number of hindered acids (mesitoic, pivalic) authentic *O*-acyluronium salts have been isolated and identified. For the mesitoic acid derivative this species is more reactive than the OAt ester. Thus the exact timing of the possible formation of **C** and its conversion into active ester or its direct involvement in a reaction process may help to explain differences between N-HATU and N-HAPyU or between H-HATU and N-HBTU. The lifetime of intermediates such as **C** in the presence of good nucleophiles such as $OXt^{(-)}$ is believed to be low and their definite involvement in the activation process of any particular case has not yet been established. Intermediate **C** is most likely involved under conditions of no or low preactivation; these conditions are generally more favorable for both peptide assembly and low loss of configuration during segment coupling.

[25] J. Klose, M. Bienert, C. Mollenkopf, D. Wehle, C. Zhang, L. A. Carpino, P. Henklein, *Chem. Commun.* **1999**, 1847.

Capillary Electrophoresis Analysis of DNA Adducts as Biomarkers for Carcinogenesis**

Oliver Johannes Schmitz,*
Christian Christoph Theophil Wörth, Dirk Stach, and Manfred Wießler

Formation of DNA adducts through the formation of covalent DNA modifications by exogenous or endogenous reactive chemical agents appears to be one of the earliest events in the development of cancer. The formation of DNA adducts thus represents a detectable and critical step in carcinogenesis and thus may serve as an early biomarker.

To establish the pathological significance of DNA adducts in human diseases such as cancer, it is necessary to find high-sample-throughput methods for the simultaneous detection of the various classes of DNA adducts. For example, in the European Prospective Investigation into Cancer and Nutrition (EPIC) study, 470 000 people from nine European countries were interviewed about their dietary habits and lifestyles.[1] Blood samples were also taken for future analysis of biomarkers, such as DNA adducts.[2] In such studies the use of the ultrasensitive ^{32}P-postlabeling method[3] has a number of drawbacks: such as the lack of automation, the use of a strong β emitter, and the fact that the simultaneous detection of DNA adducts derived from different classes of carcinogens is not possible. Approximately 300 μg of DNA or more are necessary for the determination of DNA adducts by liquid chromatography coupled to mass spectrometry (LC-MS).[4] Although this method is very useful for characterizing unknown DNA adducts it cannot be used for routine analysis. The biggest problem is getting enough DNA for studies, because of the expensive hydrolysis of such large amounts of DNA, and the fact that only similar DNA adducts can be analyzed in a single analytical run. A new analytical method with a high-sample-throughput is therefore necessary to determine several classes of DNA adducts simultaneously in only 10 μg of DNA. Fluorescence derivatization of nucleotides for the analysis of DNA adducts has been used by the research groups of Sharma and Giese since 1988[5] to replace the ^{32}P-postlabeling method. However, neither the described conjugation of ethylenediamine at the 5′-phosphate group of nucleotides[5a] followed by the derivatization with dansyl chloride or fluorescein isothiocyanate,[5b] nor fluorescence derivatization in one step with a histidine-binding group[5c, d] fulfills the requirements for high-sample-throughput analysis.

We present here a new method to determine DNA adducts. It involves the hydrolysis of DNA, fluorescence labeling of modified and unmodified nucleotides, micellar electrokinetic chromatography, and laser-induced-fluorescence detection (CE-LIF) with 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene-3-propionylethylenediamine (BODIPY FL EDA) as the fluorescence marker. Selective conjugation of 2′-deoxynucleoside-3′-monophosphates (dNMPs) through the phosphate moiety to the amino linker of BODIPY FL EDA was conducted in the presence of the water-soluble carbodiimide 1-ethyl-3-(3′-*N*,*N*′-dimethylaminopropyl)-carbodiimide (EDC) to activate the phosphate moiety of the dNMPs.[6] However, this approach to derivatization excludes the use of fluorescence markers or buffer systems with carboxylic acid, primary amino, or phosphate groups. The only suitable buffer system for the derivatization was *N*-(2-hydroxyethyl)-piperazine-*N*′-2-ethane sulfonic acid (HEPES) at pH 6.5. The enzymatic hydrolysis of DNA was also carried out in HEPES buffer at pH 6.0 to avoid a purification step. Comparison with ^{32}P-postlabeling analysis showed that digestion of calf-thymus DNA (CT-DNA) in HEPES buffer resulted in the same amounts of dNMPs as with the routinely used procedure in sodium succinate.[7] The derivatization reaction was monitored by spectroscopic characterization (ESI-MS, ^{1}H, ^{13}C, ^{11}B, and ^{19}F NMR spectroscopy) of the conjugate formed between 2′-deoxyadenosine-3′-monophosphate and BODIPY FL EDA.

So far we have explored the application of this CE-LIF method to the detection of apurinic (AP) sites and to the determination of a number of endogenous and exogenous DNA adducts (Table 1) in various samples such as oligonucleotides, CT-DNA, or human DNA. The same conditions for derivatization and separation were used in all analyses. CE-LIF analysis of etheno-dAMP- (Figure 1 A) and 5-Me-dCMP-modified (Figure 1 B) oligonucleotides gave excellent separation of the dAMP and dCMP adducts from the four unmodified nucleotides. Etheno-DNA adducts are generated by the reaction of DNA bases with lipid peroxidation products derived from endogenous sources or from exposure to xenobiotics.[8] The exact quantitation of 5-Me-dCMP in

[*] Dr. O. J. Schmitz,[+] Dr. C. C. T. Wörth, D. Stach, Prof. Dr. M. Wießler
Deutsches Krebsforschungszentrum Heidelberg
Abteilung Molekulare Toxikologie (Germany)
E-mail: olivers@uni-wuppertal.de

[+] Current address of corresponding author:
Bergische Universität-Gesamthochschule Wuppertal
FB 9/Analytische Chemie
Gaussstrasse 20, 42119 Wuppertal (Germany)
Fax: (+49) 202-4393915

[**] We thank H. Schmeiser, E. Frei, and W. V. Turner for many helpful discussions, E. Eder for Hex-dGMP, W. E. Hull for NMR spectroscopic studies, and the Deutsche Bundesstiftung Umwelt for financial support.

Supporting information for this article is available on the WWW under http://www.angewandte.com or from the author.

 © WILEY-VCH Verlag GmbH, 69451 Weinheim, Germany, 2002 1433-7851/02/4103-0445 $ 17.50+.50/0

Table 1. The corrected migration time of modified and unmodified nucleotides

Nucleotide	Corr. MT (dAMP/nucleotide)	
1,N^2-propano-2′-deoxyguanosine-3′-monophosphate (Hex-dGMP)	0.78; 0.75	($n=2$)
5-methyl-2′-deoxycytidine-3′-monophosphate (5-Me-dCMP)	0.79 ± 0.01	($n=32$)
2′-deoxycytidine-3′-monophosphate (dCMP)	0.81 ± 0.01	($n=34$)
8-hydroxy-2′-deoxyguanosine-3′-monophosphate (8-HO-dGMP)	0.83 ± 0.01	($n=6$)
2′-deoxythymidine-3′-monophosphate (dTMP)	0.938 ± 0.006	($n=42$)
2′-deoxyguanosine-3′-monophosphate (dGMP)	0.962 ± 0.004	($n=42$)
2′-deoxyadenosine-3′-monophosphate (dAMP)	1.000	
AP site	1.011 ± 0.002	($n=6$)
adducts of benzo[*a*]pyrene diol epoxide (B[*a*]P-dGMP, 2nd isomer)	1.17 ± 0.01	($n=5$)
adducts of aristolochic acid I (dA-AAI, dA-AAII, dG-AAI)	1.23; 1.19	($n=2$)
	1.20; 1.17	($n=2$)
	1.13; 1.11	($n=2$)
adducts of benzo[*a*]pyrene diol epoxide (B[*a*]P-dGMP, 1st isomer)	1.29 ± 0.01	($n=5$)
1,N^6-etheno-2′-deoxyadenosine-3′-monophosphate (etheno-dAMP)	1.38; 1.36	($n=2$)

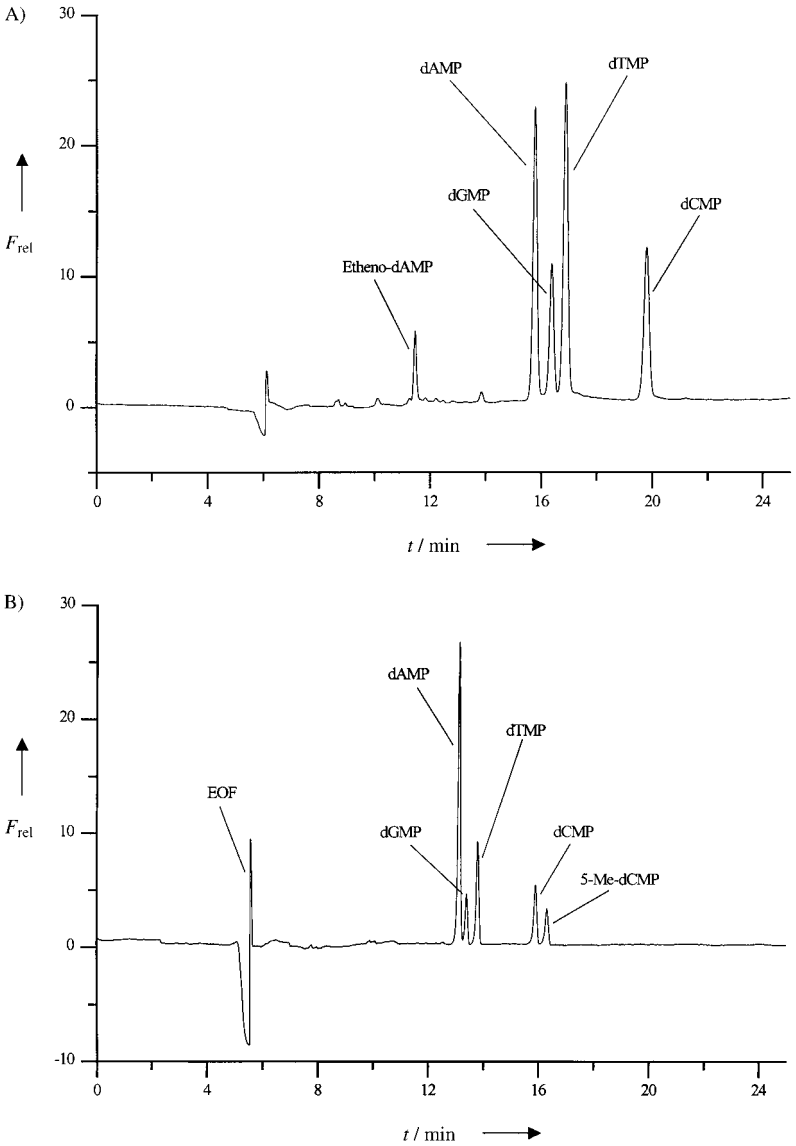

Figure 1. A) Analysis of etheno-dAMP: 10 μg of a synthesized oligonucleotide with the sequence 5′-GAG.TCT.TCC.A*GT.GTG.ATG.AT-3′ (A*: etheno-dAMP) were hydrolyzed, derivatized, and analyzed. Electrolyte: 75 mM sodium dodecyl sulfate (SDS) in 17 mM sodium phosphate buffer (pH 9.0) containing 15% (v/v) methanol; fused-silica capillary: total length: 50 cm; length to the detection window: 45.4 cm; inner diameter: 50 μm; injection: 20 psi; temperature: 25 °C; applied voltage: 20 kV. The sample was diluted 10000-fold with water. B) Analysis of 5-Me-dCMP: 10 μg of a synthesized oligonucleotide with the sequence 5′-AGA.GC*G.AGA.TTC.CAT.CA-3′ (C*: 5-Me-dCMP) were analyzed. The sample was diluted 10000-fold with water. Separation conditions were as described in (A).

normal and tumor tissue will be an important application of this analytical method because hypomethylated DNA seems to play a key role in cancer.[9, 10] Figure 2 shows the analysis of an oligonucleotide modified with 8-HO-dGMP, a biomarker of oxidative stress.[11] The determination of 8-HO-dGMP is very difficult, because a time-dependent decomposition of 8-HO-dGMP to AP sites occurs. We believe that this is one reason for the imprecise data on 8-HO-dGMP, as discussed in the literature.[12]

Another sample investigated, CT-DNA, consisted of various adducts of aristolochic acid (AA-dNMP) which are established biomarkers for Chinese-herb nephropathy.[13] Figures 3A and B show the analysis of CT-DNA and CT-DNA incubated with aristolochic acid I under zinc activation. A parallel ^{32}P-postlabeling analysis confirmed the presence of three DNA adducts specific for aristolochic acid (part of Figure 3b) as reported previously.[13] In contrast to ^{32}P-postlabeling analysis, the method introduced here allowed the simultaneous determination of unmodified nucleotides as well as the three adducts of aristolochic acid I: 5-Me-dCMP, AAI-dGMP, AAI-dAMP, and AAII-dAMP. A simultaneous determination of DNA adducts and 5-Me-dCMP is very interesting because of the suspected demethylation of CpG islands during cancerogenesis.

The reproducibility of the migration time (MT) is often poor in capillary electrophoresis because of the influence of the electroosmotic flow in uncoated fused-silica capillaries. To characterize DNA adducts in unknown samples we therefore standardized the MT of all unmodified and modified nucleotides to that of dAMP and obtained excellent reproducibility (standard deviation less than 3% by the use of different of capillaries and buffer charges). Table 1 lists the corrected MTs of all the analyzed nucleotides and the values are given as the mean and one standard deviation. A

 © WILEY-VCH Verlag GmbH, 69451 Weinheim, Germany, 2002 1433-7851/02/4103-0446 $ 17.50+.50/0 *Angew. Chem. Int. Ed.* **2002**, *41*, No. 3

difference of 0.02 in the corrected MT values between two nucleotides is necessary to ensure a baseline separation.

The reproducibility of the hydrolysis, derivatization, and separation was determined by analysis of aliquots of CT-DNA. The use of fluorescein as an internal standard made it possible to correct the peak areas and led to a relative standard deviation between 5.6 and 8.4% ($n = 29$) for the nucleotides.

The relative fluorescence quantum yield (QY) of each fluorescence-labeled nucleotide must be determined for an exact quantitation. The QY of the nucleotides were investigated and standardized to that of dAMP, which led to quantum factors of 1.00, 0.55, 0.97, and 0.98 for dAMP, dGMP, dTMP, and dCMP, respectively. The much lower quantum factor of dGMP arises from a quenching effect, which is dependent on the base.[14, 15] With the use of these factors the analysis of CT-DNA showed that 6.43 ± 0.13% (RSD = 2.1%; $n = 6$) of all cytosine residues were methylated. Since no standard of 5-Me-dCMP was available, the QY of dCMP was used for both unmodified and modified cytosine.

The strength of the ^{32}P-postlabeling procedure is the high sensitivity that is made possible by the enrichment of modified nucleotides by butanol extraction or digestion with nuclease P1. The strength of the CE-LIF method described here is its excellent selectivity. The samples must be diluted 100 fold because of the high salt concentration and the great surplus of unmodified nucleotides. This procedure leads to a relatively high detection limit of 850 pM (determined for dAMP), which means in turn a relative adduct labeling (RAL) of 2 DNA adducts per 10^6 unmodified nucleotides (normals). The sensitivity could be improved to 50 pM or 1.4 DNA adducts per 10^7 unmodified nucleotides by using electrostacking with reversed field[16] as a focussing step.

The higher detection limit is nevertheless sensitive enough for the detection of many endogenous DNA adducts and the exact quantitation of DNA

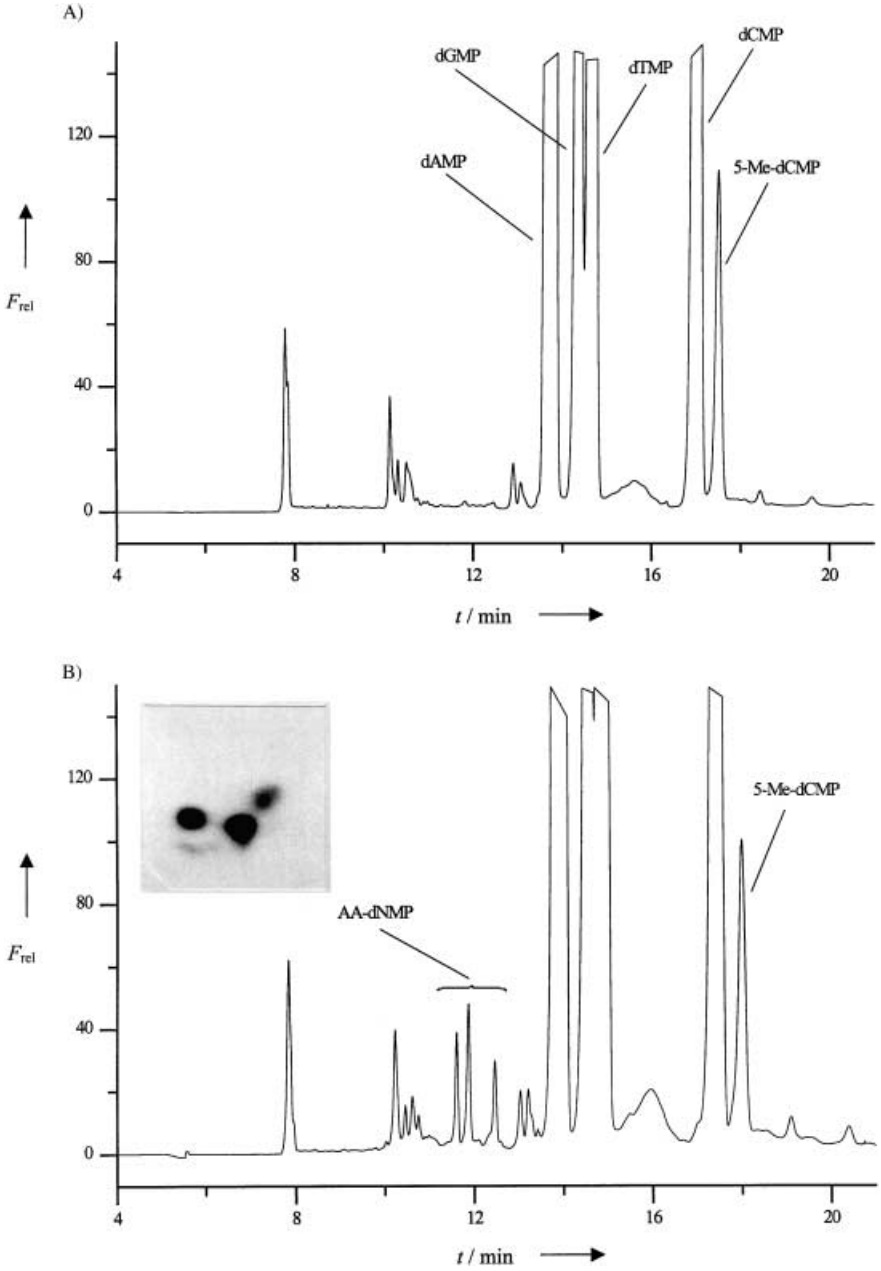

Figure 3. CE-LIF and ^{32}P-postlabeling analysis of CT-DNA incubated with aristolochic acid I (AAI) under zinc activation: 10 μg of normal CT-DNA (A) and 10 μg of a CT-DNA incubated with AAI (B) were analyzed, and the electropherograms were compared with the results of the ^{32}P-postlabeling method. The samples were diluted 100-fold with water. Separation conditions were as described in Figure 1.

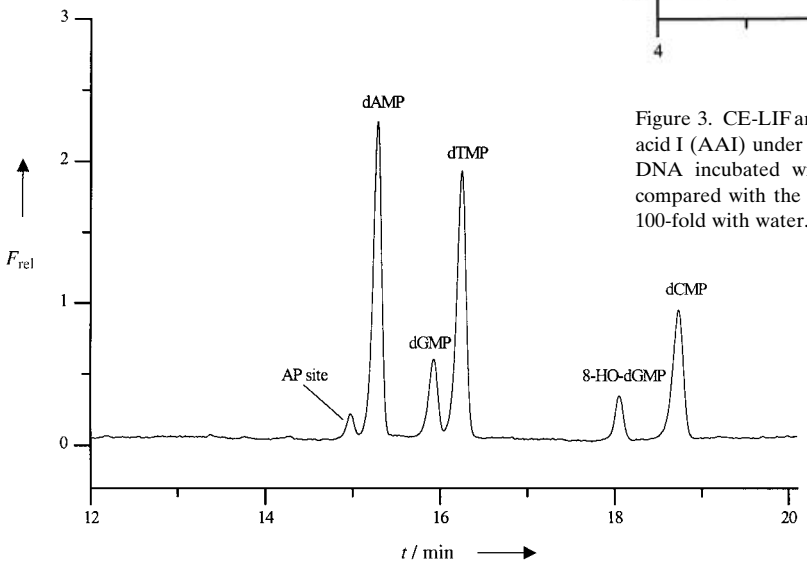

Figure 2. Analysis of 8-HO-dGMP and AP sites: 10 μg of a synthesized oligonucleotide with the sequence 5′-GAG.TCT.TCC.AGT.G*TG*.ATG.AT-3′ (G*: 8-HO-dGMP) were analyzed. The sample was diluted 10000-fold with water. Separation conditions were as described in Figure 1.

 © WILEY-VCH Verlag GmbH, 69451 Weinheim, Germany, 2002 1433-7851/02/4103-0447 $ 17.50+.50/0

methylation (5-Me-dCMP). We are continuing to improve the method by optimizing sample preparation to enrich modified nucleotides.

Experimental Section

DNA hydrolysis: DNA (10 μg) was diluted in water (5.2 μL) and hydrolyzed by incubation for 3 h at 37 °C with an enzyme mixture (4.0 μL; micrococcal nuclease: 150 mU μL^{-1} and spleen phosphodiesterase: 2.5 mU μL^{-1}) and buffer (0.80 μL; 250 mM HEPES, 100 mM $CaCl_2$, pH 6.0).

Fluorescence derivatization: 1.8 M EDC (30 μL; in 800 mM HEPES buffer, pH 6.5), 25 mM BODIPY FL EDA (30 μL; in 800 mM HEPES buffer, pH 6.5), and 800 mM HEPES buffer (20 μL) at pH 6.5, were added to the hydrolysate and incubated for 25 h at 25 °C in the dark.

Received: July 2, 2001 [Z17407]

[1] F. P. Perera, *J. Natl. Cancer Inst.* **1996**, *88*, 496 – 509.
[2] E. Riboli, R. Kaaks, *Am. J. Epidemiol.* **2000**, *151*, 371 – 376.
[3] K. Randerath, M. V. Reddy, R. C. Gupta, *Proc. Natl. Acad. Sci. USA* **1981**, *78*, 6126 – 6129; R. C. Gupta, M. V. Reddy, K. Randerath, *Carcinogenesis* **1982**, *3*, 1081 – 1092; M. V. Reddy, R. C. Gupta, E. Randerath, K. Randerath, *Carcinogenesis* **1984**, *5*, 231 – 243.
[4] J. R. Soglia, R. J. Turesky, A. Paehler, P. Vouros, *Anal. Chem.* **2001**, *73*, 2819 – 2827.
[5] a) D. J. Kelman, K. T. Lilga, M. Sharma, *Chem. Biol. Interact.* **1988**, *66*, 85 – 100; b) A. N. Al-Deen, D. C. Cecchini, S. Abdel-Baky, N. M. A. Moneam, R. W. Giese, *J. Chromatogr.* **1990**, *512*, 409 – 414; c) P. Wang, R. W. Giese, *J. Chromatogr. A* **1998**, *809*, 211 – 218; d) R. W. Giese, P. Wang, *Anal. Chem.* **1993**, *65*, 3518 – 3520.
[6] M. G. Ivanovskaya, M. B. Gottikh, Z. A. Shabarova, *Nucleosides Nucleotides* **1987**, *6*, 913 – 934.
[7] D. H. Phillips, M. Castegnaro, *Mutagenesis* **1999**, *14*, 301 – 315.
[8] H. Bartsch, *Mutat. Res.* **2000**, *462*, 255 – 279.
[9] S. B. Baylin, J. G. Herman, *Trends Genet.* **2000**, *16*, 168 – 174.
[10] B. H. Ramsahoye, D. Biniszkiewicz, F. Lyko, V. Clark, A. P. Bird, R. Jaenisch, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 5237 – 5242.
[11] H. Bartsch, J. Nair, I. Velic, *Eur. J. Cancer Prev.* **1997**, *6*, 529 – 534.
[12] M. Zeisig, T. Hofer, J. Cadet, L. Möller, *Carcinogenesis* **1999**, *20*, 1241 – 1245.
[13] J. L. Nortier, M.-C. Muniz Martinez, H. H. Schmeiser, V. M. Arlt, C. A. Bieler, M. Petein, M. F. Depierreux, L. de Pauw, D. Abramowicz, P. Vereerstraeten, J.-L. Vanherweghem, *N. Engl. J. Med.* **2000**, *342*, 1686 – 1692.
[14] U. Lieberwirth, J. Arden-Jakob, K.-H. Drexhage, D. P. Herten, R. Müller, M. Neumann, A. Schulz, S. Siebert, G. Sagner, S. Klingel, M. Sauer, J. Wolfrum, *Anal. Chem.* **1998**, *70*, 4771 – 4779.
[15] M. Torimura, S. Kurata, K. Yamada, T. Yokomaku, Y. Kamagata, T. Kanagawa, R. Kurane, *Anal. Sci.* **2001**, *17*, 155 – 160.
[16] R. L. Chien, D. S. Burgi, *Anal. Chem.* **1992**, *64*, 1046 – 1050.

Structure of Bis(pentafluorophenyl)xenon, $Xe(C_6F_5)_2$**

Harald Bock, Dirk Hinz-Hübner, Uwe Ruschewitz,* and Dieter Naumann*

Dedicated to Professor Peter Sartori on the occasion of his 70th birthday

Recently, we synthesized the first homoleptic organoxenon(II) compound with two Xe–C bonds, $Xe(C_6F_5)_2$ (**1**), through the fluoride-initiated reaction of $(CH_3)_3SiC_6F_5$ and XeF_2.[1] An alternative route to **1** is the reaction of C_6F_5XeF and $[Cd(C_6F_5)_2]$.[2] Compound **1** is obtained as a white solid which explosively decomposes above −20 °C. However, as single crystals could not be grown, **1** was characterized exclusively in solution by ^{129}Xe, ^{19}F, and ^{13}C NMR spectroscopy.

The development of apparatus and methods has now advanced to the stage where it is possible to solve and refine even structures of complicated organic and organometallic compounds on the basis of X-ray powder data.[3] Since **1** is obtained as an almost single-phase microcrystalline powder, we were encouraged to determine the crystal structure from X-ray powder data despite of the extreme thermal instability of **1**.

Compound **1** was synthesized from $(CH_3)_3SiC_6F_5$ and XeF_2[1] and investigated at −223 °C (see Experimental Section).[4] The powder diffractogram was indexed in a monoclinic unit cell,[5] and the volume of the unit cell was in good agreement with the calculated volume for four formula units. On the basis of the systematic absences, the only space group that came into consideration was $P2_1/n$. Le Bail extraction[6–8] within this space group and a subsequent structure determination using direct methods[9] led to a position which was assigned to the Xe atom. As the positions of the C_6F_5 rings could not be determined by using difference Fourier syntheses, we applied the method of "simulated annealing".[10] Two "rigid bodies" were defined for the perfluorinated phenyl rings and placed on two arbitrary positions together with the position obtained for the Xe atom and used as starting parameters. After a few cycles, a reasonable structural solution was found which was refined by the Rietveld method.[11] On account of the limited quality of data as well as a large number of parameters, the C_6F_5 rings were refined as rigid bodies (C–C 1.38, C–F 1.35 Å) and isotropic temperature factors of identical elements were constrained. A stable refinement resulted which quickly converged.[12]

The resulting molecular structure of **1** is shown in Figure 1. Both Xe–C distances (Xe–C11 2.394(9) Å, Xe–C21 2.35(1) Å) are almost identical within the double standard

[*] Prof. Dr. U. Ruschewitz, Prof. Dr. D. Naumann, H. Bock, Dr. D. Hinz-Hübner
Institut für Anorganische Chemie, Universität zu Köln
Greinstrasse 6, 50939 Köln (Germany)
Fax: (+49) 221-470-5196, (+49) 221-470-5196-4899
E-mail: Uwe.Ruschewitz@uni-koeln.de, d.naumann@uni-koeln.de

[**] This work was supported by the Fonds der Chemischen Industrie.

 © WILEY-VCH Verlag GmbH, 69451 Weinheim, Germany, 2002 1433-7851/02/4103-0448 $ 17.50+.50/0