[11] F. Meyer, U. Ruschewitz, P. Schober, B. Antelmann, L. Zsolnai, *J. Chem. Soc. Dalton Trans.* **1998**, 1181 – 1186.
[12] a) F. Meyer, H. Pritzkow, *Chem. Commun.* **1998**, 1555 – 1556; b) F. Meyer, E. Kaifer, P. Kircher, K. Heinze, H. Pritzkow, *Chem. Eur. J.* **1999**, *5*, 1617 – 1630.
[13] a) F. Meyer, K. Heinze, B. Nuber, L. Zsolnai, *J. Chem. Soc. Dalton Trans.* **1998**, 207 – 213; b) F. Meyer, P. Rutsch, *Chem. Commun.* **1998**, 1037 – 1038.
[14] Crystal data of $\mathbf{1}\cdot 2PF_6$ ($C_{30}H_{64}Cu_4F_{12}N_{12}O_4P_2$, $M=1201.0$): monoclinic, space group $C2/c$, $a=21.309(1)$, $b=11.421(1)$, $c=19.174(1)$ Å, $\beta=97.562(1)^\circ$, $V=4625.8(5)$ Å^3, $Z=4$, $\rho_{calcd}=1.725$ g cm^{-3}, $\mu(Mo_{K\alpha})=1.981$ mm^{-1}, $2\theta_{max}=52.7^\circ$, 4720 independent reflections, 3342 observed with $I>2\sigma(I)$, 417 refined parameters; all H atoms have been localized and refined isotropically, $R1=0.037$ ($I>2\sigma(I)$), $wR2=0.088$ (all data), GOF = 0.924 (refinement on F^2), max./min. residual electron density 0.661/ – 0.300 e Å^{-3}. Crystal data of $\mathbf{2}\cdot 2PF_6$ ($C_{30}H_{66}Cu_4F_{12}N_{12}O_4P_2\cdot EtCN$, $M=1258.1$): monoclinic, space group $P2_1/c$, $a=11.985(1)$, $b=26.728(3)$, $c=16.114(2)$ Å, $\beta=100.505(2)^\circ$, $V=5075.3(8)$ Å^3, $Z=4$, $\rho_{calcd}=1.647$ g cm^{-3}, $\mu(Mo_{K\alpha})=1.810$ mm^{-1}, $2\theta_{max}=49.4^\circ$, 8656 independent reflections, 5111 observed with $I>2\sigma(I)$, 644 refined parameters; the O-bound H atoms have been localized and refined isotropically, all other H atoms have been included in calculated positions, $R1=0.046$ ($I>2\sigma(I)$), $wR2=0.108$ (all data), GOF = 0.878 (refinement on F^2), max./min. residual electron density 0.892/ – 0.434 e Å^{-3}. Data have been collected on a Bruker AXS CCD diffractometer, $Mo_{K\alpha}$ radiation ($\lambda=0.71073$ Å), $T=173$ K, ω scan, structures have been solved using direct methods (SHELXS-86 and SHELXL-97). Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-138151 ($\mathbf{1}\cdot(PF_6)_2$) and CCDC-138152 ($\mathbf{2}\cdot(PF_6)_2$. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk)..
[15] a) R. Stomberg, L. Trysberg, I. Larking, *Acta Chem. Scand.* **1970**, *24*, 2678 – 2679; b) I. Shweky, L. E. Pence, G. C. Papaefthymiou, R. Sessoli, J. W. Yun, A. Bino, S. J. Lippard, *J. Am. Chem. Soc.* **1997**, *119*, 1027 – 1042; c) H. J. Breunig, T. Krüger, E. Lork, *Angew. Chem.* **1997**, *109*, 654 – 655; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 615 – 617.
[16] W. Micklitz, S. G. Bott, J. G. Bentsen, S. J. Lippard, *J. Am. Chem. Soc.* **1989**, *111*, 372 – 374.

The Use of Immobilized Templates—A New Approach in Molecular Imprinting

Ecevit Yilmaz, Karsten Haupt,* and Klaus Mosbach*

Molecular imprinting is a technique that allows specific recognition sites for target molecules to be formed in synthetic polymers through the use of templates. Customary protocols for molecularly imprinted polymers (MIPs) are based on one of two distinct approaches: the "covalent approach" and the "noncovalent approach". The covalent approach was pioneered by the group of Wulff[1] and uses covalent bonds between the imprint molecules and functional monomers. The other approach, which is based on noncovalent interactions, was introduced by Mosbach and coworkers.[2] More recently, a hybrid system was proposed that comprises a covalent imprinting step and subsequent rebinding of the imprint molecule by noncovalent interactions.[3]

Molecular imprinting of small molecules has until now only been done with the template (imprint) molecules in free solution. These polymers will be referred to here as classical MIPs. Herein we present a novel imprinting method based on oriented immobilization of the template onto a solid support. After polymerization, the support is dissolved and thus sacrificed (Figure 1). Our aim is to demonstrate the feasibility

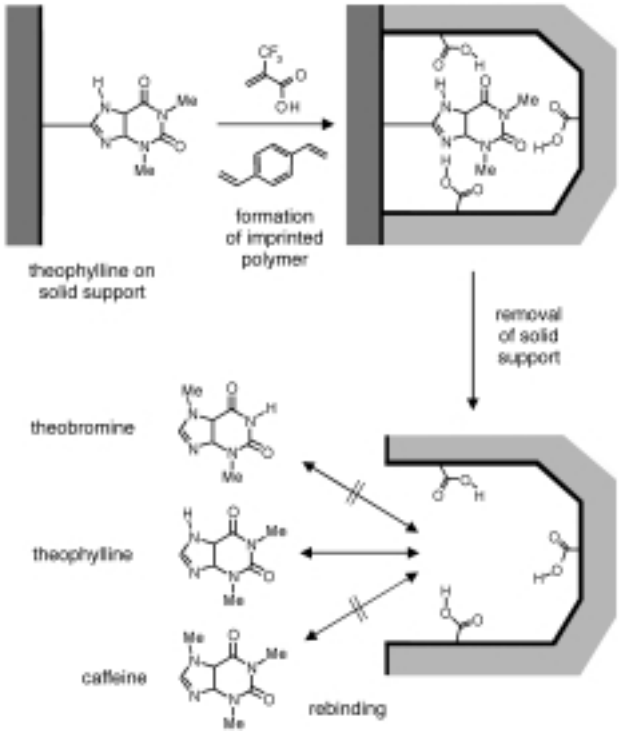


Figure 1. Schematic representation of the molecular-imprinting approach employing immobilized templates and a sacrificial solid support.

of this approach, which extends molecular imprinting technology to a new dimension. The bronchodilating drug theophylline was investigated as a model template by immobilizing its 8-carboxypropyl derivative onto a support of aminopropyl-derivatized silica gel. Immobilization of 8-carboxypropyltheophylline was achieved through the formation of amide bonds by using carbodiimide chemistry adapted from protocols used for solid-phase peptide synthesis.[4] The amount of template coupled was determined at the end of the reaction by elemental analysis (Table 1). Approximately 75% of the free aminopropyl groups on the silica surface could be coupled with 8-carboxypropyltheophylline. Acetic anhydride was added at the end of the coupling

[*] Dr. K. Haupt,[†] Prof. K. Mosbach, E. Yilmaz
Lund University
Department of Pure and Applied Biochemistry
Center for Chemistry and Chemical Engineering
PO Box 124, 22100 Lund (Sweden)
Fax: (+46) 46-2224611
E-mail: klaus.mosbach@tbiokem.lth.se.
[†] Current address:
Université Paris 12 Val de Marne
Faculté des Sciences, CRRET Laboratory
Avenue du Général de Gaulle
94010 Créteil Cedex (France)
E-mail: karsten.haupt@tbiokem.lth.se.

  0570-0833/00/3912-2115 $ 17.50+.50/0

Table 1. Elemental analysis of derivatized silica particles and the corresponding imprinted polymers.

	Aminopropyl silica gel[a]	Control silica gel[b]	Theophylline silica gel [c]	Control polymer[d]	Imprinted polymer[e]
C [%]	1.13	1.8	3.3	80.6	80.1
H [%]	0.34	0.42	0.5	7.3	6.9
N [%]	0.33	0.34	1.21	b.d.	b.d.
Si [%]	n.d.	n.d.	n.d.	b.d.	b.d.

[a] Aminopropyl-derivatized silica gel as obtained from the manufacturer. [b] Control silica obtained after acetylation of aminopropyl-derivatized silica gel with acetic anhydride. [c] Prepared by coupling carboxypropyltheophylline to aminopropyl-derivatized silica gel. [d] Control polymer obtained after imprinting with control silica gel. [e] Imprinted polymer obtained after imprinting with theophylline/silica gel. b.d. = below detection limit, n.d. = not determined.

reaction to block any remaining free aminopropyl groups that were not detectable with conventional methods, such as the Kaiser[5] or trinitrobenzenesulfonic acid (TNBS)[6] tests. The same aminopropyl-derivatized silica gel as used for the imprinted polymer was used for the preparation of control polymers, but all the aminopropyl groups were acetylated with acetic anhydride.

In previous work we described an optimized recipe for the preparation of theophylline-imprinted polymers.[7] It contains trifluoromethylacrylic acid (TFMAA), a negatively charged functional monomer, and divinylbenzene (DVB), an apolar cross-linker. This mixture yielded highly efficient polymers over a wide range of template:monomer ratios. This method was applied here for the imprinting of immobilized theophylline, with a molar ratio of approximately 1:4 between the immobilized theophylline and the TFMAA monomer used. The same molar ratio had yielded a good functioning MIP imprinted with free theophylline.[7]

In contrast to classical MIPs, where a solvent capable of forming pores (porogen) is essential to yield a macroporous structure, no porogen was used for the imprinting of immobilized theophylline. In our new system uniform macropores are generated by the removal of the silica gel, the binding sites thus being situated on the surface of the pores. It was shown, however, that the addition of toluene as a porogen to the polymerization mixture did not affect analyte recognition by the polymers obtained. The silica gel was completely removed by treatment with aqueous hydrofluoric acid (HF), as determined by elemental analysis (Table 1). The nitrogen content was also determined by elemental analysis to assertain whether theophylline molecules were still present in the polymer after removal of the silica gel. The amount of nitrogen in the polymer was found to be below the detection limit of the method. Furthermore, the elemental composition of both control and imprinted polymers was very much alike (Table 1). After removal of some incompletely polymerized material and fragments, the yield of polymer particles was approximately 80% relative to the amount of monomers initially used.

The treatment with aqueous HF is rather harsh, so its potential detrimental impact on the polymers was investigated. A polymer imprinted with free theophylline that had the same polymer composition[7] as above was also treated with aqueous HF. Binding and competitive assays were performed after the polymer had been washed and dried. No change of capacity or specificity of the polymer was observed, which demonstrates that imprinted DVB – TFMAA copolymers can withstand treatment with aqueous HF.

The porosity of the polymers was determined by nitrogen adsorption/desorption analysis. The results show a narrow pore size distribution with a mean pore diameter of 254 Å and 257 Å, and pore surface areas (by BET) of 35 $m^2 g^{-1}$ and 31 $m^2 g^{-1}$, for the imprinted and the control polymers, respectively. No larger pores or micropores were detected. In contrast, polymers prepared by the classical method had a much broader pore size distribution (30 – 1000 Å) and a larger surface area (about 500 $m^2 g^{-1}$).

Binding assays were carried out in toluene by using [^{3}H]-theophylline as a radioligand to evaluate the capacity of the polymer imprinted with immobilized theophylline and the corresponding control polymer (Figure 2). The imprinted

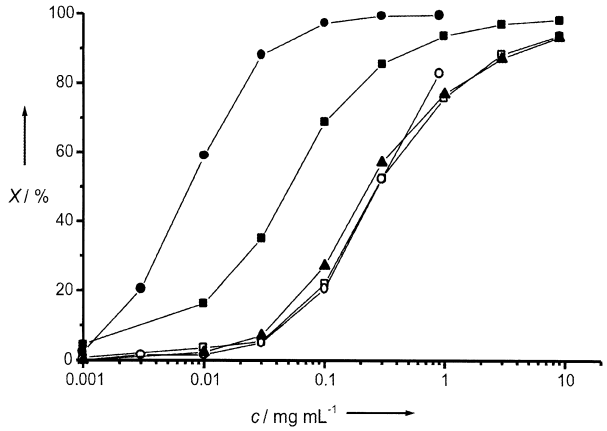


Figure 2. Adsorption of [^{3}H]theophylline as a function of the polymer concentration. The curves represent an imprinted polymer prepared with: theophylline/silica gel (■), acetylated silica gel (control polymer; □), naphthalene/silica gel (▲), free theophylline (●), and aminopropyl-derivatized silica gel (○). The points represent mean values of three measurements.

polymer has a higher capacity for theophylline than the control polymer. At a polymer concentration where the MIP binds 50% of the radioligand, the control polymer binds only 10%. To elucidate the effects of template shape and functionality on imprinting, an additional polymer was synthesized using silica gel onto which 2-naphthylacetic acid was coupled. Naphthalene is about as bulky as theophylline, but contains no functional groups or heteroatoms. As can be seen in Figure 2 this polymer had a binding capacity that was only slightly higher than the control polymer prepared with acetylated silica gel. This result indicates that the functionalities on the theophylline molecule are responsible for the observed imprinting effect.

The binding assays of MIPs prepared in the classical way using free theophylline are shown for comparison in Figure 2. The classical MIP binds more radiolabeled theophylline than the MIP prepared with immobilized theophylline. However, a direct comparison of the capacities of the two systems is difficult because of the differences in their porosities.

  0570-0833/00/3912-2116 $ 17.50+.50/0

Interestingly, both control polymers have very similar (low) binding capacities. Apparently, the presence of silica gel during the synthesis of the polymer does not cause increased nonspecific binding. Thus, the difference in binding between the MIP and the control polymers prepared in the presence of silica gel should be caused by the template immobilized on the silica gel surface.

The theophylline-imprinted polymer shows a high selectivity for theophylline over the related compounds theobromine and caffeine (Figure 3), which have cross-reactivities of less than 2% relative to theophylline. This result is similar to that obtained with polymers imprinted with the free template.[7]

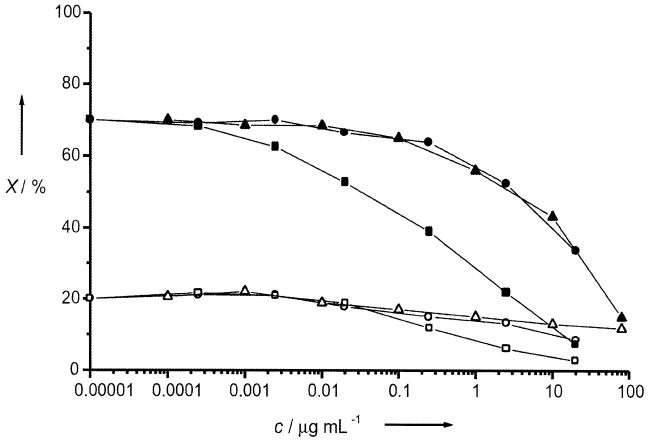


Figure 3. Competition of [^{3}H]-theophylline binding to the imprinted polymer (filled symbols) and the control polymer (empty symbols) as a function of the concentration of nonlabeled theophylline (squares), theobromine (circles), or caffeine (triangles). Polymer concentration: 70 μg mL^{-1}.

Similar studies were conducted earlier in our group using latex and nonporous silica nanobeads as a solid support, but these only had limited success. This was found to be a consequence of the low surface area of nonporous beads, and of the incompatibility of latex particles with most of the common imprinting solvents. On the other hand, we anticipated silica gel to be a suitable material for our approach because of its stability in organic solvents and its higher density, which facilitates handling. Moreover, it can be readily removed from the imprinted polymer, for example by treatment with HF. We chose a porous silica material for its larger internal surface area, which allows for a higher template load. Recently, the possibility of using silica particles as a sacrificial material in combination with DVB-based polymers was reported by another research group.[8] Although this study was not concerned with molecular imprinting, it was shown that these polymer–silica composites, after dissolution of the silica gel with HF, allow for the fine tuning of the polymer porosity.

There are several potential advantages in using immobilized templates for imprinting. Templates that are not soluble in the polymerization cocktail can be immobilized and then brought into contact with the monomers. The imprinting of insoluble templates would thus be made possible if they can be immobilized.

The possibility has been raised by others that aggregation of templates in pre-polymerization mixtures may occur.[9, 10] The formation of such aggregates can be prevented by immobilizing the template on an appropriate solid support, which at the same time might provide a useful tool for further investigations of this phenomenon. Oriented immobilization of the imprint molecule should also result in a better orientation and homogeneity of the binding sites, more so as immobilization reduces the tumbling rate of the template molecule during the imprinting polymerization step.

Since the pre-polymerization mixture contained no porogen, the pore structure of the obtained polymers is formed only by uniform macropores generated by the dissolved silica gel backbone. All imprinted binding sites should therefore be located at, or close to, the surface of the pores and so greatly facilitate diffusion of the analyte to the binding sites.

The present study also opens new possibilities for the application of MIPs. For example, the template molecule can be tagged with various markers, whereby the problem of steric hindrance, which is most often encountered with classical MIPs where the binding sites are buried in the polymer structure, is avoided. This will greatly increase the usefulness of MIPs for immunoassays and related applications.

The described composite pair silica gel/acrylate, in which the silica gel acts as a dissolvable template carrier, is one of several possible composite pairs, for example, latex/acrylates, polysaccharides/acrylates, and chitosan/acrylates.

In conclusion we have demonstrated for the first time that the imprinting of immobilized templates is feasible and constitutes an alternative way of producing MIPs. Nevertheless, the phenomena involved during imprinting at liquid–solid interphases remain to be studied in more detail.

Experimental Section

Materials: Toluene, dimethylformamide (DMF), and dichloromethane (DCM; all anhydrous) were purchased from Labscan Ltd. (Dublin, Ireland). All other solvents (analytical or HPLC grade) and HF (40% aqueous solution), were obtained from Merck (Darmstadt, Germany). 8-Carboxypropyltheophylline, theophylline, caffeine, theobromine, [8-^{3}H]-theophylline (specific activity: 0.8 mCi mol^{-1}), and diisopropylcarbodiimide (DIC) were purchased from Sigma (St. Louis, MO, USA). Aminopropyl-derivatized silica gel (7 μm, 300 Å, 0.7 mL g^{-1}) was obtained from Macherey-Nagel (Düren, Germany) and had an aminopropyl content of about 0.25 mmol per gram of silica gel. The radical polymerization initiator 2,2′-azobis(2,4-dimethylvaleronitrile) was obtained from Wako Pure Chemicals Industries (Osaka, Japan). TFMMA and technical grade DVB containing 20% ethylvinylbenzene was obtained from Aldrich (Steinheim, Germany), and was treated with basic alumina (Merck) immediately prior to use to remove polymerization inhibitors. TNBS (1% solution in DMF) and 2-naphthylacetic acid were obtained from Fluka (Buchs, Switzerland).

Preparation of the aminopropyl-derivatized silica gel surfaces for imprinting: A) Theophylline/silica gel: 8-carboxypropyltheophylline (266 mg, 1 mmol) and DIC (450 μL, 3 mmol) were dissolved in anhydrous DMF/DCM (1/1, 10 mL). Dry aminopropyl-derivatized silica gel (1 g) was then added and the suspension was shaken on a rocking-table for a minimum of 18 h at room temperature. The coupling reaction was allowed to continue until both Kaiser[5] and TNBS[6] tests were negative, which indicated that most aminopropyl groups on the silica surface had reacted. Subsequently, acetic anhydride (100 μL, 1 mmol) was added and the mixture incubated for another 2 h to acetylate any remaining aminopropyl groups. The silica gel was then washed on a G4-glass filter funnel with DMF, DCM, and methanol, before drying for 6 h at 45 °C and then in vacuo for a further 6 h. B) Control acetylated silica gel: aminopropyl silica (1 g) was treated with

  0570-0833/00/3912-2117 $ 17.50+.50/0

an excess of acetic anhydride (1 mL, 10 mmol), to obtain complete acetylation of the amino groups. The control silica gel was processed as described earlier. C) Naphthalene/silica gel: The same procedure was used as for theophylline/silica gel (A), but using 2-naphthylacetic acid (186 mg, 1 mmol) instead of 8-carboxypropyltheophylline.

Preparation of the polymers: A pre-polymerization mixture consisting of DVB (2.14 mL, 12 mmol), TFMAA (336 mg, 2.4 mmol), and 2,2′-azo-bis(2,4-dimethylvaleronitrile) (20 mg) was prepared in a glass vial. According to the pore volume of the silica (ca. 0.65 mL g^{-1}), the amount of the mixture required to fill the pores was added to the silica gel (theophylline/silica gel, control silica gel, or naphthalene/silica gel) and gently stirred with a stainless steel spatula.[11] The vial was flushed gently with N_2 for 2 min and the mixture was then allowed to polymerize overnight at 45 °C. After polymerization was completed (this was monitored by polymerizing a portion of the pre-polymerization mixture without silica gel) the polymer/silica gel composite was gently wet-milled in acetone with a manual mortar and pestle to disintegrate any particle aggregates. The composite was then transferred into a plastic tube with a screw cap, suspended in acetone (2 mL), and cooled in a water/ice bath. Aqueous HF (4 mL, 40 %) was added portionwise whilst shaking the mixture to dissolve the silica matrix of the composite. The suspension was then allowed to react overnight on a rocking-table at room temperature. The remaining polymer was washed extensively on a G4-glass filter funnel with 20 % acetone in deionized water (ca. 2 L) until the filtrate had a neutral pH value, and finally washed with methanol (0.25 L). The polymer particles were then dried in an oven at 45 °C for 6 h and in vacuo for a further 6 h.

Elemental analysis: Flash combustion elemental analysis coupled to gas chromatography was performed at MikroKemi AB (Uppsala, Sweden).

Radioligand binding assays: The polymer particles were suspended in toluene and appropriate volumes were added into 1.5-mL polypropylene test tubes, followed by the radioligand [^{3}H]-theophylline, varying amounts of a solution of a competing ligand if appropriate, and toluene to give a total volume of 1 mL. The samples were incubated on a rocking-table for 12 h at room temperature. Particles were removed by centrifugation and supernatant (500 μL) was withdrawn and added to scintillation liquid (10 mL, Ecoscint O, National Diagnostics, Atlanta, GA, USA). The radioactivity was measured by liquid scintillation counting with a Rackbeta 1219 counter (LKB Wallac, Turku, Finland). This assay is similar to that described previously.[12]

Received: September 8, 1999 [Z13982]
Publication delayed at authors request

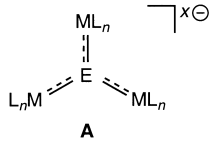
[1] G. Wulff, *Angew. Chem.* **1995**, *107*, 1958 – 1979; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1812 – 1832.
[2] K. Mosbach, O. Ramström, *Bio/Technology* **1996**, *14*, 163 – 170.
[3] M. J. Whitcombe, M. E. Rodriguez, P. Villar, E. N. Vulfson, *J. Am. Chem. Soc.* **1995**, *117*, 7105 – 7111.
[4] F. Albericio, L. A. Carpino, *Methods Enzymol.* **1997**, *289*, 104 – 126.
[5] E. Kaiser, R. L. Colescott, C. D. Bossinger, P. I. Cook, *Anal. Biochem.* **1970**, *34*, 595 – 598.
[6] W. S. Hancock, J. E. Battersby, *Anal. Biochem.* **1976**, *71*, 260 – 264.
[7] E. Yilmaz, K. Mosbach, K. Haupt, *Anal. Commun.* **1999**, *36*, 167 – 170.
[8] S. A. Johnson, P. J. Ollivier, T. E. Mallouk, *Science* **1999**, *283*, 963 – 965.
[9] H. S. Andersson, J. G. Karlsson, S. A. Piletsky, A.-C. Koch-Schmidt, K. Mosbach, I. A. Nicholls, *J. Chromatogr. A* **1999**, *848*, 39 – 49.
[10] A. Katz, M. E. Davis, *Macromolecules* **1999**, *32*, 4113 – 4121.
[11] M. Glad, P. Reinholdsson, K. Mosbach, *React. Polym.* **1995**, *25*, 47 – 54.
[12] G. Vlatakis, L. I. Andersson, R. Müller, K. Mosbach, *Nature* **1993**, *361*, 645 – 647.

Synthesis and Reactivity of $[\{(CO)_5Cr\}_3Pb]^{2-}$, an Unsaturated Compound with Trigonal-Planar Coordinated Lead

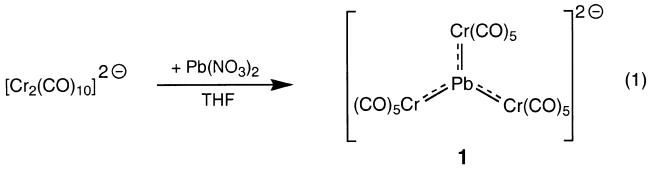
Peter Rutsch und Gottfried Huttner*

Dedicated to Professor Arndt Simon on the occasion of his 60th birthday

Compounds **A**, which contain a main group element bound in a trigonal-planar environment to three organotransition metal 16-valence electron fragments L_nM (e.g. $L_nM = (CO)_4Fe$, E = In, $x = 3$;[1] $L_nM = (CO)_4Fe$, E = Sn, Pb, $x = 2$;[2] $L_nM = (CO)_5Cr$, E = Sn, $x = 2$;[3] $L_nM = (CO)_5Cr$, E = Sb, $x = 1$;[4] $L_nM = Cp(CO)_2Mn$, E = Te, $x = 0$[5]), are in the broadest sense isoelectronic analogues of well known four-center, six-π-electron systems such as NO_3^- and CO_3^{2-}. The $p_\pi - p_\pi$ interaction in systems such as CO_3^{2-} corresponds to the metal – d_π-main group element – p_π interaction in the organometal derivatives.[6] The unsaturated character of these organometallic π systems becomes apparent from the short M–E bonds[1–5] as well as from their spectroscopic behavior.[3] The NMR signals of the trigonal-planar coordinated main group elements are each shifted to low field.[3, 7] This is also true for **1**, which is obtained from the reaction of disodium decacarbonyldichromate with lead nitrate [Eq. (1)]. The ^{207}Pb NMR



A

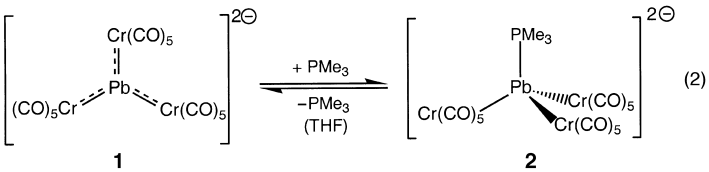


(1)

1

signal of **1** is shifted to low field at $\delta = 7885$ and supports, in agreement with the structural data (Figure 1),[8] the unsaturated character of **1**.

Chemical evidence for the unsaturated character of π systems of the type **A** was hitherto unknown. We have now found that **1** in presence of PMe_3 is in equilibrium with its base adduct **2** [Eq. (2)]. From the temperature dependence of the ^{31}P NMR spectra of **2**, the following thermodynamic parameters are obtained for the position of the formation equilibrium



(2)

1 **2**

[*] Prof. Dr. G. Huttner, Dipl.-Chem. P. Rutsch
Anorganisch-chemisches Institut der Universität Heidelberg
Im Neuenheimer Feld 270, 69120 Heidelberg (Germany)
Fax: (+49) 6221-545-707
E-mail: g.huttner@indi.aci.uni-heidelberg.de

  0570-0833/00/3912-2118 $ 17.50+.50/0