Rational Catalyst Design

DOI: 10.1002/ange.201000907

Phosphorus Ligand Imaging with Two-Photon Fluorescence Spectroscopy: Towards Rational Catalyst Immobilization**

Fabrizio Marras, Alexander M. Kluwer, Joanna R. Siekierzycka, Alessandro Vozza,*
*Albert M. Brouwer, and Joost N. H. Reek**

Catalyst recovery is an important topic in homogeneous catalysis, since product/catalyst separation is one of the main obstacles towards application of this type of catalysis. So far, several strategies for catalyst recycling have been explored, but a general strategy remains elusive.[1–3] A widely studied approach to facilitate catalyst/product separation is attachment of homogeneous catalysts to soluble or insoluble supports, which can consist of organic polymers[4–7] or dendrimers,[8,9] inorganic materials,[10,11] or hybrids thereof. Inorganic materials have proved to be particularly suited as solid supports for homogeneous catalysts because of their physical strength and chemical inertness, and many such immobilized catalyst systems have been reported. Common drawbacks, however, are the generally lower activity and selectivity compared to the homogeneous counterpart. Surprisingly, however, an in-depth investigation of the effect of the immobilization process on the performance of the catalyst appears to be lacking. To gain more insight into immobilization of transition metal catalysts we set out to design a method for the detection of bis-phosphine ligands on surfaces. The ligand studied acts as a fluorescence probe, and detection is achieved by two-photon excitation fluorescence microscopy. In principle, this allows the immobilization product to be imaged with high spatial resolution (down to single molecules). Here we report the findings of the first study on the immobilization process employing an intrinsically fluorescent ligand imaged on a submicrometer level, which indicated that precondensation of ligands takes place prior to immobilization under the standard immobilization conditions, a conclusion supported by analysis of the liquid phase. These results directly translate to simple procedures that do not have these precondensation problems. The resulting immobilized catalysts show superior performance in hydroformylation catalysis, and open the way toward rational catalyst immobilization.

Two-photon excitation (TPE) fluorescence microscopy has shown astonishing potential, but application has been mainly restricted to imaging biological samples.[12] Two-photon absorption (TPA) is a process in which two photons are absorbed simultaneously. Such a process only occurs at a very high flux of photons by focusing a pulsed near-infrared laser, thus restricting the excitation to a very small focal volume, with no appreciable off-focal fluorescence. Generally, the two-photon selection rule yields low background fluorescence, and hence high contrast in the images can be produced.[12,13]

As part of a broader effort to study the immobilization of transition metal catalysts,[14–17] we were curious whether catalyst immobilization could be studied by fluorescence imaging. For this purpose we envisioned the class of xanthene-based phosphorus ligands, also known as Xantphos, suitable for various reasons. Metal complexes of these ligands have outstanding catalytic properties in various catalytic reactions (e.g., Rh-catalyzed hydroformylation, Pd-catalyzed cross-coupling), and the ligands show facile coordination to various metals and are highly stable and easy to modify.[18,19] In addition, some members of the xantphos family, such as nixantphos (**1**, see Scheme 1) display strong fluorescence upon excitation and have previously been used for ligand immobilization.[14–17] This led us to study the photophysical properties of **1** and particularly the possibility to use the immobilized ligand as a two-photon excitation probe to study the immobilization process. For this purpose, *N*-functionalized nixantphos **2** was immobilized on glass cover slips and on activated silica. Both of these materials have similar SiOH groups at the surface for attachment of the ligand, allowing us to study the immobilization process spectroscopically and to correlate the results to the catalytic properties of the immobilized catalyst.

Ligand **1** possesses many useful chemical and spectroscopic characteristics, including a well-defined linear absorption spectrum. The spectrum shows a strong absorption at 280 nm ($\varepsilon = 1.7\times10^{4}\,\text{M}^{-1}\,\text{cm}^{-1}$) and a weaker absorption at 340 nm ($\varepsilon = 7.7\times10^{3}\,\text{M}^{-1}\,\text{cm}^{-1}$; Figure 1). In addition, **1** shows a high fluorescence quantum yield ($\lambda_{em} = 428$ nm, $\Phi = 0.33$) and short lifetime of emission ($\tau = 1.4$ ns).

Initial two-photon excitation experiments revealed that parent ligand **1** displays strong fluorescence upon excitation

---

[*] F. Marras, Dr. A. M. Kluwer, J. R. Siekierzycka, A. Vozza,
Prof. A. M. Brouwer, Prof. J. N. H. Reek
Van't Hoff Institute for Molecular Sciences, University of Amsterdam
Nieuwe Achtergracht 166, 1018 WV Amsterdam (The Netherlands)
Fax: (+31) 20-525-5604
E-mail: a.m.kluwer@uva.nl
j.n.h.reek@uva.nl
Homepage: http://www.science.uva.nl/research/imc/HomKat

[**] This research is supported by the National Research School Combination-Catalysis (NRSC-C), The Netherlands Organisation for Scientific Research (NWO)-STW and NanoNed (AMM, 6980), a national nanotechnology program coordinated by the Dutch Ministry of Economic Affairs. We acknowledge H. J. van Ramesdonk for technical assistance with the confocal microscope setup and Dr. E. R. H. van Eck of the solid-state NMR facility for advanced materials science, jointly supported by the Netherlands Organisation for Scientific Research (NWO) and Radboud University Nijmegen, for acquiring the solid-state NMR spectra.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201000907.

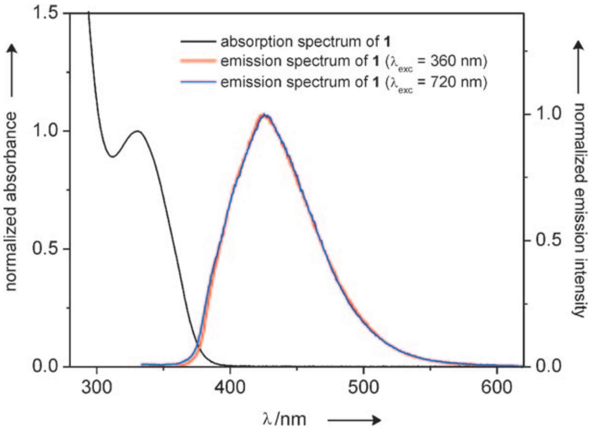
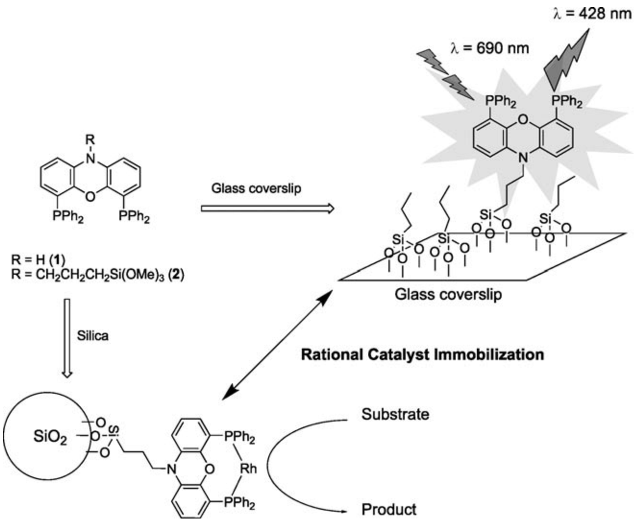


Figure 1. Normalized absorption spectrum, steady-state fluorescence spectrum, and two-photon fluorescence spectrum of **1**.



Scheme 1. Immobilization of nixantphos **2** on glass cover slips and silica for imaging and catalysis with the aim of correlating the immobilization process to the catalysis results.

at 720 nm. The recorded spectrum is virtually identical to the one-photon-induced emission spectrum (Figure 1). A quadratic relationship between emission intensity and excitation power was found, thus confirming that the recorded fluorescence is indeed the result of TPA (see the Supporting Information). The two-photon excitation spectrum was obtained by changing the excitation wavelength (at constant power) from 780 to 690 nm and measuring the emission intensity at 428 nm. The shape is identical to that of the absorption spectrum at half the wavelength. By comparison with fluorescein,[20] the TPE cross-section δ (i.e., the probability of a transition to a given state) could be determined to be approximately 1.4 GM at 690 nm ($1\ \mathrm{GM} = 10^{-50}\ (\mathrm{cm}^4\,\mathrm{s})/\mathrm{photon}$). Such a TPE cross-section is modest, but together with the fluorescence quantum yield of 0.33 it is adequate for imaging.

For immobilization of this ligand a trimethoxysilane moiety is introduced, which is known to attach covalently via the silanol groups present on the glass surface or silica support. According to the standard immobilization process (method A), clean borosilicate microscope cover slips were immersed in a refluxing toluene solution of ligand **2** (Scheme 1) at 0.1 mM concentration. After 18 h, capping agent (*n*-propyl(trimethoxy)silane) was added to ensure all silanol groups are silylated. The cover slips were cleaned in a sonicator twice with fresh toluene and once with methanol to remove all nonbonded fluorophore, dried under vacuum, and stored under argon. The increase in the static water-contact angle of the glass cover slips from 50 to 69° indicated coverage of the surface by the hydrophobic phosphorus ligand.

Two-photon scanning images recorded on the cover slip with immobilized **2** showed, besides uniformly dispersed ligand giving rise to the background emission, also more intense fluorescence spots (Figure 2, left). The two-photon fluorescence spectra recorded in these spots are identical to those of parent compound **1**. The recorded fluorescence lifetime (0.8 ns) is significantly shorter than the lifetime of the uniformly dispersed ligand (1.4 ns), and this effect is ascribed to self-quenching or concentration quenching.[21] Furthermore, the intense spots are gradually photobleached on irradiation, thus indicating that these spots are due to fluorescence of higher-concentration immobilized ligand. Clearly, the standard immobilization process does not yield a homogeneous distribution of the ligand but also larger clusters of ligands. Such a high concentration of catalyst is anticipated to affect the catalytic results (vide infra).

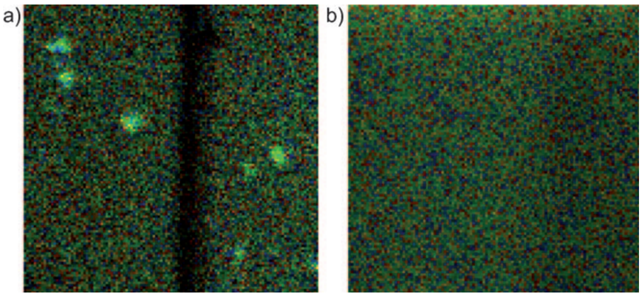


Figure 2. Two-photon scanning images (65×65 μm) of nixantphos immobilized on microscope cover slips by a) direct addition (method A) of **2** and cover slips prepared by b) dropwise-diluted (method B) addition of **2** and propyl(trimethoxy)silane. The bright green spots on the cover slips prepared by method A correspond to higher concentrations of fluorophore indicating clusters of ligand **2**. Images were acquired with similar average number of counts per pixel (see the Supporting Information for details).

To obtain a homogeneous distribution of ligands on the glass support, a mixture of **2** and *n*-propyl(trimethoxy)silane in toluene solution was slowly added over 24 h to a refluxing toluene solution containing the glass cover slips (method B). Images of the glass cover slips subjected to the new immobilization procedure do not display intense fluorescence spots (Figure 2, right). Analysis of the fluorescence lifetime images gave a typical lifetime equal to that of compound **1**. The two-photon fluorescence spectra taken at arbitrary points of the scanned area are identical to the emission spectra shown in Figure 1 (see the Supporting Information), thus showing that the ligands are well spaced. The two immobilization methods were also applied to a second fluorescent ligand system, namely, triethoxysilylpropyl-modified tetraphenyl porphyrin (see the Supporting Information). In this case method A also led to clustering of the tetraphenyl porphyrin, while method B produced well-spaced coverage of the surface. Thus new immobilization method B appears to provide homogenously distributed ligands, in contrast to the commonly used method A, which causes clustering of ligands. As this seems to be related to the immobilization strategy (independent of the ligand used), it is likely that a common mechanism is involved, which was further studied by complementary measurements on the solution phase during immobilization.

We anticipated that cluster formation was not occurring on the solid support (glass or silica) but is the result of a condensation reaction of the trialkoxysilane groups in solution, a process that also takes place during sol–gel synthesis.[17,18] To investigate this we performed immobilization experiments in which ligand **2** was covalently anchored on silica (particle size 200–400 μm; pore size 60 Å) by methods A and B[22] and sampled the reaction solution over time. The samples were analyzed by recording fluorescence intensity traces, and by gel permeation chromatography (GPC). The intensity traces recorded during immobilization by method A show, besides the background fluorescence of dissolved **2**, also more intense peaks. The intensity and frequency of the fluorescence bursts increase further with increasing reaction time (up to 65 times compared to steady-state fluorescence of **2**, see Figure 3 and the Supporting Information), which confirms that condensation of **2** takes place in solution during the immobilization process. Scans recorded on solution samples from immobilization method B show almost no emission bursts, thus indicating that all ligand **2** remains monomeric during the immobilization process. These results are in line with the GPC measurements on these samples, which show formation of larger species for method A that grow larger with reaction time (broad peak at 12 min shifting to smaller retention times), while samples taken during immobilization with method B show only monomeric ligand **2** (see Figure 4).

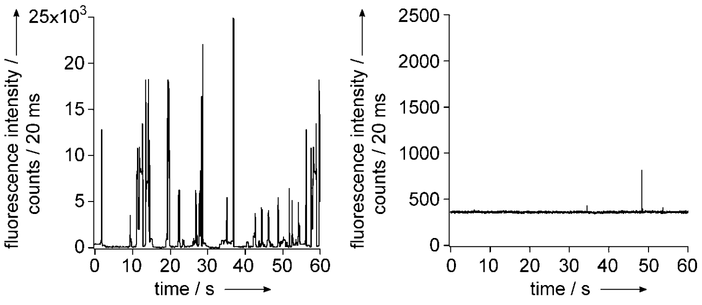


Figure 3. Fluorescence intensity traces (410–480 nm) from the focus of a diffraction-limited laser beam recorded in the reaction solvent during immobilization of **2** on silica. a) Method A, sample taken at 370 min. b) Method B, sample taken at 290 min (vertical scale is adjusted by 10× multiplication; for all other samples measured, see the Supporting Information).

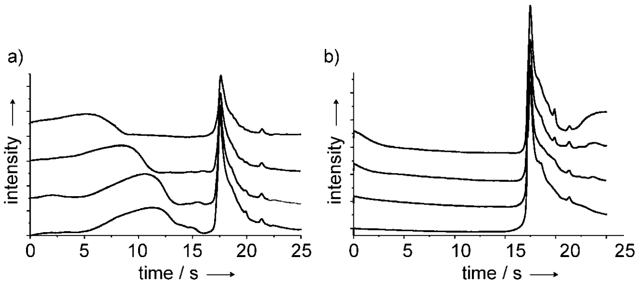
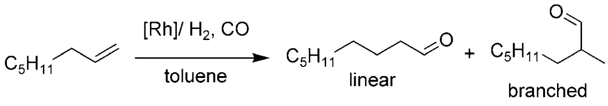


Figure 4. GPC traces of the reaction solution during immobilization of **2** on silica. A) For method A, GPC samples were taken at 130 (bottom), 190, 250, and 310 min (top). B) For method B GPC samples were taken at 110 (bottom), 170, 230, and 290 (top). The peak at 17.5 min is that of parent **2**.



Scheme 2. Hydroformylation of 1-octene by rhodium catalyst to give the linear and the branched aldehyde (nonanal and 2-methyloctanal, respectively).

From the silica-supported nixantphos material, the corresponding Rh catalysts were prepared[15] and studied in the industrially important hydroformylation reaction (Scheme 2). The results of the two different immobilization techniques were compared. The method B supported catalyst was separated after the reaction by simple filtration and the catalyst material was reused in ten consecutive batch reactions. As previously mentioned, immobilized transition metal catalysts often display activities that are one order of magnitude lower than those of their homogeneous counterparts, frequently accompanied by lower selectivities.[23] Indeed, compared to the homogeneous-phase reaction (Table 1, entries 1 and 2) the silica-immobilized catalyst prepared according to method A clearly shows deteriorated activity (Table 1, entry 3; 15 times lower activity) and lower chemo- and regioselectivity. As established by fluorescence imaging of the support we know that ligands are also attached as clusters, and we therefore ascribe part of the lower activity and selectivity to the presence of these clusters. It is well established that a high concentration of catalyst promotes formation of inactive dirhodium complexes with bridging carbonyl ligands, which explains how clusters of ligands lead to a lower activity.[24] Although formation of this dormant state is reversible, the number of active catalyst species will be reduced by cluster formation on silica, and hence the reaction rate is decreased by this process. Furthermore, a high local density of active catalyst species on a porous surface can also lead to local depletion of substrates (CO, H_2, alkene), which generally leads to isomerization side reactions as a consequence of the absence of CO. Such local substrate depletion potentially initiates catalyst decomposition (e.g., formation of metal clusters)

Table 1: Rhodium-catalyzed hydroformylation of 1-octene.[a]

Entry	Material	P [bar]/T [°C]	Conversion[b] [%]	l [%][c]	l/b[d]	TOF[e]	Cumulative TON
1[f]	homogeneous	50/80	33.9	92.9	40.8	120	–
2[f]	homogeneous	20/80	53.5	94.5	58.7	149	–
3[g]	support A	50/80	24.0	85.5	19.0	8	–
4	support B (1)	50/80	79.4	74.2	36.8	76	529
5[h]	support B (2)	50/80	84.0	82.6	37.4	62	1113
6[h]	support B (3)	50/80	85.1	87.1	34.5	102	1789
7[h]	support B (4)	20/80	95.1	91.8	54.1	130	2580
8[h]	Support B (5)	20/80	96.6	94.0	54.1	97	3331
9[h]	support B (6)	20/80	95.7	93.2	55.2	104	4031
10[h]	support B (7)	20/60	53.6	93.1	57.3	38	4346
11[f]	support B (8)	50/60	44.2	92.3	37.5	15	4624
12	support B (9)	20/80	95.0	91.3	39.9	115	5318
13	support B (10)	20/80	94.3	91.2	39.8	–	5979

[a] Catalysis performed with [Rh] = 10^{-5} mol on 1 g of silica support ([Rh] = 0.67 mM in 15 mL volume), ligand/Rh = 15, under CO/H_2 (1/1) pressure in 13 mL of toluene as solvent, 1 mL of 1-octene ([1-octene] = 0.42 M, substrate/Rh = 634) as substrate and 1 mL of *n*-decane as internal standard. [b] Conversions were measured after 23 h. Samples were analyzed by GC. [c] Percentage of nonanal (linear product) in the product mixture. [d] l/b is the linear/branched product molar ratio. [e] Turnover frequencies were calculated as moles product per mole catalyst per hour at 20–40% conversion; variations in TOF stem from differences in conversion and product distributions (full table is given in the Supporting Information). [f] [Rh] = 0.67 mM, ligand/Rh = 15, under CO/H_2 (1/1) pressure, 13 mL of toluene as solvent, 1 mL of 1-octene ([1-octene] = 0.42 M, substrate/Rh = 634) as substrate, and 1 mL of *n*-decane as internal standard; conversion was measured after 2 h. [g] Data taken from ref. [15]; [h] 1 mL of 2-propanol and 12 mL of toluene were used as solvent.

and degrades the outcome of the hydroformylation reaction.[24,25]

Interestingly, the catalyst supported according to method B, with the ligands more homogenously distributed on the support, showed much better performance in the hydroformylation of 1-octene. At 80 °C and 50 bar of syngas about 80% conversion of the 1-octene was obtained with a linear/branched (l/b) regioselectivity of around 36, thus demonstrating that both the activity and selectivity were better than with the catalyst prepared by method A (Table 1, entries 3 and 4–6). In the first run we also observed the formation of 1-nonanol, formed by a hydrogenation side reaction of the aldehyde product, which reduces the total amount of linear aldehyde. Previously, we suppressed this side reaction by adding 2-propanol as co-solvent. Subsequent catalytic reactions with the recycled catalyst were therefore performed in the presence of 2-propanol to restore the high chemoselectivity.[15]

The turnover frequencies obtained with catalyst B are increased at least eightfold compared to catalyst A. The new catalyst also shows excellent performance when the syngas pressure is reduced to 20 bar (Table 1, entries 4–7), conditions that were previously shown to yield irreproducible results as a consequence of isomerization side reactions. Under these conditions, catalyst B appears very active with TOFs comparable to those of the homogenous catalyst (130 and 149 $\mathrm{mol\,mol^{-1}\,h^{-1}}$, respectively) while the chemo- and regioselectivities are identical (94% linear aldehyde and l/b = 55). This catalytic reaction was run to nearly full conversion without affecting the catalyst performance in subsequent reactions. Importantly, rhodium analyses by means of inductively coupled plasma optical emission spectroscopy (ICP-OES) measurements on each of the ten reaction solutions show no detectable amounts of rhodium (< 3 ppb). Lowering the reaction temperature to 60 °C only slows down the reaction, while the selectivity is maintained (Table 1, entries 9 and 10). These experiments show that we now have an immobilized hydroformylation catalyst, obtained by a rational immobilization strategy, that gives similar activity and selectivity to the analogous homogeneous catalyst and is sufficiently stable to recycle and use for numerous consecutive catalysis experiments.

In conclusion, we have demonstrated that ligand immobilization can be monitored by means of two-photon fluorescence microscopy, by using nixantphos ligand **1** as fluorophore for imaging purposes. The fluorescence images reveal that under standard immobilization conditions larger clusters of ligands are supported. Moreover, confocal intensity traces and GPC measurements on the reaction solvent show that the clustering occurs already in solution, likely by a condensation reaction that is also observed in sol–gel processes, and that the prepolymerized ligand clusters are subsequently immobilized on the solid support. To reduce such clustering the immobilization process was performed with a low steady-state concentration of both **2** and capping agent to give a homogeneous distribution of ligand on the support (glass or silica). This homogeneity clearly translates to better catalyst performance, as the immobilized hydroformylation catalyst prepared by the new procedure gives much higher activity and selectivity than that obtained by the standard procedure. We believe that the new immobilization strategy will be applicable to a wide range of catalysts and, depending on the mechanism and conditions applied, these catalysts may benefit from the reported methodologies.

Received: February 12, 2010
Revised: April 9, 2010

Keywords: fluorescence microscopy · fluorescent probes · immobilization · P ligands · supported catalysts

[1] A. F. Trindade, P. M. P. Gois, C. A. M. Afonso, *Chem. Rev.* **2009**, *109*, 418 – 514.

[2] *Catalyst Separation, Recovery and Recycling, Catalysis by Metal Complexes, Vol. 30* (Eds.: D. J. Cole-Hamilton, R. P. Tooze), Springer, Dordrecht, **2006**.

[3] D. J. Cole-Hamilton, *Science* **2003**, *299*, 1702 – 1706.

[4] N. E. Leadbeater, M. Marco, *Chem. Rev.* **2002**, *102*, 3217 – 3274.

[5] C. A. McNamara, M. J. Dixon, M. Bradley, *Chem. Rev.* **2002**, *102*, 3275 – 3300.

[6] W. Keim, B. Driessen-Hölscher in *Handbook of Heterogeneous Catalysis, Vol. 1* (Eds.: G. Ertl, H. Knözinger, J. Weitkamp), Wiley-VCH, Weinhein, **1997**, p. 231.

[7] Y. Iwasawa in *Tailored Metal Catalysts, Catalysis by Metal Complexes, Vol. 7* (Eds.: Y. Iwasawa), Reidel, Dordrecht, **1986**, pp. 87 – 140.

[8] E. de Jesús, J. C. Flores, *Ind. Eng. Chem. Res.* **2008**, *47*, 7968 – 7981.

[9] G. E. Oosterom, J. N. H. Reek, P. C. J. Kamer, P. W. N. M. van Leeuwen, *Angew. Chem.* **2001**, *113*, 1878 – 1901; *Angew. Chem. Int. Ed.* **2001**, *40*, 1828 – 1849.

[10] For a thematic issue on catalyst immobilization, see *Chem. Rev.* **2002**, *102*, 120.

[11] R. Anwander in *Handbook of Heterogeneous Catalysis* (Eds.: G. Ertl, H. Knözinger, J. Weitkamp), Wiley-VCH, Weinhein, **1997**, pp. 583 – 614.

[12] A. Diaspro, G. Chirico, M. Collini, *Q. Rev. Biophys.* **2005**, *38*, 97 – 166.

[13] P. T. C. So, C. Y. Dong, B. R. Masters, K. M. Berland, *Annu. Rev. Biomed. Eng.* **2000**, *02*, 399 – 429.

[14] P. W. N. M. van Leeuwen, A. J. Sandee, J. N. H. Reek, Paul C. J. Kamer, *J. Mol. Catal. A* **2002**, *182–183*, 107 – 123.

[15] A. J. Sandee, J. N. H. Reek, P. C. J. Kamer, P. W. N. M. van Leeuwen, *J. Am. Chem. Soc.* **2001**, *123*, 8468 – 8476.

[16] N. J. Meehan, M. Poliakoff, A. J. Sandee, J. N. H. Reek, P. C. J. Kamer, P. W. N. M. van Leeuwen, *Chem. Commun.* **2000**, 1497 – 1498.

[17] A. J. Sandee, L. A. van der Veen, J. N. H. Reek, P. C. J. Kamer, M. Lutz, A. L. Spek, P. W. N. M. van Leeuwen, *Angew. Chem.* **1999**, *111*, 3428 – 3432; *Angew. Chem. Int. Ed.* **1999**, *38*, 3231 – 3235.

[18] P. W. N. M. van Leeuwen, C. P. Casey, G. T. Whiteker in *Rhodium Catalyzed Hydroformylation, Catalysis by Metal Complexes, Vol. 22* (Eds.:P. W. N. M. van Leeuwen, C. Claver), Kluwer, Dordrecht, **2000**, pp. 63 – 96.

[19] P. C. J. Kamer, P. W. N. M. van Leeuwen, J. N. H. Reek, *Acc. Chem. Res.* **2001**, *34*, 895 – 904.

[20] M. A. Albota, C. Xu, W. W. Webb, *Appl. Opt.* **1998**, *37*, 7352 – 7356.

[21] Y. Kawamura, J. Brooks, J. J. Brown, H. Sasabe, C. Adachi, *Phys. Rev. Lett.* **2006**, *96*, 017404.

[22] Instead of the *n*-propyl(trimethoxy)silane capping agent used in immobilization of **2** on glass surfaces, dimethyl(dimethoxy)silane was used for the immobilization reaction on silica.

[23] Trace amounts of water in the toluene, as determined by Karl Fisher titration, initiated the sol–gel process in solution, leading to oligomerization of the ligand. In line with this, signals of methanol formed during the initial hydrolysis are detected by ^{1}H NMR spectroscopy. See also J. D. Wright, N. A. J. M. Sommerdijk in *Sol-Gel Materials: Chemistry and Applications, Advanced Chemistry Texts, Vol. 4*, CRC, Boca Raton, **2001**.

[24] J. N. H. Reek, P. W. N. M. van Leeuwen, A. G. J. van der Ham, A. B. de Haan in *Catalyst Separation, Recovery and Recycling, Catalysis by Metal Complexes, Vol. 30* (Eds.: D. Cole-Hamilton, R. Tooze), Kluwer, Dordrecht, **2006**, pp. 39 – 72.

[25] P. W. N. M. van Leeuwen, *Appl. Catal. A* **2001**, *212*, 61 – 81.

[26] P. C. J. Kamer, J. N. H. Reek, P. W. N. M. van Leeuwen in *Rhodium Catalyzed Hydroformylation, Catalysis by Metal Complexes, Vol. 22* (Eds: P. W. N. M. van Leeuwen, C. Claver), Kluwer, Dordrecht, **2000**, pp. 35 – 62.