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Covalent and Noncovalent Interactions for [Metal(dien)nucleobase]$^{2+}$ Complexes with L-Tryptophan Derivatives: Formation of Palladium–Tryptophan Species by Nucleobase Substitution under Biologically Relevant Conditions

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The interaction of the complexes [Pd(dien)(1-MeCyt)]$^{2+}$ (**2**) and [Pd(dien)(9-EtGH)]$^{2+}$ (**3**) with the amino acids L-tryptophan (Trp) and *N*-acetyltryptophan (*N*-AcTrp) was studied and compared with the previously studied platinum analogues [Pt(dien)(1-MeCyt)]$^{2+}$ (**4**) and [Pt(dien)(9-EtGH)]$^{2+}$ (**5**). Solid-state structures for **2** and **4** are reported. For the palladium complexes, the interaction is pH sensitive. Below pH 5, the noncovalent interaction with stacking between the aromatic amino acid residue and the metalated nucleobase was observed. Fluorescence quenching experiments indicated similar association constants for platinum and palladium derivatives **2**–**5**. Unusual substitution of the model nucleobases 1-methylcytosine (1-MeCyt) and 9-ethylguanine (9-EtGH) by tryptophan was observed in the range of pH 5–11. The resulting species [Pd(dien)(Trp)]$^{+}$ (**6**) and [Pd(dien)(*N*-AcTrp)]$^{+}$ (**7**) were characterized using ^{1}H NMR, ^{13}C NMR, and ESI-MS spectroscopy with coordination indicated through the amino and deprotonated amido nitrogens, respectively. Complexes **6** and **7** were also obtained from a solution of [Pd(dien)Cl]$^{+}$ (**1**) incubated with either Trp or *N*-AcTrp, respectively.

Introduction

Nucleobase coordination to platinum centers in [Pt(dien)(nucleobase/nucleotide)]$^{2+}$ complexes significantly enhances its $\pi-\pi$ stacking interactions with L-tryptophan as measured through fluorescence-quenching experiments.[1] This interaction may represent a novel motif for biomolecule targeting and molecular recognition by metal−nucleobase (nucleic acid) complexes. The study of new chemical structures may also lead to hitherto unrecognized novel biological functions, and to fully exploit the structural motif, it is necessary to explore the extent of the kinetic and structural flexibility within any given system. In the case of the [Pt(dien)(nucleobase/nucleotide)]$^{2+}$ system, the effect of metal center and kinetic lability on $\pi-\pi$ stacking interactions may be studied using isoelectronic and isostructural palladium analogues. Palladium(II) complexes with chelating ligands such as ethylenediamine (en) or diethylenetriamine (dien) have been extensively used to model the covalent interaction of analogous platinum(II) complexes with nucleobases, nucleosides, and nucleotides. The high reactivity exhibited by the palladium systems (up to 10^6 faster hydration kinetics) makes possible the study of binding equilibria which are sometimes difficult to determine for inert platinum(II) complexes.[2−6]

Palladium−peptide interactions are of relevance because of the hydrolysis of the peptide bond catalyzed by palladium complexes.[7] The effect of ligand {Pd(en)$^{2+}$, Pd(terpy)$^{2+}$} has been extensively studied for steric effects as well as peptide substrate specificity. Additionally, palladium(II) and platinum(II)−tryptophan interactions are of specific significance because of the highly regioselective hydrolysis of tryptophan-

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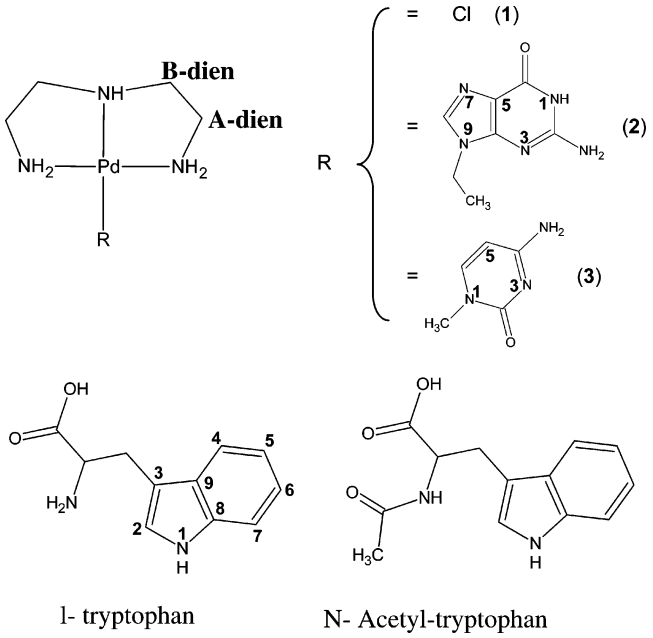
Chart 1. Representation of Structures for Compounds Used on This Study with Numbering Scheme



l- tryptophan N- Acetyl-tryptophan

containing peptides promoted by bifunctional $[M(en)]^{2+}$ (M = Pd, Pt) complexes.[8,9] More recently, the use of the $[Pd(en)]^{2+}$ moiety as a metal clip to stabilize peptide turns and short α-helices in 5−15 residue nonhelical peptides is made possible by the correct combination of structure and the reactivity of the palladium center.[10−12] In addition, the use of phosphine-modified peptides as ligands in palladium catalysts to create artificial secondary structures, such as the *β*-turn, modulates the enantioselectivity of allylation reactions with cyclic substrates.[13]

The interactions of metal−nucleobase/nucleotide complexes, often produced and studied in situ, with other relevant biologic substrates and the properties of ternary metal−nucleobase−peptide species in general have not been extensively studied.[14−16] In this work, we study the interaction between $[Pd(dien)nucleobase]^{2+}$ complexes and the amino acid L-tryptophan and *N*-acetyl tryptophan (Chart 1). Comparison is made with their platinum analogues, and evidence for a novel covalent interaction with L-tryptophan through nucleobase substitution and the structural characterization of the palladium-tryptophan moiety are reported.

Experimental Section

Reagents and Solvents. L-Tryptophan, *N*-acetyl-tryptophan, 1-methylcytosine, 9-ethylguanine, and diethylenetriamine were obtained from Aldrich and used as received. Water was double distilled and deionized on a Barnstead ultrapure water system model NANOpure. Complexes $[Pt(dien)(1\text{-}MeCyt)](NO_3)_2$ (**4**) and $[Pt(dien)(9\text{-}EtGH)](NO_3)_2$ (**5**) were synthesized from [Pt(dien)Cl]Cl according to reported procedures and characterized through elemental analysis, $^1H/^{195}Pt$ NMR and ESI-MS.[1]

Instrumentation. pH measurements were performed on a Corning pH meter model 340, equipped with an Accumet microelectrode with calomel reference, model 13-620-095. ^1H and ^{13}C NMR experiments were performed on a Varian INOVA (400 MHz) spectrometer. Chemical shifts were referenced to the residual peak of water at 4.69 ppm in ^1H NMR and 2D NMR COSY experiments, while dioxane (67.19 ppm) was used for ^{13}C NMR experiments. A mass spectrometer Micromas Q-Tof 2 equipped with a quadrupole/time-of-flight detector was used for mass spectra. X-ray data were collected on a Bruker SMART APEX CCD diffractometer with an area detector system.

X-ray Structure Determination. Colorless plates of **2** were obtained upon slow cooling (3 weeks, 0 °C) of a saturated DMF solution after the addition of diethyl ether. Colorless plates of **4** were obtained by the hanging drop method in a water/2-methyl-2,4-pentanediol mixture. The structures were solved by direct methods with subsequent refinements using SHELXS-97 and SHELXL-97.[17] Analysis of short contacts was performed with the Mercury 1.2.1. software from Cambridge Crystallographic Date Centre (CCDC).

Fluorescence Experiments. In a typical experiment, 3 mL of *N*-AcTrp (5 μM) were titrated with aliquots of the corresponding quenching compound (7.5 mM) in the range [quencher]/[*N*-AcTrp] = 10−100; 20 mM Tris-HCl buffer adjusted with a few drops of HNO_3 was used in all experiments (pH 4.8). The maximum intensity of the spectrum (ca. 362.9 nm) was measured for each addition, and the association constants for each system were obtained from the analysis of the Eadie−Hofstee plots.[18] Measurements were made at 20 °C, and the reported association constants (K_a) were averaged over a number of six different experiments; the significance of results was compared using an unpaired t-test. Fluorescence spectra were recorded in the range of 295−450 nm with a scan rate of 120 nm/min.

Synthesis of Complexes. [Pd(dien)Cl]Cl (1). Complex **1** was synthesized by a modification of the reported procedure.[19] $PdCl_2$ was suspended in 2 mL of water and reacted with dien in a 1:2 molar ratio at 90 °C until a yellow solution resulted (∼15 min). The pH of this solution was then decreased from 6.9 to 4.8 by addition of concentrated HCl, and half of the solvent was evaporated. After this, a few drops of ethanol were added, and yellow crystals appeared, which were filtered, washed with cold ethanol/ether, and dried in vacuo (60% yield). Anal. Calcd for $C_4H_{13}N_3Cl_2Pd$: C, 17.13; H, 4.68; N, 14.98. Found: C, 17.26; H, 4.40; N, 14.71.^1H NMR (D_2O): δ 2.90 (8 H, m).

$[Pd(dien)(1\text{-}MeCyt)](NO_3)_2$ (2). Two equivalents of $AgNO_3$ were added to a solution of **1** in water, and the mixture was stirred overnight to remove the chloride ligands and produce the complex $[Pd(dien)NO_3]NO_3$ in situ. The solution was then filtered and

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1-MeCyt was added in equimolar amounts, and the mixture was stirred at room temperature for 24 h. The off-white solid was obtained upon solvent evaporation, and the powder was then washed with methanol and ether to remove unreacted 1-MeCyt (80% yield). Anal. Calcd for $C_9H_{20}O_7N_8Pd$: C, 23.55; H, 4.40; N, 24.43. Found: C, 23.69; H, 4.05; N, 24.28. ^{1}H NMR (D_2O): δ 7.45 (1H, d, J = 7.3 Hz), 5.82 (1H, d, J = 7.3 Hz), 3.27 (3H, s), 2.95 (8H, m). ESI-MS: m/z = 333.41 [Pd(dien)(1-MeCyt) − H^+]$^+$.

[Pd(dien)(9-EtGH)](NO$_3$)$_2$ (3). Complex **3** was prepared in a manner similar to that of **2** using 9-EtGH as a nucleobase; the white product was then recrystallized in a water/methanol solution to obtain the final complex (60% yield). Anal. Calcd for $C_{11}H_{22}O_7N_{10}$-Pd: C, 25.76; H, 4.33; N, 27.32. Found: C, 24.69; H, 4.05; N, 27.28. ^{1}H NMR (D_2O): δ 7.98 (1 H, s), 3.97 (2H, q, J = 7.3 Hz), 3.02 (8H, m), 1.29 (3H, t, J = 7.3 Hz). ESI-MS: m/z = 387.31 [Pd(dien)(9-EtGH) − H^+]$^+$.

Incubation Experiments (NMR Spectroscopy). In a typical experiment, 7−10 mM solutions of the complexes (**1**−**5**) were incubated with Trp or *N*-AcTrp, at room temperature for 30 min (Pd) or overnight (Pt). pD was adjusted by addition of NaOD solution, NMR spectra were referenced to the residual signal of D_2O (^{1}H, 4.69 ppm) or dioxane (^{13}C, 67.19 ppm).

Results and Discussion

I. Crystal Structures of [Pd(dien)(1-MeCyt)](NO$_3$)$_2$ (2) and [Pt(dien)(1-MeCyt)](NO$_3$)$_2$ (4). The cations of complexes **2** and **4** are very similar except for the torsional angle formed between the nucleobase plane and the Pt,Pd-diethylenetriamine moiety, Figure 1.

The 1-MeCyt ligand is not completely perpendicular to the coordination square plane, and appreciable deviations of 12.22 and 23.51° for **2** and **4** are found, respectively. Reported crystal structures for the analogous complexes with the cytosine ligand featured smaller deviations from perpendicularity for the nucleobase, 9.14 (Pd) and 3.81° (Pt).[20] Interestingly, these latter complexes favor the opposite rotamer of that observed for the complexes discussed here (N4C-endo, Figure 1), which is generated because of the hindered rotation in the Pd,Pt−N bond. The presence of the methyl moiety modifies the intermolecular hydrogen-bonding pattern between cations in the solid state to a significant extent, since Cyt/Cyt (N1H−O2) interactions are replaced by dien/1-MeCyt (O2C−N2H) interactions. Also, the stacking interaction among 1-MeCyt's is seen with an approximate internucleobase distance of 3.4 (**2**) and 3.5 Å (**4**), which is not exhibited in the cytosine analogues (Supporting Information, Figures S2−S4). This fact could correlate with recent calculations performed at the second-order perturbation theory (MP2) level showing that the presence of substituents on an aromatic ring contribute favorably to face-to-face stacking interactions via better distribution of the electrostatic potential in the surface of the nucleobase.[21] The ring−plane overlap is appreciable since a ring overlap of less than 30% is the common feature for an offset or slipped stacking mode of interaction, according to a recent geometrical analysis on complexes with aromatic nitrogen heterocycles as ligands

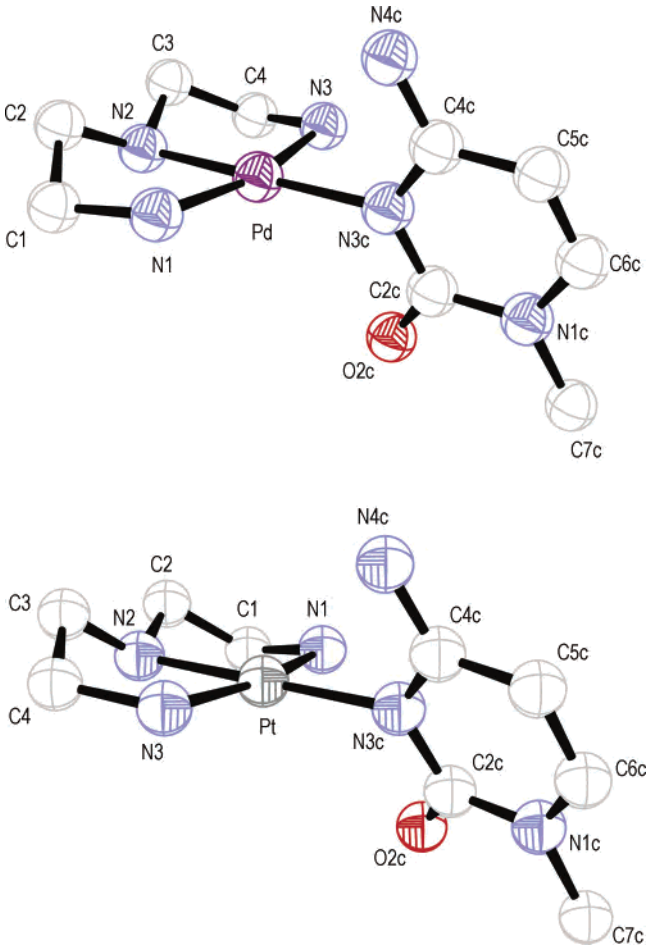

Figure 1. ORTEP diagram of [Pd(dien)(1-MeCyt)]$^{2+}$ (top) and [Pt(dien)-(1-MeCyt)]$^{2+}$ (bottom) with numbering scheme (50% probability ellipsoids). Hydrogen atoms omitted for clarity.

(7620 data sets). The contribution from $\pi-\sigma$ attraction as stated in the Hunter−Sanders rule is not discarded since a slight offset between the nucleobase's planes is observed.[22] Bond lengths and bond angles are as expected. Table 1 summarizes the crystallographic data and details of refinement for [M(dien)(1-MeCyt)](NO$_3$)$_2$ (M = Pd, **2**; M = Pt, **4**). Selected interatomic distances and angles are shown in Table 2.

II. Study of the Tryptophan/*N*-Acetyltryptophan Interaction with [M(dien)(nucleobase)](NO$_3$)$_2$ (M = Pt, Pd). The complexes [Pd(dien)Cl]Cl (**1**), [Pd(dien)(1-MeCyt)]-(NO$_3$)$_2$ (**2**), and [Pd(dien)(9-EtGH)](NO$_3$)$_2$ (**3**) were synthesized and characterized through microanalysis and spectroscopic techniques. The chloride complex was used as a control for palladium−peptide interactions in the absence of nucleobase. The chemical behavior of **1**−**3**, as well as **4** and **5**, toward Trp and *N*-AcTrp was examined over a range of pH.

II.a. Evidence for Formation of the Covalent [Pd(dien)-(L-Tryptophan)]$^+$ Complex, 6. Mass Spectrometry. ESI-MS spectra of the systems **1**−**3**/Trp taken at pD > 9.0 (pD = pH* + 0.4,[23] where pH* is the measurement of the pH

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Table 1. Crystallographic Data and Details of Refinement for Compounds **2** and **4**

	2	**4**
formula	$C_9H_{20}N_8O_7Pd$	$C_9H_{20}N_8O_7Pd$
fw	458.73	547.42
cryst color and habit	colorless plate	colorless plate
temp (K)	153 (2)	153 (2)
cryst size (mm)	0.31 × 0.28 × 0.12	0.28 × 0.26 × 0.08
cryst syst	triclinic	triclinic
space group	$P\bar{1}$	$P\bar{1}$
a (Å)	9.6993 (5)	8.9086 (5)
b (Å)	9.8688 (5)	10.4677 (6)
c (Å)	10.2130 (5)	10.6379 (6)
α (deg)	66.6210 (10)	61.3910 (10)
β (deg)	67.1570 (10)	67.7770 (10)
γ (deg)	74.0060 (10)	73.1150 (10)
vol (Å^3)	818.38 (7)	798.87 (8)
Z	2	2
D_{calcd} (Mg/m$^{3)}$	1.862	2.276
μ (Mo Kα) (mm^{-1})	1.188	8.838
F(000)	464	528
θ range for data collection	2.27−32.54	2.24−32.49
reflns collected	5398	5634
reflns observed	4823	4289
no of params refined	306	302
R_{int}	0.015	0.051
R_1 (observed data)	0.0242	0.0447
R_2 (observed data)	0.0519	0.0754
residual$_{\rho max,\ \rho min}$ (e Å^{-3})	0.851, −0.638	3.891, −2.483

Table 2. Selected Interatomic Distances (Å) and Angles (deg) for **2** and **4**

	2	**4**
Pd/Pt−N1	2.0494(16)	2.039(5)
Pd/Pt−N2	2.0001(14)	2.018(5)
Pd/Pt−N3	2.0429(15)	2.056(5)
Pd/Pt−N3c	2.0462(13)	2.045(5)
N2−Pd/Pt−N3	84.16(6)	83.4(2)
N2−Pd/Pt−N3c	178.40(6)	179.6(2)
N3−Pd/Pt−N3c	94.31(6)	97.0(2)
N2−Pd/Pt−N1	84.17(6)	84.4(2)
N3−Pd/Pt−N1	167.10(6)	167.7(2)
N3c-Pd/Pt−N1	97.40(6)	95.2(2)

meter), showed signals corresponding to the starting complexes (i.e., **1** [Pd(dien)Cl]$^+$ (*m*/*z* = 246.0), **2** [Pd(dien)(1-MeCyt)-H$^+$]$^+$ (*m*/*z* = 333.4), and **3** [Pd(dien)(9-EtGH)-H$^+$]$^+$ (*m*/*z* = 387.2)) along with a strong signal at *m*/*z* = 412.3(1) which was assigned to the species [Pd(dien)(Trp)]$^+$ because of (i) the presence of the characteristic isotopic distribution for the palladium atom and (ii) a single net-positive charge, determined from the average *m*/*z* value between peaks in the signal. Figure 2 shows the ESI-MS spectrum for the system **2**/Trp where, in addition to the signals from the Pd−nucleobase and Pd−Trp complexes, an additional signal also exhibiting the isotopic distribution of palladium is assigned to the species [Pd(dien)-H$^+$]$^+$ (*m*/*z* = 208.5). The loss of a proton from the diethylentriamine ligand was a general observation in all the palladium complexes studied here and has been reported to sometimes occur under ESI-MS conditions for other metal complexes, as well.[24]

For [Pd(dien)(1-MeCyt)]$(NO_3)_2$, further ESI-MS signals could be assigned to the free nucleobase forming protonated adducts such as [1-MeCyt + H$^+$]$^+$ (*m*/*z* = 126.6) and [(1-MeCyt)$_2$ + H$^+$]$^+$ (*m*/*z* = 251.6). The corresponding sodium adducts, [1-MeCyt + Na$^+$]$^+$ and [(1-MeCyt)$_2$ + Na$^+$]$^+$ (*m*/*z* = 148.6 and 273.6 respectively), were also observed and are very likely caused by the NaOD added to increase the solution pD, Figure 2. Signals due to nucleobase adducts with protons and sodium were also observed for system **3**/Trp with 9-EtGH: [9-EtGH + H$^+$]$^+$ (*m*/*z* = 180.6); [(9-EtGH)$_2$ + H$^+$]$^+$ (*m*/*z* = 359.4); [9-EtGH + Na$^+$]$^+$ (*m*/*z* = 202.5); and [(9-EtGH)$_2$+Na$^+$]$^+$ (*m*/*z* = 381.3), Figure S5.

Nuclear Magnetic Resonance Spectroscopy. Monitoring of the ^{1}H NMR spectra of equimolar solutions of **2** and Trp also suggested the formation of the complex [Pd(dien)(Trp)]$^+$ (**6**), generated by the substitution of a nucleobase ligand by the amino acid, Figure 3. The reaction is pH dependent, and above a pH/pD value of 5, new signals corresponding to free nucleobase, as well as coordinated tryptophan, appear with a concomitant decrease in the signals of the starting complex and free peptide. The peptide signals undergo a moderate downfield shift in all aromatic signals, as well as some significant variations in the chemical shifts for the α- and *β*-proton signals, with the α proton signals shifted by almost 1 ppm.

These new signals were consistent upon changes in temperature (20−45 °C) and time (two weeks), but as stated, the intensities exhibited a strong pD dependence. The appearance of signals corresponding to coordinated tryptophan occurs in parallel with signals corresponding to the appearance of free nucleobase. Coordination of 1-MeCyt to the [Pd(dien)]$^{2+}$ group does not cause an appreciable shift of its proton signals, but shifts for the methyl group of 1-MeCyt were easier to follow since it appeared as a strong singlet: 3.29 ppm (coordinated) and 3.25 ppm (free).[25] Despite the reported stability of [Pd(dien)(nucleoside/nucleotide)] complexes even under very acidic conditions, complex **2** was found to exhibit 1-MeCyt displacement to some extent even at pD = 2.0. In this case, the signals for free nucleobase corresponded to the protonated form and appeared at 7.71 (^3J = 7.7 Hz) and 6.03 (^3J = 7.7) for H6 and H5, a net downfield displacement of 0.23 and 0.17 ppm, respectively. In this case, a substitution pathway involving hydrolysis through protonation of the 1-MeCyt ligand is indicated.

Identical spectral changes of the tryptophan moiety were seen for [Pd(dien)(L)]$(NO_3)_2$ (L = Cl$^-$ (**1**) or 9-EtGH (**3**)), and the appearance of free nucleobase was also observed in the latter case. The initial pD values were ca. 4.1, 4.8, and 5.0 for **1**/Trp, **2**/Trp, and **3**/Trp, respectively. The singlet corresponding to the H8 proton in **3** appeared at 7.60 ppm with a downfield shift of 0.38 ppm compared to the free nucleobase. The shift of this signal is a very characteristic diagnostic tool for assessing metalation/demetalation in guanine-derived molecules such as 9-EtGH.[26] For the system **1**/Trp, no additional signals, excluding those for free and

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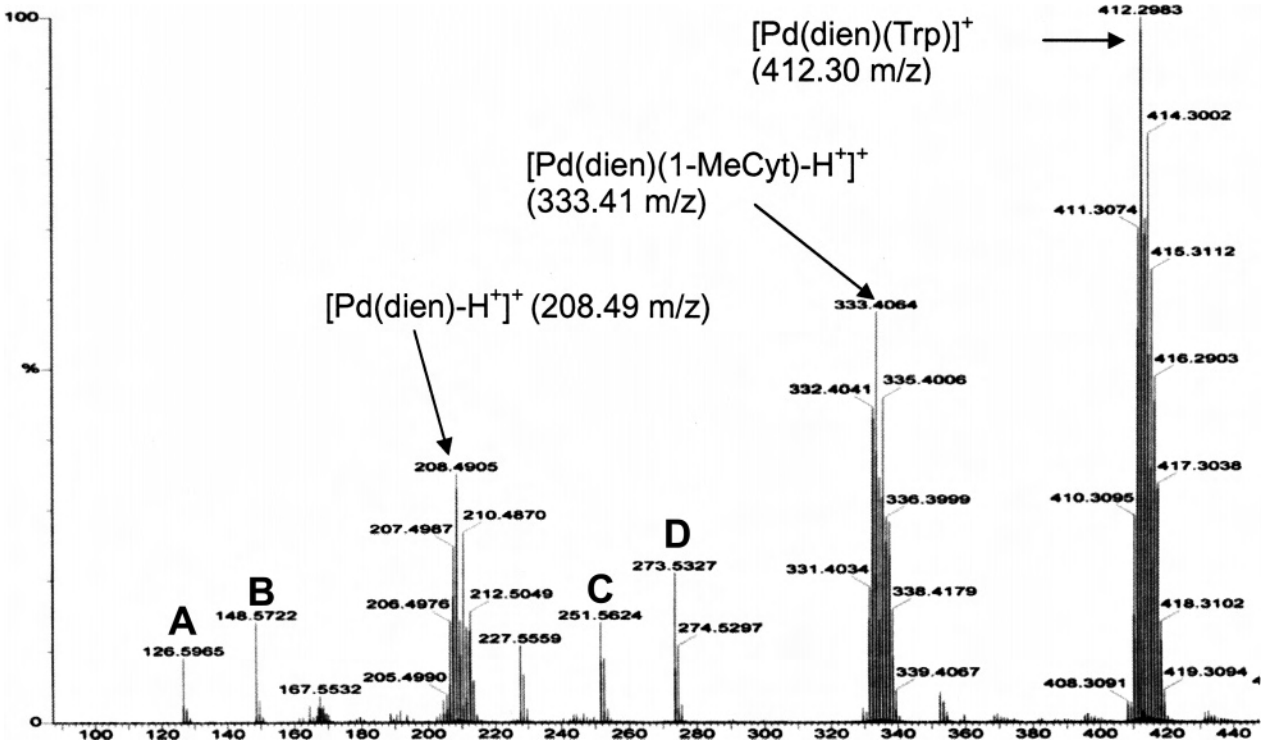

Figure 2. ESI-MS spectrum of a $[Pd(dien)(1\text{-MeCyt})]^{2+}$/Trp solution (pD 9.0); major species are indicated, and minor signals were assigned as follows: A = $[1\text{-MeCyt} + H^+]^+$ (126.6 *m*/*z*), B = $[1\text{-MeCyt} + Na^+]^+$ (148.6 *m*/*z*), C = $[(1\text{-MeCyt})_2 + H^+]^+$ (251.6*m*/*z*), and D = $[(1\text{-MeCyt})_2 + Na^+]^+$ (273.6 *m*/*z*).

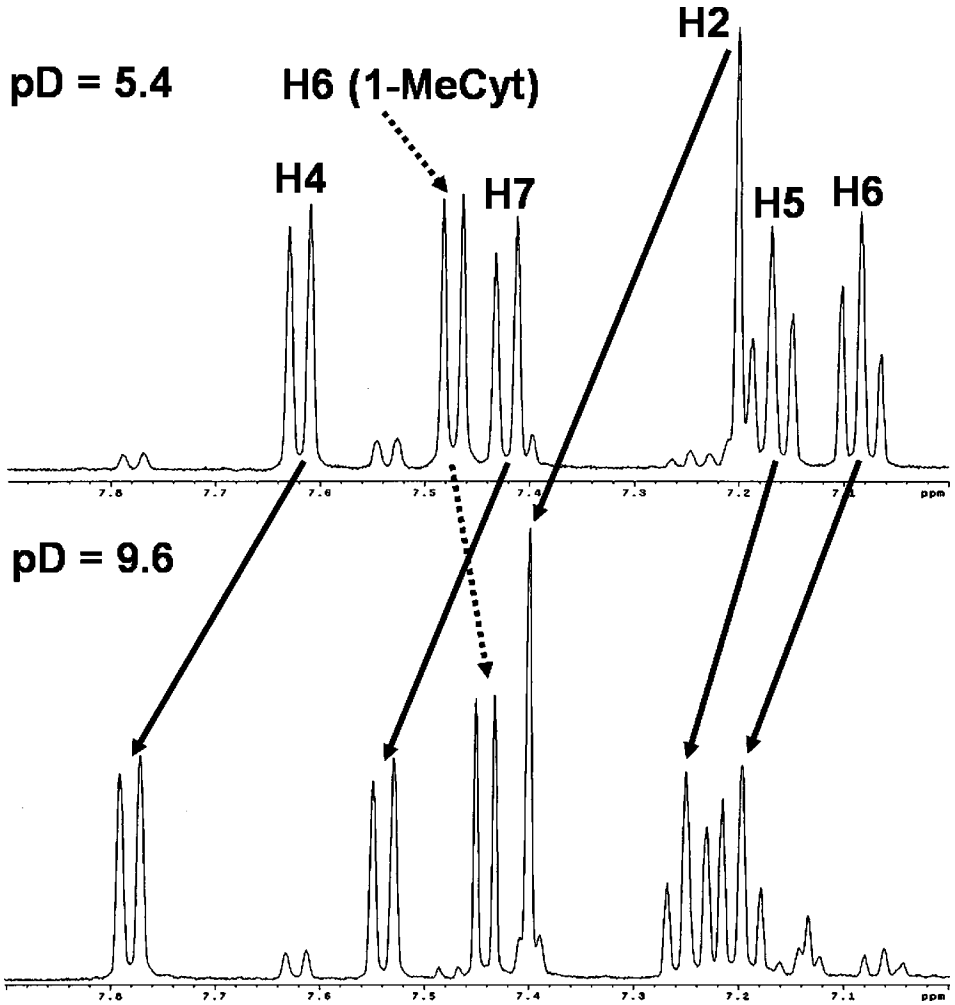

Figure 3. Influence of pD on ^1H NMR spectrum from a $[Pd(dien)(1\text{-MeCyt})]^{2+}$/Trp solution; arrows indicate the shift for coordinated Trp in $[Pd(dien)(Trp)]^+$. The peak marked with the dashed arrow corresponds to 1-methylcytosine.

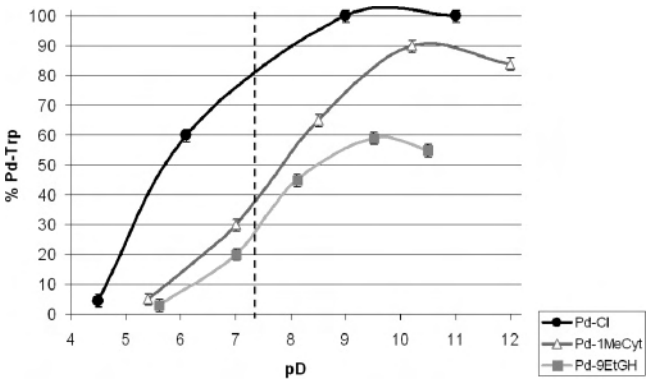

Figure 4. pD-dependent formation of complex [Pd(dien)(Trp)]$^+$ from different starting complexes; physiological pH (7.4) is shown as a dashed line.

Table 3. ^{1}H NMR − Chemical Shifts Observed for Coordinated and Free Trp/N−AcTrp in **6** and **7**

	Trp (ppm)	Pd−Trp (ppm)	Δδ (ppm)	*N*-AcTrp (ppm)	Pd−*N*-AcTrp (ppm)	Δδ (ppm)
H4	7.62	7.78 dd, J = 7.9 Hz	+0.16	7.56	7.69 dd, J = 8.1 Hz	+0.13
H7	7.42	7.53 dd, J = 7.9 Hz	+0.11	7.39	7.41 dd, J = 8.1 Hz	+0.02
H2	7.20	7.40 s	+0.20	7.14	6.97 s	−0.17
H5	7.17	7.25 t, J = 7.5 Hz	+0.08	7.13	6.88 t, J = 7.4 Hz	−0.25
H6	7.08	7.19 t, J = 7.5 Hz	+0.11	7.06	6.99 t, J = 7.4 Hz	−0.07
Hα	3.88	2.87 m	−1.01	4.40	4.32 m	−0.08
Hβ'	3.32	3.36 m	+0.04	3.24	3.14 m	−0.10
Hβ	3.16	3.36 m	+0.20	3.20	3.00 m	+0.20
CH_3				1.74	1.74 s	0.00

coordinated Trp, were observed confirming the proposed substitution reaction and ruling out other possible side reactions.

The chemical shifts for free and coordinated Trp are compared in Table 3. 2D NMR COSY experiments confirmed the assignments (Figures S6 and S7 in Supporting Information). The signal for the H4 proton in coordinated Trp was used as a good diagnostic to monitor the progress of the formation of complex **6** in the pD range tested. A direct comparison between chloride and nucleobase substitution could be made using the integration for this doublet. Substitution of the chloride ligand occurs at a lower pD value than those for both nucleobases suggesting a higher stability in solution for complexes **2** and **3**. Approximately 80% of the starting material, **1**, reacted to form **6**, [Pd(dien)(Trp)]$^+$, whereas 40 and 25% are formed from **2** and **3**, respectively, around the physiological pD (7.4), Figure 4. There is a consistently lower rate of [Pd(dien)(Trp)]$^+$ formation from complex **3** than that from **2** at similar pD values.

The release of the steric demands of the exocyclic groups on 1-methylcytosine may be one feature contributing to the more efficient displacement of this ligand by tryptophan. At pD > 9, the formation of [Pd(dien)(Trp)]$^+$ is reduced considerably in the case of the **3**/Trp system in comparison to **2**, explained by additional equilibria in solution for 9-EtGH, involving both N1 and N7 coordination in the nucleobase. The observation of an additional signal in the ^{1}H NMR spectrum attributable to the dinuclear species {[Pd(dien)]$_2$(9-EtGH-*N1*,*N7*)}, previously reported for high pH values when N1 is deprotonated,[16,25,27] supports this possibility.

Table 4. Summary of ^{13}C NMR Chemical Shifts Observed in the Aliphatic Region for coordInated and Free Trp, *N*-AcTrp, and Diethylenetriamine (dien)

	Trp	[Pd(dien)(Trp)]$^+$	Δδ (ppm)
Cα (ppm)	56.10	60.48	+ 4.68
Cβ (ppm)	27.21	29.64	+ 2.43
	N-AcTrp	[Pd(dien)(*N*-AcTrp)]$^+$	Δδ (ppm)
Cα (ppm)	54.52	56.55	+ 2.03
Cβ (ppm)	27.34	28.05	+ 0.71
	[Pd(dien)Cl]$^+$	[Pd(dien)(Trp)]$^+$	[Pd(dien)(*N*-AcTrp)]$^+$
dien-A CH_2 (ppm)	54.28	52.83	53.22
dien-B CH_2 (ppm)	48.28	48.35	49.23
Δδ (ppm)	6.00	4.48	3.99

II.b. Characterization of the [Pd(dien)(L-tryptophan)]$^+$Complex, 6. The ^{13}C NMR spectra from systems **1**−**3**/Trp compared to free Trp at the same pD revealed that shifts in aromatic carbons were not as pronounced as in the case of the aliphatic carbons. The principal shift changes are summarized in Table 4. In the aliphatic part of the ^{13}C NMR spectrum (data extracted from the simple system **1**/Trp), the two inequivalent sets of carbons in the diethylenetriamine ligand can be seen along with the signals for the α- and β-carbons for complex **6** (Figure S8). The α- and β-carbons suffered downfield shifts of 4.68 and 2.43 ppm, respectively, upon coordination to palladium, and the separation of the two ^{13}C NMR signals of the diethylenetriamine ligand was decreased to 1.5 ppm compared to free Trp, which has been correlated with displacement of chloride in the coordination sphere of the complex (vide infra).[28] Thus, the combined ^{1}H and ^{13}C NMR data are strongly suggestive of coordination through the amino group. This conclusion is in agreement with the reported thermodynamic preference of the palladium(II) ion for ligands with S- or N- donors over O-donors.[29]

The process of nucleobase (aromatic N-donor) substitution by tryptophan (aliphatic N-donor) may be correlated with the properties of the putative spectator ligand diethylenetriamine. The nature of the ligands bound to the metal ion can affect donor atom preference in substitution reactions, for example, [Pt(terpy)Cl]$^+$ was found to react selectively with the imidazole ring of histidine residues in proteins, whereas the complex was unexpectedly unreactive to methionine, defying the HSAB principle.[30] In a similar manner, [Pd(terpy)Cl]$^+$ with 3 aromatic nitrogens was reported to be

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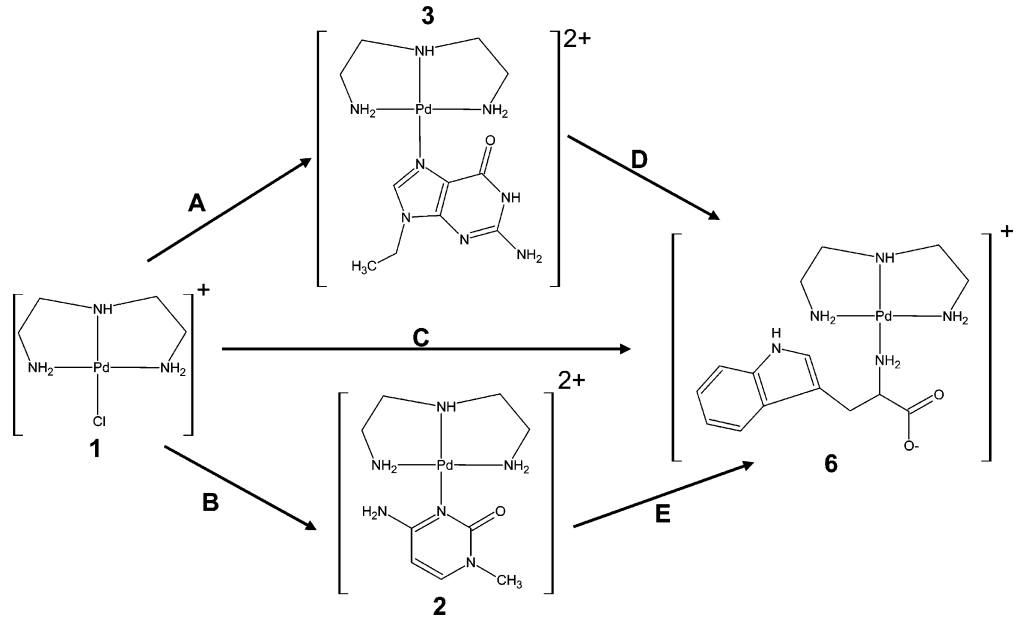
Scheme 1. Schematic Representation of the Reactions Performed on This Work Showing the Three Substitution Paths for the Formation of Complex **6**



A/B, nucleobase + $AgNO_3$; C/D/E, Trp + NaOH.

unreactive toward *N*-acetyllysine and glycine over a range of pH, further suggesting the unfavorable situation of simultaneous aromatic/aliphatic coordination of nitrogens around the palladium(II) center.[25] The formation of **6** from the various complexes studied here is shown in Scheme 1.

II.c. Formation of [Pd(dien)(*N*-acetyltryptophan)]$^+$ complex, 7. The reaction of complexes **1**−**3** with *N*-AcTrp was also followed by ESI-MS and ^{1}H NMR spectroscopy to evaluate the differences between an amino group and the more representative amide bond of proteins. In all cases, coordination of *N*-AcTrp to the palladium center was confirmed by the presence of a signal in the mass spectrum at m/z = 454.12, which was assigned to the species [Pd(dien)(*N*-AcTrp)]$^+$,**7** (see Figure S9, Supporting Information). The protonated and sodium adducts of the free 1-MeCyt and 9-EtGH nucleobases observed previously in the case of Trp were also observed for **2** and **3**, respectively, confirming the nucleobase substitution by *N*-AcTrp. ^{1}H NMR spectra of systems **1**−**3**/*N*-AcTrp exhibited the presence of new signals attributed to coordination as the pD of the solution was increased above pD 8 (Figure S10, Supporting Information). The chemical shifts for free and coordinated *N*-AcTrp are compared in Table 3. 2D NMR COSY experiments confirmed the assignments, Figure S11. Some differences with respect to chemical shift changes were noted in comparison to the tryptophan case (i.e., only H4 and H7 protons undergo upfield shifts and all other aromatic signals exhibited a downfield shift indicating a different electronic influence on the indole ring upon coordination). Additionally, signals in the aliphatic part of the spectrum corresponding to protons Hα and Hβ exhibited a smaller change in **7** compared to that in **6**.

The formation of complex **7** was monitored using the corresponding integrals for H4 in the ^{1}H NMR spectrum. Coordination of *N*-AcTrp is not as favored as for the tryptophan system: the formation of **7** from **1** is approximately 50% at pD 9.2 compared to the almost quantitative conversion of **6** at the same pD. However the trend observed for ligand displacement from the Pd(II) complex (i.e., Cl^- > 1-MeCyt > 9-EtGH) was maintained, giving percentages for nucleobase substitution of 15% for 1-MeCyt and <10% for 9-EtGH at pD 10.6. Attempts to produce complex **7** in a greater than 50% yield by raising the pD above 11 were unsuccessful, since at very high pD, a more complex equilibrium is produced. Species such as [Pd(dien)(OH)]$^+$ (m/z = 226.48) were detected via ^{1}H NMR and ESI-MS suggesting a displacement of the coordinated nucleobase or chloride by the more nucleophilic hydroxyl ligand, contrasting with recent results obtained for the platinum analogue under highly basic conditions.[31]

II.d. Characterization of the [Pd(dien)(*N*-acetyltryptophan)] Complex. Monitoring of the ^{13}C NMR spectrum showed that the downfield shift experienced by the α- and β-carbons was not as large as that in the case for Trp, although separations of more than 2 ppm were observed (Table 4), correlating well with the modest proton chemical shifts. The separation between the ^{13}C NMR signals of the diethylenetriamine ligand has been used as a diagnostic to determine the nature of L in [Pt(dien)L]$^{+n}$ complexes. For **1** with L = Cl^-, the separation of these signals was found to be 6.00 ppm, whereas complexes **6** and **7** have separations ($\Delta\delta$) of 4.48 and 3.99 ppm, respectively, which are typical values for N-donors such as nitriles or amides (Table 4). Coordination through oxygen also induces similar shift differences of the dien C atoms, although in this case, values close to 5 ppm and above have been observed.[28] In addition, the ^{13}C NMR shift of the C=O carbonyl moiety undergoes

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an upfield shift upon *N*-AcTrp coordination and formation of **7**, in contrast to reported downfield shifts for binding through the oxygen atom of a carbonyl moiety.[28] The combination of spectral data thus suggests that the amide nitrogen is the coordination site. The coordination through the oxygen atom in any of the carbonyl moieties was further ruled out because of the weak coordination exhibited by these functionalities with a metal center. Coordination via a deprotonated amido nitrogen, in the case of *N*-AcTrp, can be understood given the high pD values (8 for complex **1** and >9 for **2** or **3**) needed for the formation of **7**. Glycine is reported to coordinate through O-coordination at low pH (3.6) but the N-bound species is exclusively formed at pH 7.4 for $[Pd(dien)(H_2O)]^+$ and glycine.[29] Bifunctional binding through formation of an N,O-chelate has been reported for other amino acids in addition to Trp.[32] The thermodynamic tendency to form the chelate leads even to the formation of an organometallic bond with indolyl C(3): O coordination when the model compound indole-3-acetamide is used instead of Trp.[33] The possibility of coordination via the indole N1 is also ruled out because of the small chemical shifts observed for the aromatic signals (Table 3) and its high pK_a value (16.8). Interestingly, coordination through this site has been reported only after previous coordination of an organometallic (η^6-cymene)Ru(II) fragment, which induce a drastic increase in the acidity of N1 by more than 8 orders of magnitude.[34] In summary, we propose that *N*-AcTrp coordinates via the deprotonated amido moiety to the Pd(II) center, and experiments aiming to obtain the solid structure of **7** and confirm the proposed structure are currently underway.

II.e. Reaction of [Pt(dien)(Nucleobase)]$(NO_3)_2$ (4, 5) with L-Tryptophan and *N*-acetyl Tryptophan. No nucleobase substitution was detected for Pt−nucleobase complexes **4** and **5** even after 72 h at pD 10.2. Slight chemical shifts in the range of 0.01−0.07 ppm could be detected for *N*-AcTrp, but they were attributable to stacking interactions as have been found previously.[1,35]

III. Study of Noncovalent Interactions in [M(dien)(nucleobase)]$(NO_3)_2$ (M = Pt, Pd) Complexes. The initial purpose of this study was to compare the effects of palladium and platinum in [M(dien)(nucleobase)] compounds on the enhancement of the stacking interaction with tryptophan and derivatives. Experimental conditions were chosen to eliminate the observed interactions of palladium by using a pH (4.8) that was low enough to limit any covalent binding, even for the more reactive Trp molecule. The degree of quenching experienced by Trp and *N*-AcTrp in complexes **2**−**5** was analyzed as done previously through Eadie−Hofstee plots (ΔF vs ΔF/[quencher]) which yielded the association constant value (K_a) for each system from its slope (Table 5). In the first place, the good agreement between the K_a values obtained for *N*-AcTrp and Trp suggests that the *N*-acetyl moiety doess not affect the stacking interaction with the nucleobase considerably, and it confirms the inertness of platinum−nucleobase complexes to substitution side reactions with the Trp substrate, a result that very likely would have been compromised in the case of the palladium(II) complexes. In general, a significant enhancement of the stacking interaction was observed for metal-coordinated nucleobases in comparison that of the free ones, in agreement with our previous results.

Table 5. Association Constants Obtained from Eadie−Hofstee Plots of *N*-AcTrp with Different Quenchers[a]

$$\Delta F = -\frac{1}{K_a}\frac{\Delta F}{[\text{quencher}]} + \Delta F_c$$

quencher	K_a	std deviation.
1-MeCyt	6.2 (6.0)	0.3 (0.1)
$[Pt(dien)(1\text{-MeCyt})]^{2+}$	8.8 (8.8)	0.3 (0.2)
$[Pd(dien)(1\text{-Mecyt})]^{2+}$	7.3	0.2
9-EtGH	3.5 (3.3)	0.2 (0.1)
$[Pt(dien)(9\text{-EtGH})]^{2+}$	7.0 (6.8)	0.1 (0.3)
$[Pd(dien)(9\text{-EtGH})]^{2+}$	5.1	0.3

[a] Values in parenthesis obtained for L-Trp from ref 1. Eadie−Hofstee equation, where $\Delta F = F - F_0$, is the difference between fluorescence intensities of *N*-AcTrp in the presence (F) and absence (F_0) of the quencher. ΔF_c is the difference of the *N*-AcTrp completely complexed with the quencher.

Conclusions

The kinetic lability of the Pd(II) center compared to that of Pt(II) resulted in a novel nucleobase substitution and Pd−amino acid complex formation. The increased steric hindrance inherent in metal−cytosine compounds suggests that the 1-MeCyt compound should be *less susceptible* to nucleophilic substitution by hindering (in the classic explanation) the approach of the incoming nucleophile. It is possible that the substitution is favored by the correct orientation of the amino acid through the enhanced stacking interaction; the higher association constants observed for cytosine over guanine derivatives are in agreement with this possibility. Palladium−peptide conjugates are proposed intermediates in the catalytic cycle involving peptide bond hydrolysis catalyzed by species such as $[Pd(en)(H_2O)_2]^{2+}$. The application of the chemistry reported here to tryptophan-containing peptides and proteins is ongoing.

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Supporting Information Available: CIF files and complementary figures for the solid state and spectroscopic characterization of the systems studied. This material is available free of charge via the Internet at http://pubs.acs.org.

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