Lipase-Catalyzed Optical Resolution of (1*RS*,7*RS*)-2-Oxotricyclo[2.2.1.0^{3,5}]heptane-7-carboxylic Acid Methyl Ester: Precursor for Large Scale Synthesis of Non-Racemic Prostaglandins

E. Wolfgang Holla,* Heinz-Peter Rebenstock, Bernd Napierski, Gerhard Beck
Hoechst AG, Zentrale Pharmaforschung, Postfach 800320, D-65926 Frankfurt/Main, Germany
Fax +49(69)331399
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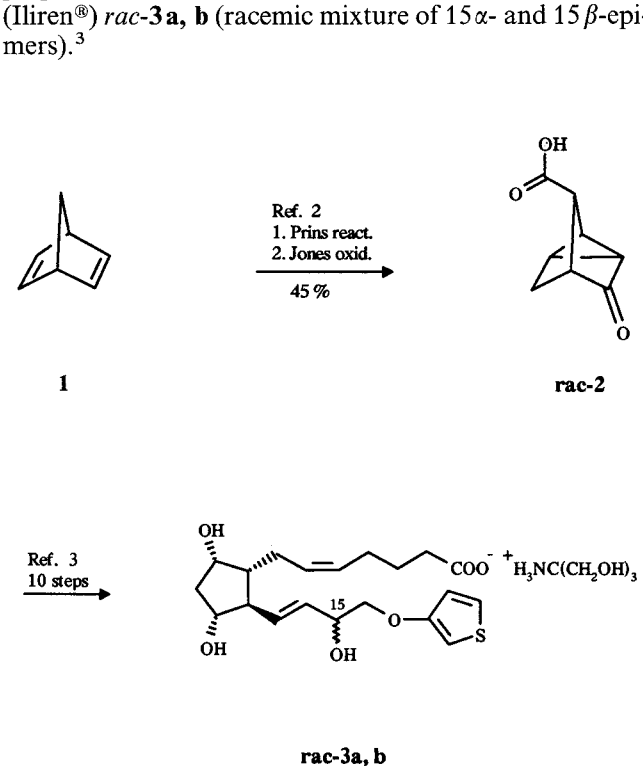
As a result of screening several lipases, esterases and proteases for the ability to resolve ester derivatives of the racemic title compound *rac*-**2**, a lipase-catalyzed enantioselective hydrolysis of methyl ester *rac*-**4** was developed on a 200 g scale. Exposure of the readily available racemic bicyclo[2.2.1]heptane derivative *rac*-**4** to thermostable lipase SP 526 from *Candida antarctica* in buffered solution affords the desired (+)-enantiomer as (remaining) ester **4** with ≥ 99% enantiomeric excess in 82% yield.

Bicyclo[2.2.1]heptane derivatives are valuable starting materials for the synthesis of prostaglandins.[1,2] Racemic keto acid *rac*-**2** (**2** + *ent*-**2**), for example, which is readily available from Prins reaction of norbornadiene **1** and subsequent Jones oxidation, is used for the large scale preparation of the luteolytic prostaglandin Tiaprost (Iliren®) *rac*-**3a, b** (racemic mixture of 15α- and 15β-epimers).[3]



Scheme 1

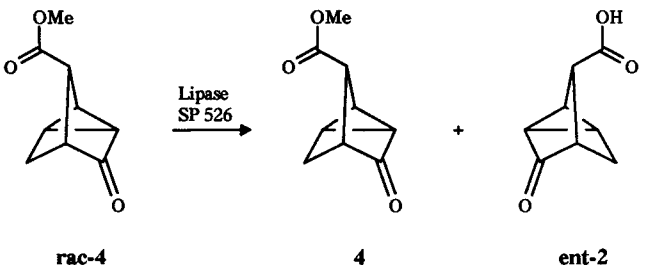
It has been known for more than 20 years that the optical resolution of (1*RS*,7*RS*)-2-oxotricyclo[2.2.1.0^{3,5}]heptane-7-carboxylic acid (*rac*-**2**) can be achieved with (*S*)-(−)-phenylethylamine. According to the literature,[4] it takes three to five recrystallizations to obtain the **2**-salt in optically pure form. This procedure is unsuitable for technical purposes. In order to investigate a "racemic switch"[5] to (+)-Iliren as epimeric mixture **3a, b** or as 15α-epimer **3a**, we studied the feasibility of an enzyme-catalyzed kinetic resolution[6] of keto acid *rac*-**2** and its esters.

In an initial screening, several esters of keto acid *rac*-**2**, namely methyl ester *rac*-**4**, ethyl ester *rac*-**5**, vinyl ester *rac*-**6**, allyl ester *rac*-**7**, hexyl ester *rac*-**8**, and benzyl ester *rac*-**9**, were subjected to 27 commercially available lipases, esterases and proteases in 0.1 M phosphate buffer, pH = 7.0, at 20–25 °C. In a second part of the screening, tetrahydrofuran, acetone, dimethoxyethane and dichloromethane were used as cosolvents. Monitoring of these reactions by TLC showed that 13 enzymes were able to hydrolyze esters of *rac*-**2**.

Due to practical reasons we focussed our efforts on the hydrolysis of methyl and vinyl ester *rac*-**4** and *rac*-**6**, respectively, with lipase P from *Pseudomonas cepacia*, porcine pancreas lipase, lipase OF from *Candida rugosa*, lipases SP 525 and SP 526 from *Candida antarctica*, porcine liver esterase, cholesterol esterase from *Pseudomonas fluorescens*, bovine pancreas acetone powder and alcalase from *Bacillus licheniformis*. After determination of the extent of conversion of the racemic substrate and the optical purities of the resulting acid and and the remaining ester by HPLC and GC, respectively, it was obvious that in almost all of the selected enzymatic reactions conversion as well as enantiomeric ratio E[7] were very poor. Thermostable lipase SP 526 was identified as the most efficient biocatalyst: the E values of the hydrolysis of vinyl and methyl ester *rac*-**6** and *rac*-**4** at room temperature were found to be 10–20.

A marked improvement in enantioselectivity was observed when the hydrolysis of the methyl ester *rac*-**4** was performed at a higher temperature. At 50 °C, the lipase SP 526-catalyzed kinetic resolution possesses an E value of ≥ 60.

The stereochemical preference of lipase SP 526-catalyzed enantio-differentiating methyl ester hydrolysis is shown in Scheme 2. The unwanted (−)-enantiomer *ent*-**4** is the fast-reacting isomer yielding (−)-acid *ent*-**2** whereas the (+)-methyl ester **4** remains unchanged and has to be cleaved by chemical means to afford the desired prostaglandin precursor **2**.



Scheme 2

In order to establish a process suitable for an optical resolution of methyl ester *rac*-**4** on multi 100 g scale we studied the lipase SP 526-catalyzed hydrolysis in more detail. Using automatic titration the reaction parameters were examined on a 2–5 g scale. The main variables are: temperature, pH value, amount of enzyme and cosolvent, concentration of buffer and racemic substrate. As a result of this optimization we were able to achieve an optical resolution of methyl ester *rac*-**4** on a 200 g scale using lipase SP 526 at 50 °C and pH 7.0 in 0.05 M potassium phosphate buffer–dimethoxyethane (9.2:1, v/v). Extractive workup after 54% conversion of hydrolysis afforded the desired methyl ester **4** with ≥ 99% ee in 82% theoretical yield. The unwanted (−)-acid *ent*-**2** was isolated from the remaining aqueous mixture with 85% ee in almost quantitative yield based on conversion.

The hydrolysis of optically pure methyl ester **4** to the (+)-acid **2** was achieved using sodium hydroxide in water/methanol/*tert*-butyl methyl ether at room temperature. This reaction which has not yet been optimized afforded the prostaglandin precursor **2** in 88–93% yield.

As a consequence of the stereochemical preference of *Candida* lipase SP 526 the enzymatic esterification of racemic keto acid *rac*-**2** seems to be an interesting alternative. This reaction is under investigation.

In summary, we have developed a process to perform a racemic switch to (+)-Iliren (**3a, b**). Key step is the first enantioselective hydrolysis of (1*RS*,7*RS*)-2-oxotricyclo-[2.2.1.0^{3,5}]heptane-7-carboxylic acid methyl ester (*rac*-**4**) catalyzed by thermostable lipase SP 526 from *Candida antarctica* on 200 g scale which occurs with ≥ 99% ee and 82% yield. For a technical synthesis of (+)-Iliren (**3a, b**) on multikilogram scale the described resolution process has to be further scaled up with immobilized *Candida* lipase SP 526.

Alkyl esters *rac*-**5**, *rac*-**7**, *rac*-**8**, *rac*-**9** were prepared by 4-(dimethylamino)pyridine (DMAP)-catalyzed esterification of *N,N*-dicyclohexylcarbodiimide (DCC)-activated carboxylic acid.[8] The vinyl ester *rac*-**6** was obtained from acid *rac*-**2** via Na_2PdCl_4 or Li_2PdCl_4 catalyzed transvinylation in commercially available vinyl acetate.[9]

Lipase P (*Pseudomonas cepacia*) was obtained from Amano Pharmaceutical Co., lipase OF (*Candida rugosa*) from Meito Sangyo Co., Ltd., porcine pancreas lipase, porcine liver esterase, cholesterol esterase (*Pseudomonas fluorescens*) and bovine pancreas acetone powder were purchased from Sigma Chemie GmbH, lipases SP 525 and SP 526 (both *Candida antarctica*) and alcalase (protease from *Bacillus licheniformis*) were supplied by Novo Nordisk Biotechnologie GmbH.

Melting points were determined on a Büchi 535 melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra (internal standard TMS) were recorded in $CDCl_3$ or DMSO-d_6 on a Varian Gemini 200, a Bruker AM 270 and a Bruker ARX 500 spectrometer and are reported in ppm. Mass spectra were determined at 70 eV on a Fisons Instruments VG TRIO 2000 mass spectrometer. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. Determination of ester/acid ratio by HPLC was performed on Lichrospher 100 RP18 5 μm (E. Merck, Darmstadt), monitoring at 210 nm and 254 nm, eluting with H_2O/MeCN (9:1 + 0.1% KH_2PO_4 + 0.1% camphorsulfonic acid; flow rate: 1 mL/min; t_{ret}: 3.04 min [**2**/*ent*-**2**], 9.73 min [**4**/*ent*-**4**]). For determination of optical purity by HPLC, methyl ester **4** was analyzed on Chiralpak AD 25 cm × 0.46 cm (Daicel Chemical Industries, Ltd.), monitoring at 215/286 nm, eluting with hexane/propan-2-ol (9:1; flow rate: 1 mL/min; 40 °C; t_{ret}: 6.45 min [**4**], 7.95 min [*ent*-**4**]).

(1*RS*,7*RS*)-(±)-2-Oxotricyclo[2.2.1.0^{3,5}]heptane-7-carboxylic Acid Methyl Ester (*rac*-4):

K_2CO_3 (387.0 g, 2.80 mol) which had been dried overnight at 150 °C in vacuo was added to a solution of white, crystalline *rac*-**2** (210.0 g, 1.38 mol) in acetone (2.1 L). After stirring the resultant suspension for 15 min, Me_2SO_4 (392.6 mL, 522.2 g, 4.14 mol) was added dropwise at r.t. within 15–20 min. Stirring at r.t. was continued for 3 h, then the solids were filtered and washed with acetone (600 mL). The combined acetone solutions were cooled to 10 °C and Et_3N (500 mL, 365.3 g, 3.61 mol) was added dropwise at 10–20 °C within 45–60 min (exothermic reaction). The temperature was allowed to rise to 22–25 °C and stirring was continued for 1 h. The resultant mixture was evaporated under reduced pressure and acidified with 0.02 M HCl (900 mL). Extraction with *tert*-butyl methyl ether (3 × 300 mL), drying ($MgSO_4$) and evaporation in vacuo gave *rac*-**4** (215–220 g) as a pale yellow oil which could be used for the enzymatic resolution without further purification. Vacuum distillation afforded *rac*-**4** (212.0 g, 92.5%) as a clear colourless liquid; bp 75–80 °C/0.07 mbar.

^{1}H NMR ($CDCl_3$, 270 MHz): δ = 1.50 (dt, 1 H, *J* = 1, 5.5 Hz), 1.88 (dt, 1 H, *J* = 11, 1 Hz), 1.98 (dt, 1 H, *J* = 11, 1 Hz), 2.2–2.3 (m, 2 H), 2.42 (m, 1 H), 3.02 (br s, 1 H), 3.72 (s, 3 H).

^{13}C NMR ($CDCl_3$, 125 MHz): δ = 19.29, 19.57, 21.10, 28.58, 40.60, 45.50, 51.58, 171.20, 209.37.

IR (neat): ν = 1763.0, 1735.5 cm^{-1}.

MS (DCI/MeOH): *m/z* (%) = 167 ($M + H^+$, 100).

(1*R*,7*R*)-(+)-2-Oxotricyclo[2.2.1.0^{3,5}]heptane-7-carboxylic Acid Methyl Ester (4):

Ester *rac*-**4** (230.0 g, 1.38 mol) was stirred with lipase SP 526 (34.5 g) from *Candida antarctica* in 0.05 M potassium phosphate buffer, pH = 7.0 (2.76 L), and 1,2-dimethoxyethane (300 mL) at 48–51 °C. The pH was kept at 7.0 for 22 h by automatic addition of 1.0 M NaOH (750 mL). The reaction mixture was filtered (Seitz filter sheets supra 200), almost 2 L of dimethoxyethane–water were removed under reduced pressure, and the remaining aqueous mixture was extracted with *tert*-butyl methyl ether (3 × 300 mL). Drying (Na_2SO_4) and evaporation of the solvent in vacuo afforded (+)-ester **4** as pale yellow oil (94.0 g, 82%, ≥ 99% ee); $[\alpha]_D^{20}$ + 89 (*c* = 1, dioxane).

^{1}H NMR ($CDCl_3$, 270 MHz): δ = 1.50 (dt, 1 H, *J* = 1, 5.5 Hz), 1.88 (dt, 1 H, *J* = 11, 1 Hz), 1.98 (dt, 1 H, *J* = 11, 1 Hz), 2.2–2.3 (m, 2 H), 2.42 (m, 1 H), 3.02 (br s, 1 H), 3.72 (s, 3 H).

MS (DCI/MeOH): *m/z* (%) = 167 ($M + H^+$, 100), 134 ($M^+ - MeOH$, 10).

Distillation of a sample afforded a colourless oil which crystallized at 15–20 °C; mp 26–27 °C; $[\alpha]_D^{20}$ + 96 (*c* = 1, dioxane).

(1*S*,7*S*)-(−)-2-Oxotricyclo[2.2.1.0^{3,5}]heptane-7-carboxylic Acid (*ent*-2):

After extractive workup of the desired (+)-methyl ester **4** from the above reaction, the remaining aqueous mixture was acidified to pH 1 and evaporated in vacuo. Trituration of the solid residue with water (300–350 mL) and filtration by suction afforded *ent*-**2** (111 g, 85% ee) as an off-white crystalline solid.

^{1}H NMR, MS and IR data of the unwanted (−)-acid *ent*-**2** are in accordance with data of starting material *rac*-**2** and (+)-acid **2**.

(1*R*,7*R*)-(+)-2-Oxotricyclo[2.2.1.0^{3,5}]heptane-7-carboxylic Acid (2):

To a mechanically stirred mixture of (+)-methyl ester **4** (120.0 g, 0.72 mol), water (400 mL), MeOH (400 mL), and *tert*-butyl methyl ether (400 mL) were added NaOH pellets (34.5 g, 0.86 mol). After stirring for 3 h the reaction mixture was acidified to pH 1 with conc. HCl (80–90 mL) and concentrated under reduced pressure. The solid residue was suspended in water (200 mL), cooled to 0–5 °C and then filtered under suction to give crude **2** (120–125 g). Recrystallization from toluene/acetone (~ 16:1, 110–120 mL) gave the desired keto acid **2** (101.6 g, 93%) in optically pure (≥ 99.8%

ee) form; mp 143–144 °C (Lit.[4a] mp 137–138 °C); $[\alpha]_D^{20}$ +97.5 ($c = 1$, dioxane) (Lit.[4a] $[\alpha]_D^{25}$ +74 ($c = 1$, MeOH).

^{1}H NMR (DMSO-d_6), 200 MHz): $\delta = 1.46$ (dt, 1 H, $J = 1$, 5.5 Hz), 1.74 (dt, 1 H, $J = 11$, 1 Hz), 1.86 (dt, 1 H, $J = 11$, 1 Hz), 2.04 (br s, 1 H), 2.22–2.40 (m, 2 H), 2.98 (s, 1 H), 12.52 (s, 1 H).

IR (KBr): $\nu = 3041.4$, 1734.2 (br) cm^{-1}.

MS (DCI/MeOH): m/z (%) = 153 ($M + H^+$, 100), 134 ($M^+ - H_2O$, 6).

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