[8] T. P. Kogan, B. Dupré, K. M. Keller, I. L. Scott, H. Bui, R. V. Market, P. J. Beck, J. A. Voytus, B. M. Revelle, D. Scott, *J. Med. Chem.* **1995**, *38*, 4976 – 4984.
[9] a) J. Fejzo, W. M. Westler, J. L. Markley, S. Macura, *J. Am. Chem. Soc.* **1992**, *114*, 1523 – 1524; b) C. Zwahlen, S. J. F. Vincent, L. D. Bari, M. H. Levitt, G. Bodenhausen, *J. Am. Chem. Soc.* **1994**, *116*, 362 – 368; c) S. J. F. Vincent, C. Zwahlen, G. Bodenhausen, *J. Biomol. NMR* **1996**, *7*, 169 – 172; d) S. J. F. Vincent, C. Zwahlen, C. B. Post, J. W. Burgner, G. Bodenhausen, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 4383 – 4388.
[10] T. Scherf, J. Anglister, *Biophys. J.* **1993**, *64*, 754 – 761.
[11] D. Henrichsen, I. Brockhausen, H. Schachter, T. Peters, *Glycoconjugate J.*, submitted.
[12] The method for the E-selectin bioassay is found in reference 17 in: G. Thoma, J. L. Magnani, R. Öhrlein, B. Ernst, F. Schwarzenbach, R. O. Duthaler, *J. Am. Chem. Soc.* **1997**, 7414 – 7415.
[13] Sialyl Lewis^x tetrasaccharide **1** was used as reference for the ELISA tests. Its relative IC_{50} was adjusted to 1 mM (the absolute values lie between 0.9 and 1.5 mM). The sialyl Lewis^x mimetic **2** exhibits a relative IC_{50} of 0.09 mM.[2a] A relative IC_{50} of 0.66 mM has been reported for **4**,[8] however, in the ELISA test it exhibits a relative IC_{50} of >10 mM and is therefore classified as inactive. The remaining components of the compound library, **3** and **5** – **11,** with relative IC_{50} values of >10 mM, are also regarded as inactive.
[14] B. Ernst, unpublished results.

Discovery of Peptide – Zirconium Complexes That Mediate Phosphate Hydrolysis by Batch Screening of a Combinatorial Undecapeptide Library**

Albrecht Berkessel* and David A. Hérault

Dedicated to Professor Bernt Krebs on the occasion of his 60th birthday

The search for new low-molecular weight catalysts is one of the most fascinating fields of contemporary organic chemistry. One source of inspiration is the spatial arrangement of functional groups in the active site of enzymes, that is the design and synthesis of enzyme models.[1, 2] Many enzymes harbor a metal ion in the active site. The success in modeling a metalloenzyme—and in constructing a novel metal catalyst in general—heavily relies on the matching of the ligand(s) with the central metal ion and with the substrate. Although this problem is widely recognized, it was only recently that parallel ligand synthesis and rapid screening methods were introduced to the field of catalyst research.[3–6]

Herein, we describe our approach to artificial hydrolase activity which consists of a) the split-mix synthesis[7] of a ligand library containing 625 solid-phase-bound undecapeptides, b) complexing of the ligand library with Lewis acidic transition metals, and c) screening of the library with chromogenic test substrates. As it turned out, solid-phase-bound undecapeptides ligands could be identified that mediate the hydrolysis of phosphates. The sequences of the most active (and inactive) peptides were determined by means of Edman degradation, and the catalytic activity or inactivity could be confirmed in homogeneous solution.

The general structure of our ligand library is shown in Figure 1. Initially Fmoc-L-Phe was attached to TentaGel S – NH_2 by using PyBOP as the coupling agent (Fmoc = 9-fluorenylmethoxycarbonyl).[8] After splitting the polymer into five fractions, the second amino acid was introduced. After this step L-Arg, L-His, L-Tyr, L-Trp, or L-Ser was found at position **X**. After pooling the fractions, the spacer Gly was added twice. Seven more coupling steps, including three split-mix additions of **X** and a final deprotection protocol completed the synthesis. In other words, the 625 polymer-bound undecapeptides thus produced contain four variable positions separated by three spacers Gly – Gly and occupied by L-Arg, L-His, L-Tyr, L-Trp, or L-Ser in a combinatorial fashion. Arginine was chosen because a typical motif encountered, for example, in the X-ray crystal structures of staphylococcal nuclease[9] and other enzymes that catalyze phosphoryl transfer,[10] is the activation of the anionic phosphate moiety towards nucleophilic attack by water by ion-pair formation with the cationic guanidinium moiety. This structural theme has inspired a lot of work aimed at catalytically active mono- and bis-guanidinium receptors, which serve as enzyme models for the hydrolysis of phosphoric esters.[11, 12] The imidazole moiety of histidine is a ligand for numerous transition metal ions in metalloenzymes, as is tyrosine.[12, 13] It was hoped that the electron-rich indole system of tryptophan would display $\pi-\pi$ stacking interactions with the aromatic esters that were envisaged as test substrates. Finally, the hydroxy group of serine might serve as the acceptor for an acyl/phosphoryl group in a transesterification reaction.[11–12]

Figure 1. General structure of the undecapeptide library. PEG = polyethylene glycol.

Our approach to the simulation of hydrolase activity bears some resemblance to the work of Zouhair Atassi and Manshouri, which was aimed at the active sites of serine esterases and proteases:[14, 15] The components of the catalytic triad (Asp-His-Ser) were scaffolded onto cyclic 29-mer peptides, separated by Gly spacers, and designed to reproduce the spatial arrangement of the catalytically active functional groups in the enzymes' active sites. Unfortunately, the remarkable activities and selectivities reported by Zouhair Atassi and Manshouri could not be reproduced by others.[16] Most likely, the 29-mers do not adopt the conformation in which the catalytic triad is in the desired orientation.[16] Instead of merely matching structures, our approach includes the combinatorial shuffling of functional groups plus the restriction of conformational mobility by addition of metal ions that are expected to coordinate the peptides (see below).

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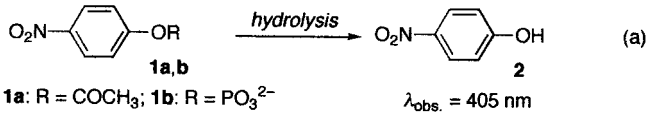
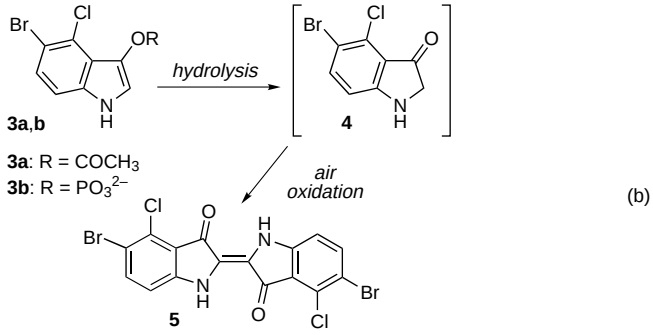
To screen the library for hydrolase activity we employed chromogenic test substrates. Upon hydrolysis, standard substrates like *para*-nitrophenyl esters **1a** and **1b** liberate *para*-nitrophenol (**2**) [Eq. (a)], which can be easily determined



(a)

quantitatively by UV/Vis spectroscopy. However, since **2** is a freely diffusible species, the information on which polymer bead(s) it was formed, is lost. We therefore turned our attention to the 3-hydroxyindolyl derivatives **3a**, **b** [Eq. (b)]:[17] When the ester functions are hydrolyzed, the



(b)

resulting indoxyl derivative **4** is oxidized in the aerated solution to afford the turquoise and insoluble indigo dye **5**. In the screening experiments, 1 mM solutions of Cu^{2+}, Zn^{2+}, Fe^{3+}, Co^{3+}, Eu^{3+}, Ce^{4+}, or Zr^{4+} (or without metal salts) were added to about 2500 of the polymer beads in EPPS buffer at pH 5.6.[18] Activity was found with the 3-hydroxyindolyl phosphate **3b** in the presence of Zr^{4+}: In this assay, about 20 of the about 2500 beads showed the typical turquoise color of the indigo dye **5** (Figure 2a). The three most intensively colored beads were isolated and subjected to Edman degradation, together with two control beads that did not show an indigo stain at all. The sequences found are listed in Figure 3. The top sequence **A** in Figure 3 is the one determined from the most deeply stained bead in the assay.[19] This peptide **A** was re-synthesized first in polymer-bound form as described above. When the TentaGel beads carrying **A** were exposed to the Zr^{4+} ion and the substrate **3b**, they all turned deeply turquoise (Figure 2b). Alternatively, when the control sequence **D** was re-synthesized on solid support and subjected to the same assay conditions, none of the beads adopted a turquoise stain (Figure 2c).

Next, the catalytic activity of the peptide **A** and the inactivity of peptide **D** were probed in homogeneous solution. For this purpose, the peptides were synthesized on Wang resin and cleaved off. In fact, when the test substrate **3b** was exposed to peptide **A** in the presence of Zr^{4+}, the hydrolysis of **3b** was nicely indicated by the precipitation of the indigo dye **5**, whereas no significant hydrolysis occured when **D** was employed. Since the low solubility of **5** prohibited a quantitative assessment of the kinetic parameters of the hydrolysis,

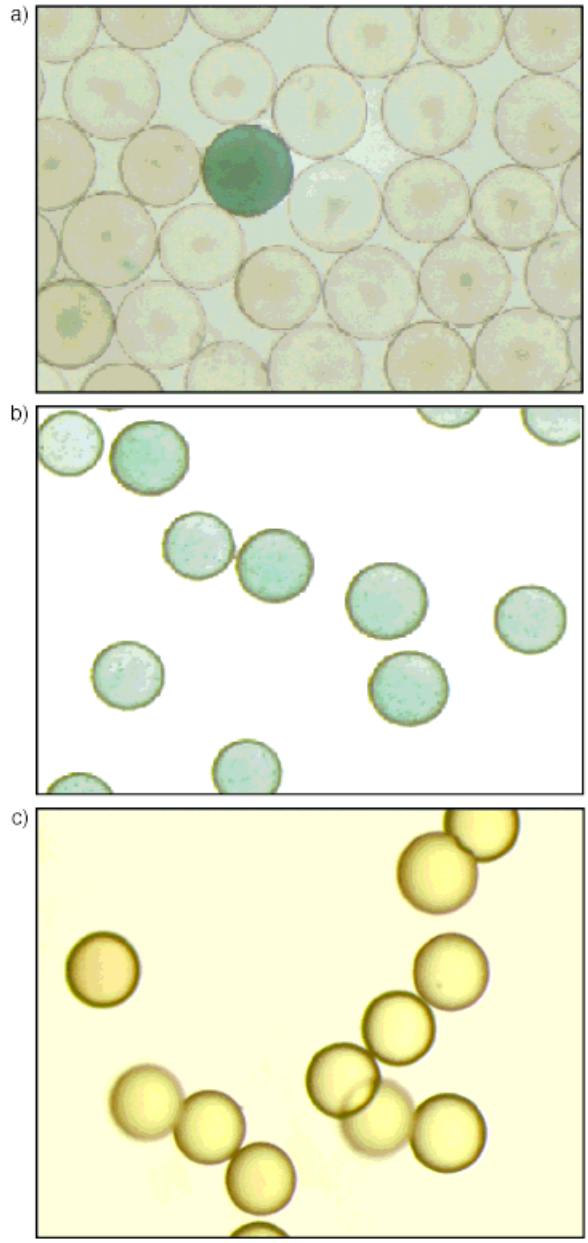


Figure 2. TentaGel beads exposed to Zr^{4+} and **3b**, carrying the following undecapeptides: a) library; b) sequence **A**; c) sequence **D**.

Active sequences:

H_2N-**Ser**-Gly-Gly-**His**-Gly-Gly-**Arg**-Gly-Gly-**His**-Phe-CO_2H **A**

H_2N-**Ser**-Gly-Gly-**Ser**-Gly-Gly-**Ser**-Gly-Gly-**His**-Phe-CO_2H **B**

H_2N-**Ser**-Gly-Gly-**Arg**-Gly-Gly-**His**-Gly-Gly-**His**-Phe-CO_2H **C**

Inactive sequences:

H_2N-**Trp**-Gly-Gly-**Arg**-Gly-Gly-**Trp**-Gly-Gly-**Arg**-Phe-CO_2H **D**

H_2N-**Arg**-Gly-Gly-**Ser**-Gly-Gly-**Arg**-Gly-Gly-**Trp**-Phe-CO_2H **E**

Figure 3. Hit and control sequences found by screening of the undecapeptide library with Zr^{4+} and **3b**.

we resorted to *para*-nitrophenyl phosphate (**1b**). The results are depicted in Figure 4 (see Experimental Section for conditions): When incubated with Zr^{4+} and the control peptide **D**, only a very slow liberation of *para*-nitrophenol

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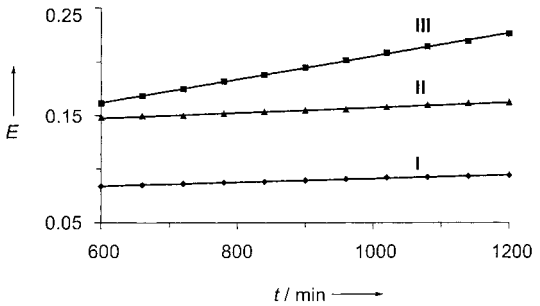

Figure 4. UV/Vis monitoring of the hydrolysis of **1b** (20 °C, 67 mM MES-buffer,[20] pH = 5.4, $\lambda_{obs} = 405$ nm). Trace I: **1b** (3.3 mM); trace II: **1b** (3.3 mM); $ZrCl_4$ (1.7 mM), peptide **D** (1.7 mM); trace III: **1b** (3.3 mM), $ZrCl_4$ (1.7 mM), peptide **A** (1.7 mM).

(**2**) was observed. The rate of hydrolysis in the presence of **D** is virtually the same as that of the background hydrolysis of *para*-nitrophenyl phosphate (**1b**; Figure 4, traces II and I, respectively). In contrast, in the presence of the peptide **A**, the hydrolysis of *para*-nitrophenyl phosphate (**1b**) proceeded about five times faster (Figure 4, trace III).[21] Interestingly, rapid hydrolysis of phosphates was recently reported for Zr^{4+} alone and in the presence of amino or hydroxy ligands.[22, 23]

In summary, we have combinatorially synthesized a library of 625 undecapeptides. On-bead screening of this library with the test substrates **3a**,**b** in the presence of a variety of transition metal ions enabled the identification of members of this library that—in combination with Zr^{4+}—mediate phosphate hydrolysis. The activity (and inactivity of the control peptides) was not an exclusive feature of the polymer-bound state, but was also reproduced in homogenous solution when the peptides were cleaved off the resin. The inactivity of the combination Zr^{4+}/control peptide **D** suggests that our combinatorial approach apparently identified peptide ligands that do not totally prevent binding/activation of the anionic phosphate to the positively charged zirconium cation (cleavage of the neutral ester **3a** was not observed). This is an important starting point for the further development of ligands that allow Zr^{4+} to perform phosphate hydrolysis in biological matrices. We presently do not attempt to explain the activity of, for example, **A** and the inactivites of **D** and **E** on the basis of their mere sequences—the absence of structural data for the peptide–zirconium complexes of **A**–**C** (and **D**,**E**, respectively) precludes a sound rationalization. Nevertheless, it is interesting to note, for example, that the inactive sequences **D** and **E** carry two positive charges (2 × Arg), whereas **A** and **C** carry one arginine residue and **B** none at all. Possibly, the combination of histidine as a neutral Zr^{4+}-binding motif[24] and serine as the phosphate acceptor may be seen as the common feature of the active sequences **A**–**C**. To the best of our knowledge, this is the first case of a truely combinatorial approach to the discovery of a well defined metal–ligand system: The ligand diversity was generated in a combinatorial manner, the active members of the library were selected by bulk screening of the library, and the structures of the hits (and controls) were analyzed subsequently.

Experimental Section

Microscopy was done using a Zeiss Axioplan 2 microscope, equipped with a MC-3250 CCD-camera and KS 100 software for video processing. Peptide sequences were determined from single beads using an Applied Biosystems Procise 494 Protein Sequencer. TentaGel S–NH_2 resin was purchased from Rapp Polymere, Tübingen, 5-bromo-4-chloroindolyl butyrate (**3a**) and 5-bromo-4-chloroindolyl phosphate (**3b**) from Molecular Probes Europe BV, Leiden, The Netherlands. Peptide synthesis was performed on TentaGel S–NH_2 following a standard procedure consisting of the repetition of the following four steps: 1) Coupling with 2 equiv of an Fmoc-protected L-amino acid by using 2 equiv of PyBOP[8] in 3% *N*-methylmorpholine in absolute DMF; 2) washing with absolute DMF; 3) deprotection with 20% piperidine in absolute DMF; 4) washing with absolute DMF. The efficiencies of the coupling steps were determined by UV/Vis spectroscopic quantification of the subsequent deprotections. After the last cycle, the resins were treated with TFA/EtSH/DMS/phenol 80:10:5:5 (TFA = trifluoroacetic acid, DMS = dimethyl sulfide). In the split-mix synthesis of the library, the coupling of the functional amino acids **X** was carried out in five separate reactors, using Fmoc-L-Arg(Pmc), Fmoc-L-Trp, Fmoc-L-His(Trt), Fmoc-L-Ser(*t*Bu) and Fmoc-L-Tyr(*t*Bu), respectively. For the addition of the Gly units, the resins were pooled in one reactor.

Screening: The resin (5 mg, ca. 2500 beads, corresponding to ca. 1.2 μmol peptide) was treated with 1 mL of a 1 mM solution of 5-bromo-4-chloroindolyl butyrate (**3a**) or 5-bromo-4-chloroindolyl phosphate (**3b**) in EPPS buffer (10 mM, pH 5.6) and 1 mL of 1 mM solutions of the following salts in EPPS buffer (10 mM, pH 5.6): $CuCl_2$, $FeCl_3 \cdot 6H_2O$, $ZnCl_2$, $EuCl_3 \cdot 6H_2O$, $ZrCl_4$, $(NH_4)_2Ce(NO_3)_6$, and $Na_3[Co(NO_2)_6]$. In an additional experiment, buffer was added without a metal salt. The turquoise stain of the indigo dye **5** developed exclusively in the assay using 5-bromo-4-chloroindolyl phosphate (**3b**), and only in the presence of Zr^{4+}. About 20 stained beads were found after incubation for one day at about 20 °C. The three most deeply stained beads were selected, together with two control beads that did not show any stain. Edman degradation afforded the sequences **A**–**C** and **D**,**E**, respectively. For experiments in homogeneous solution, the peptides **A** and **D** were synthesized on Wang resin by the above Fmoc-technique and cleaved off the solid support by treatment with TFA. The kinetic runs shown in Figure 4 were carried out in a Beckman DU-640 spectrophotometer at 20 °C and at a total sample volume of 3 mL. In the case of peptide **A** (Figure 4, trace III), 20 h reaction time corresponded to about 7% conversion of *para*-nitrophenyl phosphate (**1b**). The degree of conversion was determined by calibration using *para*-nitrophenol (**2**). Extinction/time profiles were monitored for up to two days and remained linear ($\rho \geq 0.998$) during this period of time. In multiple experiments, slopes were reproduced within ±5%.

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[1] R. Breslow, *Acc. Chem. Res.* **1995**, *28*, 146–153.
[2] a) A. J. Kirby, *Angew. Chem.* **1996**, *108*, 770–790; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 705–724; b) Y. Murakami, J. Kikuchi, Y. Hisaeda, O. Hayashida, *Chem. Rev.* **1996**, *96*, 721–758.
[3] L. C. Hsieh-Wilson, X.-D. Xiang, P. G. Schultz, *Acc. Chem. Res.* **1996**, *29*, 164–170.
[4] a) S. J. Taylor, J. P. Morken, *Science* **1998**, *280*, 267–270; b) K. D. Shimizu, B. M. Cole, C. A. Krueger, K. W. Kuntz, M. L. Snapper, A. H. Hoveyda, *Angew. Chem.* **1997**, *109*, 1782–1785; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1704–1707; c) B. M. Cole, K. D. Shimizu, C. A. Krueger, J. P. A. Harrity, M. L. Snapper, A. H. Hoveyda, *Angew. Chem.* **1996**, *108*, 1776–1779; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1668–1671; d) S. R. Gilbertson, X. Wang, *Tetrahedron Lett.* **1996**, *37*, 6475–6478; e) K. Burgess, H.-J. Lim, A. M. Porte, G. A. Sulikowski, *Angew. Chem.* **1996**, *108*, 192–194; *Angew. Chem. Int. Ed. Engl.* **1996**,

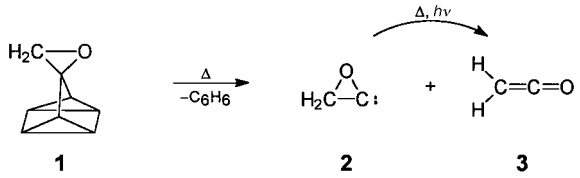
  1433-7851/99/3801-0104 $ 17.50+.50/0

35, 220 – 222; f) G. Liu, J. A. Ellman, *J. Org. Chem.* **1995**, *60*, 7712 – 7713.
[5] For an approach to phosphatase activity by grafting catalytically active groups onto polyallylamine and complexation with metal ions, see ref. [6].
[6] F. M. Menger, A. V. Eliseev, V. A. Migulin, *J. Org. Chem.* **1995**, *60*, 6666 – 6667.
[7] a) K. S. Lam, M. Lebl, V. Krchnák, *Chem. Rev.* **1997**, *97*, 411 – 448; b) F. Balkenhohl, C. von dem Bussche-Hünnefeld, A. Lansky, C. Zechel, *Angew. Chem.* **1996**, *108*, 2436 – 2488; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2288 – 2337; c) A. Furka, F. Sebestyén, M. Asgedom, G. Dibó, *Int. J. Pept. Protein Res.* **1991**, *37*, 487 – 493; d) R. A. Houghten, C. Pinilla, S. E. Blondelle, J. R. Appel, C. T. Dooley, J. H. Cuervo, *Nature* **1991**, *354*, 84 – 86; e) K. S. Lam, S. E. Salmon, E. M. Hersh, V. J. Hruby, W. M. Kazmiersky, R. J. Knapp, *Nature* **1991**, *354*, 82 – 84.
[8] PyBOP: benzotriazol-1-yloxy-tripyrrolidinophosphonium hexafluorophosphate; J. Coste, D. Le-Ngueyen, B. Castro, *Tetrahedron Lett.* **1990**, *31*, 205 – 208.
[9] a) F. A. Cotton, E. E. Hazen, Jr., M. J. Legg, *Proc. Natl. Acad. Sci. USA* **1979**, *76*, 2551 – 2555; b) P. W. Tucker, E. E. Hazen, Jr., F. A. Cotton, *Mol. Cell. Biochem.* **1979**, *23*, 67 – 86.
[10] N. Sträter, W. N. Lipscomb, T. Klabunde, B. Krebs, *Angew. Chem.* **1996**, *108*, 2158 – 2191; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2024 – 2055.
[11] a) M.-S. Muche, M. W. Göbel, *Angew. Chem.* **1996**, *108*, 2263 – 2265; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2126 – 2129; b) V. Jubian, A. Veronese, R. P. Dixon, A. D. Hamilton, *Angew. Chem.* **1995**, *107*, 1343 – 1345; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1237 – 1239; c) D. M. Perreault, L. A. Cabell, E. V. Anslyn, *Bioorg. Med. Chem.* **1997**, *5*, 1209 – 1220; d) T. Oost, M. Kalesse, *Tetrahedron* **1997**, *53*, 8421 – 8438; e) R. Hettich, H.-J. Schneider, *J. Am. Chem. Soc.* **1997**, *119*, 5638 – 5647; f) P. Hurst, B. K. Takasaki, J. Chin, *J. Am. Chem. Soc.* **1996**, *118*, 9982 – 9983.
[12] D. M. Perreault, E. V. Anslyn, *Angew. Chem.* **1997**, *109*, 470 – 490; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 432 – 451.
[13] a) S. J. Lippard, J. M. Berg, *Principles of Bioinorganic Chemistry*, University Science Books, Mill Valley, USA, **1994**; b) J. Reedijk in *Bioinorganic Catalysis* (Ed.: J. Reedijk), Dekker, New York, USA, **1993**, pp. 1 – 10.
[14] M. Zouhair Atassi, T. Manshouri, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 8282 – 8286.
[15] Examples of designed catalytically active peptides: a) K. Johnsson, R. K. Allemann, H. Widmer, S. A. Benner, *Nature* **1993**, *365*, 530 – 532; b) K. S. Broo, H. Nilsson, J. Nilsson, A. Flodberg, L. Baltzer, *J. Am. Chem. Soc.* **1998**, *120*, 4063 – 4068.
[16] a) B. W. Matthews, C. S. Craik, H. Neurath, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 4103 – 4105; b) D. R. Corey, M. A. Phillips, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 4106 – 4109; c) J. A. Wells, W. J. Fairbrother, J. Otlewski, M. Laskowski, Jr., J. Burnier, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 4110 – 4114.
[17] Commercially available from Molecular Probes Europe BV, Leiden, The Netherlands.
[18] EPPS: 3-[4-(2-hydroxyethyl)-1-piperazino]propanesulfonic acid. The polymer (ca. 5 mg, ca. 1.2 μmol peptide) was incubated with the metal salt (1 μmol) and the test substrates **3a**,**b** (1 μmol).
[19] To exclude sequence-specific adsorption effects, the following control experiments were carried out: a) The hydroxyindolyl phosphate **3b** was hydrolyzed enzymatically and oxidized to the indigo dye **5** in an aerated solution of alkaline phosphatase from *bovine intestinal mucosa* (EC 3.1.3.1; Sigma; 20 U mg^{-1}). Exposure of about 2500 beads of the undecapeptide library to the resulting deeply colored suspension did not afford stained beads. b) When TentaGel S – NH_2 was swollen in an aqueous solution of the above alkaline phosphatase, exposure to the reagent **3b** resulted in a deep turquoise stain of all beads.
[20] MES: 2-morpholinoethanesulfonic acid.
[21] Mixtures of L-Arg, L-His, and L-Ser of equivalent concentrations did not effect an acceleration.
[22] a) R. Ott, R. Krämer, *Angew. Chem.* **1998**, *110*, 2064 – 2067; *Angew. Chem. Int. Ed.* **1998**, *37*, 1957 – 1960; b) R. A. Moss, J. Zhang, K. G. Ragunathan, *Tetrahedron Lett.* **1998**, *39*, 1529 – 1532.
[23] Uncomplexed Zr^{4+} as the cause of the observed acceleration appears very unlikely according to the following control experiments: At constant Zr concentration, a duplication of the peptide concentration does not result in a decrease of the rate of hydrolysis. On the other hand, at constant peptide concentration, an increase of the Zr concentration results in an increase of the rate of hydrolysis. Complex formation is furthermore indicated by resonance shifts observed in the NMR spectra of peptide **A** upon addition of Zr^{4+}.
[24] R. C. Fay in *Comprehensive Coordination Chemistry, Vol. 3* (Eds.: G. Wilkinson, R. D. Gillard, J. A. McClaverty), Pergamon, Oxford, **1987**, p. 363 – 451.

Oxiranylidene**

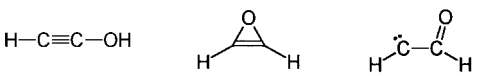
Günther Maier,* Hans Peter Reisenauer, and Michael Cibulka

Fourteen years ago we could generate cyclopropenylidene by high vacuum flash pyrolysis of a quadricyclane derivative, synthesized by Hoffmann et al.,[1] and identify it spectroscopically in a rare gas matrix.[2] This was the starting point for intensive studies on the C_3H_2 potential-energy surface.[3] Consequently we tried to synthesize oxiranylidene (**2**) by using the quadricyclane derivative **1** as a precursor. This compound in particular was used because Hoffmann and Schüttler[4] had detected ketene (**3**) on thermolysis of **1** in an earlier experiment. The question as to whether oxiranylidene (**2**) is the primary product of this reaction could not be answered at that time. It was logical to resume the search for **2**



1 **2** **3**

with techniques available today (high vacuum flash pyrolysis in combination with matrix-isolation). This was especially tempting because of the great theoretical interest in oxiranylidene (**2**). Ab initio calculations predict that it should be a minimum on the C_2H_2O potential-energy surface with a considerable energy barrier to isomerization.[5, 6] Therefore it should be possible to detect **2** in a cryogenic matrix. Herein we show that besides ketene (**3**) and ethynol (**4**),[7] oxiranylidene (**2**) is indeed an observable C_2H_2O species.



4 **5** **6**

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