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New Peptide Labels Containing Covalently Bonded Platinum(II) Centers as Diagnostic Biomarkers and Biosensors

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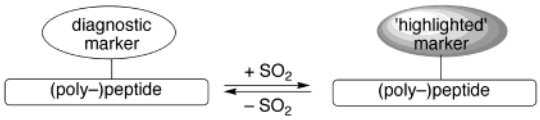
ABSTRACT



Water- and acid-resistant arylplatinum(II) complexes have been covalently bonded to the N-terminus of L-valine, thus providing organometallic biomolecules with excellent stability properties. Owing to the ^{195}Pt nucleus ($I = {}^1/_2$), these building blocks are potentially versatile biomarkers (e.g., MRI). Moreover, they display efficient in vitro biosensor characteristics since they detect SO_2 gas selectively and fully reversibly by an instantaneous change of the spectroscopic properties including a diagnostic ^{195}Pt NMR signal.

The selective folding of primary polypeptide sequences into unique and predetermined 3D structures with complex shape and functionalities is one of the most fascinating observations in contemporary biochemistry.[1] Up to now, only few dominant forces such as hydrophobicity[2] have been identified to contribute to the formation of tertiary structures. Details of the folding process are, however, still far from being fully understood.[3] Peptide labeling is an important concept to monitor such folding processes and also to investigate the mode of action of complex polypeptides such as proteins and (co-)enzymes in real time.[4] Robust organometallic markers[5] are particularly attractive, since transition metal complexes usually exhibit high selectivity and signals that are easily differentiated from the organic bulk.[6] Appropriate organometallic biomarkers may be introduced either by peptide side-chain modifications[7] or by labeling of one of the terminal amino acids of a protein.[8] Essential to both these approaches are (i) the full stability of the diagnostic site under physiological conditions, (ii) the emission of a characteristic signal remote from the signals of the biomolecule, and (iii) no inhibitive interference of the biomarker with an amino acid (sequence) of the labeled polypeptide.

Here, we report on the broad application potential of organoplatinum(II) complexes [PtX(NCN-R)] (NCN-R is the abbreviation for the terdentate coordinating monoanionic

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(8) For organometallic peptide labeling at the N-terminus, see, for example: (a) Eckert, H.; Forster, B.; Seidel, C. *Z. Naturforsch. B* **1991**, *46*, 339. (b) Amiens, C.; Balavoine, G.; Guibé, F. *J. Organomet. Chem.* **1993**, *443*, 207. (c) Hungate, R. W.; Miller, F.; Goodman, M. S. *Tetrahedron Lett.* **1988**, *27*, 4273. For labeling of the C-terminus, see, for example: (d) Brosch, O.; Weyhermüller, T.; Metzler-Nolte, N. *Eur. J. Inorg. Chem.* **2000**, 323.

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"pincer" ligand $[C_6H_2(CH_2NMe_2)_2\text{-}2,6\text{-}R\text{-}4]^-$ containing a functional group R, see Figure 1)[9] when covalently bonded

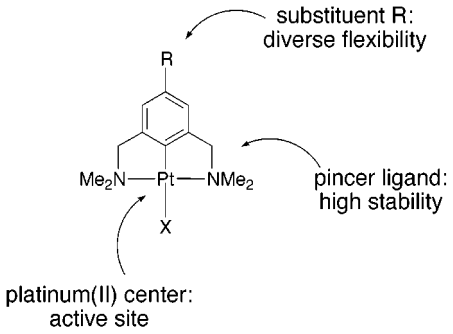


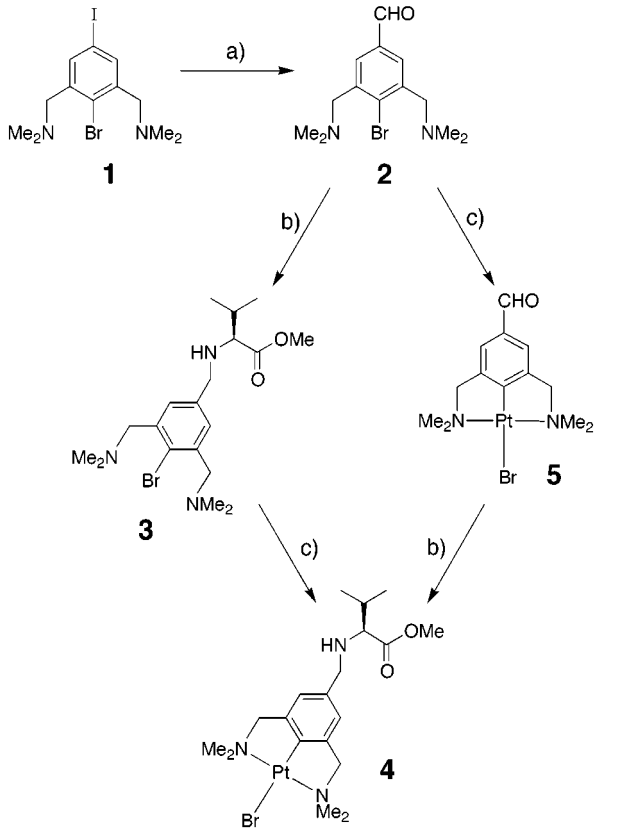
Figure 1. Organoplatinum complexes comprising a diagnostic platinum(II) center bound to the rigidly terdentate coordinating, monoanionic "pincer" ligand. Various functional groups may be introduced into the aryl moiety of the ligand system.

to peptides (R = amino acid sequence), since these bio-organometallic species combine various attractive concepts: First, the ^{195}Pt nucleus displays a characteristic MRI activity (natural abundance 33.8%, $I = {}^1/_2$).[10] Peptide functionalization of the platinum(II) complexes hence provides a diagnostic organometallic biomarker for medical and biochemical applications. This is particularly intriguing since chemical shift values as well as coupling constants are direct consequences of the steric and electronic environment of the observed nuclei and may therefore provide additional chemical information. A second concept emanates from the sensor activity of related platinum(II) complexes containing pincer-type ligands. The metal center reversibly binds SO_2, which is readily indicated by various spectroscopic techniques.[11] Therefore, such biofunctionalized organoplatinum sites can display, simultaneous to their biomarker properties (MRI), also potential biosensor activity (reversible gas detection).

A successful protocol for the covalent binding of the organometallic synthon [PtX(NCN-R)] on peptides invokes appropriate functionalization of the substituent R on the pincer ligand and starts from the metal-free bifunctional ligand precursor [NC(Br)N-I-4] **1** (Scheme 1) containing reactive $C_{aryl}-I$ and $C_{aryl}-Br$ bonds.[12]

Selective lithiation of the designated 4-position by lithium/iodide exchange was achieved at −100 °C using *t*-BuLi as lithiating agent. The aryllithium species was quenched in situ with DMF which afforded, after aqueous workup, the correspond aldehyde [NC(Br)N-CHO-4)], **2**. Aldehydes have

Scheme 1[a]



[a] Reagents and conditions: (a) *t*-BuLi (2 equiv), THF (−100 °C, 10 min), then DMF (rt, 12 h); (b) L-Val-OMe·HCl, NEt_3, $MgSO_4$ (rt, 12 h), then HOAc, $NaBH_3CN$ MeOH (10 °C to rt, 2 h); (c) $[Pt(tol\text{-}4)_2(SEt_2)]_2$, C_6H_6 (reflux, 3 h).

been frequently used for both the synthesis and functionalization of amino acids.[13] Indeed, in the presence of methoxy-protected L-valine, L-Val-OMe, the aldehyde functionality in **2** was readily converted into the corresponding Schiff base.[14] Reduction of the resulting imine intermediate with $NaBH_3CN$ afforded [NC(Br)N-CH_2-Val-OMe], **3**, a C-protected α-amino acid containing a pincer-functionalized amine site at the N-terminus (87% yield).

Metalation of the ligand precursor **3** with $[Pt(tol\text{-}4)_2(SEt_2)]_2$[15] resulted in the clean formation of the organoplatinum-labeled L-valine [PtBr(NCN-CH_2-Val-OMe)], **4** (Scheme 1). Full metalation was unambiguously confirmed by multinuclear NMR spectroscopy: in the ^{1}H NMR spectrum, for example, the signals due to the $ArCH_2N$ and the NMe_2 protons were characteristically shifted to lower field and were located at 4.15 and 3.09 ppm, respectively (benzene-d_6 solution). Also, a single resonance at −3183 ppm was resolved by ^{195}Pt NMR spectroscopy.

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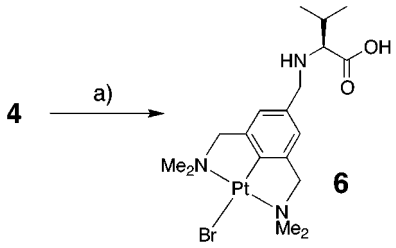
The excellent stability properties of the organoplatinum unit (e.g., in terms of reaction conditions required for chemical modifications of the peptide support) was illustrated by an alternative synthesis of the metalated amino acid **4**. Platination of the aldehyde **2** using a procedure similar to that for the peptide-functionalized ligand precursor (i.e., $[Pt(tol\text{-}4)_2(SEt_2)]_2$ in benzene) led to the metalated aldehyde **5** (Scheme 1). In the presence of a primary amine such as L-Val-OMe, a Schiff base reaction was promoted. The resulting imine was subsequently reduced with $NaBH_3CN$ at low temperature (below 10 °C) to give the functionalized amino acid **4** without significantly affecting the organometallic site.

Preliminary experiments suggested that deprotection of the C-terminus of the organoplatinum-labeled valine **4** can be achieved by hydrolysis with methanolic NaOH. Saponification of the ester group and formation of the N-terminal functionalized amino acid **6** (Scheme 2) was strongly

Scheme 2[a]

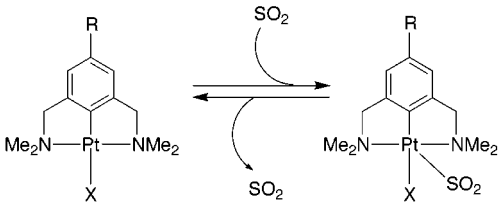


[a] Reagents and conditions: (a) NaOH (1 M), MeOH (rt, 8 h).

indicated by the absence of the singlet at $\delta_H = 3.97$ in the ^1H NMR spectrum, assigned to the protons of the methoxy group in **4**. The metalorgano-substituted amino acid **6** may be considered as the archetype of N-labeled peptides containing an MRI active biomarker. Currently, we are studying the use of **6** and its protected versions in standard peptide synthesis using DCC or related coupling agents[13] in order to construct polypeptides, which contain a labeling sequence that is easily detected in solution (^{195}Pt MRI activity) or in the solid state (X-ray diffraction).

The second concept is based on the observations that similar organoplatinum(II) complexes (e.g., complex **7**, Scheme 3) are diagnostic sensor materials for the selective and reversible detection of SO_2 both in solution[11] or in the solid state.[16] The working principle of this SO_2 recognition process has been investigated in detail, and various characteristic signals are observed as a direct response on the

Scheme 3[a]



R = H	X = Cl	**7a**	(–3150 ppm)	**8a**	(–1988 ppm)
	X = Br	**7b**	(–3178 ppm)	**8b**	(–2022 ppm)
	X = I	**7c**	(–3212 ppm)	**8c**	(–2060 ppm)
R = CH_2-Val-OMe	X = Br	**4**	(–3183 ppm)	**9**	(–2031 ppm)

[a] In parentheses, the ^{195}Pt NMR chemical shift values are given (64.5 MHz, benzene-d_6, 298 K, referenced to external K_2PtCl_4, $\delta_{Pt} = -1630$).[17]

presence of SO_2, such as a strong color change from colorless to bright orange, and various spectroscopic modifications in the material (IR, UV−vis, NMR).[11a] Addition of SO_2 induces a low-field shift of the resonances due to the CH_2N (e.g., from $\delta_H = 4.02$ in **7** to $\delta_H = 4.23$ in **8**) and NMe_2 protons in the ^1H NMR spectrum (benzene-d_6 solution).

These effects are much more pronounced in the ^{195}Pt spectrum, which reveals a downfield shift of the platinum resonance from −3178 ppm in **7b** to −2022 ppm in **8b** (Scheme 3). This indicates a strong deshielding of the platinum nucleus upon SO_2 adsorption and is in accordance with a platinum-to-ligand charge transfer.[18] The nature of the metal-bound halide, in particular its π-acceptor/donor properties, is reflected in small but significant chemical shift differences. The presence of SO_2 is readily indicated by the platinum-labeled amino acid **4** since an instantaneous change of the UV−vis spectroscopic properties of the solution was noted, which is indicative for the formation of the corresponding adduct $[PtBr(NCN\text{-}CH_2\text{-}Val\text{-}OMe)(SO_2)]$, **9** (Scheme 3). Similar to the nonfunctionalized organoplatinum species **7** and **8**, a strong colorization of the solution from colorless to bright orange was observed. Moreover, the resonance of the platinum nucleus in the ^{195}Pt NMR spectrum shifted characteristically from −3183 ppm (in **4**) to −2031 ppm upon coordination of SO_2 in **9**. Importantly, these materials are characterized by various outstanding sensor properties such as an instantaneous signal transduction, short response times,[19] and full reversibility. These features correspond well with the established bonding principles, which involve a nucleophilic platinum center in **7** and a metal-to-ligand charge transfer as the principal interaction for SO_2 binding.[16,18−20] Moreover, these results confirm earlier observations which demonstrated that functionalization of the pincer ligand at C_{para} does not substantially affect the physical properties of the platinum site for gas sensoring. This identifies the ^{195}Pt NMR resonance as a diagnostic probe for both, the presence of a [PtX(NCN)] unit at a given

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position in the peptide structure and also for its physical location by labeling the platinum position as the corresponding Pt−SO_2 complex.[21]

Hence, organoplatinum(II)-labeled peptides such as **4** represent promising candidates for applications both as SO_2 biosensors and as diagnostic biomarkers. Intriguing results may emerge from a combination of the labeling properties and the sensor activity of these materials, in particular with respect to the physiological relevance of SO_2.[22]

Such biosensor applications may be envisaged owing to the excellent chemical and physical (e.g., up to 150 °C) stability of the organometallic entity. Neither the Pt−N nor the Pt−C bond of such cycloplatinated pincer complexes was cleaved when exposed to aqueous solutions (either neutral, basic or acidic) for prolonged time periods. Notably, conditions that lead to rapid protein degradation (e.g., pH < 1, 50 °C, 5 h) did not lead to any detectable bond cleavage or metal decoordination. The covalent metal−carbon bond in complexes of type **7** and **4** increases the stability of the metal−ligand arrangement considerably when compared to alternative bioinorganic agents, where ligand dissociation and release of (heavy) metals due to noncovalent (weak) coordination is an important issue.[23]

In summary, we have demonstrated the successful biofunctionalization of organoplatinum(II) sites by covalently linking the N-terminus of amino acids with organoplatinum(II) complexes.[24] This provides access to a diagnostic peptide labeling sequence with a broad application potential, owing to the excellent physical and chemical stability of the organometallic site paired with the diagnostic MRI activity. Remarkably, this organoplatinum marker combines an effective labeling system with a selective molecular recognition site for the detection of SO_2 gas, thus providing *functional* biomarkers which may concomitantly be used also as biosensors. In biological systems, such organoplatinum-labeled peptides may therefore be traced and located specifically by "highlighting" the platinum site with SO_2 gas. Current studies are aimed at the use of the organoplatinum marker under physiological conditions. It is noteworthy that both the low solubility of [PtX(NCN)] in water and the stability of SO_2 in aqueous media most likely prevent in vivo but not in vitro biosensor applications. However, incorporation of peptides containing such organoplatinum markers in a hydrophobic pocket could provide an indication of whether SO_2 or its sulfite(s) are present in a hydrophobic microenvironment. Finally, the platinated aldehyde **5** represents a versatile probe for the identification of primary amines in polypeptide structures, both in the main chain (end group determination) and in the side chains (recognition of Lys and Arg residues). The availability of polyfunctional peptide labels, in particular the combination of sensor and marker properties as demonstrated with the labeled peptide **4**, may contribute to an improved understanding of physiologically relevant substances, e.g., by unraveling their influence on peptide folding or on the mode of action of complex biomolecules.

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Supporting Information Available: Experimental procedures and characterization for compounds **2**−**5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) The exact chemical shift value is directly correlated to the actual SO_2 concentration, and low concentrations induce only a partial downfield shift. This allows not only for qualitative but also for quantitative monitoring of the SO_2 concentration, which is a particularly important characteristic in view of sensor applications in complex systems.

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