Hydrogenation of lipid mixtures catalysed by palladium(II)-alizarin red and ruthenium(II)-carbene complexes in aqueous solutions

PhD thesis

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Introduction

Lipids are important constituents of cell membranes. Certain properties of the cell can be altered by the catalytic hydrogenation of the unsaturated lipids found in the cell membrane. These properties include for example heat shock tolerance and different transport processes that depend on the fluidity of the membrane. The more saturated lipids the membrane consists of, the more compact it is, because of the close packing of the molecules.

The most important aim of this work was to prepare new water soluble catalysts that are applicable in the hydrogenation of lipids under mild conditions tolerable even by live cells. In the case of live systems only catalysts having considerable activities at low temperatures can be used. The solubility of the catalysts in water is extremely important, since the application of other solvents usually lead to the necrosis of the cell. Furthermore, catalyst are required to exhibit selectivity toward the hydrogenation of the C=C bonds of fatty acid residues compared to C=O and other unsaturated groups.

Having studied the available literature on the chemistry of carbene-complexes, Ru(II)-N-heterocyclic carbene complexes were synthetized. In these compounds the metal-carbon bond between the central metal ion and the carbene carbon atom of the ligand has enhanced stability compared to the metal-phosphorus bond of the phosphine analogues. Therefore carbene complexes are usually less sensitive to water and oxigen than the corresponding phosphine complexes.

Cell membranes consist of various types of lipids. Individual lipids of these lipid types differ in the length of fatty acid residues and in the number of double bonds. One of the aims of this study was to develop a fast and reliable method for the detection of various lipids and for the determination of the reactivity of individual lipid molecules in lipid mixtures upon hydrogenation. Most analytical methods are unappropriate to study the reactivity of individual lipids and to detect differences in the reactivities of different lipid types.

Applied experimental techniques

At low pressures lipid hydrogenation reactions were carried out in a Schlenk-vessel, whereas in the case of higher pressures (2-10 bar) a pressure resistant glass tube reactor was applied. Conversions of individual lipids in lipid mixtures were obtained on a BRUKER Biflex III MALDI-TOF mass spectrometer in the presence of 2,5-dihydroxi-benzoic acid

(DHB) matrix. The total conversions of mixtures consisting of several lipids (e.g. soy-bean lecithin) were determined on a HEWLETT-PACKARD 5890 Series II gas chromatograph. By the application of this technique only the ratio of the fatty acid residues of lipids can be obtained, however no information is gained on the reactivity of individual lipids.

The hydrogenation of ketones, aldehydes and olefines, the redox isomerisation of allyl-alcohol, and the hydrogen transfer reaction between 2-propanol and acetophenone were followed by ^{1}H-NMR measurements on a BRUKER DRX 360 instrument.

The identification of new Ru(II)-NHC-carbene complexes was carried out by ^{1}H-, ^{13}C-, and ^{31}P-NMR techniques (BRUKER DRX 360) and by mass spectrometry (BRUKER BIOTOF II ESI-TOF). Infra red spectra were recorded on a PE Paragon FT 1000 instrument in KBr pastilles. The formation of Ru(II)-carbene-phosphine complexes was studied by UV-VIS spectrophotometry on a Hitachi U2000 device. pH potentiometric titrations were carried out on an automatically controlled Metrohm 702 S14 titration system equipped with a Metrohm 6.0234.100 combined electrode. X-ray crystallographic data of $[RuCl_2L(C_{10}H_{14})]$ (L = 1-butyl-3-methylimidazol-2-ylidene, $C_{10}H_{14}$: *p*-cymene) (**1**) were collected on an Enraf Nonius MACH3 diffractometer.

Abbreviations

BMIMCl: 1-butyl-3-methylimidazolium chloride
DGDG: digalactosyl diacylglycerol
DHB: 2,5-dihydroxy benzoic acid
DOPC: dioleyl phosphatidylcholine
DOPE: dioleyl phosphatidylethanolamine
ESI: electron spray ionisation
MALDI: *m*atrix *a*ssisted *l*aser *d*esorption and *i*onisation
MGDG: monogalactosyl diacylglycerol
NHC-carbene: N-heterocyclic carbene
Pd(QS)$_2$: palladium(II)-alizarin red
PG: phosphatidylglicerol
PTA: 1,3,5-triaza-7-phosphaadamantane
SL: sulpholipid
TOF: Turnover Frequency:
(reacted substrate) (catalyst×time)$^{-1}$,
(mol/mol h)
***m*TPPMS**: diphenyl-(3-sulphophenyl)-phosphine
$[RuCl_2L(C_{10}H_{14})]$ ⇒
L: 1-butyl-3-methylimidazol-2-ylidene
$C_{10}H_{14}$: *p*-cymene (4-isopropyl-toluene)

New scientific results

The new scientific results of my work are summarized in the following *nine* points:

I. A fast and reliable analytical method was developed for the detection of individual lipids in lipid mixtures and for the determination of the reactivity of individual lipid components.

MALDI-TOF-MS proved to be applicable in the determination of the reactivity of DOPC (dioleyl-phosphatidyl choline), a lipid containing two double bonds, in hydrogenation reactions (*figure 1*).

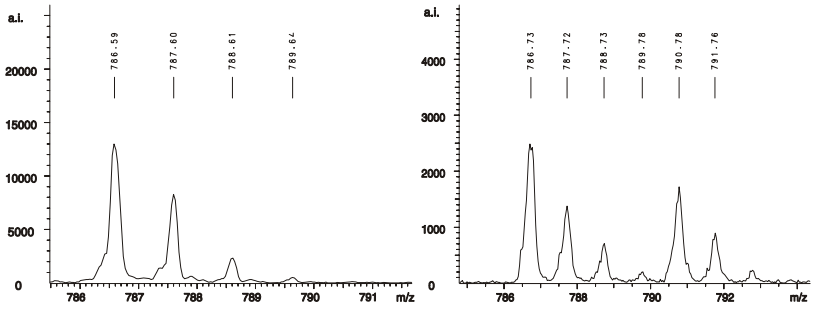


Figure 1 MALDI-TOF-MS spectra of DOPC and hydrogenated DOPC
[$Pd(QS)_2$]=12.1μM, [DOPC]=127μM, T=30°C, p(H_2)=106 kPa, t=2 min

In the hydrogenation reaction catalysed by palladium(II)-alizarin red ($Pd(QS)_2$) the half saturated product (788 Da) formed in a small amount compared to the fully saturated lipid (790 Da).

The detection of the individual lipids of DOPE/DOPC mixtures of different ratios and of lipid mixtures isolated from the thylakoid membrane of *Synechocystis PCC 6803* meets difficulties, since the presence of some certain lipids (e.g. DOPC) in the mixture prevents the detection of other lipid components. It was observed in our experiments that the use of DHB as a matrix and the addition of saturated NaCl/EtOH solutions to the lipid samples ensured comparable intensities of the $[M+Na]^+$ signals of different components in the spectrum. Based on this observation a quantitative analytical method has been worked out for the determination of the reactivity of individual lipids in lipid mixtures.

II. In the hydrogenation of DOPE-DOPC mixtures of different ratios catalysed by $Pd(QS)_2$ the conversions obtained for the individual lipids did not differ significantly, whereas in the case of Synechocystis PCC 6803 significant differences in the reactivities of the components were determined.

Irrespective of the ratio of the components in the hydrogenation reaction, the reactivity of DOPE (dioleyl-phosphatidyl ethanolamine) and DOPC (dioleyl-phosphatidyl choline) did not differ significantly. On the addition of cholesterol the reactivity of both lipids decreased. This phenomenon is explained by the effect of cholesterol causing a rigid structure of the vesicles. The reactivity of DOPE and DOPC decreased by about the same extent (*figure 2*).

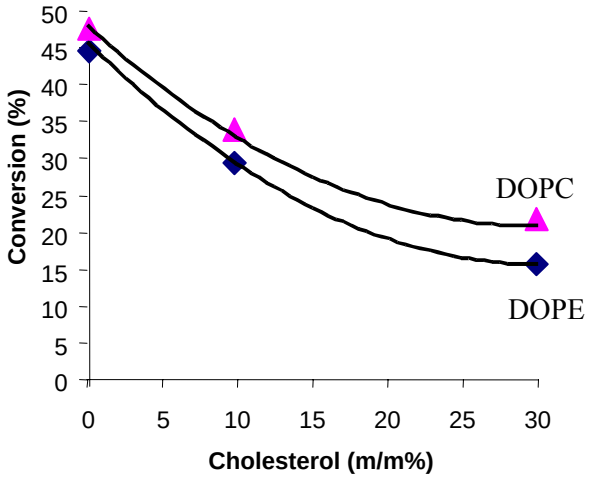


Figure 2 Hydrogenation of DOPE:DOPC = 1:1 mixtures in the presence of cholesterol
$[Pd(QS)_2]$= 12.1 μM, [lipid] ([DOPE]+[DOPC]) = 127 μM, T = 30°C, $p(H_2)$ =109 kPa, t = 5 min

In the hydrogenation of the four component lipid mixture isolated from the thylakoid membrane of *Synechocystis PCC 6803* the conversions for MGDG and DGDG were 23.0 and 24.4 % respectively, whereas only 12.0 % of SL and 11.4 % of PG were reacted.

*III. A new ruthenium(II)-N-heterocyclic carbene complex {$[RuCl_2L(C_{10}H_{14})]$ (L: 1-butyl-3-methylimidazol-2-ylidene, $C_{10}H_{14}$: p-cymene) (**1**)} was prepared. The complexes existing in the aqueous solution of **1** were identified.*

The two step carbene transfer reaction of 1-butyl-3-methylimidazolium chloride (BMIMCl) and $[RuCl_2(C_{10}H_{14})]_2$ via a silver(I)-dicarbene particle leads to the formation of the stable water soluble **1** complex (*figure 3*).

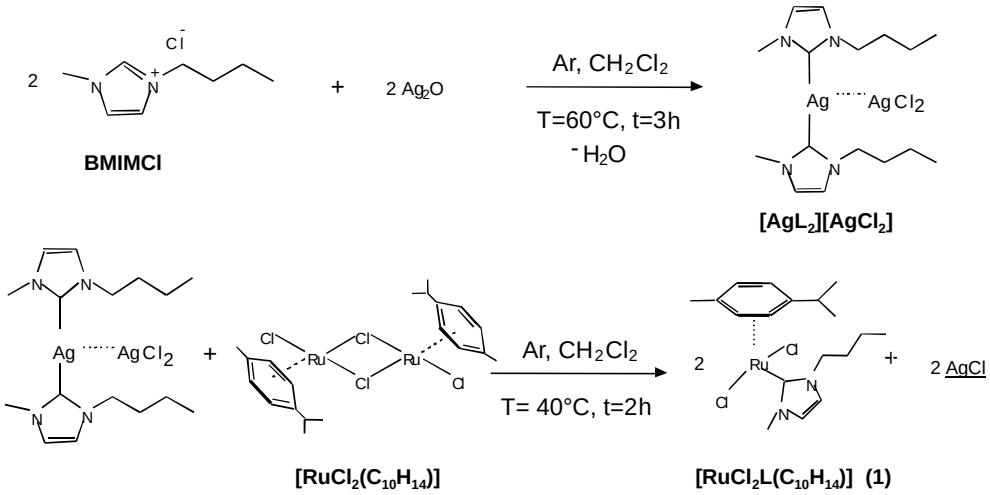


Figure 3 Preparation of $[RuCl_2L(C_{10}H_{14})]$ by the carbene transfer methodology

The carbene transferring particle in the reaction is the extremely unstable $[AgL_2][AgCl_2]$.

In a D_2O solution of **1** two singlet ^{13}C resonances are seen in the carbene region of the spectrum referring to the $[RuCl(H_2O)L(C_{10}H_{14})]^+$ (**2a** – 169.8 ppm) and $[Ru(H_2O)_2L(C_{10}H_{14})]^{2+}$ (**2b** – 169.5 ppm) complex cations. In a 0.1 M KCl solution of **1** the dominant particle is **2a**, whereas the presence of **2b** is not observed. In concentrated (0.1-0.5 M) KCl solutions the undissociated **1** is also detectable by ESI-TOF-MS.

*IV. New carbene-phosphine mixed ligand complexes were prepared in situ in the aqueous solution of **1**. Complex-formation reactions were followed by spectrophotometry.*

The addition of equivalent amounts of PTA (*figure 4.a.*) to the aqueous solution of **1** resulted in the formation of $[RuCl(PTA)L(C_{10}H_{14})]^+$ (**4a**) and $[Ru(H_2O)(PTA)L(C_{10}H_{14})]^{2+}$ (**4b**). The complexes were identified by ^{13}C-NMR measurements (**4a**: -35.5 ppm; **4b**: -36.1 ppm). In 0.1 M KCl solutions the dominant particle is **4a**.

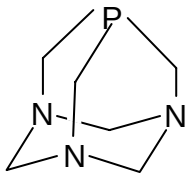


Figure 4.a The structure of 1,3,5-triaza-7-phosphaadamantane (PTA)

The reaction, accompanied by a colour change from yellowish brown to yellow, was followed by UV-VIS spectrophotometry. PTA was added in small portions, and after each addition, the spectrum of the solution was recorded in the region of 370-550 *nm (figure 4.b*).

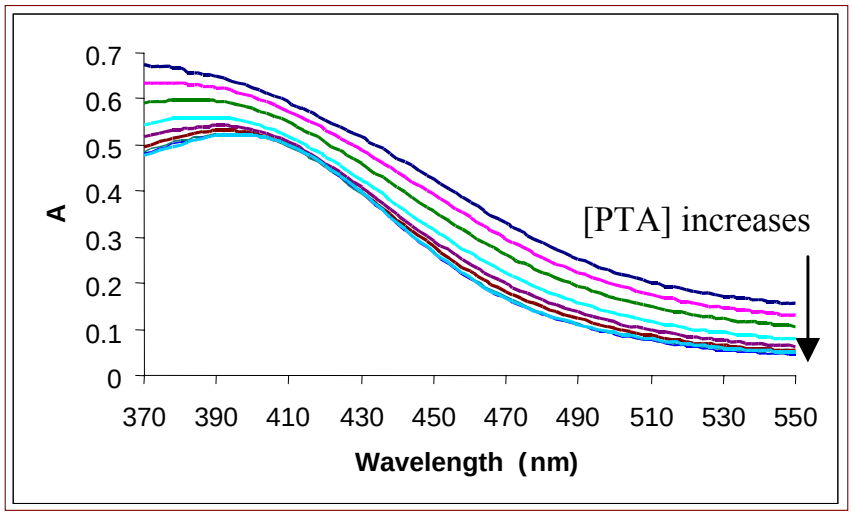


Figure 4.b Examination of the formation of Ru(II)-carbene-PTA complexes by UV-VIS spectrophotometry
[Ru] = 0.98 mM, [PTA-stock solution] = 16.25 mM, T = 25°C

The addition of PTA caused a decrease in the absorbances at every point of the examined range of wavelengths, however when the ruthenium to PTA ratio reached 1, no further spectral changes could be detected. On the basis of this finding it is concluded that only one PTA per Ru is coordinated in the reaction, and the new complexes are stable to water. This provides a possibility to apply *in situ* prepared complexes in catalytic processes.

V. The acid-base properties of ***1*** *were determined by pH-potentiometric titration and the distribution of the complexes formed in the solution was calculated as a function of the pH.*

The pH-potentiometric titration of **1** was carried out in a 0.1 M KCl solution, in which the predominant species is **2a**. According to ^{13}C-NMR measurements the Ru-C bond is stable in a wide (2-12) pH range, furthermore no sign of polimer or colloid formation was observed under the conditions of titrations. *Figure 5* shows the distribution of the species formed in the solution on the addition of KOH, as a function of the pH.

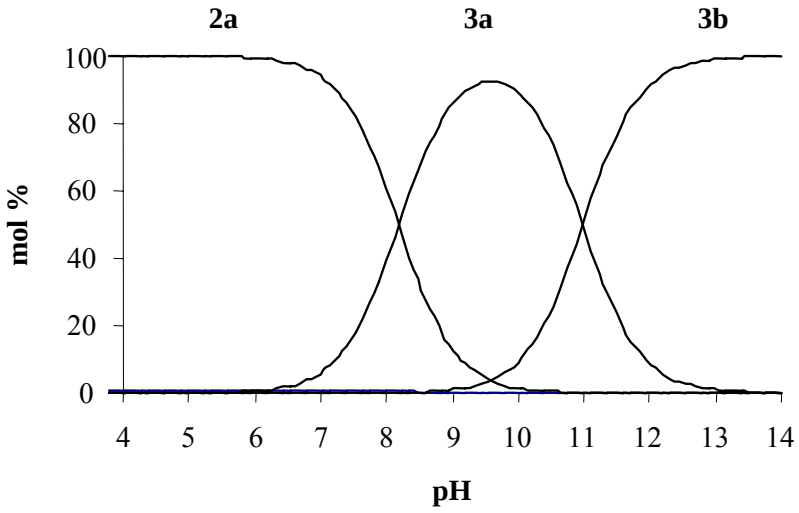
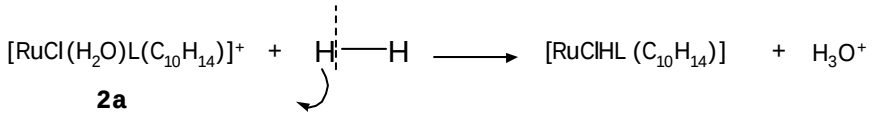


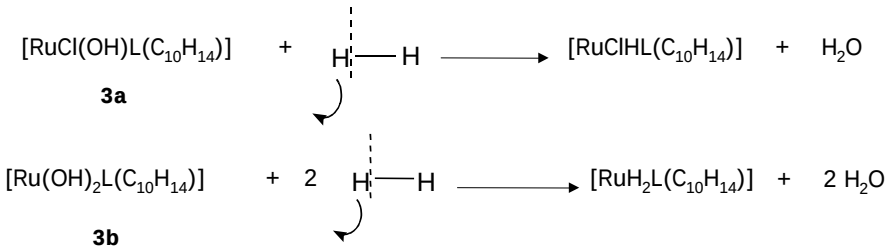
Figure 5 Distribution of complexes formed on the addition of KOH, as a function of the pH
[KOH] = 0.1950 M, $[Cl^-]_{total}$= 0.1 M, [HCl] = 9.09x10^{-3} M, [Ru] = 3.67x10^{-3} M, T = 25°C

Under pH 6 the only species in the solution is **2a**. As the concentration of the base increases, at pH 7 [RuCl(OH)L($C_{10}H_{14}$)] (**3a**) appears as a result of the H_2O – OH exchange, or the deprotonation of the coordinated water ligand in the coordination sphere. Between pH 9 and pH 10 **3a** is by far the most abundant species in the solution, however the further increase of the pH gives rise to the formation of the dihydroxo-complex ([Ru$(OH)_2$L($C_{10}H_{14}$)] – **3b**) by a Cl – OH exchange. At pH 12 exclusively **3b** exists in the solution.

It was concluded on the basis of pH-static titrations that the hydrogen molecule is activated by a heterolytic bond splitting in the reaction with the studied Ru-complexes. The heterolytic H-H bond splitting in neutral solutions (pH 7.5; the dominant species is **2a**) results in the production of H^+ ions:



In basic solutions (pH 10.8; **3a** and **3b** are detected) the formation of protons is not observed:



These are the first pH-potentiometric studies of the acid-base properties of N-heterocyclic carbene complexes.

VI. The new Ru(II)-NHC carbene complexes showed catalytic activity in the hydrogenation of ketones, aldehydes and olefines under mild conditions.

The hydrogenation conversions of various substrates and the turnover frequencies (TOF) of the applied catalysts {**1** and **4** (**1**+PTA: **4a** +**4b**)} are listed in *table 1*.

Table 1 Hydrogenation of various substrates catalyzed by **1** and **4**
$p(H_2)$ = 10 bar, T = 80°C, [Ru] = 4.73 mM, [substrate] = 667 mM, t = 1 hour, pH = 6.9

Substrate	Catalyst			
	$[RuCl_2L(C_{10}H_{14})]$ (**1**)		**1**+PTA (**4**)	
	Conversion (%)	TOF (h^{-1})	Conversion (%)	TOF (h^{-1})
Acetone	33,6	47	98,2	139
Acetophenone	28,4	40	46,1	65
Allyl alcohol	85,6	121	95,6	135
Benzylidene-acetone	18,7	26	42,3	60
Cinnamaldehyde	27,3	39	42,0	59
Propanal	78,3	110	86,2	122
4-styrenesulfonic acid Na-salt	3,5	5	27,4	39

At 80°C and 10 bar H_2 pressure **4** proved to exhibit higher catalytic activity than **1** in the hydrogenation of all applied substrates. **4** selectively hydrogenated the C=C double bond of benzylideneacetone with a conversion of 42.3 % (selectivity: 91.9 %). In the presence of **1** the conversion was as small as 18.7 % with 61.7 % selectivity. It is remarkable that the hydrogenation of ketones was not observed with the phosphine complexes analogous to **1** ($[RuCl_2(PR_3)(C_{10}H_{14})]$, PR_3 = PTA or *m*TPPMS).

VII. The Ru(II)-NHC carbenes studied in this work are the first transition metal – NHC complexes that catalyse redox isomerization. The detailed study of the redox isomerization reaction of allyl alcohol was carried out, which included the determination of the changes in the concentration of the reactants in the function of time, and the temperature dependence of the process.

The hydrogenation of allyl alcohol led to the formation of two products: propanol (formed upon hydrogenation) and propanal (formed by redox isomerization). The formation of propanal could be detected exclusively in the presence of hydrogen gas. For this reason it is supposed that the catalytically active species in redox isomerization is a hydrido complex.

In the reaction until about 90 % total conversion propanol and propanal were produced in approximately 1:1 ratio. Following that propanal was formed on the expense of propanol (*figure 7*).

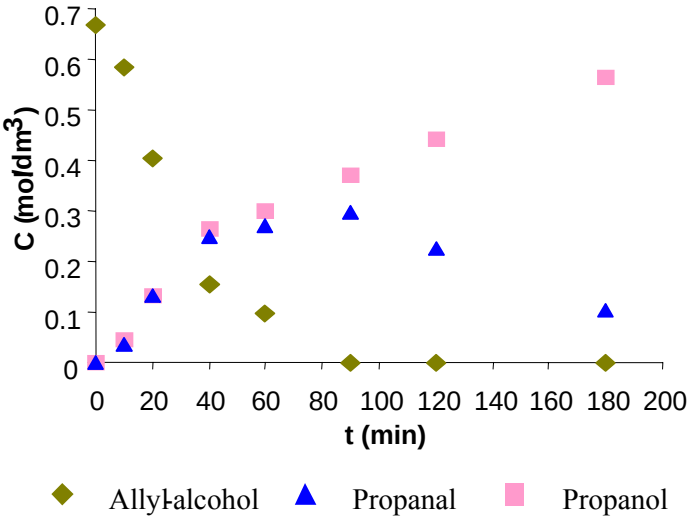


Figure 7 Hydrogenation and redox isomerization of allyl alcohol –change of the concentration of the reactants in the function of time; $p(H_2)$ = 10 bar, T = 80°C, [**1**] = 4.73 mM, [allyl-alcohol] = 667 mM, pH = 6.9

Examining the temperature dependence of the reaction it was found that allyl-alcohol neither hydrogenated nor took part in redox isomerisation in the presence of **1** at 40°C. However at 80 °C almost total conversion was reached in 1 hour.

VIII. The new Ru(II)-carbene complexes were found to catalyse the hydrogen transfer reaction between 2-propanol and acetophenone. The effects of the reaction conditions and that of the applied base were determined.

The hydrogen transfer reaction between 2-propanol and acetophenone resulted in higher conversions (catalyst: **4**) when KOH was used as a base instead of K_2CO_3 (*table 2*). The increase in the reactivity can probably be explained by KOH being a stronger base that plays important role in the deprotonation of the hydrogen donor molecule.

Table 2 Hydrogen transfer reaction between 2-propanol and acetophenone catalysed by **4** in the presence of KOH and K_2CO_3; t = 2.5 hour, [**4**] = 25.74 mM, [acetophenone] = 1.00 M, [base] = 475.54 mM, $V_{\text{reaction mixture}}$ = 1.12 ml, added water: 0.1 ml

Base	Added water	Conversion (%)	TOF (h^{-1})
K_2CO_3	-	67.97	9.44
K_2CO_3	+	8.11	1.13
KOH	-	96.33	13.38
KOH	+	0.21	0.03

The addition of water decreased the conversions of hydrogen transfer reactions. In the case of KOH the reaction was almost totally blocked in the presence of water.

*IX. The new Ru(II)-NHC carbene complexes catalysed the hydrogenation of DOPC, soy-bean lecithin and Synechocystis lipid mixtures under mild conditions in aqueous dispersions. Catalyst **1** proved to be more active at low temperatures (37°C), while **4** showed higher activities at elevated temperatures (50-80°C).*

In the case of hydrogenating DOPC by **1**, the composition of the hydrogenated mixture was different from that obtained by the use of $Pd(QS)_2$ as a catalyst (section *I.*). The concentration of the half-saturated product (788 Da) was significant in this case (*figure 9*).

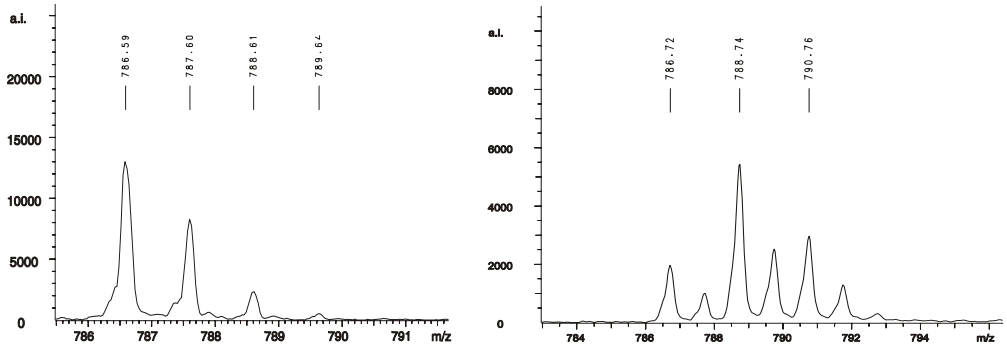


Figure 9 MALDI-TOF-MS-spectra of DOPC and hydrogenated DOPC
[**1**]=1.88mM, [DOPC]=1.27mM, T=37°C, $p(H_2)$=10 bar, t=3 hours

Differences were detected in the reactivities of individual lipid types in the hydrogenation of *Synechocystis* lipid mixtures. The reactivity differences were especially significant when **1** was used as a catalyst; MGDG and DGDG reached 23.3 and 18.1 % conversions respectively, whereas only 3.1 % of SL was reacted. The hydrogenation of PG was not detectable.

The hydrogenation of soy-bean lecithin catalysed by **1** at 37°C resulted in a conversion of 11.6 %, whereas in the presence of **4** the conversion was only 2.1 %. However, at 80°C a conversion of 17.5 % was detected in the reaction catalysed by **1**, while the application of **4** resulted in a conversion of 99.8 %.

It is concluded on the basis of this work that the water-soluble Ru(II)-N-heterocyclic carbene complexes catalyse the hydrogenation, the redox isomerisation and the hydrogen transfer reactions of olefines and oxo-compounds under mild conditions and are suitable for the hydrogenation of the aqueous dispersions of lipids. The hydrogenation of lipids can be quantitatively detected by MALDI-TOF-MS.

List of publications

Articles connected to the topic of this Thesis:

1. ***P. Csabai**, F. Joó*: Reactivity of the individual lipid classes in homogeneous catalytic hydrogenation of model and biomembranes detected by MALDI-TOF mass spectrometry, *Catalysis Communications*, **2003***, 4,* 275-280.

2. ***P. Csabai**, F. Joó*: Synthesis and catalytic properties of new water-soluble Ru(II)-N-heterocyclic carbene complexes, *Organometallics,* **2004**, *23*, 5640-5643

Conferences:

1. ***Csabai P**., Joó F.*: *Biológiai membránok módosításának vizsgálata MALDI-TOF-MS technikával*, XXIV. Kémiai Előadói Napok, Szeged, 2001. október 29-31.

2. ***Csabai P**., Joó F.*: *Lipidkeverékek hidrogénezése hagyományos és fém – karbén komplex katalizátorokkal*, IX. Nemzetközi Vegyészkonferencia, Kolozsvár, 2003. November 14-16.

3. ***P. Csabai**, F. Joó*: *Hydrogenation of aqueous lipid dispersions followed by MALDI-TOF mass spectrometry*, 28th International Conference on Solution Chemistry, Debrecen, August 23-28, 2003

4. ***P. Csabai**, F. Joó*: *Synthesis and catalytic properties of new water-soluble Ru(II)-N-heterocyclic carbene complexes*, 14th International Symposium on Homogeneous Catalysis, Munich, July 5-9, 2004

5. ***Csabai P**., Joó F.*: *Vízoldható ruténium-N-heterociklusos karbén komplexek előállítása és katalitikus tulajdonságaik vizsgálata*, XXXIX. Komplexkémiai Kollokvium, 2004. május 26-28, Agárd-Gárdony

6. ***P. Csabai**, M. Fekete, F. Joó*: *Synthesis and catalytic properties of new water-soluble Ru(II)-N-heterocyclic carbene complexes*, Green Solvents For Synthesis, Bruchsal, Germany, October 3-6, 2004

7. ***P. Csabai**, M. Fekete, G. Papp, H. Horváth, Á. Kathó, F. Joó*: Synthesis and catalytic properties of new water-soluble organometallic Ru(II)-complexes, *COST D30 Workshop, Tarragona, Spain, November 19-20, 2004.*