% yield (Table 1, Entry 3). Total recovery of the pure components from the substrate mixture t/c-**1** was estimated to be 84% for the *trans*-component (as t-**2**) by a twice-repeated operation, and to be 83% for the *cis*-component (as c-**1**) by a single operation (Scheme 2).



Scheme 2

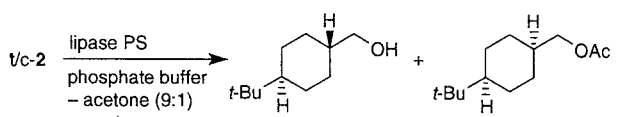
We next carried out the hydrolytic treatment on the acetate mixture t/c-**2** in a 9:1 mixture of phosphate buffer and acetone in the presence of the same lipase used above at room temperature (Scheme 3). The reaction proceeded

Table 1. Diastereoselective Acylation of a Diastereomeric Mixture of the Alcohol **1**[a]

Entry	Substrate (**1**) (*trans*/*cis*)	Vinyl Acetate (mol. equiv)	Time (h)	Products	
				Acetate (**2**) (yield) t-**2**/c-**2** (%)	Alcohol (**1**) (yield) c-**1**/t-**1** (%)
1	56 : 44	25.0	38	89 : 11 (60)	> 99 : < 1 (35)
2	56 : 44	7.5	100	90 : 10 (58)	> 99 : < 1 (34)
3	91 : 9	5.0	100	> 99 : < 1 (80)	53 : 47 (15)
4	28 : 72	3.0	80	83 : 17 (31)	> 99 : < 1 (65)

[a] Reaction was carried out at r.t. using lipase PS on Celite (10 mg/mmol of **1**) in *tert*-butyl methyl ether (10 mL/mmol of **1**).

rather slowly to give the *cis*-major acetate c-**2** (c/t = 74 : 26) and the pure *trans*-alcohol (t-**1**) (> 99 % de) in yields of 53 and 37 % even after 10 days (Table 2, Entry 2). A more satisfactory result could be obtained after 20 days to give the highly enriched *cis*-acetate c-**2** (88 % de) and the pure *trans*-alcohol t-**1** (> 99 % de) in yields of 42 and 51 % (Table 2, Entry 3). Total recovery of the pure components from the substrate mixture t/c-**2** under the latter conditions was estimated to be 91 % for the *trans*-component (as t-**1**) and 95 % for the *cis*-component (as c-**2**) in a single operation though the latter separation was a little unsatisfactory (88 % de). However, the acylative treatment of the *cis*-major alcohol (c-**1**) (t/c = 26 : 74), obtained by methanolysis from the *cis*-major acetate c-**2** (t/c = 26 : 74), with three molar excess of vinyl acetate in *tert*-butyl methyl ether in the presence of the same lipase furnished the pure *cis*-alcohol (c-**1**) (> 99 % de) in 65 % yield leaving the *trans*-major acetate t-**2** (t/c = 83 : 17) in 31 % yield (Table 1, Entry 4).



Scheme 3

Table 2. Diastereoselective Deacylation of a Diastereomeric Mixture of the Acetate **2**[a]

Entry	Substrate (**2**) (*trans*/*cis*)	Time (day)	Products	
			Alcohol (**1**) (yield) t-**1**/c-**1** (%)	Acetate (**2**) (yield) c-**2**/t-**2** (%)
1	57 : 43	4	> 99 : < 1 (15)	53 : 47 (70)
2	57 : 43	10	> 99 : < 1 (37)	74 : 26 (53)
3	57 : 43	20	> 99 : < 1 (51)	94 : 6 (42)

[a] Reaction was carried out at room temperature using lipase PS on Celite (10 mg/mmol of **2**) in a phosphate buffer–acetone mixture (9 : 1) (10 mL/mmol of **2**).

Although it is not unexpected that a lipase catalyzes both acylation and deacylation at the same particular center,[7] the present observation that both reactions occurred invariably at the equatorial side chain are worthy of note.

In conclusion, we have established an efficient method for the separation of a diastereomeric mixture of *trans*- and *cis*-4-*tert*-butylcyclohexanemethanols by enzymatic kinetic acylation–deacylation procedure using the same lipase and found that both the acylation and the deacylation have selectively occurred at the equatorial side chain in these conformationally locked substrates.

IR spectra were recorded on a JASCO-IR-700 spectrometer. ^{1}H NMR spectra were recorded on a Hitachi R-3000 (300 MHz). Mass spectra were measured on a JEOL JMS-DX303 instrument.

Diastereomeric Mixture of Methyl 4-*tert*-Butylcyclohexancarboxylate (t/c-4):
To stirred MeOH (160 mL) was added acetyl chloride (1.6 mL, 22.5 mmol) at 0 °C, then after 10 min, a diastereomeric mixture of 4-*tert*-butylcyclohexanecarboxylic acid (**3**; 5.36 g, 29.1 mmol) (Aldrich, *trans*/*cis*-mixture) was added at the same temperature and the mixture was stirred for 44 h at r.t. The mixture was evaporated under reduced pressure and the residue was dissolved in Et_2O (100 mL). The organic layer was washed with 5 % $NaHCO_3$ (100 mL × 2), brine (10 mL), dried ($MgSO_4$) and evaporated under reduced pressure. The residue was chromatographed on silica gel (90 g, eluent: EtOAc/hexane, 1 : 9) to give the methyl ester t/c-**4** as a colorless oil which was estimated to be a 57 : 43 mixture based on ^{1}H NMR analysis; yield: 5.61 g (97 %).

IR (neat): $\nu = 1737\ cm^{-1}$.

^{1}H NMR ($CDCl_3$): $\delta = 3.69$ (s, 0.43 × 3 H), 3.66 (s, 0.57 × 3 H), 2.24–1.98 (m, 2 H), 1.86–0.87 (m, 8 H), 0.84 (s, 0.57 × 9 H), 0.82 (s, 0.43 × 9 H).

MS: $m/z = 198$ (M^+), 57 (100 %)

HRMS: m/z calc. for $C_{12}H_{22}O_2$ 198.1620, found 198.1614.

Diastereomeric Mixture of 4-*tert*-Butylcyclohexanemethanol (t/c-1):
To a stirred suspension of $LiAlH_4$ (1.07 g, 28.3 mmol) in THF (50 mL) was added the ester t/c-**4** (5.61 g, 28.3 mmol) at 0 °C and the mixture was stirred for 30 min at r.t. The reaction was quenched by addition of a minimum amount of 30 % NH_4OH and the mixture was filtrated through a Celite pad. The filtrate was evaporated under reduced pressure and chromatographed on silica gel (90 g, eluent: EtOAc/hexane, with 1 : 2) to give the alcohol t/c-**1** as a colorless oil which was estimated to be a *trans*/*cis* mixture (57 : 43) based on ^{1}H NMR analysis; yield: 4.06 g (93.5 %).

IR (neat): $\nu = 3336\ cm^{-1}$.

^{1}H NMR ($CDCl_3$): $\delta = 3.64$ (q, 0.43 × 2 H, $J = 5.5$ Hz), 3.44 (t, 0.57 × 2 H, $J = 6.0$ Hz), 1.85–1.77 (m, 3 H, 1 H exchangeable with D_2O), 1.55–1.36 (m, 4 H), 1.09–0.86 (m, 4 H), 0.84 (s, 0.57 × 9 H), 0.83 (s, 0.43 × 9 H).

MS: $m/z = 170$ (M^+), 57 (100 %).

HRMS: m/z calc. for $C_{11}H_{22}O$ 170.2951, found 170.1639.

Diastereomeric Mixture of 4-*tert*-Butylcyclohexanemethyl Acetate (t/c-2):
To a stirred solution of the alcohol mixture t/c-**1** (84 mg, 0.494 mmol) and Et_3N (0.2 mL, 1.435 mmol) in CH_2Cl_2 (5 mL) was

added Ac_2O (0.2 mL, 2.120 mmol) at 0°C and the mixture was stirred for overnight at r.t. The mixture was evaporated under reduced pressure and chromatographed on silica gel (5 g, eluent: EtOAc/hexane, 1:9) to give the acetate mixture t/c-**2** as a colorless oil which was estimated to be a *trans*/*cis* mixture (57:43) based on ^{1}H NMR analysis; yield: 84 mg (80%).

IR (neat): $\nu = 1743\ cm^{-1}$.

^{1}H NMR ($CDCl_3$): $\delta = 4.08$ (d, 0.43×2 H, $J = 8.0$ Hz), 3.87 (d, 0.57×2 H, $J = 6.6$ Hz), 2.06 (s, 0.43×3 H), 2.05 (s, 0.57×3 H), 1.81–1.71 (m, 4H), 1.58–1.41 (m, 2H), 1.10–0.86 (m, 4H), 0.84 (s, 0.57×9 H), 0.83 (s, 0.43×9 H).

MS: $m/z = 213$ ($M^+ + 1$), 57 (100%).

HRMS: m/z calc. for $C_{13}H_{25}O_2$ 213.1855, found 213.1851.

Lipase-Mediated Kinetic Acylation of the Alcohol Mixture t/c-1; Typical Procedures:

Table 1, Entry 1: A solution of the alcohol mixture t/c-**1** (t/c = 57:43; 217 mg, 1.28 mmol), vinyl acetate (0.6 mL, 6.4 mmol), and lipase PS on Celite (13 mg) in *t*-BuOMe (22 mL) was stirred at r.t. for 25 h. After filtration through a Celite pad, the mixture was evaporated under reduced pressure and chromatographed on silica gel (20 g) to give the pure *trans*-acetate t-**2** [> 99% *trans*, 95 mg (35%)] from EtOAc/hexane (1:8) fraction and the *cis*-enriched alcohol c-**1** [t/c = 33:67; 130 mg (60%)] from EtOAc/hexane (1:2) fraction.

Table 1, Entry 2: A solution of the alcohol mixture t/c-**1** (t/c = 57:43; 161 mg, 0.95 mmol), vinyl acetate (0.7 mL, 7.1 mmol), and lipase PS on Celite (9 mg) in *t*-BuOMe (16 mL) was stirred at r.t. for 100 h and treated as above to give the *trans*-enriched acetate t-**2** [91% *trans*, 117 mg (31%)] and the pure *cis*-alcohol c-**1** [> 99% *cis*, 98 mg (65%)].

Table 1, Entry 3: A solution of the *trans*-enriched alcohol t-**1** (t/c = 91:9; 140 mg, 0.82 mmol), vinyl acetate (0.4 mL, 4.3 mmol), and lipase PS on Celite (8 mg) in *t*-BuOMe (14 mL) was stirred at r.t. for 100 h and treated as above to give the pure *trans*-acetate t-**2** [> 99% *trans*, 140 mg (80%)] and the alcohol mixture t/c-**1** [t/c = 53:47; 21 mg (15%)].

Table 1, Entry 4: A solution of the *cis*-enriched alcohol c-**1** (t/c = 28:72; 150 mg, 0.88 mmol), vinyl acetate (0.2 mL, 2.1 mmol), and lipase PS on Celite (9 mg) in *t*-BuOMe (15 mL) was stirred at r.t. for 80 h and treated as above to give the *trans*-enriched acetate t-**2** [t/c = 83:17; 58 mg (31%)] and the pure *cis*-alcohol c-**1** [> 99% *cis*, 98 mg (65%)].

*cis-Alcohol (c-**1**):*

IR (neat): $\nu = 3258\ cm^{-1}$.

^{1}H NMR ($CDCl_3$): $\delta = 0.83$ (s, 9H), 0.94–1.09 (m, 3H), 1.20 (br s, 1H), 1.41–1.59 (m, 4H), 1.76–1.87 (m, 3H), 1.20 (br s, 1H) 1.41–1.59 (m, 4H), 1.76–1.87 (m, 3H), 3.64 (d, 2H).

MS: $m/z = 170$ (M^+), 57 (100%).

HRMS: m/z = calc. for $C_{11}H_{22}O$ 170.1671, found 170.1664.

*trans-Acetate (t-**2**):*

IR (neat): $\nu = 1745\ cm^{-1}$.

^{1}H NMR ($CDCl_3$): $\delta = 0.84$ (s, 9H), 0.89–1.05 (m, 5H), 1.47–1.62 (m, 1H), 1.74–1.87 (m, 4H), 2.05 (s, 3H), 3.87 (d, 2H).

MS: $m/z = 213$ ($M^+ + 1$), 57 (100%).

HRMS: m/z calc. for $C_{13}H_{25}O_2$ 213.1855, found 213.1810.

***cis*-4-*tert*-Butylcyclohexanemethyl Acetate (c-2):**

To a stirred solution of the *cis*-alcohol c-**1** (> 99% *cis*: 53 mg, 0.312 mmol) in CH_2Cl_2 (1.5 mL) was added Ac_2O (44 µL, 0.468 mmol) and Et_3N (65 µL, 0.488 mmol) at r.t. and the mixture was kept stirring for 12 h at r.t. The mixture was diluted with Et_2O (10 mL), washed with brine, dried ($MgSO_4$), and evaporated under reduced pressure. The residue was chromatographed on silica gel (10 g, eluent: EtOAc/hexane, 1:8) to give the *cis*-acetate c-**2** [> 99%, *cis*: 66 mg (100%)].

Lipase-Mediated Kinetic Deacylation of the Acetate Mixture (t/c-2); Typical Procedure:

Table 2, Entry 3: A solution of the acetate mixture t/c-**2** (t/c = 57:43; 199 mg) and lipase PS on Celite (9 mg) in a 9:1 mixture of 0.2 M phosphate buffer and acetone (9.4 mL) was stirred at r.t. for 20 d. After filtration through a Celite pad, the filtrate was extracted with EtOAc (3×20 mL) and the extract was washed with brine ($10\ mL \times 2$), dried ($MgSO_4$), and evaporated under reduced pressure. The residue was chromatographed on silica gel (50 g) to give the acetate mixture t/c-**2** [t/c = 60:40; 105 mg (42%)] from EtOAc/hexane (1:8) fraction and the pure *trans*-alcohol t-**1** [99% *trans*: 102 mg (51%)] from EtOAc/hexane (1:2) fraction.

*trans-Alcohol (t-**1**):*

IR (neat): $\nu = 3324\ cm^{-1}$.

^{1}H NMR ($CDCl_3$): $\delta = 0.80–1.06$ (m, 4H), 0.85 (s, 9H), 1.33–1.49 (m, 2H), 1.73 (br s, 1H), 1.77–1.88 (m, 4H), 3.43 (d, 2H).

MS: $m/z = 170$ (M^+), 57 (100%).

HRMS: m/z calc. for $C_{11}H_{22}O$ 170.1671, found 170.1673.

(1) For a preceding example of separation of diastereomeric mixture by lipase, see: Geresh, S.; Elbaz, E.; Glaser, R. *Tetrahedron* **1993**, *49*, 4939.

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