ammonium ion were observed in trace quantities along with an insoluble black solid. These products were evidently formed by the oxidation of the diamine portion of the nylon-6,6 backbone since similar products were observed from the oxidation of hexamethylenediamine under the standard conditions.

Both polymethyl methacrylate and polyacrylic acid were oxidized under standard conditions to give oligomeric materials with drastically reduced molecular weights. For example, polymethyl methacrylate with $M_w = 96200$ and a polydispersity of 1.5 was oxidized to products with $M_w = 985$ and a polydispersity of 1.7, as observed by gel permeation chromatography in chloroform.

In conclusion, a wide variety of addition and condensation polymers can be oxidatively degraded under relatively mild conditions by a mixture of nitrogen oxides and dioxygen. In the particular cases of polystyrene, high- and low-density polyethylene, and perhaps polypropylene useful organic compounds are produced in moderate to good yields. The procedure is less useful for condensation polymers, such as nylon-6,6, since these can in principle be hydrolyzed back to the starting monomers.

Experimental Section

The reactions were carried out in 125-mL Parr pressure reactors with glass liners. Typically, the polymeric material (approx. 0.25 g) was added to a glass liner and then placed into the reactor, which was sealed. The reactor was purged with N_2, and then pressurized to 275 kPa with NO, to 3445 kPa with N_2, and finally to 4135 kPa with O_2. The reactor was heated to 170 °C for 16 h, following which it was cooled and the pressure released. The product mixture was then removed and analyzed by one or more of the following techniques: NMR spectroscopy, mass spectrometry, and gel permeation chromatography.

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Investigation of the Molecular Recognition of Amino Acids by Cyclopeptides with Reflectometric Interference Spectroscopy**

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Molecular recognition plays a crucial part in many biological processes where guest molecules are bound very selectively and reversibly by their host. These mostly enantioselective recognition processes, in which chiral ligands interact with their receptors, can be imitated with synthetic molecules.[1] Synthetic receptors have various possible applications in the medical and pharmaceutical field, as new separation-phase material or sensitive coatings.[2] In fundamental research, important insights into the type of interaction in host – guest complexes are gained.[3] A multitude of different macrocyclic receptors such as crown ethers, cyclophanes, cyclodextrins, calixarenes, and cyclopeptides has already been synthesized.[4] With the cyclopeptides cyclo(Pro-Gly)$_3$ and cyclo(Pro-Gly)$_4$ the distinction between D and L amino acid salts in $CDCl_3$ became possible with NMR spectroscopic methods.[5] With macrocyclic peptides, chiral amines were distinguished,[6] and zwitterionic amino acids were complexed with chiral crown ethers.[7] These examinations of interactions between cyclopeptides and analyte were carried out with complicated methods.

We now report on novel sensor modules with surface-bound cyclopeptides as molecular receptors. In earlier work we were able to prove that cyclohexapeptide libraries can be used as chiral selectors in capillary electrophoresis.[8] With combinatorial solid-phase synthesis a host of such detector molecules can be produced.[9] Compared with singular procedures, as for calixarenes and cyclodextrins, our strategy shows a higher diversity by several orders of magnitude (virtual library: 10^{16} cyclohexapeptides from commercially available starting materials) and therefore a better chance of finding a receptor lead structure. Cyclohexapeptides are limited in their conformation,[10] and many basketlike structures can be synthesized by modifying the side chains. Based on these findings we immobilized the cyclopeptides **1** – **3** (Figure 1) on glass surfaces that function as transducers. The interaction between the cyclopeptide and the analyte is examined with reflectometric interference spectroscopy (RIfS).[11] In this optical method of detection, the reflection and the interference of light at phase boundaries are used for the time-resolved determination of changes in the optical film thickness (product of refractive index n and physical film thickness d) of thin transparent films from the resulting interference

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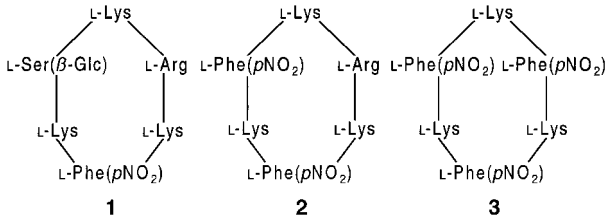

Figure 1. Amino acid sequences of the cyclohexapeptides **1**—**3** used as chemoreceptors.

pattern.[12] Adsorption or bonding processes on thin cyclopeptide layers can thus be registered directly, and a large amount of analytes and receptors can be examined in a short period of time.

To prepare our chemosensors, a monolayer of the cyclohexapeptides **1**–**3** was covalently bound to a glass support previously treated with epoxysilane through the amino functionalities of three lysine side chains (Figure 2). For this, the positions 1, 3, and 5 in the sequence were chosen as anchoring sites. L-Arginine, 4-nitro-L-phenylalanine and O-glycosidically modified serine were used in the positions 2, 4, and 6 of the sequence. L-arginine has proved itself as an important amino acid in cyclopeptide libraries. In the tricyclic peptide antibiotic vancomycin, used as chiral separatory phase material, sugar units and aromatic residues play an important part.[13] The properties of the cyclopeptides were modified as follows by means of sequence alterations: In the cyclopeptide **2**, the O-glycosidically modified serine of **1** is replaced by 4-nitro-L-phenylalanine. In addition, L-arginine is exchanged against 4-nitro-L-phenylalanine in the cyclopeptide **3**. To examine the amino acid/cyclopeptide interaction, solutions of different amino acids in neutral, buffer-free water were pumped over the sensitive layers, and the resulting optical film thickness was determined after a state of equilibrium was reached. Such a time-resolved course of the film thickness for different amino acids is shown in Figure 3; in this, the peak height is the signal utilized.

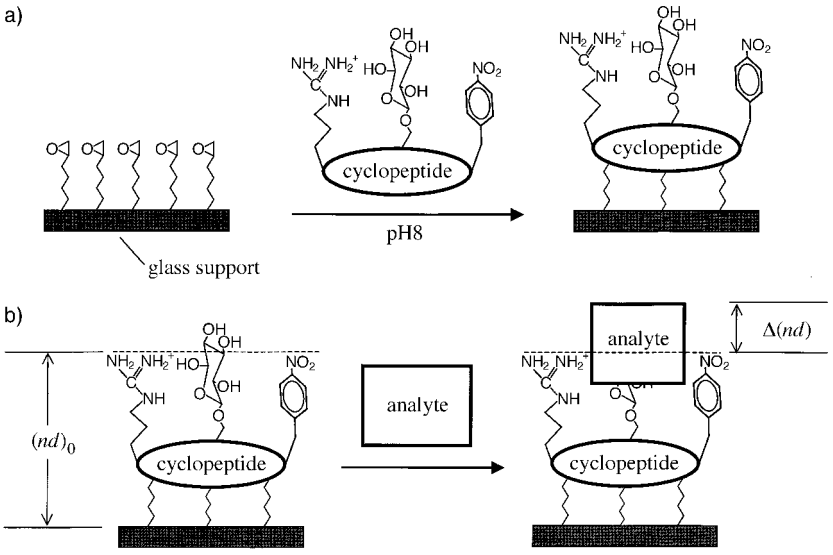

Figure 2. a) Linking of the cyclohexapeptides to the transducer surface. b) Change in optical film thickness ($\Delta(nd)$) after interaction with the analyte.

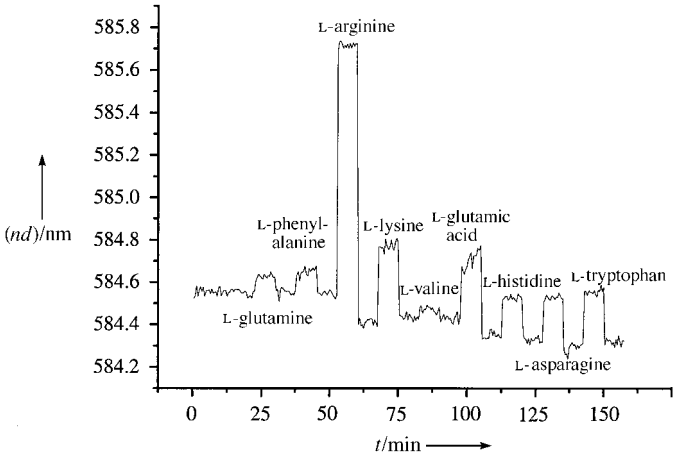

Figure 3. Course of the film thickness at the optical transducer for the interaction of **1** after the injection of different L-amino acids (1000 ppm each) in neutral, buffer-free water. The unit ppm corresponds to 1 mg amino acid per liter of water.

Furthermore, a test set of twelve L-amino acids with all three surface-bound cyclopeptides in neutral, buffer-free water was examined. The sensor signals thus obtained clearly show that all three cyclopeptides preferentially bind L-arginine but not L-lysine, which also contains two amino groups (Figure 4). For all amino acids, the RIfS signals are much more intense for the cyclopeptide **1** layer than for the coverage with **2** or **3**. Therefore the influence of the glucose group has to be considerable. The sensor signal for L-arginine is almost twice as high for cyclopeptide **1** as for the cyclopeptides **2** and **3**, which proves the selective interaction.

To examine a possible enantiomer recognition by the cyclohexapeptide layers, calibration measurements were carried out with L- and D-arginine in the concentration range from 0–880 mg L^{-1} (Figure 5). The two calibration graphs do not differ significantly; in the region of higher concentration a possible indication for the discrimination of enantiomers can be seen. For quantification purposes, a Langmuir adsorption curve was calculated from the experimental values using nonlinear regression according to Equation (1).[14]

$$Y = A + \frac{b\,c\,x}{1 + b\,x} \qquad (1)$$

Here, Y describes the measured signal, x the concentration, A the intercept, b the ratio of the constants for adsorption and desorption and c the saturation signal. With this function the detection limit for arginine can be determined if the tripled noise of the baseline for the film thickness is used as the Y value. The detection limits (x values) for L- and D-arginine thus obtained for all three chemoreceptors are summarized in Table 1. With the cyclopeptide **1**, a concentration of about 15 μmol L^{-1} arginine can be detected in neutral, buffer-free water.

A survey of the influence of different matrices on the interaction between cyclo-

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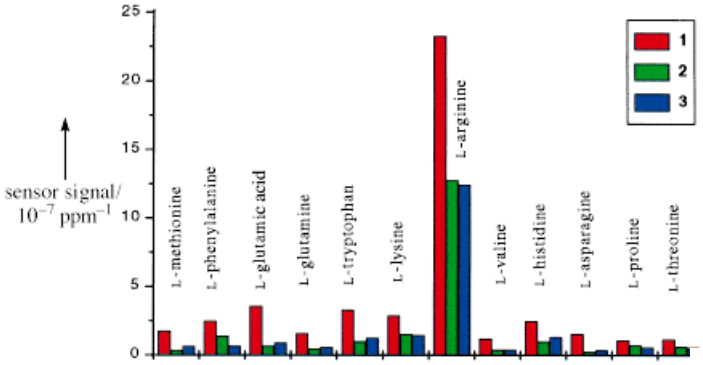

Figure 4. Comparison of the sensor signals of all three sensitive layers for different amino acids in water.

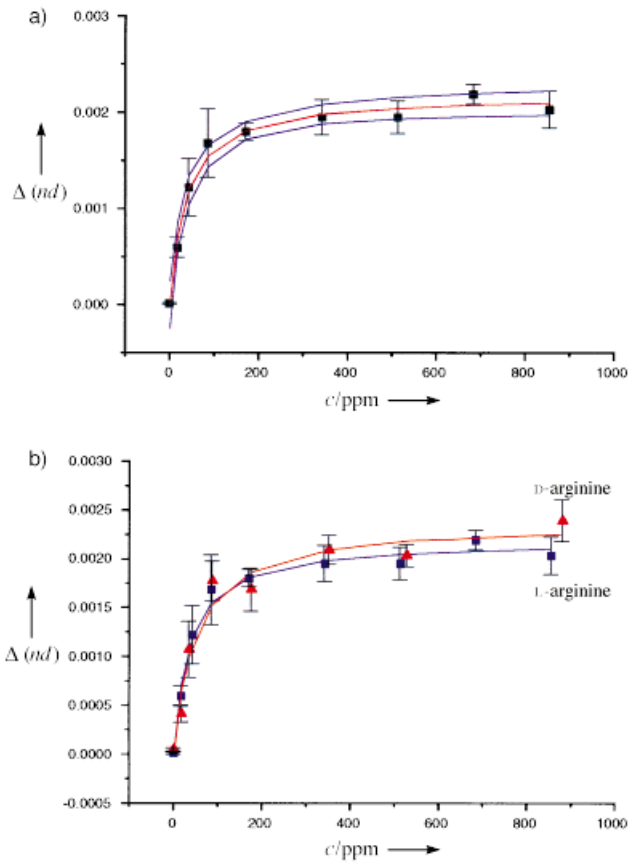

Figure 5. Calibration curves for both arginine enantiomers (▲: D-arginine, ■: L-arginine), determined for immobilized **1**. a) Progress of the calibration with L-arginine (layer **1**), course of the Langmuir function and confidence region of the calibration. b) Comparison of the calibrations for both isomers.

Table 1. Detection limits for L- and D-arginine.[a]

cyclohexapeptide	L-arginine [μmol L^{-1}]	D-arginine [μmol L^{-1}]
1	14.4	17.2
2	40.2	31.6
3	20.1	20.1

[a] For arginine, 1 μmol L^{-1} corresponds to 0.174 mg L^{-1}.

peptide and amino acid led to a different result when PBS buffer (*p*hosphate *b*uffered *s*aline, pH 7.4) was used as solvent. In PBS buffer the α-amino acids mainly are zwitterionic. The change in medium yields a completely different selectivity (Figure 6): From a test set of 14 amino acids, L-glutamine is now the most easily recognized one by all three sensors and is also distinguished from closely related L-asparagine. The signal-amplifying influence of the sugar unit again becomes apparent here. Although the two cyclopeptides **2** and **3** only differ in one recognition element, the signal height changes

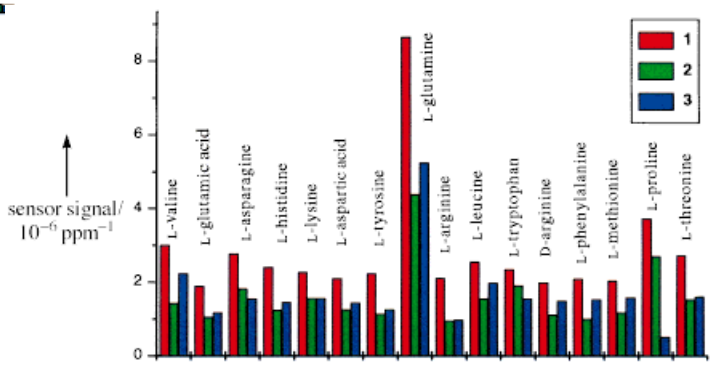

Figure 6. Signals of the three cyclopeptide sensors for different amino acids, measured in PBS buffer.

for different amino acids. This indicates that by varying the cyclopeptides and the constitution of the buffer solution further selectivities for other amino acids can specifically be researched.

In combination with reflectometric interference spectroscopy, optical transducers with surface-bound cyclohexapeptides allow the selective and fast detection of low concentrations of amino acids in aqueous solutions. The detection is possible without derivatization. With combinatorial solid-phase chemistry a multitude of new receptors can be synthesized. Not only single receptors, but also receptor libraries can be immobilized which can be deconvolutively adapted. At present we are selectively looking for receptors for certain analytes with sensor arrays. Owing to the narrow concentration range and the selectivity obtained, the use of the sensor is possible in a broad spectrum of biotechnological sensor applications and for medical diagnosis.

Experimental section

1–**3**: Multiple parallel peptide syntheses (15 μmol each) are carried out on a 2-chlorotritylchloride resin with 9-fluorenylmethoxycarbonyl (Fmoc) amino acids and diisopropylcarbodiimide activation. The hexapeptides, which are protected at the side chains, are cleaved with hexafluoro-2-propanol (30% in dichloromethane) and cyclized in solution (1 mM in DMF). The cyclization reagents are extracted, and the side-chain protecting groups are cleaved with trifluoroacetic acid/water/triisopropylsilane (95/2.5/2.5; 3 h). Then the peptides are precipitated with diethyl ether, centrifuged, dissolved in *tert*-butyl alcohol/water (4/1), and lyophilized. Peptide **1** is taken up in anhydrous methanol, and pH 10–11 is established by adding sodium methoxide (0.5 M in methanol). After 3 h the solution is neutralized with acetic acid and evaporated to dryness, and the residue is dissolved in *tert*-butyl alcohol/water (4/1) and lyophilized. **1**: ESI-MS: $[M+H]^+$: 979.0. **2**: ESI-MS: $[M+H]^+$: 925.5. **3**: ESI-MS: $[M+H]^+$: 962.0; HPLC purity >90% (RP-18, acetonitrile/H_2O, 214 nm).

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Immobilization of **1**–**3** on the transducer surface: The surface of the glass is cleaned with a freshly prepared Piranha solution (H_2SO_4/H_2O 2/1) (1 h), washed with water, and dried at room temperature (RT). The silanization takes place with a solution of 3-glycidoxypropyltrimethoxysilane (20% in toluene; 4 h at 110 °C). To link the cyclopeptides to the surface, a solution of **1**, **2**, or **3** (0.1 mg mL^{-1}) in phosphate buffer (50 mM, pH 8) is added (24 h, RT). Then it is washed with brine (1M) and water. To characterize the cyclopeptide-functionalized glass supports, static contact-angle measurements were carried out with six different solvents and the polar (σ^P) and disperse share (σ^D) in the surface energy were determined. Immobilized cyclopeptide **1**: $\sigma^D = 23.51$ mN m^{-1}, $\sigma^P = 21.37$ mN m^{-1}. Immobilized cyclopeptide **2**: $\sigma^D = 23.18$ mN m^{-1}, $\sigma^P = 23.49$ mN m^{-1}. Immobilized cyclopeptide **3**: $\sigma^D = 23.27$ mN m^{-1}, $\sigma^P = 25.63$ mN m^{-1}.

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FT-IR Mapping—A New Tool for Spatially Resolved Characterization of Polymer-Bound Combinatorial Compound Libraries with IR Microscopy**

Wolfgang J. Haap,* Tilmann B. Walk, and Günther Jung*

A decisive advantage of combinatorial organic solid-phase chemistry[1–5] for finding lead structures is its higher throughput compared to previous synthetic methods, as easily automated reactions are preferentially carried out on polymer resins.[6] Further improvement of the efficiency is directed to an increasing miniaturization of the synthesis as well as screening of the prepared compounds. Resin-bead analysis plays an important role in the development of such technologies. With the techniques that have now been established for analyzing single resin beads—such as NMR spectroscopy,[7, 8] matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry, and FT-IR microscopy[9–11]—only polymer-bound single compounds could be analyzed so far. In combinatorial chemistry, however, techniques are needed with which a large number of compounds can be analyzed simultaneously.

Owing to its high sensitivity, the simplicity of the apparatus, and the high sample throughput, IR microscopy is suited for parallel analysis. The combination of a FT-IR microscope with a motor-driven *x*-*y* stage allows the automatic spectral mapping of surfaces and objects embedded in them. If single resin beads are embedded in a KBr window by pressing, they can be visualized with this measuring arrangement in the form of an IR map. The superpositioning of the visible video image of several resin beads with the corresponding IR map (Figure 1) shows the good correlation of both types of representations. In this case, for the IR reconstruction the typical polystyrene combination vibration at 1942 cm^{-1} was chosen.

Through mapping of larger areas, hundreds of embedded resin beads can be detected and the compounds bound to them identified. To exhibit the potential of this method, a model library of isoxazolidines[12] on Rink amide ARAM resin was synthesized by the split-and-combine method[13] (Scheme 1). The polymer-bound library obtained consists of a total of 18 compounds. If one considers the different building blocks of the synthesis, a nitro group or the carboxamidomethyl residue is found in 50% each of the

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