Keywords: crystal engineering · hydrogen bonds · N ligands · nickel · supramolecular chemistry

[1] J.-M. Lehn, *Supramolecular Chemistry*, VCH, Weinheim, **1995**.
[2] a) G. M. Whitesides, J. P. Mathias, C. T. Seto, *Science* **1991**, *254*, 1312 – 1319; b) J. Rebek, Jr., *Angew. Chem.* **1990**, *102*, 261 – 272; *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 245 – 255.
[3] a) S. Subramanian, M. J. Zaworotko, *Coord. Chem. Rev.* **1994**, *137*, 357 – 401; b) O. Ermer, *J. Am. Chem. Soc.* **1988**, *110*, 3747 – 3754.
[4] a) M. C. Etter, *Acc. Chem. Res.* **1990**, *23*, 120 – 126; b) C. B. Aakeröy, K. R. Seddon, *Chem. Soc. Rev.* **1993**, *26*, 397 – 407.
[5] I. Weissbuch, R. P. Biro, M. Lahav, L. Leiserowitz, *Acta Crystallogr. Sect. B* **1995**, *51*, 115 – 148.
[6] a) G. R. Desiraju, *Crystal Engineering: The Design of Organic Solids*, Elsevier, Amsterdam, **1989**; b) G. R. Desiraju, *Angew. Chem.* **1995**, *107*, 2541 – 2558; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2311 – 2327.
[7] J. C. MacDonald, G. M. Whitesides, *Chem. Rev.* **1994**, *94*, 2383 – 2420.
[8] a) M. J. Zaworotko, *Chem. Soc. Rev.* **1994**, *137*, 283 – 288; b) A. D. Burrows, C.-W. Chan, M. M. Chowdhry, J. E. McGrady, D. M. P. Mingos, *Chem. Soc. Rev.* **1995**, *138*, 329 – 339.
[9] S. W. Kaiser, R. B. Saillant, W. M. Butler, P. G. Rasmussen, *Inorg. Chem.* **1976**, *15*, 2681 – 2687.
[10] M. Tadokoro, J. Toyoda, K. Isobe, T. Itoh, A. Miyazaki, T. Enoki, K. Nakasuji, *Chem. Lett.* **1995**, 613 – 614.
[11] M. M. Chowdhry, D. M. P. Mingos, A. J. P. White, D. J. Williams, *Chem. Commun.* **1996**, 899 – 900.
[12] Crystal structure determinantions. General: Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-103 363 – 103 366. 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). a) Crystal data for **1**: $C_{22}H_{31}O_2N_{13}Ni_2$, $M_r = 568.27$, trigonal, space group $P\bar{3}$ (no. 147), $a = 11.167(2)$, $c = 13.206(4)$ Å, $V = 1426.1(3)$ Å^3, $Z = 2$, $\rho_{calcd} = 1.323$ g cm^{-3}, $F(000) = 596$, 106 parameters; $R_1 = 0.060$, $R_w = 0.080$, GOF = 1.55 for all 1613 data ($I > 3\sigma(I)$); max./min. residual electron density: 0.79/ – 0.36 e Å^{-3}. Diffractometer: Rigaku AFC5R; $\mu(Mo_{K\alpha}) = 7.24$ cm^{-1}; Lp correction; 3094 reflections collected; the structure was solved with the program PATTY, and refined with the program DIRDIF92; reflections were refined based on F_o by full-matrix least squares. b) Crystal data for **2**: $C_{62}H_{94}O_2N_{26}Ni_2$, $M_r = 1353.00$, orthorhombic, space group $Pca2_1$ (no. 29), $a = 18.667(2)$, $b = 23.7236(8)$, $c = 16.786(1)$ Å, $V = 7433.6(8)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.209$ g cm^{-3}, $F(000) = 2880$, 829 parameters; $R_1 = 0.065$, $R_w = 0.079$, GOF = 1.63 for all 2803 data ($I > 3\sigma(I)$); max./min. residual electron density: 0.44/ – 0.27 e Å^{-3}. Diffractometer: Nonius CAD4; $\mu(Cu_{K\alpha}) = 10.90$ cm^{-1}; Lp correction; 7729 relections collected; the solution was solved with the program SAPI91, and refined with the program DIRDIF94; reflections were refined based on F_o by full-matrix least squares. c) Crystal data for **3**: $C_{41}H_{67}O_{11}N_{12}NiK$, $M_r = 1001.85$, monoclinic, space group $C2/m$ (no. 12), $a = 19.077(3)$, $b = 29.074(3)$, $c = 9.769(3)$, $\beta = 110.39(2)^\circ$, $V = 5078(1)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.310$ g cm^{-3}, $F(000) = 2128$, 287 parameters; $R_1 = 0.057$, $R_w = 0.073$, GOF = 1.77 for all 1484 data ($I > 3\sigma(I)$), max./min. residual electron density 0.29/ – 0.29 e Å^{-3}. Diffractometer: Rigaku AFC7R; $\mu(Cu_{K\alpha}) = 18.38$ cm^{-1}; Lp correction; 4004 reflections collected; the structure was solved with the program PATTY, and refined with the program DIRDIF94; reflections were refined based on F_o by full-matrix least squares. d) Crystal data for **4**: $C_{60}H_{84}O_2N_{30}Ni_2$, $M_r = 1374.92$, tetragonal, space group $P4_12_12$ (no. 92), $a = 19.061(1)$, $c = 38.474(2)$ Å, $V = 13977(1)$ Å^3, $Z = 8$, $\rho_{calcd} = 1.307$ g cm^{-3}, $F(000) = 5808$, 778 parameters; $R_1 = 0.075$, $R_w = 0.109$, GOF = 1.83 for all 3213 data ($I > 3\sigma(I)$), max./min. residual electron density 0.80/ – 0.36 e Å^{-3}. Diffractometer: Rigaku AFC7R; $\mu(Cu_{K\alpha}) = 11.89$ cm^{-1}; Lp correction; 5374 reflections collected; the structure was solved with the program SHLXS86, and refined with the program DIRDIF94; reflections were refined based on F_o by full-matrix least squares.
[13] J. S. Moore, S. Lee, *Chem. Ind.* **1994**, 556 – 560.
[14] L. Carlucci, G. Ciani, D. M. Proserpio, A. Sironi, *J. Am. Chem. Soc.* **1995**, *117*, 4562 – 4569.

Bioaffinity NMR Spectroscopy: Identification of an E-Selectin Antagonist in a Substance Mixture by Transfer NOE**

Dirk Henrichsen, Beat Ernst, John L. Magnani, Wei-Tong Wang, Bernd Meyer,* and Thomas Peters*

Leukocytes play a central role in the body's resistance to tissue lesions and microbial infections. So to be able to exercise their defense function, they must selectively enter the area of the affected tissue. The first step in this inflammatory cascade is the cytokine-induced expression of P-selectin and E-selectin, which, located on the surface of endothelial cells, interact specifically with ligands on leukocyte surfaces. With E-selectin this is the ESL – 1 ligand, with P-selectin the PSGL – 1 ligand.[1] The specific interactions between selectins and ligands first initiate a "rolling" of the leukocytes, which leads to further specific interactions with other membrane proteins and, ultimately, to leukocyte migration into the affected tissue. In pathological situations such as myocardial infarction, transplantation, or rheumatoid arthritis, suppression of the inflammatory cascade is desirable. Considerable effort has therefore been expended on the preparation of potent P- and E-selectin antagonists.[2] In the case of E-selectin, the bioactive conformation of the sialyl Lewis^x mimetic **2** was recently elucidated by transfer NOE experiments.[3] It emerged that the bioactive conformation of this antagonist has much in common with that of the sialyl Lewis^x **1** (see Scheme 1).[4]

As always in the search for active compounds, the search for sialyl Lewis^x mimetics requires screening procedures that allow the rapid identification of lead compounds. A procedure we have described recently, "bioaffinity NMR spectroscopy", is based on the selective detection of transfer NOEs (trNOEs).[5] It makes use of the observation that small molecules (relative molecular mass up to about 2 kDa) exhibit strong negative trNOEs when bound to receptor proteins, and can thus be differentiated from nonbinding molecules with weak positive NOEs. Isotope labeling of the

[*] Prof. Dr. B. Meyer
Institut für Organische Chemie der Universität Hamburg
Martin-Luther-King-Platz 6, D-20146 Hamburg (Germany)
Fax: (+49) 40-4123-2878
E-mail: bernd.meyer@sgi1.chemie.uni-hamburg.de
Prof. Dr. T. Peters, Dipl.-Chem. D. Henrichsen
Institut für Chemie der Medizinischen Universität
Ratzeburger Allee 160, D-23538 Lübeck (Germany)
Fax: (+49) 451-500-4241
E-mail: tp@chemie.mu-luebeck.de
Prof. Dr. B. Ernst[+]
Novartis Pharma AG, Basel (Switzerland)
Dr. J. L. Magnani, Dr. W.-T. Wang
GlycoTech Corp., Rockville, MD (USA)
[+] Present Address:
Universität Basel, Institut für Pharmazie
Basel (Switzerland)

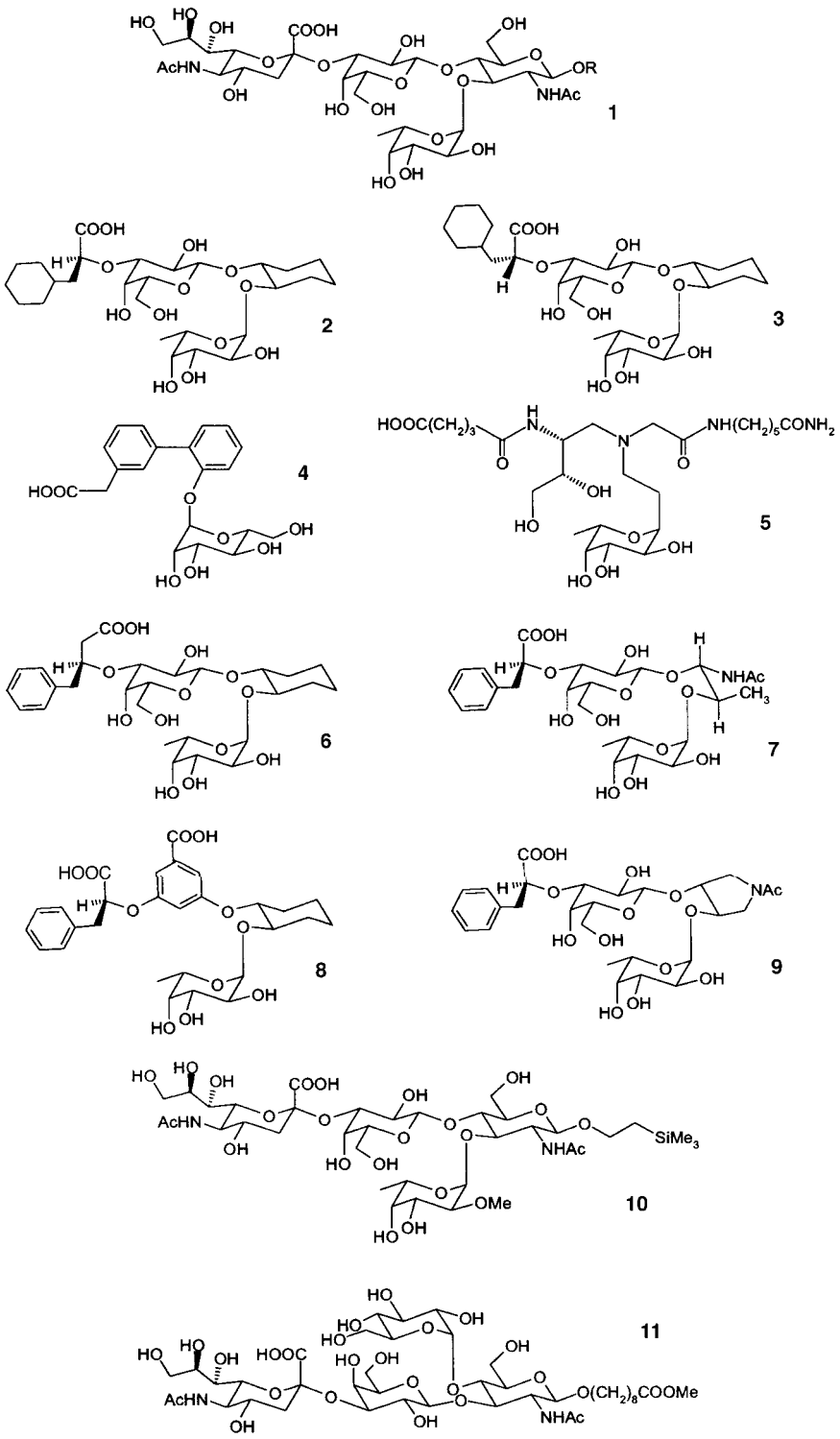
[**] This work was supported by BMBF (FKZ 031161 and 0311395), the Deutschen Forschungsgemeinschaft (Sonderforschungsbereich 470, Teilprojekt B3), and the Fonds der Chemischen Industrie. We thank Dr. T. Keller and Dr. G. Wolff (Bruker Analytik GmbH, Rheinstetten) for excellent support.

  1433-7851/99/3801-0098 $ 17.50+.50/0

protein is not necessary, nor is there any limitation to the relative molecular mass of the receptor protein. Lead compounds in pharmacological research ideally have relative molecular masses below 1 kDa, and they are therefore, in principle, readily identifiable by the cited procedure. Since trNOEs are ideally suited to the analysis of bioactive conformations,[6] the analysis of the bioactive conformations of ligands in compound mixtures should also be possible.

Another procedure that has been introduced recently for small proteins (< 30 kDa) is based on ligand-induced changes in ^{15}N chemical shifts of amino acids at the binding site measured by ^{15}N HSQC spectroscopy.[7] However, we were unable to use this method since recombinant E-selectin, which is present as an IgG chimera, has a relative molecular mass of about 220 KDa, which is well outside the range accessible to ^{15}N HSQC spectroscopy.

Here we show that bioactive components can be identified by bioaffinity NMR spectroscopy, even if structurally related ligands are present in a library. The compound library selected consisted of ten compounds (**2**–**11**, Scheme 1), seven of which have a basic structure derived from a Lewis^a or Lewis^x pattern (**2**, **3**, **6**, **7**, **9**–**11**). The tetrasaccharides **10** and **11** are sialyl Lewis^x and sialyl Lewis^a tetrasaccharide derivatives with only minor structural differences. Compound **10** differs from sialyl Lewis^x **1** only in that the 2-hydroxyl functional group of L-fucose is replaced by a methoxy group. The sialyl Lewis^a derivative **11** contains an L-galactose group in place of the L-fucose group. Compounds **4**,[8] **5**, and **8** are further simplified sialyl Lewis^x mimetics that contain merely the fucose residue and the carboxyl group of the natural product **1** as potential active groups.



Scheme 1. Schematic drawings of compounds **2**–**11** present in the compound library compared with the sialyl Lewis^x base structure **1**.

The NOESY spectra of this ligand library were measured at several temperatures. It emerged that a few ligands showed weak negative NOEs at room temperature, but most exhibited the weak positive NOEs that are typical of low molecular weight compounds. No negative NOEs were observed at 310 K, so at this temperature a clear differentiation between trNOES (negative value) and NOEs (positive value) was possible. As expected, the NOESY spectrum of the compound mixture exhibited a complex signal pattern that precluded identification of individual components (Figure 1, left).

In the trNOE experiments, recombinant E-selectin/hIgG was deglycolsylated enzymatically, since it was known that the signals of covalently bound sialyl *N*-acetyllactosamine chains interfere with trNOE measurements.[4] Treatment with glycopeptidase F (PNGase F) and neuraminidase from *Clostridium perfringens* reduced the fraction of covalently bound carbohydrate to about one-sixth of the original value. More than

  1433-7851/99/3801-0099 $ 17.50+.50/0

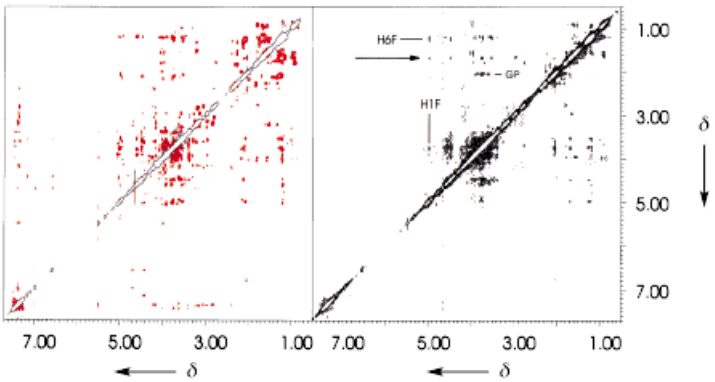


Figure 1. Left: 2D NOESY spectrum of the compound mixture in D_2O at 310 K. The mixing time was 900 ms. Only positive NOEs (red) appeared. Right: 2D trNOESY spectrum of the compound mixture in the presence of E-selectin at a mixing time of 300 ms and a temperature of 310 K. A spin lock filter[10] of 15 ms was used. The molar ratio of binding sites to ligands was 1:15 (see Experimental Section). The cross signals shown are negative (trNOEs) and can be assigned to the bioactive component **2**. Only negative cross signals (black) are illustrated. Cross signals that are caused by spin diffusion are indicated with an arrow. GP indicates the signals of the glycan moiety of E-selectin.

90% of the sialic acid residues were removed. A final treatment with β-galactosidase from *E coli* reduced E-selectin activity by about 90%, so further deglycosylation was not possible. The NOESY spectra of the deglycosylated E-selectin showed that the normally intense ^{1}H NMR signals of the sialic acid residues have almost disappeared, whereas the *N*-acetyllactosamine signals still represented a disruptive factor.

The trNOESY spectra of the ligand library at different levels of excess relative to deglycosylated E-selectin were measured at 310 K. This was to ensure that favorable ratios were present for the detection of potential trNOES of components that were present in the mixture at varying concentrations. The ratios were 5:1, 8:1, 12:1, 15:1, and 20:1, and in all cases trNOEs were observed. The ratio of 15:1 emerged as the most favorable (maximum trNOEs).

A comparison of the NOESY spectrum of the free library (Figure 1, left) with that of the trNOESY of the library in the presence of E-selectin (Figure 1, right) shows that the majority of cross signals in the trNOESY spectrum is absent (under these experimental conditions they appeared as weak positive NOEs, and thus could be readily differentiated from the strong negative trNOEs). For example, no trNOEs are observed in the region of the chemical shifts of the aromatic protons, so that compounds **4** and **6**–**9** are eliminated as bioactive compounds. The typical signal patterns for *N*-acetylneuraminic acid residues, for example the cross signals between the geminal protons on C3 of the *N*-acetylneuramic acid units, are also absent. Thus, **10** and **11** were also rejected as bioactive compounds. Compound **5**, which contains L-fucose as a C-glycosidic linkage, is characterized by the presence of 13 CH_2 groups. In the trNOESY spectrum there is, however, no sign of this number of CH_2 groups. Therefore, the trNOESY cross signal pattern observed is assigned to either **2** or **3**. Both are derived from the natural sialyl Lewis^x structure **1,** in which the *N*-acetylglucosamine residue is replaced by a cyclohexanediol ring (Cyc), and the *N*-acetylneuraminic acid by a cyclohexyllactic acid residue (Lact). In **2** the cyclohexyllactic acid has an *S* configuration, and in **3** an *R* configuration. A differentiation between these two compounds was not possible on the basis of the trNOE cross signal pattern. A comparison of the NOESY spectra was necessary, which demonstrated that **2** was the active component, as will be discussed in more detail in the following.

A complete assignment of the ^{1}H NMR signals for the sialyl Lewis^x mimetic **2** has already been made, [3] and the bioactive conformation was determined by trNOE experiments. Therefore, in the following only the assignment of a number of the more important signals will be discussed briefly. Figure 2 illustrates the sections from the NOESY (left) and trNOESY (right) spectra that are especially characteristic for the bioactive compound **2**. There are a series of interglycosidic trNOEs between the cyclohexanediol ring (Cyc) on the one

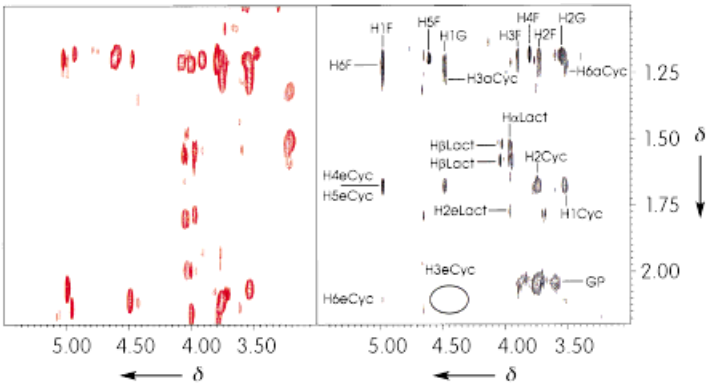


Figure 2. Left: section from the 2D NOESY spectrum in Figure 1. Right: section from the 2D trNOESY spectrum in Figure 1. The typical cross signal patterns for **2** are clearly recognizable, and several of them are labeled. Interglycosidic trNOEs between the anomeric protons of fucose and galactose and the cyclohexane ring lie under these cross signals. The encircled region indicates the extinction of an interglycosidic trNOE by superimposition with positive NOEs of the free library (see text).

hand, and fucose (F) and galactose (G) on the other, a number of which are labeled in Figure 2. Starting from H1^G, there are, for example, interglycosidic trNOEs to H3a^{Cys}, H4a^{Cys}, H4e^{Cys}, and H5e^{Cys}, of which the latter three are caused by spin diffusion in the cyclohexane ring. The interglycosidic trNOE between H2^G and H6^F is also unambiguously recognizable. A further characteristic trNOE between Hα^{Lact} and H3^G is also observed (not shown in the section in Figure 2). Other typical cross signals for **2** are observed within the cyclohexane ring as well as the cyclohexyllactic acid residue, as is illustrated in Figure 2. Starting from H1^F, there are interglycosidic trNOEs to H6a^{Cyc}, H6e^{Cyc}, H4a^{Cyc}, H4e^{Cyc}, H5e^{Cyc}, and H3a^{Cyc}. Since only the effects towards H6a^{Cyc} and H6e^{Cyc} may be considered to be "direct" trNOEs, a high fraction of spin diffusion is also present here. The cross signal for the connectivity between H1^F and the protons H4e^{Cyc} and H5e^{Cyc}, which is transmitted by spin diffusion, lies on a trace of further cross signals (this is labeled by an arrow in Figure 1, right), which is almost exclusively attributable to spin diffusion. This becomes clear from a comparison with the NOESY spectrum of the free library (Figure 2, left), since there these cross signals are absent. Equally, the fact that all protons of fucose show

  1433-7851/99/3801-0100 $ 17.50+.50/0

intraglycosidic trNOEs to the C6 methyl group of fucose is attributable to spin diffusion (Figure 2, right). Whereas spin diffusion is a disadvantage for the structural characterization of biological macromolecules, here it greatly simplifies unambiguous assignment of the bioactive component **2**. However, superposition of positive NOE and negative trNOE cross signals also lead to extinction of one interglycosidic NOE, namely, that between $H1^{G}$ and $H3e^{Cyc}$ (encircled region in Figure 2, right).

The trace of cross signals labeled "GP" in Figures 1 and 2 is not observed for the free library. These are signals which must be assigned to the *N*-acetyl groups of the covalently bound *N*-acetyllactosamine residues in E-selectin. This becomes immediately clear by comparison with a NOESY spectrum of E-selectin. Apparently, the spin lock pulse[10] that is applied at the beginning of the t_1 evolution period during the measurement of the trNOESY spectra effectively filters the interfering protein signals, but not, however, the signals of the mobile, covalently bound carbohydrate chains. This effect may be used directly to observe carbohydrate chains of glycoproteins selectively, but here it is undesirable.[11]

Overall the NMR experiments show that the library contains only one component with binding activity towards E-selectin, namely, compound **2**. Generally it may be said that identification of ligands with dissociation constants K_D in the range 10^{-7} to 10^{-3} is possible with bioaffinity NMR spectroscopy. In conclusion, it should also be mentioned that derivative **4**, which has been described as a potential E-selectin mimetic,[8] shows no trNOEs. The same applies to compound **5**, which is closely related to fucopeptides, for which good binding activity was reported.[2d] In spite of the structural similarity, no trNOEs were observed for **5**. Thus, the K_D values of **4** and **5** must be greater than 10^{-3} *m*.

To verify our results, we carried out independent ELISA activity measurements.[12] These confirm the results of our trNOE experiments.[13]

In total, the investigations show that the bioaffinity NMR procedure we have recently described[5] is well suited for the identification of bioactive components, even where significant structural similarities lead to extensive signal overlap. In contrast to current procedures, bioaffinity NMR spectroscopy has a number of advantages which should be highlighted: 1) Separation of the components of a library or, in general, of any mixture prior to activity tests is not necessary. 2) Small amounts of protein suffice since the ligands are added in a 10- to 20-fold excess. 3) The protein tested can be used for further tests after dialysis. 4) Neither the protein nor the ligand must be immobilized. 5) The procedure may be used in competitive experiments and, in particular, the investigation of allosteric effects.

Experimental Section

Recombinant E-selectin/hIgG was obtained as previously described;[4a] compounds **2** – **11** were synthesized.[14]

Deglycosylation of E-selectin/hIgG with PNGase F and neuraminidase: E-selectin/hIgG (10 mg, 1.75 mg per mL phosphate buffered solution (PBS)) was adjusted to pH 8.6. PNGase F (Sigma, G-8031; 40 units, 200 μL) was added, and the mixture was incubated for 16 h at 37 °C. Deglycosylation was followed by gel filtration chromatography and SDS-PAGE. When conversion was complete, separation was achieved with a Sephacryl S-400 column in PBS at pH 7.4. Incubation with agarose-immobilized neuraminidase from *Clostridium perfringens* was carried out by the same method at pH 4.5 and 37 °C. The activity was tested against HSA-coupled sialyl Lewis^a (HSA = human serume albumine).

For NMR investigations, E-selectin/hIgG (2.5 mg, 11.25 nmol) was dialyzed against deuterated imidazole (100 mM) and $CaCl_2$ (1 mM) in D_2O at pH 7.4 and concentrated to a sample volume of 0.5 mL with Centricon YM-50 (Amicon). Compounds **2** – **11** were present in the mixture in the following amounts: **2** (M_{rel} = 600.64) 1.66 μmol, **3** (M_{rel} = 600.64) 1.91 μmol, **4** (M_{rel} = 412.38) 3.12 μmol, **5** (M_{rel} = 614.63) 2.74 μmol, **6** (M_{rel} = 586.6) 0.99 μmol, **7** (M_{rel} = 592.58) 1.66 μmol, **8** (M_{rel} = 590.54) 1.76 μmol, **9** (M_{rel} = 623.59) 1.84 μmol, **10** (M_{rel} = 957.0) 1.25 μmol, **11** (M_{rel} = 1037.05) 1.205 μmol. The compound mixture was used to prepare the following E-selectin:ligand mole ratios (each E-selectin/hIgG chimera contains two binding sites for ESL – 1): 1:5, 1:8, 1:12, 1:15, 1:20. The data for the ligands relate in each case to compound **11**.

All spectra were measured on a Bruker DRX 500 NMR spectrometer (500.13 Mhz). The phase-sensitive 2D NOESY spectra of the substance mixture were measured at 300 or 310 K with 512 increments in t_1 (TPPI), each with 32 scans of 2 K data points in t_2. The spectral width was 10 ppm in both dimensions. Prior to the Fourier transformation, the data matrix was zero-filled to 2 K × 1 K data points. For measurement of the 2D trNOESY spectra of the compound library in the presence of E-selectin, a spin lock pulse (3.2 kHz) of 15 ms duration was applied after the first π/2 pulse.[10] A total of 48 scans per t_1 increment was recorded.

Received: August 5, 1998 [Z12253]
German version: *Angew. Chem.* **1999**, *111*, 106 – 110

Keywords: compound libraries · NMR spectroscopy · oligosaccharides · selectins

[1] a) M. P. Bevilacqua, S. Stengelin, M. A. Gimbrone, Jr., B. Seed, *Science* **1989**, *243*, 1160 – 1165; b) L. A. Lasky, *Science* **1992**, *258*, 964 – 969; c) A. Levinovitz, J. Muhloff, S. Isenmann, D. Vestweber, *J. Cell. Biol.* **1993**, *121*, 449 – 459; d) M. Lenter, A. Levinovitz, S. Isenmann, D. Vestweber, *J. Cell. Biol.* **1994**, *125*, 471 – 481; e) K. L. Moore, N. L. Stults, S. Diaz, D. F. Smith, R. D. Cummings, A. Varki, R. P. McEver, *J. Cell. Biol.* **1992**, *118*, 445 – 456.

[2] a) H. C. Kolb, B. Ernst, *Pure Appl. Chem.* **1997**, *69*, 1879 – 1884; b) H. C. Kolb, B. Ernst, *Chem. Eur. J.* **1997**, *3*, 1571 – 1578; c) K. E. Norman, G. P. Anderson, H. C. Kolb, K. Ley, B. Ernst, *Blood* **1998**, *91*, 475 – 483; d) T. F. Lampe, G. Weitz-Schmidt, C.-H. Wong, *Angew. Chem.* **1998**, *110*, 1761 – 1764; *Angew. Chem. Int. Ed.* **1998**, *37*, 1707 – 1711; e) T. Uchiyama, V. P. Vassilev, T. Kajimoto, W. Wong, H. Huang, C.-H. Wong, *J. Am. Chem. Soc.* **1995**, *117*, 5395 – 5396; f) E. E. Simanek, G. J. McGarvey, J. A. Jablonowski, C.-H. Wong, *Chem. Rev.* **1998**, 833 – 862.

[3] W. Jahnke, H. C. Kolb, M. J. J. Blommers, J. L. Magnani, B. Ernst, *Angew. Chem.* **1997**, *109*, 2715 – 2719; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2603 – 2607.

[4] a) K. Scheffler, B. Ernst, A. Katopodis, J. L. Magnani, W.-T. Wang, R. Weisemann, T. Peters, *Angew. Chem.* **1995**, *107*, 2034 – 2037, *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1841 – 1844; b) K. Scheffler, J.-R. Brisson, R. Weisemann, J. L. Magnani, W.-T. Wang, B. Ernst, T. Peters, *J. Biomol. NMR* **1997**, *9*, 423 – 436; c) R. M. Cooke, R. S. Hale, S. G. Lister, G. Shah, M. P. Weir, *Biochemistry* **1994**, *33*, 10591 – 10596; d) L. Poppe, G. S. Brown, J. S. Philo, P. V. Nikrad, B. H. Shah, *J. Am. Chem. Soc.* **1997**, *119*, 1727 – 1736.

[5] a) B. Meyer, T. Weimar, T. Peters, *Eur. J. Biochem.* **1997**, *246*, 705 – 709; b) T. Peters, B. Meyer, DE-B 19649359, **1996**.

[6] a) H. N. B. Moseley, W. Lee, C. Arrowsmith, N. R. Krishna, *Biochemistry* **1997**, *36*, 5239 – 5299; b) H. N. B. Moseley, E. V. Curto, N. R. Krishna, *J. Magn. Reson. B* **1995**, *108*, 243 – 261; c) F. Ni, *Prog. NMR Spectrosc.* **1994**, *26*, 517 – 606; d) T. Peters, B. M. Pinto, *Curr. Opin. Struct. Biol.* **1996**, *6*, 710 – 720.

[7] a) S. B. Shuker, P. J. Hajduk, R. P. Meadows, S. W. Fesik, *Science* **1996**, *274*, 1531 – 1534; b) H. Kessler, *Angew. Chem.* **1997**, *109*, 857 – 859; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 829 – 831.

  1433-7851/99/3801-0101 $ 17.50+.50/0

[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).

[*] Prof. Dr. A. Berkessel, Dr. D. A. Hérault
Institut für Organische Chemie der Universität
Greinstrasse 4, D-50939 Köln (Germany)
Fax: (+49) 221-470-5102
E-mail: berkessel@uni-koeln.de

[**] This work was supported by the Fonds der Chemischen Industrie. The authors wish to thank the BASF AG, in particular Dr. H. Platsch, T. Brugger, and W. Weishaar, for carrying out the Edman degradations.

  1433-7851/99/3801-0102 $ 17.50+.50/0