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Mutti, Francesco G.; Orthaber, Andreas; Schrittwieser, Joerg H.; de Vries, Johannes G.; Pietschnig, Rudolf; Kroutil, Wolfgang
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Simultaneous Transition Metal Catalytic Oxidation and Enzymatic Reduction using Orthogonal Reagents

Francesco G. Mutti, Andreas Orthaber, Joerg H. Schrittwieser, Johannes G. de Vries, Rudolf Pietschnig, and Wolfgang Kroutil

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S1. General information

Chemicals and Enzymes: (S)-2-chloro-1-phenylethanol (2a), chloroacetophenone (3a), acetone, chloroacetone, potassium tert-butoxide and sodium formate were purchased either from Sigma-Aldrich or Acros. The iridacycle catalyst (1) was synthesised as reported in literature, starting from commercially available [Cp*IrCl₂]₂ (Sigma-Aldrich), potassium hexafluorophosphate, and N-methylbenzylamine. Racemic 2-chloro-1-phenylethanol (2a), 1-phenoxy-3-chloroethanol (2b), 1-chloro-2-octanol (2c)² and 6,6-dimethyl-2-chlorocyclohexanone (3f)³ were synthesised according to literature. Toluene was dried with 3 Å molecular sieves. Distilled water was used in the preparation of all aqueous buffers.

ADH-A from *Rhodococcus ruber* was overexpressed in *E. coli* BL23(DE3)/pET22b as previously reported, the cells were disrupted and enzyme was partially purified by heat treatment (60°C, 20 min; then centrifugation). NAD-specific formate dehydrogenase 002 from *Candida boidinii* and β-NADH disodium salt were purchased from Codexis.

All GC analyses were carried out with an Agilent 7890 A GC system, equipped with standard FID detector and using a Chrompack Chirasil Dex-CB column (25m, 320 μm, 0.25 μm). Helium was used as carrier gas and EtOAc was used as solvent.

The following sterical demanding α-haloketones were investigated as hydrogen acceptors: 3-chloro-2-norbornanone, 2-chlorocyclopentanone, 2-chlorocyclohexanone, 2,8-dichlorocyclooctanone, 2-bromocyclohexanone, 2-chloro-α-tetralone, 6,6-dimethyl-2-chlorocyclohexanone 3f.

S2. Racemisation experiment

a) in toluene: Iridacycle (1) (2.11 mg, 3.33 μmol) and KOtBu (0.45 mg, 4.00 μmol) were placed into a 2 mL vial with cap. After everything was transferred into a glove-box, dry
toluene (1.0 mL) was added. The solution became shining purple within 15 minutes, indicating activation of the catalyst. Finally, the substrate (S)-2-chloro-1-phenylethanol (2a) (8.81 μL, 66 μmol) was added and the vial was thoroughly closed. The sample was shaken at 1000 rpm in an Eppendorf thermomixer in vertical position, at 21°C for 1 h. The sample was filtered using Rotilabo ®-Spritzenfilter (13 mm, 0.2 μm, Nylon) and analysed by GC.

b) in toluene / aqueous buffer: 1 (2.11 mg, 3.33 μmol) was activated in dried toluene (300 μL) as described above in a glove-box. Then distilled water (700 μL) was put into a second vial and the organic solution containing the activated catalyst was gently pipetted onto the aqueous phase. Finally, the substrate (S)-2a (8.81 μL, 66 μmol) was added and the vial was thoroughly closed. Samples were vigorously shaken for 2 h at 21°C. The reaction was stopped by extraction with ethyl acetate (2 × 500 μL) and the organic layer was separated from the aqueous phase by centrifugation (2 min, 13000 rpm) and dried with Na₂SO₄. Conversion was determined by GC analysis.

S3. Oxidation of racemic chlorohydrins with iridacycle catalyst

1 (2.11 mg, 3.33 μmol, 5 mol% or 0.85 mg, 1.33 μmol, 2 mol%) and KOTBu (0.45 mg, 4.00 μmol or 0.18 mg, 1.6 μmol) were placed into a 2 mL vial with cap. Then, everything was transferred into a glove-box and dry toluene (1 mL) was added. The solution became shining purple within 15 minutes, indicating activation of the catalyst. Then, the oxidant [e.g. acetone (3d), chloroacetone (3e), 6,6-dimethyl-2-chlorocyclohexanone (3f)] and the substrate (S)-2a (8.81 μL, 66 μmol) were added. The vial was thoroughly closed and reaction was run at 21°C. Maximum conversion is reached after approximately 3 h. The sample was filtered using Rotilabo ®-Spritzenfilter (13 mm, 0.2 μm, Nylon) and analysed by GC.

S4. Reduction of chloroacetophenone with iridacycle catalyst

The aim of this experiment is to prove that 1 can reduce o-chloroacetophenone (3a) in
combination with a reducing reagent such as 2-propanol.

![Iridacycle (1)](image)

1 (2.11 mg, 3.33 μmol, 5 mol%) was activated with KOTBu (0.45 mg, 4.00 μmol) in dry toluene (200 μL) in a glove-box by stirring for 15 minutes at 21°C. The reaction was carried out in a 2 mL dark glass vial filled with aqueous buffer Tris-HCl (700 μL, pH 7.5, 50 mM). The organic phase containing the activated catalyst was transferred onto the aqueous solution and 2-propanol (200 μL) and substrate acetophenone (3a) (10.2 mg, 66 μmol) were added. The reaction was run for 22 hours at 21°C at 1000 rpm on an Eppendorf thermomixer kept in vertical position. The reaction was stopped by extraction with ethyl acetate (2 × 500 μL) and the organic layer was separated from the aqueous phase by centrifugation (2 min, 13000 rpm) and dried with Na₂SO₄. Conversion was determined by GC analysis.

S5. Reduction of ω-chloroacetophenone 3a with ADH-A

The activity of the ADH-A preparation was tested in the reduction of chloroacetophenone 3a:

![Reduction of ω-chloroacetophenone 3a with ADH-A](image)

ADH-A preparation (500 μL, 25 U) and a solution of NADH (300 μL) in buffer (Tris-HCl, pH 7, 50 mM) were mixed to give a NADH concentration 1 mM. Then substrate 3a (10.2 mg,
66 μmol) and 2-propanol 2d (200 μL) were added. Reactions were shaken at 30 °C for 24 h at 120 rpm on a rotary shaker plate and stopped by extraction with ethyl acetate (2 × 500 μL). The organic layer was separated from the aqueous phase by centrifugation (5 min, 13000 rpm) and dried with Na₂SO₄. Conversion was determined by GC. Quantitative conversion and perfect e.e. (> 99%) was obtained within 24 hours.

S6. Reduction of ω-chloroacetophenone 3a with ADH-A in the presence of iridacycle 1

a) NADH recycling with 2-propanol: a stock solution of activated 1 (2.11 mg, 3.33 μmol, 5 mol%) was prepared by treatment with KOTBu (0.45 mg, 4.00 μmol) in dry toluene (200 μL) in a glove-box by stirring for 15 minutes at 21°C. A 0.5 mol% solution of 1 was prepared by dilution with toluene. A solution of ADH-A in buffer (Tris-HCl, pH 7.5, 50 mM, 450 μL, 500 U) was mixed with a NADH solution in buffer Tris-HCl (pH 7.5, 50 mM, 150 μL) to reach NADH concentration 4 mM. The organic phase containing 1 was poured onto the aqueous phase containing ADH-A and NADH. Then, 2-propanol (200 mL) and substrate 3a (10.2 mg, 66 μmol) were added. The reaction was run for 1 hour at 30 °C on a rotary shaker plate at 250 rpm and stopped by extraction with ethyl acetate (2 × 500 μL). The organic layer was separated from the aqueous phase by centrifugation (5 min, 13000 rpm) and dried with Na₂SO₄. The reduction proceeded with 35% conversion and 96% e.e. The GC chromatogram is depicted below:
b) NADH recycled with FDH-sodium formate: ADH-A (500 μL, 500 U) in Tris-HCl buffer (pH 7.5, 50 mM) was mixed with FDH (1 mL, 550 U). NADH (5.7 mg, 4 mM) and sodium formate (18 mg, 265 μmol) were added. A toluene solution of activated 1 (500 μL, 1 mol%) was poured onto the aqueous phase and 3a was added (5.1 mg, 33 μmol). The reaction was shaken on a rotary plate at 120 rpm, for 4 h, at 30°C and stopped by extraction with ethyl acetate (2 × 500 μL). The organic layer was separated from the aqueous phase by centrifugation (5 min, 13000 rpm) and dried with Na₂SO₄. The reduction proceeded with 82% conversion and 97% e.e.

S7. Combination of iridacycle catalyst and ADH-A for simultaneous oxidation – reduction

Solutions of ADH-A (500 U, 500 μL, pH 7.5, 50 mM), FDH (140 μL, 30 U) and NADH (110 μL, final concentration 1 mM) in Tris-HCl buffer were combined. A toluene solution of activated 1 (150 μL; 2 mol % or 5 mol%) was poured onto the aqueous phase. Then, a toluene solution of 2,2-dimethyl-6-chloro cyclohexanone (4) (150 μL,) was added. The amount of oxidant was varied from 2 eq. (66 μmol, 10.6 mg), 5 eq. (165 μmol, 26.5 mg ) to 10 eq. (330 μmol, 53.0 mg). Finally, the substrate rac-2a (4.4 μL, 33 μmol) or rac-2b (5.1 μL, 33 μmol) or rac-2c (7 μL, 33 μmol) was added. Reactions were shaken on a rotary plate at 120 rpm for 16 h at 30°C and stopped by extraction with ethyl acetate (2 × 500 μL). The organic layer was
separated from the aqueous phase by centrifugation (5 min, 13000 rpm) and dried with Na$_2$SO$_4$.

Some typical chromatograms are shown below:

(1) 1 (2 mol%), 4 (2 eq.), substrate 2a, reaction mixing by vigorously shaking.

Starting from racemic 2a, enantoienriched mixture of the (R)-enantiomer (R:S = 65:35; e.e. = 30%) was obtained. The amount of remaining intermediate chloroacetophenone 3a was negligible.

(2) 1 (2 mol%), 4 (2 eq.), substrate 2a, reaction mixing by gentle stirring, maintaining 2-phase system.
Starting from racemic \(2a\), we obtained an enantioenriched mixture of the \((R)\)-enantiomer (R:S = 45:30; e.e. = 20\%). The amount of remaining intermediate chloroacetophenone \(3a\) was herer significant (26\%) (see main text). Therefore, when the reaction is performed with phases separated, the diffusion of \(3a\) from the organic phase to the aqueous phase is the rate limiting step. The diffusion is faster, when the reaction is shaken and no phases separation is observed (exp. 1).

\[(3)\ 1\ (2\ \text{mol\%}),\ 4\ (2\ \text{eq.}),\ \text{substrate}\ 2c,\ \text{reaction mixing by shaking}\]

Starting from racemic 1-chloro-2-octanol (2c), we obtained an enantioenriched mixture of the Prelog R enantiomer (R:S = 59:41; e.e. = 17\%). The amount of remaining intermediate 1-chloro-2-octanone (3c) was negligible.

S8. Analytics

**Method A:** GC program parameters; injector 200°C; constant flow 1.5 mL/min.; temperature program 60°C/hold 1 min.; 140°C/rate 10°C per min/hold 15 min; 180°C/rate 10°C per min/hold 0 min.

**Method B:** GC program parameters; injector 200°C; constant flow 1.5 mL/min; temperature program 100°C/hold 1 min.; 145°C/rate 1°C per min/hold 0 min; 180°C/rate 10°C per min/hold 0 min.
Method C: GC program parameters; injector 200°C; constant flow 1.5 mL/min; temperature program 60°C/hold 1 min; 110°C/rate 10°C per min/hold 0 min; 124°C/rate 0.5°C per min/hold 0 min; 180°C/rate 10°C per min/hold 0 min.

![2a](image)

GC/Method A: (S)-2a 16.9 min.; (R)-2a 17.5 min.; the absolute configuration was assigned by comparison of elution order on GC and coinjection with commercially available (S)-2a and (R)-2a.

![3a](image)

GC/Method A: 3a 12.9 min.; the retention time was compared to commercially available 3a.

![2b](image)

GC/Method B: (R)-2b 48.7 min.; (S)-2b 48.9 min.; the absolute configuration was assigned by the comparison of elution order on GC with chlorohydrins previously obtained.

![3b](image)

GC-method A: 3b 19.8 min; reference compound was obtained according to literature.

![2c](image)

GC/Method C: (S)-2c 19.5 min.; (R)-2c 19.8 min.; the absolute configuration was assigned by the comparison of elution order on GC with chlorohydrins previously obtained.
References


