

Organoplatinum(II) Complexes as a Color Biomarker in Solid-Phase Peptide Chemistry and Screening

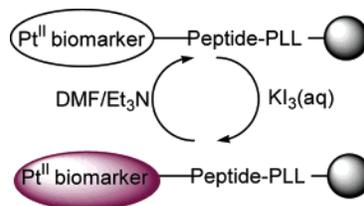
Gabriela Guillena,[†] Koen M. Halkes,[‡] Gema Rodríguez,[†] Guido D. Batema,[†]
Gerard van Koten,^{*,†} and Johannis P. Kamerling[‡]

Debye Institute, Department of Metal-Mediated Synthesis, Utrecht University,
Padualaan 8, 3584 CH Utrecht, The Netherlands, and Bijvoet Center for Biomolecular
Research, Department of Bio-Organic Chemistry, Section of Glycoscience and
Biocatalysis, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

g.vankoten@chem.uu.nl

Received February 26, 2003

ABSTRACT



A novel organoplatinum(II) biomarker is introduced to facilitate the solid-phase screening of combinatorial libraries for substrates and inhibitors of enzymes and receptors. The robust organoplatinum(II) biomarker can be incorporated, on amine functions, in peptides using standard peptide coupling techniques. The chemistry, stability, and (reversible) coloration process with KI_3 of the organoplatinum(II) biomarker was investigated.

Peptide and peptide-like structures are increasingly viewed as useful tools in all areas of biomedical research and as effective immunodiagnostic and therapeutic agents. Both drug discovery and fundamental approaches to the elucidation of various biological processes rely on the availability of pure and well-defined ligands, substrates, and inhibitors of the receptors and enzymes that are under investigation. Combinatorial chemistry has become more and more instrumental in identifying and supplying the biomedical field with such compounds.¹ The development of the portion-mixing methodology² for library synthesis allows for the rapid generation of millions of compounds for high-throughput screening. The introduction of water-compatible resins, such as PEGA,³ has facilitated the screening of resin-bound

compounds. Color biomarkers (e.g., Disperse Red 1),⁴ chromophores (e.g., 7-amino-4-methylcoumarin or *p*-nitroanilide), and the internal fluorescence-quenched assay are used, among others, for the identification of substrates⁵ and inhibitors⁶ of receptors and enzymes from solid-phase combinatorial libraries. The development of combinatorial library synthesis in combination with high-throughput screening of resin-bound compounds has sparked interest in the development of highly specific and robust biomarkers that can be easily introduced on the solid-phase. In this sense, the application of organometallic complexes as labeling agents for biomolecules is particularly attractive, since

(3) Meldal, M. *Tetrahedron Lett.* **1992**, *33*, 3077–3080.

(4) Lowik, D. W. P. M.; Weingarten, M. D.; Broekema, M.; Brouwer, A. J.; Clark Still, W.; Liskamp, R. M. J. *Angew. Chem., Int. Ed.* **1998**, *37*, 1846–1850; *Angew. Chem.* **1998**, *110*, 1947–1950.

(5) St. Hilaire, P. M.; Willert, M.; Juliano, M. A.; Juliano, L.; Meldal, M. *J. Comb. Chem.* **1999**, *1*, 509–523.

(6) St. Hilaire, P. M.; Alves, L. C.; Herrera, F.; Renil, M.; Sanderson, S. J.; Mottram, J. C.; Coombs, G. H.; Juliano, M. A.; Juliano, L.; Arvelo, J.; Meldal, M. *J. Med. Chem.* **2002**, *45*, 1971–1980.

[†] Debye Institute.

[‡] Bijvoet Center for Biomolecular Research.

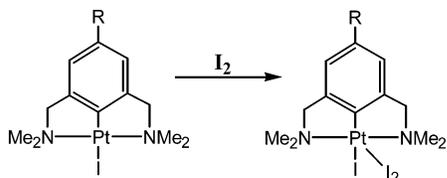
(1) Graven, A.; St. Hilaire, P. M.; Sanderson, S. J.; Mottram, J. C.; Coombs, G. H.; Meldal, M. *J. Comb. Chem.* **2001**, *3*, 441–452.

(2) Furka, A.; Sebestyen, F.; Asgedom, M.; Dibo, G. *Int. J. Pept. Protein Res.* **1991**, *37*, 487–493.

transition-metal complexes exhibit signals that can be easily differentiated from the organic bulk.^{7,8} Examples of organometallic labels and their detection on the solid-phase have been reported recently.⁹

We have reported the use of organoplatinum(II) complexes of the type [PtX(NCN-R)] [NCN-R is the abbreviation of the terdentate, monoanionic “pincer” ligand [C₆H₂(CH₂NMe₂)₂-2,6-R-4]⁻, which have been attached to the *N*- or *C*-terminus as well as to the α -carbon of several α -amino acids.¹⁰ The choice of platinum(II) complexes as biomarkers was based on the NMR activity of the ¹⁹⁵Pt nucleus¹¹ and the SO₂ recognition capability of platinum(II), as indicated by a instantaneous change of the solution from colorless to orange.¹² In addition, NCN–platinum(II) complexes react with I₂ to give an end-on (η^1) I₂ complex (Scheme 1).¹³ This complex is stable in air and becomes

Scheme 1. Formation of η^1 -I₂ Organometallic



intensely red/dark brown. Finally, the NCN–platinum(II) biomarker is considerably smaller than the organometallic biomarkers reported so far, which is expected to result in less steric and electronic interference with biological recognition and binding processes.

In this paper, we report the facile introduction of NCN–platinum complexes (**1**¹⁰ and **2**^{11b}) (Figure 1) onto the amine-terminus of resin-bound peptides and their stability toward the acidic conditions that are necessary for the deprotection of the side-chain protecting groups of the different α -amino acids. In addition, it will be shown that the biomarker-

(7) (a) Metzler-Nolte, N. *Angew. Chem.* **2001**, *113*, 1072–1076; *Angew. Chem., Int. Ed.* **2001**, *40*, 1040–1043. (b) Le Bideau, F.; Salmain, M.; Top, S.; Jaouen, G. *Chem. Eur. J.* **2001**, *7*, 2289–2294. (c) Ahn, D.-R.; Kim, T.; Hong, J.-I. *J. Org. Chem.* **2001**, *66*, 5008–5011. (d) Salmain, M.; Licandro, E.; Baldoli, C.; Maiorana, S.; Tran-huy, H.; Jaouen, G. *J. Organomet. Chem.* **2001**, *617–618*, 376–382. (e) Dialer, H.; Steglich, W.; Beck, W. *Tetrahedron* **2001**, *57*, 4855–4861. (f) Xu, Y.; Kraatz, H.-B. *Tetrahedron Lett.* **2001**, *42*, 2601–2603.

(8) Severin, K.; Bergs, R.; Beck, W. *Angew. Chem.* **1998**, *110*, 1722–1743; *Angew. Chem., Int. Ed.* **1998**, *37*, 1635–1654. (b) Jaouen, G.; Vessieres, A. *Acc. Chem. Res.* **1993**, *26*, 361–369.

(9) Salmain, M.; Fischer-Durant, N.; Cavalier, L.; Rudolf, B.; Zakrewski, J.; Jaouen, G. *Bioconjugate Chem.* **2002**, *13*, 693–698.

(10) (a) Albrecht, M.; Rodríguez, G.; Schoenmaker, J.; van Koten, G. *Org. Lett.* **2000**, *2*, 3461–3464. (b) Guillena, G.; Rodríguez, G.; Albrecht, M.; van Koten, G. *Chem. Eur. J.* **2002**, *8* (23), 5368–5376.

(11) (a) Pregosin, P. S. *Coord. Chem. Rev.* **1982**, *44*, 247–291. (b) Slagt, M. Q.; Rodríguez, G.; Grutters, R.; Klein Gebbink, J. M.; Klopper, W.; Lutz, M.; Spek, A. L.; van Koten, G. Manuscript in preparation.

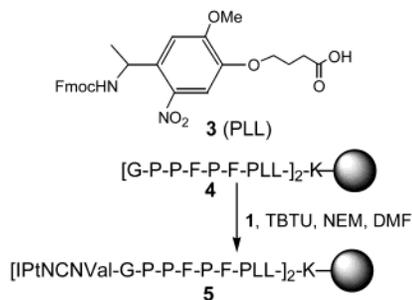
(12) (a) Albrecht, M.; Lutz, M.; Spek, A. L.; van Koten, G. *Nature* **2000**, *406*, 970–974. (b) Albrecht, M.; Gossage, R. A.; Lutz, M.; Spek, A. L.; van Koten, G. *Chem. Eur. J.* **2000**, *6*, 1431–1445. (c) Albrecht, M.; van Koten, G. *Adv. Mater.* **1999**, *11*, 171–174. (d) Albrecht, M.; Gossage, R. A.; Spek, A. L.; van Koten, G. *Chem. Commun.* **1998**, 1003–1004.

(13) Gossage, R. A.; Ryabov, A. D.; Spek, A. L.; Stufkens, D. J.; van Beek, J. A. M.; van Eldik, R.; van Koten, G. *J. Am. Chem. Soc.* **1999**, *121*, 2488–2497.

containing peptides can be simply highlighted by the addition of an aqueous KI₃ solution to yield visually detectable colored resin beads.

Since it was intended to develop a biomarker that could function in solid-phase screening assays, a resin was chosen that combined good characteristics for organic synthesis and the screening in aqueous buffer solutions. For this purpose, PEGA₁₉₀₀ resin [a copolymer of bis(2-aminopropyl)poly(ethylene glycol)/acrylamide] was used as the solid support. First, a lysine residue was coupled to the resin to double its loading capacity (0.13 mmol/g resin), and subsequently, the photolabile linker¹⁴ **3** (Scheme 2) was introduced.¹⁵ This

Scheme 2. Introduction of *N*-Labeled Platinum(II) Valine **1** (IPt(NCN)Val) on Resin-Bound Peptide Sequence



versatile linker was used to facilitate the mild cleavage of the organoplatinum(II)-containing lead structures from the resin and to permit expedient in situ analysis via matrix-assisted laser desorption ionization-time-of-flight (MALDI-TOF) mass spectrometry.¹⁶ The peptide sequence Gly-Pro-Pro-Phe-Pro-Phe (GPPFPF) was synthesized on the photolabile linker, using syringe technology¹⁷ and Fmoc/OPfp-derivatized amino acids, which were activated with 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (Dhbt-OH) to give the modified resin bead **4** (Scheme 2). To the *N*-terminus of the resin-bound peptide was incorporated, initially, the *N*-protected platinum(II) valine residue **1** (IPt(NCN)Val, Figure 1), using *O*-benzotriazol-1-yl-*N,N,N,N*-tetramethyluronium tetrafluoroborate (TBTU) and *N*-ethylmorpholine (NEM) activation. The organoplatinum(II)-containing peptide **5** (Scheme 2) was obtained quantitatively, as confirmed by MALDI-TOF mass spectrometry.

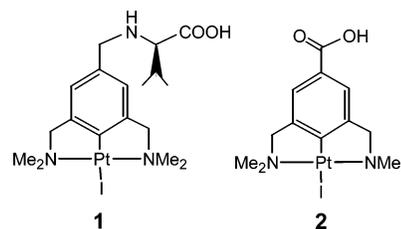


Figure 1. Organoplatinum(II) complexes intended for use as biomarkers in solid-phase screening assays.

Treatment of resin-bound **5** with SO₂ gas yielded orange beads; however, this color dissipated instantaneous after removal of the beads from the SO₂ atmosphere. Beads with a persistent purple/black color were obtained upon treatment of **5** with an aqueous solution of KI₃ (Figure 2).

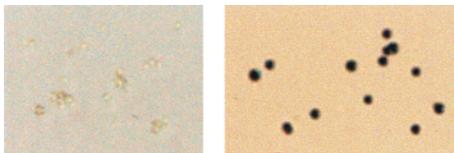
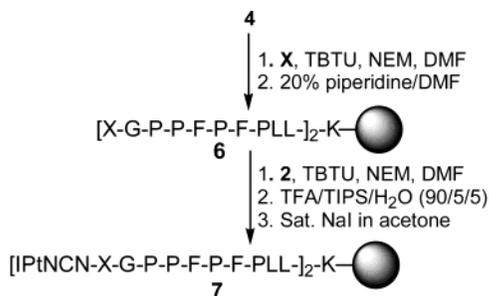


Figure 2. Detection of organoplatinum(II)-containing peptides. The left panel shows resin beads **5** in aqueous solution. The right panel shows resin beads **5** after treatment with KI₃.

As the mild and more persistent KI₃ coloration process represents a new approach for highlighting the NCN–Pt biomarker, it was explored in more detail in this study. Because the initial experiments with resin-bound **5** showed that the secondary amine in the organoplatinum(II) valine residue was cleaved under acidic peptide deprotection protocols (95% TFA/H₂O for 30 min removes approximately 30% of the biomarker), compound **2** (Figure 1), lacking the labile secondary amine function, was chosen to replace **1**.

To prove the general applicability of biomarker **2**, it had to be shown that it could be coupled onto all natural α -amino acids, is stable toward acidic peptide deprotection protocols, and finally, that the KI₃ coloration method is compatible with all the side-chain functions of α -amino acids. To this end, resin-bound peptide **4** (400 mg) was distributed equally over the 20 wells of a peptide synthesizer. In parallel, each well of the synthesizer was reacted with one of the 20 naturally occurring, suitably protected, α -amino acids (Scheme 3).

Scheme 3. Introduction of Organoplatinum(II) **2** (IPtNCN) on Different α -Amino Acid-Containing Resin-Bound Peptide Sequences



X = any of the natural α -amino acids. **6** and **7** depict the general structure of the formed products

After removal of the Fmoc-protecting group from each of the 20 peptides, biomarker **2** (2 equiv) was introduced using TBTU/NEM activation (Scheme 3). To remove all side-chain

protecting groups (*t*-Bu, Boc, and Trt), each organoplatinum(II)-containing peptide was treated for 2 h with a mixture of TFA/Tri-isopropylsilane/H₂O (90/5/5 v/v/v). By irradiation with a Hg lamp (UV light) of a few resin beads of each well, the MALDI-TOF mass spectra of the different, released organoplatinum(II)-containing peptides were recorded. The obtained mass spectra (for a representative example see Figure 3) of each of the 20 different peptides showed that

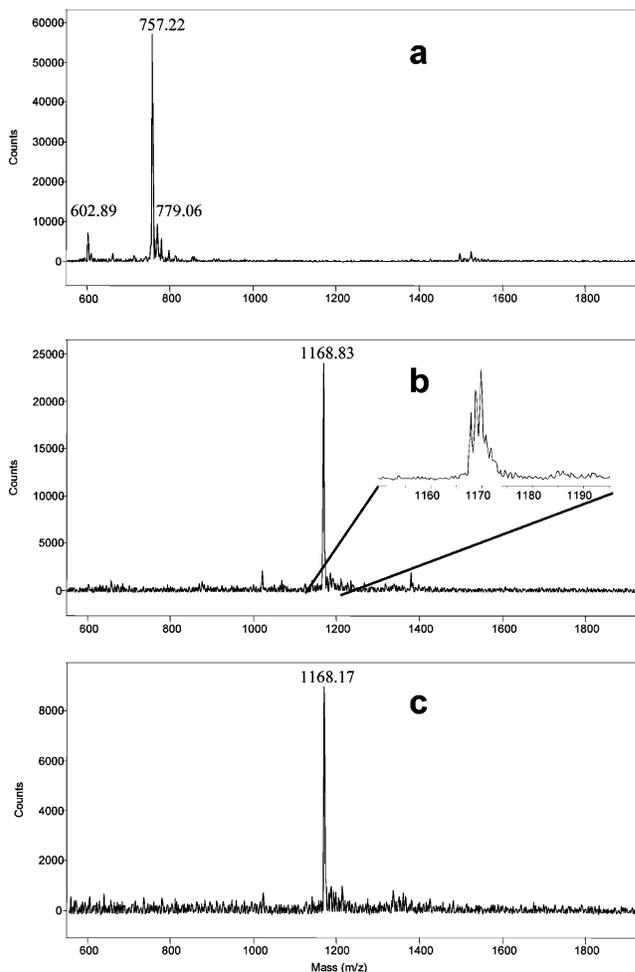


Figure 3. MALDI-TOF mass spectra of the peptide sequence: (a) **6** when X = Pro; (b) **7** when X = Pro the insert in the spectrum shows the characteristic isotope pattern; (c) **7** when X = Pro after treatment with KI₃ followed by washing with deionized water, cf. Scheme 3 for X. Note that during ionization in the mass spectrometer direct loss of I (spectrum b) or I₃ (spectrum c) occurs.

the biomarker **2** was quantitatively introduced and that no degradation occurred under the acidic deprotection conditions (the cysteine-containing organoplatinum(II) peptide could only be visualized using high laser power). In all MALDI-

(14) The partial cleavage of this linker during the screening process of fluorescence-based assays and its fluorescence-quenching capabilities in the internal fluorescence-quenched assay⁵ hampers its use in this type of solid-phase assays. However, the KI₃-highlighting of the organoplatinum(II) biomarker is completely compatible with the photolabile linker **3**.

(15) Holmes, C. P.; Jones, D. G. *J. Org. Chem.* **1995**, *60*, 2318–2319.

TOF mass spectra the characteristic isotope pattern of the Pt-nucleus is clearly visible.

The 20 resin-bound organoplatinum(II)-labeled peptides were treated for 2 min with a 2.5 mM solution of KI_3 in $\text{H}_2\text{O}/\text{MeOH}$. All 20 compounds obtained a striking purple/black color (Figure 4) that could be easily distinguished from

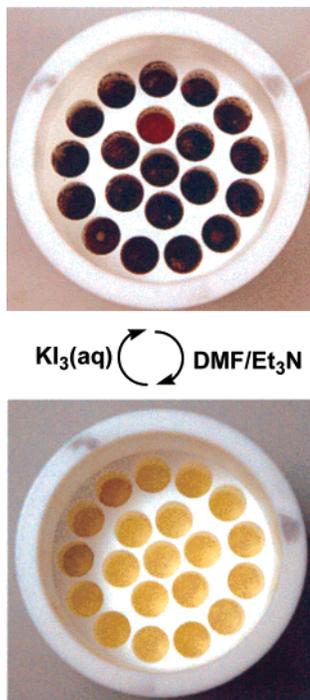


Figure 4. Picture of 20-well peptide synthesizer: (a) (top) **7** upon KI_3 treatment; (b) (bottom) **7** upon KI_3 treatment, followed by $\text{DMF}/\text{Et}_3\text{N}$ washings.

the slightly yellow color that beads obtain that do not contain the organoplatinum(II) biomarker. The MALDI-TOF mass spectra of each individual organoplatinum(II)-containing peptide, before and after the KI_3 treatment, showed them to be identical. This indicates that the KI_3 coloration method did not interfere with any of the side chains of the different α -amino acids (for a representative example see Figure 3). After the KI_3 treatment, the beads were extensively washed

(16) Halkes, K. M.; St. Hilaire, P. M.; Jansson, A. M.; Gotfredsen, C. H.; Meldal, M. *J. Chem. Soc., Perkin Trans. 1* **2000**, 2127–2133.

(17) Meinjohanns, E.; Meldal, M.; Jensen, T.; Werdelin, O.; Galli-Stampino, Mouritsen, S.; Bock, K. *J. Chem. Soc., Perkin Trans. 1* **1997**, 871–884.

with water which did not give rise to any color bleaching. The original transparent color of the organoplatinum(II)-labeled peptide beads could be regained by washing the beads with a $\text{DMF}/\text{Et}_3\text{N}$ or $\text{DMF}/\text{morpholine}$ solution (Figure 4). Upon treatment of the same, decolorized beads with a saturated NaI in acetone solution (10 min), followed by 2.5 mM KI_3 in $\text{H}_2\text{O}/\text{MeOH}$ (2 min), the purple/black color reappeared. This process of “switching on and off” the color of the beads could be repeated at least three times without affecting either the color intensity or the structure of the compounds, as indicated by identical MALDI-TOF mass spectra. The high sensitivity of the coloring process was shown in an experiment in which different amounts of biomarker **2** were introduced on the resin-bound GPPFPF peptide sequence. Capping of only 6% of the available amine-termini of the peptide with the biomarker resulted in colored beads that could be easily distinguished from beads containing no biomarker (see the Supporting Information).

In conclusion, we have demonstrated that the organoplatinum(II) biomarker **2** can be routinely introduced on the *N*-terminus of any α -amino acid in a resin-bound peptide and is completely stable toward the acidic peptide deprotection protocols (as opposed to biomarker **1**).

In addition to the already known SO_2 highlighting, the presence of platinum(II) in peptide chains can be detected by treatment with an aqueous solution of KI_3 , causing an almost instantaneous change of color from colorless to purple/black. Capping of only 6% of the available amine-termini of a resin-bound peptide sufficed for a clearly distinguishable color change of the resin bead. The purple/black color is stable in water, but can be easily removed by washing with $\text{DMF}/\text{Et}_3\text{N}$ or $\text{DMF}/\text{morpholine}$ solutions. The robustness of **2**, its compatibility with the photolabile linker **3**, the mild and reversible (“switching on and off”) character of the coloration process, and its intense purple color are all characteristics that will make this novel type of organoplatinum(II) biomarker a valuable addition to the existing arsenal of coloring agents used in solid-phase screening assays.

Acknowledgment. G.G. and G.R. are Marie Curie fellows and thank the European Commission for Marie Curie Grants (Contract Nos. HPMF-CT-2000-00472 and HMPF-CT-1999-00236, respectively).

Supporting Information Available: Detailed description of the experimental procedure for quantification of coloring intensity. This material is available free of charge via the Internet at <http://pubs.acs.org>.

OL034337S