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Note

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Asymmetric Synthesis of 1-Phenylethylamine from Styrene via Combined Wacker-Oxidation and Enzymatic Reductive Amination

Florian Uthoff, Harald Gröger*

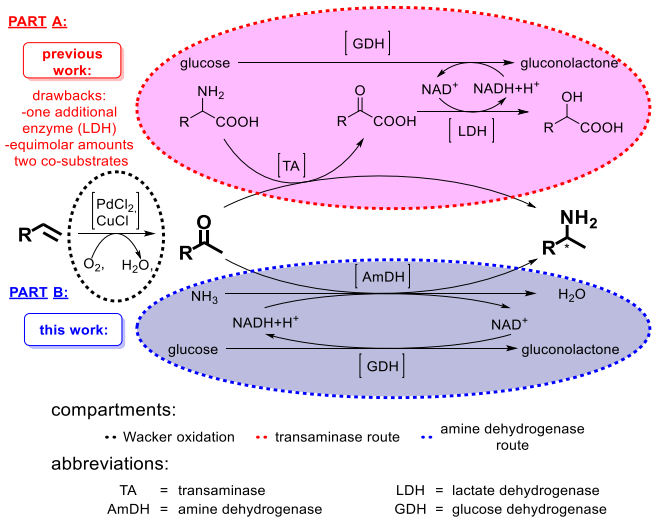
Chair of Organic Chemistry I, Faculty of Chemistry, Bielefeld University, Universitätsstr. 25, 33615 Bielefeld, Germany

KEYWORDS: Amine dehydrogenase, chemoenzymatic synthesis, enzyme catalysis, one-pot synthesis, Wacker-oxidation.

ABSTRACT: An enantioselective chemoenzymatic two-step one-pot transformation of styrene to 1-phenylethylamine has been developed based on combining an initial Pd/Cu-catalyzed Wacker-oxidation of styrene with a subsequent reductive amination of the *in situ*-formed acetophenone. As a nitrogen source only ammonia is needed. The incompatible catalysts were separated by means of a polydimethylsiloxane membrane, thus leading to quantitative conversion and excellent enantiomeric excess of the corresponding amine. The overall one-pot process formally corresponds to an asymmetric hydroamination of styrene with ammonia.

The synthesis of enantiomerically pure amines *via* novel retrosynthetic approaches is of high interest since, e.g., such amines are widely used in the fine chemical, agrochemical and pharmaceutical industry.[1] Recently we developed an enantioselective chemoenzymatic two-step one-pot approach to 1-phenylethylamines starting from styrenes, thus formally corresponding to an asymmetric hydroamination reaction of alkenes with ammonia (Scheme 1, Part A).[2] In detail, this process is based on combining a Pd/Cu-catalyzed Wacker-oxidation[3] of styrenes and a subsequent enzymatic transamination of the ketones formed as intermediates. Due to deactivation of the transaminase (TA) by the Cu-component, the catalyst components have been embedded in different compartments within one reactor by means of PDMS-membranes according to a concept, which was developed by the Bowden group[4] for combining non-compatible chemical reactions and introduced by our group to the field of chemoenzymatic synthesis.[5] Although high conversion and enantioselectivities were obtained in this one-pot amine synthesis,[2] limitations of this process are the high enzyme loading for the transaminase as well as the use of the two organic reagents L-alanine and D-glucose in stoichiometric amounts in combination with the need for two further enzymes (lactate dehydrogenase (LDH), glucose dehydrogenase (GDH)) in order to shift the equilibrium towards the product side. Thus, simplification of the one-pot synthesis by minimizing the number of required enzymes as well as by replacement of L-alanine by ammonia as a more preferred nitrogen donor were considered as major tasks for process improvement. Addressing these challenges, in the following we report our achievements on realizing such a more simplified process concept being capable to convert styrene with ammonia into 1-phenylethyl-1-amine (*R*)-**3** in a more efficient and practical one-pot fashion while keeping conversion as well as enantioselectivity in an excellent range. Toward this end, we replaced the transaminase by an amine dehydrogenase (AmDH)[6] as a biocatalyst, which enables us to use ammonia instead of L-alanine and to avoid the use of a lactate dehydrogenase as a further enzyme component. The overall concept is shown in Scheme 1, Part B. Accordingly, after the Wacker-oxidation as the initial step, the formed ketones are *in situ* converted enantioselectively into the corresponding amine via reductive amination in the presence of an amine dehydrogenase. This reductive amination step requires ammonia as a nitrogen source and the reduced form of a cofactor, NADH, which gives its oxidized form, NAD^+, in this reaction. As such cofactors are very expensive and high-molecular weight molecules, their use as reducing agent in stoichiometric amount is not economical. Thus, in order to use the cofactor in catalytic amount the cofactor NADH is recycled *in situ* by means of, e.g., a glucose dehydrogenase, which catalyzes oxidation of D-glucose to D-gluconolactone and after spontaneous ring-opening to D-gluconic acid. As D-gluconic acid is neutralized with a base, the whole reaction is shifted towards the product side in an irreversible fashion, thus enabling a quantitative formation of the desired amine product.

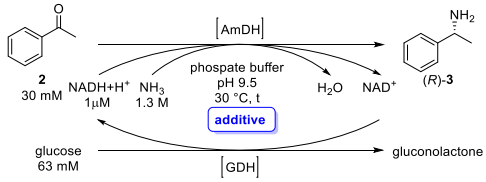
Scheme 1. Concepts for asymmetric chemoenzymatic one-pot approaches towards amines starting from alkenes



The initial step of our work consisted in identifying a suitable amine dehydrogenase for being coupled with the Wacker-catalysts, thus enabling the envisaged chemoenzymatic one-pot transformation of styrenes to 1-arylethyl-1-amines. Among the various representatives of the enzyme class of amine dehydrogenases[7] we choosed a double mutant of a leucine dehydrogenase from *Exigobacterium sibiricum* (EsLeuDH-DM) developed by Xu *et al*. as this enzyme turned out to be able for catalyzing the reductive amination of acetophenones.[7a] In addition, in recent own work we utilized this enzyme successfully in process development of the reductive amination of acetophenone **2**.[8] The initial reaction conditions for our current study with this amine dehydrogenase were based on the previously described reaction conditions.[7] A high ammonia concentration combined with an enzymatic cofactor recycling via oxidation of glucose push the equilibrium to the product side. The cofactor is thus needed only in catalytic amounts for quantitative conversion.

In the next step, the compatibility of the amine dehydrogenase with the metal components of the chemocatalytic system for the Wacker-oxidation were studied since such a compatibility represents the prerequisite for combining these two reactions in a "classic" one-pot synthesis. Previous works with an alcohol dehydrogenase and a transaminase showed that palladium salts have minor negative effects on enzymes, whereas copper causes a dramatic loss of enzyme activity.[2,5] In order to be able to determine even a slight biocatalyst deactivation, the reaction time was reduced in a way that a conversion of less than 100% was obtained. In detail, 79% conversion were obtained after 38 h in combination with 99% *ee* (Scheme 2). The presence of palladium (II) chloride leads to almost equal conversion (77%) and no decreased selectivity. However, a complete loss activity was observed when copper chloride (CuCl) was added (Scheme 2), thus being in accordance with previous studies on reduced enzyme activities in the presence of copper salts when using other enzyme classes.[2,5] The results shown in Scheme 2 illustrate that the amine dehydrogenase is not compatible with the copper component of the catalytic system for the Wacker oxidation.

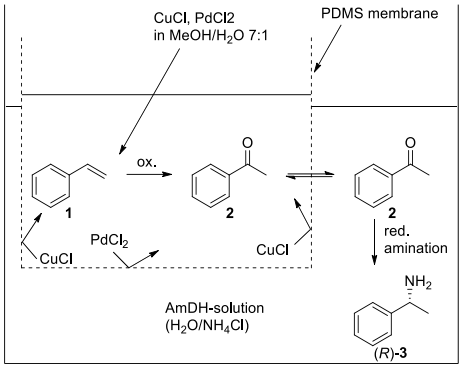
Scheme 2. Studies on the Compatibility of the Amine Dehydrogenase with Palladium and Copper Ions of the Catalyst for Wacker-Oxidation



additive	reaction time [h]	conversion [%]	enantiomeric excess [%]
-	48	97	99
-	38	79	99
$PdCl_2$ (0.14 eq)	38	77	99
CuCl (1.27 eq)	38	0	n.d.

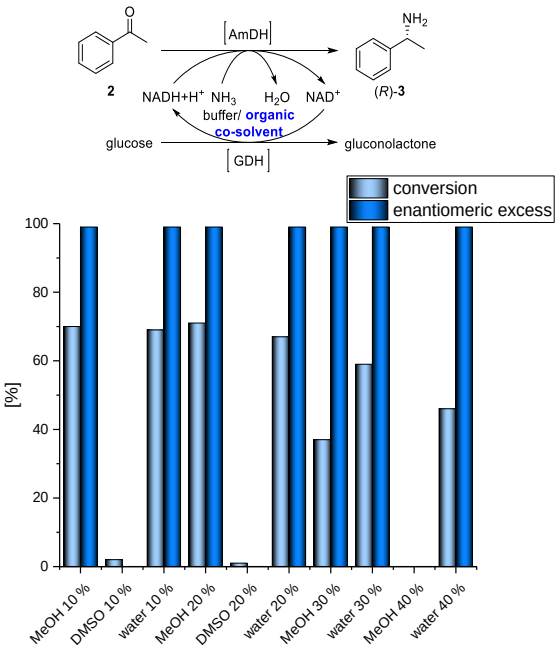
In order to overcome this hurdle of incompatibility, the compartmentalization concept applied successfully for other chemoenzymatic syntheses has been tested. This compartmentalization is based on the use of a polydimethylsiloxane (PDMS)-membrane as so-called "thimbles", which separates the reactor in two compartments. Since only hydrophobic components can pass the membrane whereas water-soluble components are retained by this membrane, the metal salts as well as the enzymes remain in their aqueous compartment. The concept for this chemoenzymatic one-pot process with compartmentalization of the catalysts by means of a PDMS-thimble is shown in Scheme 3. As mentioned above, in the field of chemoenzymatic synthesis this concept has been applied successfully by our group for the combined use of metal-type Wacker-catalysts with an alcohol dehydrogenase and transaminase.[2,5] In addition, the Greaney and Micklefield groups applied this concept for combining a halogenase with a Suzuki-cross coupling reaction.[9]

Scheme 3. Concept of Combining Wacker-Oxidation and Enzymatic Reductive Amination *via* Catalyst Compartmentalization



With regard to preparative applications of this compartmentation technique, diffusion through the membrane can represent a critical step. Previous work[2,5] showed that co-solvents enable an increased diffusion rate. However, the presence of a co-solvent might affect catalyst or substrate stability. Here, DMSO and MeOH were chosen as promising candidates known from our previous work[2] to explore the compatibility of the amine dehydrogenase with these organic co-solvents. The results are shown in Figure 1.

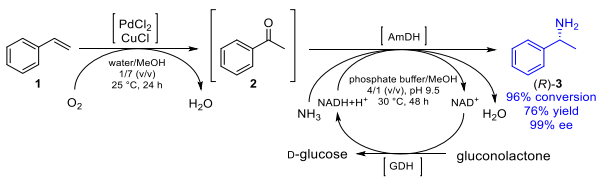
Figure 1. Impact of different Organic Co-Solvents (in [%]) on the Stability of the Amine Dehydrogenase



The amount of co-solvent was increased successively to 40% of the reaction volume. For each buffer/co-solvent mixture, a reaction was carried out with the same volume of water as reference to eliminate the effect of lower total ammonia concentration. Figure 1 shows that the selectivity of the amine dehydrogenase remains completely unaffected in the presence of methanol. At a volume fraction of 30 % of MeOH, the relative conversion begins to decrease and at 40 % no conversion was detected. For DMSO, even the addition of 10 % of the reaction volume leads to a strong decrease in conversion. The product was only detected in traces and thus could not be used for a determination of selectivity. An increase in the DMSO concentration leads to a further decrease in the range of the detection limit of the product. Thus, DMSO is not suitable as a co-solvent for this amine dehydrogenase system. For further experiments, the biotransformations have been conducted in an aqueous medium containing 20% MeOH since this is the maximum concentration of a co-solvent not showing a negative impact on the biotransformation within this enzymatic cascade.

With this optimized biotransformation towards the desired compartmentalization concept in hand, next a one-pot transformation by using a Wacker-oxidation and a biocatalytic reductive amination was performed under these buffer-MeOH conditions (Scheme 4). The advantageous solvent mixture consisting of MeOH and water was also used for the Wacker-oxidation, and both reactions were compartmentalized by means of a PDMS membrane. In Figure 2 the result of the one-pot process is shown. A quantitative conversion leads to the desired amine (*R*)-**3** in good yield and excellent enantiomeric excess of 99% *ee*. The yield of 76 % is limited by the Wacker oxidation which gives a range of by-products.

Scheme 4. Optimized One-Pot Process of a Formal Asymmetric Hydroamination by Combining Wacker-Oxidation and Reductive Amination with an Amine Dehydrogenase



Thus, an efficient transformation of styrene **1** to (*R*)-1-phenylethylamine **3** has been developed in spite of the fact that the copper ions of the Wacker-oxidation are not compatible with the amine dehydrogenase utilized in the second reaction step. The quantitative conversion and excellent *ee*-value show that by means of a PDMS-based compartmentalization such a one-pot transformation of styrene **1** to 1-phenyl-ethyl-1-amine (*R*)-**3** can be realized, leading to the desired chiral amine with high enantioselectivity when starting from a non-functionalized alkene. It should be added that in previous work re-usability of the PDMS-thimble with the interior phase bearing the Wacker oxidation catalyst system therein was demonstrated with ten reaction cycles in total and without catalyst leaching and loss of membrane performance.[5] Thus, in analogy also for this one-pot process recyclability of the Wacker oxidation catalyst (as the highest priced component of this process) can be expected.

In conclusion, an enantioselective chemoenzymatic two-step one-pot transformation of styrene to 1-phenylethyl-amine has been developed based on combining an initial Pd/Cu-catalyzed Wacker-oxidation of styrene with a subsequent reductive amination of the *in situ*-formed acetophenone. The latter step proceeds in a high enantioselective fashion in the presence of an amine dehydrogenase by consuming glucose as a co-substrate for *in situ*-cofactor recycling. As a nitrogen source only ammonia is needed. As the enzyme turned out not to be compatible with the catalytic system of the Wacker-oxidation, compartmentalization of the chemo- and biocatalysts by means of polydimethylsiloxane-based thimbles was carried out, leading to the desired amine with quantitative conversion and excellent enantiomeric excess. The overall one-pot process formally corresponds to an asymmetric hydroamination of styrene with ammonia. The tandem reaction has been demonstrated with styrene as a model substrate in this work but other amines can be expected to be accessible by this concept as well since a broad substrate scope has been already described for the individual two reactions, namely Wacker oxidation[2,5] and amine dehydrogenase-catalyzed reductive amination.[6,8] Thus, this type of process has the potential to be used as a platform technology for the synthesis of a broad range of amines when starting from different substituted styrenes within a one-pot process.

EXPERIMENTAL SECTION

General information. Chemicals for this work were purchased from *Sigma-Aldrich*, *Alfa Aesar*, *Roth*, *Merck*, *VWR*, *Acros Organics*, *Amano Enzyme Inc.* and *Oriental Yeast Co.*, *Fisher Scientific* and used without further purification. All HPLC chromatograms were recorded on a LC-Net II / ADC Machine and pumps (PU-2080) of the company *JASCO*. Chiral columns (Chiralpak AD-H) for analytical separation of the enantiomers *via* HPLC are commercially available from *Daicel Chemical Industries*.

A typical procedure of the Wacker oxidation is described in the following. Styrene **1** (115 µL, 1.00 mmol, 1.00 eq.) is added to a mixture of $PdCl_2$ (8.7 mg, 0.05 mmol, 0.05 eq) and CuCl (99.9 mg, 1.00 mmol, 1.00 eq.) in MeOH (700µL) and H_2O (100 µL). The reaction is carried out under an atmosphere of oxygen (provided *via* a balloon) at room temperature. After 24 h the reaction is terminated by adding H_2O. The mixture is extracted by methylene chloride, and the organic phase is dried with Na_2SO_4. Then, the solvent is evaporated in vacuo, and the resulting crude product is analyzed by NMR (with *tert*-BuOH as an internal standard). The conversion related to the formation of the product is 86 %. For a detailed product ratio and its explanation see Supporting Information and ref.[10]. ^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm) = 6.80-7.30 (m, 5 H, H^{ar}), 2.61 (s, 3 H). All signals are in agreement with the data reported in literature.[2,11]

Biocatalyst preparation. The construction of the biocatalyst was described before and is used in this work the same way. Also the commercial resources are exactly the same.[8]

a) Transformation of plasmid into *E. coli* (according to ref.[8]). After digestion 10 µL plasmid DNA was added to chemical competent cells (50 µL) and incubated for 30 min on ice. The cells were heated at 42 °C for 90 sec and incubated again for five minutes on ice. Afterwards 1 mL of LB media was added. The mixture was heated for three hours at 37 °C and 800 rpm. Subsequently, the cells were cultured on LB agar plates with suitable antibiotic and incubated overnight at 37 °C.

b) Protein expression (according to ref.[8]) typically in a 500 mL scale. TB medium with kanamycin (50 µg/mL) were inoculated with 1% overnight culture. The cultures were grown at 37 °C and 180 rpm. When the culture reached an OD of 0.5, cell cultures were induced with 200 µL of IPTG (1M). For expression temperature was reduced to 20 °C. Afterwards cells were harvested (4000 g,

4 °C, 30 min). For purification cells were suspended in binding buffer (25% cell suspension) and digested under ultrasound (3x3 min, 5x10 cycles). The suspension was centrifuged at 20,000 g for 20 min. The pellet was discarded and the crude extract was used for biotransformation without further purification.

c) Activity assay (according to ref.[8]). For the activity assay the oxidation of NADH to NAD+ was measured by decrease in absorbance at 340 nm at 30 °C. The enzyme activity is defined as µmol min^{-1}. The extinction coefficient is $6.3*10^3$ L mol^{-1} cm^{-1}. The reaction was performed in a 1 mL cuvette consisting of 980 µL buffer 2 M ammonium chloride buffer, pH 9.5 with ketone (5–20 mM), 10 µL NADH (10 M, final concentration 0.1 mM) and 10 µL enzyme crude extract. The activity was measured with a V-630 UV/Vis spectrophotometer from *JASCO*.

A typical procedure of the enzymatic reductive amination is described in the following. Stock solution concentrations are given in the brackets. First, ammonia (776 µL, 1.55 mmol, 2 M, pH 9.5) and glucose (50 µL, 0.05 mmol, 1 M) are dissolved in an AmDH crude extract (413 µL, 0.06 M phosphate buffer, pH 7.0, 0.23 U/µmol$_{substrate}$). To the solution are given a solution of cofactor NAD^+ (20 µL, 1 µmol, 50 mM) and glucose dehydrogenase (6 µL, 0.19 U/µmol$_{substrate}$). Then, acetophenone (**2**, 4.22 µL, 0.04 mmol) is added to the solution. The reaction mixture is stirred 48 h at 30 °C. After the complete reaction time, the mixture is extracted by methylene chloride (3 times). The combined organic layers are dried with Na_2SO_4 and separated from the solvent in vacuo. A conversion of 96 % was determined by NMR and an enantiomeric excess of 99 % was measured after derivatization on HPLC. ^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm) =1.39 (d, $^3J_{HH}$=6.7 Hz, 3 H, H-9), 4.10 (q, $^3J_{HH}$=6.7 Hz, 1 H, H-7), 7.10-7.30 (m, 5 H, H^{ar}). All signals are in agreement with the data reported in literature.[12]

For *ee*-analysis the amines were transformed to amides. The derivatization of the amine products through acylation with acetic anhydride was conducted according to a protocol reported by Kroutil *et al*.[13]: DMAP (0.8 eq.) is dissolved in acetic anhydride (20.0 eq.). The amine (1.0 eq.) is dissolved in EtOAc and given to the DMAP solution. After stirring the mixture at room temperature the reaction was quenched with water and extracted with methylene chloride. The crude product was purified by one acidic extraction at pH 1 and one basic (pH 13) extraction (3 times each of them).

As a reference for HPLC analytics a racemic mixture of 1-phenylethylamine (*rac*-**3**) was acylated after the procedure described above. The reaction was done in a 100 mg scale and leads into quantitative conversion and a very good yield (87 %). ^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm) = 7.40-7.20 (m, 5 H, H^{ar}), 5.14 (qui, $^3J_{HH}$=8.0 Hz; 1 H), 1.99 (s, 1 H), 1.50 (d, $^3J_{HH}$=5.0 Hz, 1 H). The measured signals are in agreement with the data reported in literature (ref.[10i]). Retention times (by using CO_2 / 2-propanol 95:5; 1.5 mL/min; 20 °C; 10 mPa back pressure; AD-H column; 210 nm): t_R(*R*): 15.0 min; t_R(*S*): 20.3 min.

Preparation of a PDMS thimble. The preparation of the polydimethylsiloxane (PDMS)-thimbles on a 1 mL-scale contains the following major steps: first, the surface of a glass bottle is passivated by adding five drops of trichloro-(1*H*,1*H*,2*H*,2*H*-perfluorooctyl)silane (CAS: 102488-47-1) in a desiccator and an incubation time of 3 h at 45 mbar. Sylgard 184® is mixed, degassed and kept for 5 min at a temperature of 65 °C. Then, the passivated bottle is dipped into the Sylgard solution, followed by incubating the bottle for 1 h again at a temperature of 65 °C. This procedure is repeated and after dipping the bottle the third time into the Sylgard solution, the bottle is incubated 15 min at 150 °C. After putting the bottle into *n*-hexane the bottle can be separated from the membrane. The membrane is washed one time with water and methylene chloride and then used for the synthetic transformations.[5]

Formal asymmetric hydroamination to amine (*R*-3). A typical procedure of combined reaction types (Wacker oxidation and transamination) is described in the following exemplified for styrene, **1**, as a substrate. First, styrene (56 µL, 0.5 mmol, 1.00 eq.) is added to a mixture of $PdCl_2$ (4.5 mg, 0.03 mmol, 0.05 eq) and CuCl (49.5 mg, 0.5 mmol, 1.00 eq.) in MeOH and H_2O (350 µL: 50 µL, 7:1) which is prepared in a PDMS thimble. The reaction is done under an atmosphere of oxygen (provided *via* a balloon) at RT. After 24 h the exterior volume is filled with MeOH (3.97 mL) and the complete reactor is stirred for 19 h at room temperature. Before adding the enzymatic solution, ammonia (9.7 mL, 19.38 mmol, 2 M, pH 9.5) and glucose (625 µL, 0.05 mmol, 1 M) are dissolved in an AmDH crude extract (5.16 mL, 0.06 M phosphate buffer, pH 7.0, 0.23 U/µmol$_{substrate}$). To the solution are given a solution of cofactor NAD^+ (250 µL, 1 µmol, 50 mM) and glucose dehydrogenase (75 µL, 0.19 U/µmol$_{substrate}$). Then, the enzymatic solution is filled in the exterior volume. The reaction mixture is stirred 48 h at 30 °C. After the complete reaction time, the mixture is extracted by methylene chloride (3 times). The combined organic layers are dried with Na_2SO_4 and separated from the solvent in vacuo. Conversion is calculated by NMR spectrum of the crude product is purified by extraction with hydrochloric acid (1 M). The pH of the aqueous phase is changed by sodium hydroxide solution to 14 and is extracted by methylene chloride. The enantiomeric excess is determined after derivatization to the corresponding amide by chiral HPLC. ^{1}H-NMR signals of the resulting product (*R*)-**3** within the crude product (500 MHz, $CDCl_3$): δ (ppm) =1.39 (d, $^3J_{HH}$=6.7 Hz, 3 H), 4.10 (q, $^3J_{HH}$=6.7 Hz, 1 H), 7.10-7.30 (m, 5 H, H^{ar}). The yield (46 mg, 76%) has been calculated based on the amount of obtained amine. All signals agree with them published before in literature.[11,12]

ASSOCIATED CONTENT

For HPLC chromatograms detailed product characterization of the Wacker oxidation see Supporting Information.

AUTHOR INFORMATION

Corresponding Author

* E-mail: harald.groeger@uni-bielefeld.de.

Author Contributions

The manuscript was written through contributions of all authors.

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