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# Cyclopentadienyl-Based Amino Acids (Cp-aa) As Phenylalanine Analogues for Tumor Targeting: Syntheses and Biological Properties of [(Cp-aa)M(CO)$_3$](M = Mn, Re, $^{99m}$Tc)

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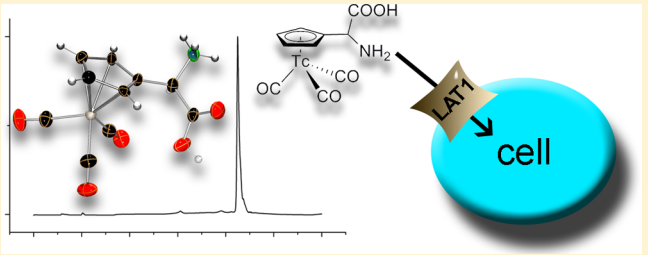
Supporting Information

**ABSTRACT:** Due to an enhanced demand for amino acids, the L-type amino acid transporter 1 (LAT1) is overexpressed in many tumor cell lines. LAT1 represents therefore an attractive target for cancer therapy and diagnosis. On the basis of our reported aqueous synthesis of [(Cp-R)$^{99m}$Tc(CO)$_3$]-type complexes,[1−5] we describe the preparation of unnatural amino acid analogues [(Cp-CH$_2$CH(NH$_2$)COOH)Mn(CO)$_3$] and [(Cp-CH(NH$_2$)COOH)M(CO)$_3$] (M = Mn, Re, $^{99m}$Tc). Starting from fully protected HC$_5$H$_5$-aa (aa = amino acid), [(Cp-aa)$^{99m}$Tc(CO)$_3$] complexes are accessible in quantitative yields and in a one-step synthesis from [$^{99m}$TcO$_4$]$^-$. The rhenium and manganese analogues were prepared and structurally characterized to confirm the authenticity of the $^{99m}$Tc complex. The inhibition constant of natural phenylalanine (phe) for LAT1 is in the range 70 ± 10 μM. The $K_i$ value of [(Cp-CH(NH$_2$)COOH)Mn(CO)$_3$] (**1a**) is 53 ± 11 μM, whereas $K_i$ for the "true" phe analogue [(Cp-CH$_2$CH(NH$_2$)COOH)Mn(CO)$_3$] (**2**) was surprisingly high at 277 ± 37 μM. Complex **1a** caused efflux when exposed to cells, underlining its active transport by LAT1 into the cell. $^{99m}$Tc analogues of small biological lead structures such as amino acids are generally not recognized anymore by their targets, in particular by trans-membrane transporters. The bioorganometallic analogues presented here are, however, actively transported and corroborate the importance of organometallic complexes as mimics of organic lead structures in life sciences.




## INTRODUCTION

Among the different modalities for diagnostic molecular imaging, single photon emission computed tomography (SPECT) with $^{99m}$Tc is, quantitatively, still the most important one and represents roughly 85% of clinically administered radiopharmaceuticals. For targeted imaging, larger biomolecules can conveniently be labeled with relatively bulky $^{99m}$Tc complexes without substantial loss of affinities.[6−11] The labeling of small biomolecules with $^{99m}$Tc complexes represents a distinct challenge since the label often governs the structure, the biological pathways, and the affinities of the vector. Central nervous system receptor ligands,[12,13] carbohydrates (glucose),[14−19] amino acids,[20] or pharmaceutically active lead structures are examples of such small molecules.[21,22] Since complex size plays a crucial role in affecting bioactivity of small molecules, the compact cyclopentadienyl (Cp) ligand, one of the smallest tridentate entities, is particularly attractive. Our recent "one-pot" syntheses of [(Cp-R)$^{99m}$Tc(CO)$_3$]-type complexes enables the application of Cp in aqueous systems as a complementation to the generally used Werner-type ligands. Besides being small, the mimicking of phenyl rings by piano-stool-type [CpM(CO)$_3$] in organic lead structures, as pioneered by, for example, Jaouen and co-workers, is an additional important feature with regard to structure recognition by receptors.[2,5,23−26]

We are particularly interested in labeling the α-amino acid function with $^{99m}$Tc complexes for targeting the L-type amino acid transporter 1 (LAT1). LAT1 was shown to be overexpressed in many tumor cell lines, such as PA-1 tetratocarcinoma cells, T24 bladder carcinoma cells, RERF-LC-MA lung small-cell carcinoma cells, and HeLa uterine cervical carcinoma cells.[27] It plays an essential role in the cellular supply of important amino acids, needed for cell growth and tissue development. LAT1 is the transporter for, for example, tyrosine and phenylalanine, mainly labeled with $^{18}$F or $^{123/125}$I but rarely with $^{99m}$Tc.[28−31] Previously, we could show that L-lysine derivatized with 2,3-diaminopropionic acid (DAP) as ligand at its ε-position and labeled with the *fac*-{$^{99m}$Tc(CO)$_3$}$^+$ core was still recognized by LAT1, albeit at a low $K_i$ = 300 μM, and actively internalized into cells.[20] Despite being the first small molecule labeled with $^{99m}$Tc and actively transported through a

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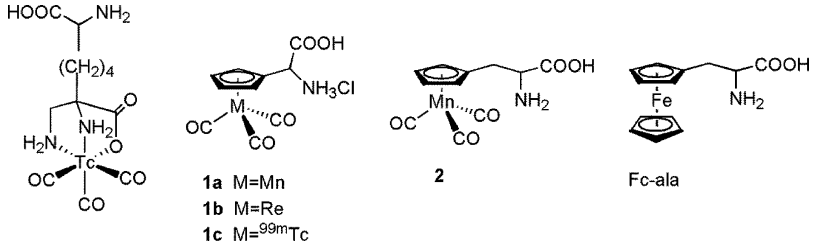
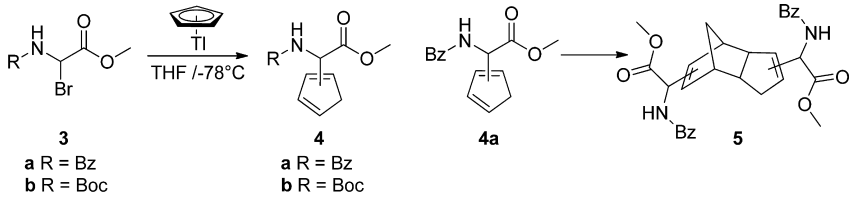
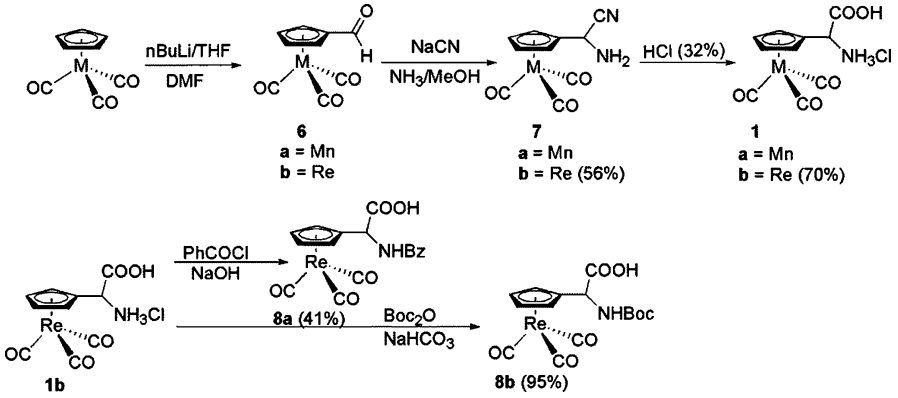
**Scheme 1. Unnatural Amino Acid Analogues Recognized and Transported by LAT1, DAP Derivative of L-Lysine (left),[20] and Ferrocene Mimic of Phenylalanine (right)[37]**




**Scheme 2. Synthesis of Cyclopentadiene-Based Unnatural Amino Acid Precursors Cp-CH(NHR)COOCH$_3$ (R = Bz, Boc) (4a,b)**




**Scheme 3. Syntheses of the Metal-Based Amino Acids 1a and 1b and the Corresponding −NH$_2$-Protected Analogues 8a and 8b**




membrane, $K_i$ values could not be improved by structural alterations. In order to achieve a better structural match between the natural substrate phenylalanine and the $^{99m}$Tc-labeled mimic, we adopted the phenyl-cyclopentadienyl analogy and replaced the phenyl ring by piano-stool-type [(Cp-R)M(CO)$_3$] complexes. A variety of such analogues, e.g., [Fc-CH$_2$-CH(NH$_2$)COOH] and others, have been prepared, but their biological activity has not been investigated.[32−37] We report here the synthesis of organometallic amino acid analogues [(Cp-CH(NH$_2$)COOH)M(CO)$_3$] (**1a**, M = Mn, **1b**, M = Re, **1c**, M = $^{99m}$Tc) and [(Cp-CH$_2$CH(NH$_2$)COOH)-Mn(CO)$_3$] (**2**) and their interactions with LAT1 in comparison to Fc-ala and the DAP derivative of L-lysine. The synthesis of the fully protected organic Cp precursor is described together with labeling studies, yielding [(Cp-CH(NH$_2$)COOH)$^{99m}$Tc-(CO)$_3$] (**1c**) in quantitative yields and under moderate conditions. Since $K_i$ with LAT1 of **1c** is slightly lower than that of phenylalanine, complex **1c** represents a true, $^{99m}$Tc-labeled amino acid analogue. The basic structures of organometallic amino acid analogues are depicted in Scheme 1.

## RESULTS AND DISCUSSION

The labeling of cyclopentadienyl derivatives with the *fac*-{$^{99m}$Tc(CO)$_3$}$^+$ core requires either a monomeric precursor or its Diels−Alder dimer. The preparation of the benzoyl-protected compound C$_5$H$_5$-CH(NHBz)COOCH$_3$ (**4a**) followed a previously published procedure and was adapted for the corresponding Boc-protected molecule **4b**.[36] Accordingly, fully protected and brominated glycine **3a** and **3b** were reacted with TlCp in THF at −78 °C, which gave **4a** and **4b** in about 75% yield (Scheme 2). The $^1$H NMR of **4a**/**b** showed an isomeric mixture of the 2′- or the 3′-substituted products in a ratio of about 1:1 (Supporting Information, SI). The olefinic Cp signals appear as multiplets from 6.6 to 6.3 ppm. Notably, whereas **4a** underwent Diels−Alder dimerization to **5** in hexane within 24 h, such a reaction was not observed for **4b**, likely due to steric constraints as imposed by the bulky Boc protecting groups. It was not possible to fully deprotect **4a** or **4b** in order to access the respective free amino acid form. Cyclopentadiene is sensitive to the deprotection conditions and underwent cross reactions such as amination of the double bonds or polymerization. For this reason, labeling studies were performed with the fully protected amino acid precursors (*vide infra*).

For structural characterization of the microscopic amounts of $^{99m}$Tc complexes ($t_{1/2}$ = 6 h), comparison of HPLC retention times of the fully characterized Re or Mn homologues with $^{99m}$Tc complexes is an accepted methodology.[38−42] Accordingly, [(Cp-CH(NH$_2$)COOH)Re(CO)$_3$] (**1b**), [(Cp-CH(NHBz)COOH)Re(CO)$_3$] (**8a**), and [(Cp-CH(NHBoc)-COOH)Re(CO)$_3$] (**8b**) were synthesized and characterized by ESI-MS, $^1$H NMR, IR, and X-ray crystallography for **1a**, **1b**,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and **7a** (Scheme 3 and SI). The Mn complex **1a** was also synthesized for studying the competitive binding and uptake with LAT1.

[(Cp-CHO)M(CO)$_3$] **6a,b** were synthesized from [CpM(CO)$_3$][43] and compounds **7a,b** from a modified Strecker synthesis[44,45] as a mixture of enantiomers in 56% yield. The $^1$H NMR spectrum of **7b** in $CDCl_3$ showed the aromatic Cp signals as multiplets at 5.69, 5.67, 5.38, and 5.31 ppm, respectively, instead of the often found pseudotriplets due to the adjacent stereocenter at the $\alpha$-position. The enantiotopic $\alpha$-proton C*H*(CN)(NH$_2$) was observed as a singlet at 4.79 ppm (SI).[44,45] Acidic hydrolysis of **7b** led to the unnatural amino acid analogue [(Cp-CH(NH$_2$)(COOH)Re(CO)$_3$] (**1b**) in 70% yield (Scheme 3). The $^1$H NMR spectrum in DMSO showed the Cp signals as multiplets at 6.02, 5.89, 5.67, and at 5.62 ppm, respectively. A singlet at 4.88 ppm indicated the $\alpha$-proton C*H*(NH$_2$)(COOH) and a broad singlet the N*H*$_2$ at 8.73 ppm. Complexes **1a**, **1b**, and **7a** were crystallized and their structures elucidated by X-ray analysis (Figure 1 and SI). An ORTEP of **1b** is given in Figure 1. The Re center is $\eta^5$-coordinated to the C$_5$H$_4$-CH(NH$_2$)(COOH) ligand, and the three CO groups complete the distorted octahedral coordination geometry. The structure of the manganese complex **1a** is isomorphous to **1b**. All three compounds crystallized in a nonracemic space group. Complexes **1a** and **1b** are the models for their $^{99m}$Tc-labeled homologues. They serve for the determination of $K_i$ values with LAT1 and for HPLC confirmation of the $^{99m}$Tc structure of complex **1c**.

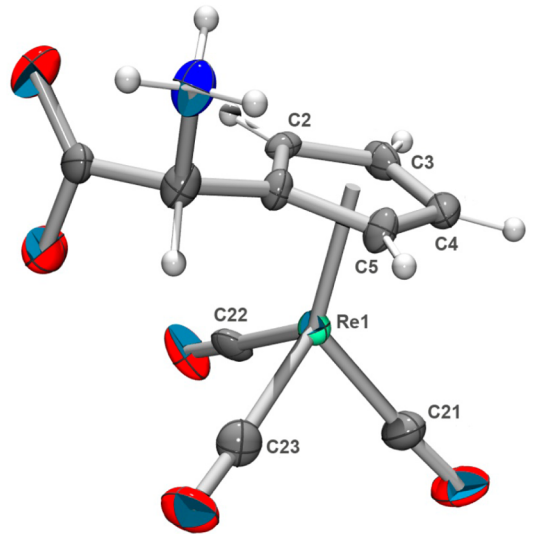


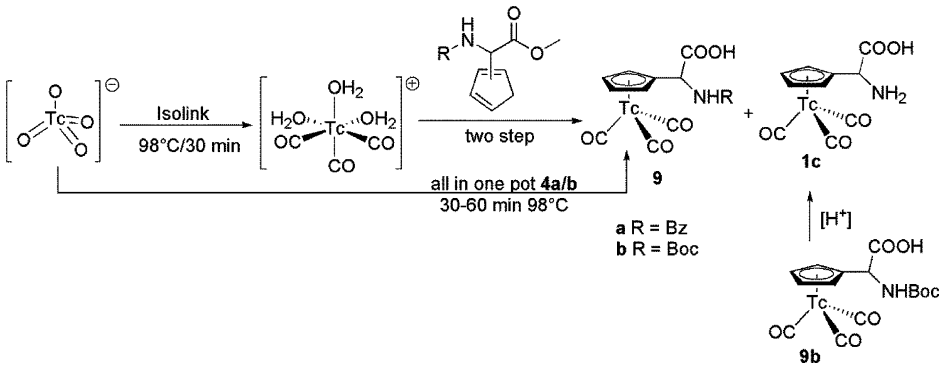

**Figure 1.** ORTEP presentation of [(Cp-CH(NH$_2$)COOH)Re(CO)$_3$] xHCl (contains one HCl) **1b**. Proton of COOH, chloride anion and water molecule omitted for clarity. Important bond lengths (Å) and angles (deg) are as follows: Re(1)−C(21) = 1.925(9); Re(1)−C(22) = 1.929(9); Re(1)−C(23) = 1.918(11); Re(1)−C(1) = 2.282(8); Re(1)−C(5) = 2.303(8); C(23)−Re(1)−C(21) = 90.5(4); C(23)−Re(1)−C(22) = 89.8(4); C(21)−Re(1)−C(22) = 89.9(4).

The reaction of **1b** with benzoyl chloride or di-*tert*-butyldicarbonate (Boc$_2$O) gave [(Cp-CH(NHBz)(COOH)Re(CO)$_3$] (**8a**) and [(Cp-CH(NHBoc)(COOH)Re(CO)$_3$] (**8b**) in 41% and 95% yield, respectively. Since we expected that labeling of **4a** or **4b** under alkaline conditions would result in ester hydrolysis but not in cleavage of the respective NH$_2$ protecting group, the carboxylate groups in **8a** and **8b** were not esterified. Compounds **8a** and **8b** represent therefore models of $^{99m}$Tc intermediates during the preparation of **1c**. For later comparison of $K_i$ values, the unnatural amino acids **2** and Fc-CH$_2$-CH(NH$_2$)COOH were prepared according to literature methods (Fc = ferrocene).[33,44,46]

The preparation of Cp complexes in water is not an obvious method. For complexes comprising $^{99m}$Tc however, direct synthesis in water is crucial in light of routine application. As reported, Diels−Alder dimers of the Cp framework are versatile starting materials for preparing [(Cp-R)$^{99m}$Tc(CO)$_3$] in water,[3−5,25] although starting directly from the corresponding monomer would be more convenient. Indeed, some cyclopentadiene derivatives such as **4b** dimerize very slowly, while others, such as **4a**, dimerize in relatively short time at rt.

For the formation of [(Cp-CH(NHBz)COOH)$^{99m}$Tc(CO)$_3$] (**9a**), [(Cp-CH(NHBoc)COOH)$^{99m}$Tc(CO)$_3$] (**9b**), and [(Cp-CH(NH$_2$)COOH)$^{99m}$Tc(CO)$_3$] (**1c**), two different methods were employed (Scheme 4). In the "one-pot" method, [$^{99m}$TcO$_4$]$^-$ is mixed with all the components as in the Isolink Kit and the cyclopentadiene derivatives **4a** or **4b**. At 90−95 °C the $^{99m}$Tc complexes were received in 70−98% yield. In the "two-step" method, [$^{99m}$Tc(OH$_2$)$_3$(CO)$_3$]$^+$ is prepared in >98% yield after 30 min at 95 °C and was then mixed with the corresponding cyclopentadiene derivative. Both methods exhibited the same labeling behavior and product distribution. It is remarkable that these organometallic piano-stool complexes are received under fully aqueous conditions and at boiling water temperature without relevant side products. It seems that, for example, product **1c** is a strong thermodynamic

**Scheme 4. Two-Step and One-Pot Syntheses of Piano-Stool Complexes with $^{99m}$Tc Yielding the Ester Hydrolyzed but −NH$_2$-Protected Acid Precursors 9a and 9b; Complete Deprotection under Mild Acidic Conditions to 1c**





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sink, representing the only (stable) product under the synthetic conditions.

The $^{99m}$Tc complex **9a** was obtained after 30 min in 91% yield by the "one-pot" method with **4a**. The reaction of **4b** with $[^{99m}Tc(OH_2)_3(CO)_3]^+$ at 90 °C for 30 min gave **9b** in 80% yield, and prolonged heating (60 min) increased the yield to 94% for both reactions. Compound **4b** reacted directly with $[^{99m}TcO_4]^-$ to give **9b** in 77% yield. About 7% Boc deprotection from **9b** occurred during the reaction, which directly led to **1c** (Scheme 4). Prolonged heating (180 min) increased the yield of **1c** (50% after 3 h and >95% after 4 h, Figure 2). Ester groups were rapidly and completely hydrolyzed

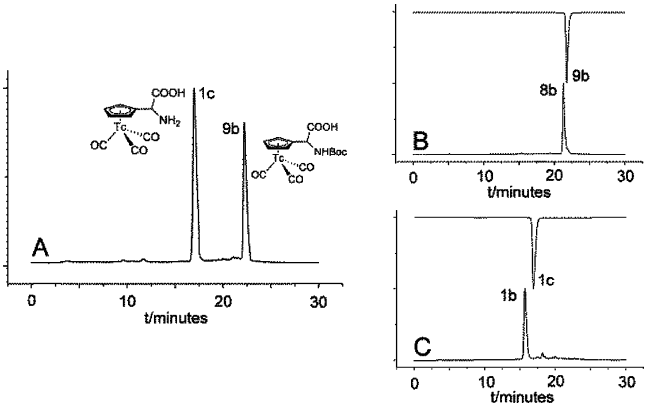


**Figure 2.** (A) HPLC $\gamma$-trace of the reaction products received after one-pot reaction (180 min, 90 °C) of $[^{99m}TcO_4]^-$ with **4b**; peak at 16.9 min represents **1c**, peak at 22.2 min **9b**; (B) co-injection of Re (bottom-up, **8b**) and $^{99m}$Tc homologue (top-down, **9b**); (C) co-injection of Re (bottom-up, **1b**) with $^{99m}$Tc (top-down, **1c**).

in all cases during the labeling procedure. We noted that the reaction with the Diels–Alder product **5** did not give any product at all, in sharp contrast to reactions with all other Thiele's acid derivatives reported so far.[3−5] The relatively bulky benzoyl protecting group is probably responsible for this unreactivity.

The authenticity of the $^{99m}$Tc complexes was confirmed by comparing the HPLC retention times with their rhenium homologues. Figure 2A shows the trace as received after the one-pot reaction of $[^{99m}TcO_4]^-$ with **4b**. The peak at about 22 min represents **9b** with the hydrolyzed ester function. The peak at 16.9 min corresponds to fully deprotected **1c**. Both peaks were separated by HPLC and co-injected with the respective rhenium homologues (Figure 2B and C). Whereas **8b** and **9b** exhibited the same retention times (difference due to detector separation), **1b** and **1c** differed by slightly more than one minute. Occasionally, $Re^I$ and $^{99m}Tc^I$ homologues are not fully identical in structure or, for example, lipophilicity, exhibiting thus different retention times and rendering authenticity questionable. To corroborate the identity of peaks **1b** and **1c** (Figure 2C), we compared the rate of acidic Boc deprotection in homologues **8b** (Re) and **9b** ($^{99m}$Tc). If the conversion of **9b** → **1c** corresponds to a reaction not involving Boc deprotection, thus leading to a compound different from **1b**, kinetics should be different as compared to conversion of **8b** → **1b**. Therefore, **9b** was isolated by HPLC, the pH was adjusted to 2−3, and the solution was heated to 90 °C for 4 h. The same procedure was applied to the Re homologue **8b**. The rates of conversion for Re and $^{99m}$Tc are identical, implying the same Boc deprotection reaction taking place and, consequently, the formation of the same products (Figure 3). Thus, **1b** and **1c** are homologues despite the differences in retention times.

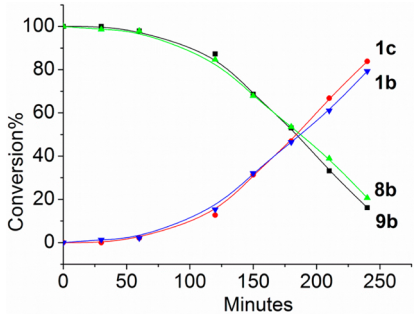


**Figure 3.** Conversion rates of **9b** → **1c** ($^{99m}$Tc) and **8b** → **1b** (rhenium).

We investigated the biological behavior by quantitatively assessing the competitive inhibition constant $K_i$ to and transport by LAT1 as described in the Experimental Section and SI. Lineweaver–Burk plots were used to study the type of inhibition of the amino acid analogue from their inhibition potential of the [$^3$H]-L-phe/L-phe couple. The apparent $K_m$ and corresponding $K_i$ values were calculated by means of Eadie–Hofstee plots. In addition, as LAT1 is an amino acid antiport system, cell uptakes of the organometallic amino acids **1a**, **2**, and Fc-ala (enantiomeric mixtures) were measured by monitoring the efflux of [$^3$H]-L-phe, preloaded into the cells before incubation with substrates. Compound **1a**, [(Cp-$CH(NH_2)COOH)Mn(CO)_3$], displayed a $K_i$ = 53 ± 11 $\mu$M, thus, a higher affinity than the natural substrate phe (73 ± 10 $\mu$M) itself.[20] Unexpectedly, the "true" phe analogue **2**, [(Cp-$CH_2CH(NH_2)COOH)Mn(CO)_3$], has a $K_i$ value of 277 ± 37 $\mu$M, about 4 times higher than phe (Figure 4). The ferrocene-

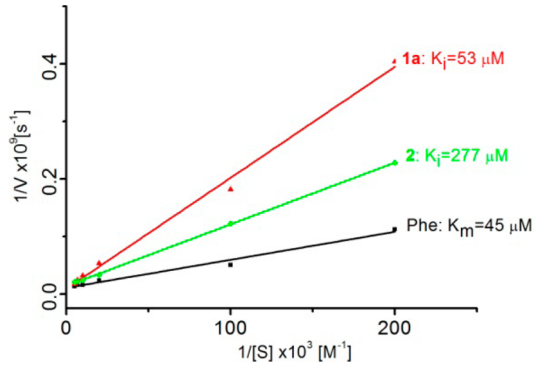


**Figure 4.** Lineweaver–Burk plot: competitive binding assays of **1a** and **2** to LAT1 ($K_i$ values). The common intercept on the *y*-axis indicates a competitive interaction between L-phe and the inhibitors **1a** and **2**, meaning they bind to the same binding pocket on the LAT1 transport sytem.

based amino acid Fc-ala on the other hand showed again a high $K_i$ value of 60 $\mu$M, well comparable with phenylalanine. Since **1a** and Fc-$CH_2$-$CH(NH_2)COOH$ stimulate efflux of [$^3$H]-L-phe, comparable to the DAP derivative of L-lysine,[20] they are actively transported into the cells. Quantitatively, the highest efflux is caused by Fc-ala (25 ± 7%), followed by **1a** or the DAP derivative (≈14 ± 4%). **2** was not actively transported into cells. We noticed that **2** reduced the efflux stimulating capacity of 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH).


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This can only point to an antagonistic effect, where **2** binds to the outward binding sites of the amino acid transport system, but fails to be actually transported across the membrane. This *in vitro* behavior clearly indicates compounds **1a** and Fc-ala to be substrates for LAT1 and not just inhibitors of the protein, whereas **2** can be regarded as a competitive inhibitor.

## CONCLUSION

Small, natural biomolecules such as amino acids generally do not tolerate structural changes without substantial or, mostly, complete loss of their biological functions. Replacing a phenyl ring in such small molecules by cyclopentadienyl complexes may reduce the structural impact to the lead structure (phenyl-Cp analogy). This has been shown by the syntheses and the biological behavior of the organometallic amino acid analogues [(Cp-CH($NH_2$)COOH)M(CO)$_3$] (M = Mn, Re, $^{99m}$Tc). This compound is recognized by LAT1 with a $K_i$ comparable to the natural substrate phenylalanine. Since the homologue with $^{99m}$Tc can be prepared in a one-pot reaction, the corresponding complex may well serve as a molecular imaging agent for visualizing rapidly growing cells such as cancer. Corresponding *in vivo* investigations are ongoing. We also extend the previously reported methods for the preparation of cyclopentadienyl complexes of technetium to derivatives that do not need to be Diels–Alder dimerized but for which the monomeric precursors can yield piano-stool complexes in water directly. With this study, we also introduce a new example for the use of identical compounds for combined therapy and imaging in a theranostics sense.[4,38,39] Cold rhenium-based compound **1b** has potential for therapy (inhibitor), and the homologous complex with $^{99m}$Tc **1c** can serve as imaging agents for single photon emission computed tomography.

## EXPERIMENTAL SECTION

**General Procedures.** Reactions were carried out in dried glassware under a $N_2$ atmosphere. Solvents were dried using standard techniques and stored over molecular sieves. All chemicals were obtained from commercial sources and used without further purification. Compounds **4a**, **5**,[36] methyl 2-bromo-2-((*tert*-butoxycarbonyl)amino)acetate,[47] and rhenium complex **7b**[43] were prepared according to literature procedures. Manganese complexes (**1a**, **6a**, **7a**) were prepared in the same way as the corresponding rhenium complexes. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker Advance 400 or 500 MHz spectrometer. Mass spectra were measured on a Bruker Esquire HCT (ESI) instrument. Elemental analyses were performed on a Leco CHNS-932 elemental analyzer. IR spectra were recorded as KBr pellets on a Perkin-Elmer BX II IR spectrometer.

RP-HPLC was performed on a Merck Hitachi LaChrom L7200 tunable UV detector and a radiodetector, separated by a Teflon tube, which causes about a 0.4–0.7 min delay compared to UV/vis detection. UV/vis detection was performed at 250 nm. The detection of radioactive $^{99m}$Tc complexes was performed with a Berthold LB 507 radiodetector equipped with a NaI(Tl) scintillation detector. Analytical separations were performed on a Nucleosil C-18 column (100 Å, 5 μm, 250 × 4 mm). Analytical columns were eluted with a flow rate of 0.5 mL min$^{-1}$ using 0.1% TFA in $H_2O$ (solvent A) and methanol (solvent B) as eluents with a variable gradient (0–3 min, 100% A; 3–3.1 min, 0 to 25% B; 3.1–9 min, 25% B; 9–9.1 min, 25% B to 34% B; 9.1–20 min, 34% B to 100% B; 20–25 min, 100% B; 25–25.1 min, 100% B to 100% A; 25.1–30 min, 100% A).

**[Cp-CH(NHBoc)COOCH$_3$] (4b).** According to the literature procedure,[36] **4b** was synthesized as follows: CpTl (435.8 mg, 1.62 mmol) was added to the THF (10 mL) solution of **3b** (412 mg, 1.54 mmol) at −78 °C. The mixture was stirred in the dark for 5 h and was then allowed to reach room temperature. Solvents were removed under reduced pressure, and the residue was purified by silica gel column chromatography ($CH_2Cl_2$) to give an isomeric mixture of **4b** as a colorless oil (72%).

$^1$H NMR (500 MHz, *d*-$CDCl_3$) δ/ppm: 6.52–6.30 (m, 6H, Cp), 5.30 (d, $J_{HH}$ = 16.50 Hz, 2H, N*H*), 5.20 (d, $J_{HH}$ = 16.50 Hz, 2H, C*H*), 3.75 (s, 6H, C$H_3$), 3.00 (s, 4H, Cp), 1.48 (s, 18H, Boc). IR (KBr): 1616 cm$^{-1}$ (−COOMe). Calcd for $M_r$ ($C_{13}H_{19}NO_4$): 253.13. ESI-MS [*m*/*z*] ($CH_3OH$, pos. mode): 276.2 [M + Na]$^+$. Anal. Calcd for $C_{13}H_{19}NO_4$: C, 61.64; H, 7.56; N, 5.53. Found: C, 61.27; H, 7.31; N, 5.25.

**[(Cp-CH($NH_2$)CN)Re(CO)$_3$] (7b).** Following a modified Strecker synthesis,[44,45] NaCN (16.33 mg, 0.33 mmol), $NH_4Cl$ (8.92 mg, 0.17 mmol), and $MgSO_4$ (20.06 mg, 0.17 mmol) were added to a saturated solution of $NH_3$ in MeOH (5 mL) and stirred at 0 °C. **6b** was added, and the reaction mixture was stirred for 1 h at room temperature and for 3 h at 30 °C. The solvent was removed *in vacuo*, and the residue was taken into diethyl ether. Filtration and evaporation of the solvent afforded an orange solid, which was purified by silica gel column chromatography (diethyl ether/hexane, 4:1) to give **7b** (56%).

$^1$H NMR (500 MHz, *d*-$CDCl_3$) δ/ppm: 5.69–5.65 (m, 2H, Cp), 5.38–5.30 (m, 2H, Cp), 4.79 (s, 1H, C*H*). IR (KBr): 2022, 1918 cm$^{-1}$ (Re-CO). Calcd for $M_r$ ($C_{10}H_{10}N_2O_3Re$): 393.02. ESI-MS [*m*/*z*] ($CH_3OH$, pos. mode): 415.9 [M + Na]$^+$. ESI-MS [*m*/*z*] ($CH_3OH$, neg. mode): 391.9 [M − H]$^-$. Anal. Calcd for $C_{10}H_{10}N_2O_3Re$: C, 30.61; H, 2.57; N, 7.14. Found: C, 30.13; H, 2.27; N, 6.98.

**[(Cp-CH($NH_3$Cl)COOH)Re(CO)$_3$] (1b).** **7b** (200 mg, 0.45 mmol) was hydrolyzed by refluxing in HCl (5 mL, 32%). The reaction was followed by HPLC. After 3 h, the mixture was filtered and extracted with methyl *tert*-butyl ether. The aqueous layer was then dried under reduced pressure, and **1b** was obtained (70%).

$^1$H NMR (400 MHz, $d_6$-DMSO) δ/ppm: 8.73 (s, 2H, N$H_2$), 6.02 (s, 1H, Cp), 5.88 (s, 1H, Cp), 5.67 (s, 1H, Cp), 5.62 (s, 1H, Cp), 4.88 (s, 1H, C*H*). $^{13}$C NMR (125 MHz, $d_6$-DMSO) δ/ppm: 168.48 (CO), 101.91 (*C*OOH), 99.66 (Cp1), 93.43 (Cp2), 87.35 (Cp3), 87.00 (Cp4), 83.11 (Cp5), 81.02 (Cp-CH). IR (KBr): 2021, 1941, 1908 cm$^{-1}$ (Re-CO), 1636 cm$^{-1}$ (−COOH). Calcd for $M_r$ ($C_{10}H_8NO_5Re$): 408.38. ESI-MS [*m*/*z*] ($CH_3OH$, pos. mode): 392.9 [M − $NH_2$ + H]$^+$, 431.9 [M + Na]$^+$. ESI-MS [*m*/*z*] ($CH_3OH$, neg. mode): 407.9 [M − H]$^-$, 364.0 [M + COOH]$^-$, 443.8 [M + Cl]$^-$. Anal. Calcd for $C_{10}H_8NO_5Re$: C, 27.00; H, 2.04; N, 3.15. Found: C, 26.94; H, 2.01; N, 3.09.

**[(Cp-CH(NHBz)COOH)Re(CO)$_3$] (8a).** A solution of **1b** (50 mg, 0.112 μmol) in NaOH (2.0 M, 25 mL) was cooled to 0 °C with an ice bath. Benzoyl chloride (15 μL, 124 μmol) was added, and the reaction mixture was allowed to warm to room temperature. After 2 h, the pH was adjusted to 5–6 by adding HCl (1.0 M), and the reaction mixture was extracted with ethyl acetate (3 × 25 mL). The combined organic phases were dried over magnesium sulfate and evaporated *in vacuo*. The solid residue was purified by silica gel column chromatography (EtOAc/hexane, 2:1) to afford **8a** (41%).

$^1$H NMR (400 MHz, $d_4$-MeOH) δ/ppm: 7.88–7.85 (m, 2H, Ar), 7.57–7.52 (m, 1H, Ar), 7.49–7.44 (m, 2H, Ar), 5.93–5.84 (m, 2H, Cp), 5.54 (s, 1H, C*H*), 5.45–5.39 (m, 2H, Cp). IR (KBr): 2020, 1921 cm$^{-1}$ (Re-CO), 1696, 1642 cm$^{-1}$ (−COOH). Anal. Calcd for $C_{17}H_{12}NO_6Re$: C, 39.84; H, 2.36; N, 2.73. Found: C, 39.63; H, 2.27; N, 2.56.

**[(Cp-CH(NHBoc)COOH)Re(CO)$_3$] (8b).** $NaHCO_3$ (56.7 mg, 674.4 μmol) and di-*tert*-butyl dicarbonate (58.9 mg, 269.8 μmol) were added consecutively to a solution of **1b** (100 mg, 224.8 μmol) in a 1:1 mixture of THF/$H_2O$ (10 mL) at 0 °C. After 45 min, the solution was allowed to reach room temperature and was stirred for 18 h. The solution was then extracted two times with methyl *tert*-butyl ether. The aqueous layer was acidified to pH = 4–5 with 0.1 M HCl at 0 °C and then extracted two times with $CH_2Cl_2$. The combined $CH_2Cl_2$ phases were dried over $MgSO_4$ and evaporated *in vacuo* to give **8b** (95%).

$^1$H NMR (400 MHz, $d_4$-MeOH) δ/ppm: 5.73–5.70 (m, 2H, H), 5.35–5.32 (m, 2H, H), 3.35 (s, 1H, H), 1.46 (s, 9H, H). IR (KBr): 2016, 1926, 1893 cm$^{-1}$ (Re-CO), 1636, 1614 cm$^{-1}$ (−COOH). Anal.


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Calcd for $C_{15}H_{16}NO_7Re$: C, 36.50; H, 4.21; N, 2.66. Found: C, 36.37; H, 4.15; N, 2.46.

**X-ray Crystallography.** Crystallographic data were collected at 183(2) K on an Oxford Diffraction Xcalibur system with a Ruby detector using Mo K$\alpha$ radiation ($\lambda$ = 0.7107 Å) that was graphite-monochromated. Suitable crystals were covered with oil (Infineum V8512, formerly known as Paratone N), mounted on top of a glass fiber, and immediately transferred to the diffractometer. The program suite CrysAlis$^{Pro}$ was used for data collection, multiscan absorption correction, and data reduction.[48] The structures were solved with direct methods using SIR97[49] and refined by full-matrix least-squares methods on $F^2$ with SHELXL-97.[50] The crystal of **1b** was nonmerohedrally twinned by a 2-fold rotation around the following vector in reciprocal space (*hkl*): 0.9131 0.000 4−0.4076. The two twin domains were present in a ratio of 76:24. The twin refinement was performed with the hklf5 command of SHELXL. CCDC 881056 (**7a**), 881057 (**1b**), and 881280 (**1a**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Center via www.ccdc.cam.ac.uk/data_request/cif.

**Biological Assay.** *Cell Cultures.* R1M rhabdomyosarcoma cells (VUB) were cultivated as described earlier.[20]

*In Vitro Experiments.* All *in vitro* experiments were carried out in six-well plates (VWR), using at least three wells for each data point. Cells were counted by means of a Bürker counting chamber. Influx and efflux were studied in a $Na^+$-free buffer (HEPES: pH 7.4; 100 mM choline-Cl (Sigma), 2 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM Hepes, 5 mM Tris, 1 g/L glucose, and 1 g/L bovine serum albumin) and MEM buffer (pH = 7.2, containing essential and nonessential amino acids of which 1.2 mM amino acids were known to be transported by the L transport system). The process was terminated by physical withdrawal of the buffer and washing three times with ice-cold phosphate-buffered saline (PBS). Subsequently, the cells were detached from the well with 2 mL of 0.1 M NaOH (VWR). The radioactivity of the samples was counted using a gamma counting system (Cobra-inspector 5003, Canberra Packard, Meriden, CT, USA).

*Inhibition of [$^3$H]-L-Phe Influx.* In these inhibition experiments the cells were incubated with 37kBq [$^3$H]-L-phe and different concentrations (10−200 $\mu$M) of L-phe for 1 min in HEPES buffer in the absence and presence of 100 $\mu$M amino acid analogues of interest.

*Trans-stimulation of [$^3$H]-L-Phe.* The cells were incubated with 37 kBq [$^3$H]-L-phe for 15 min in HEPES buffer. The incubation medium was removed, and the cells were washed three times with ice-cold PBS. Subsequently HEPES buffer containing 0.1 mM L-phe, BCH (LAT references), or amino acid analogues was added. The efflux medium was removed after 1 min, after which the cells were washed three times with ice-cold PBS, detached with 0.1 M NaOH, suspended, and counted.

## ■ ASSOCIATED CONTENT

### Supporting Information

NMR spectra and crystallographic data for compounds **1a**, **1b**, and **7a**, labeling, and HPLC conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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## ■ REFERENCES


(1) Schubiger, P. A.; Alberto, R.; Smith, A. *Bioconjugate Chem.* **1996**, *7*, 165−179.
(2) Wald, J.; Alberto, R.; Ortner, K.; Candreia, L. *Angew. Chem., Int. Ed.* **2001**, *40*, 3062−3066.
(3) N'Dongo, H. W. P.; Liu, Y.; Can, D.; Schmutz, P.; Spingler, B.; Alberto, R. *J. Organomet. Chem.* **2009**, *694*, 981−987.
(4) Can, D.; Spingler, B.; Schmutz, P.; Mendes, F.; Raposinho, P.; Fernandes, C.; Carta, F.; Innocenti, A.; Santos, I.; Supuran, C. T.; Alberto, R. *Angew. Chem., Int. Ed.* **2012**, *51*, 3354−3357.
(5) Liu, Y.; Spingler, B.; Schmutz, P.; Alberto, R. *J. Am. Chem. Soc.* **2008**, *130*, 1554−1556.
(6) Bartholoma, M. D.; Louie, A. S.; Valliant, J. F.; Zubieta, J. *Chem. Rev.* **2010**, *110*, 2903−2920.
(7) Achilefu, S. *Chem. Rev.* **2010**, *110*, 2575−2578.
(8) Yang, D. J.; Kim, E. E.; Inoue, T. *Ann. Nucl. Med.* **2006**, *20*, 1−11.
(9) Schwochau, K. *Angew. Chem., Int. Ed.* **1994**, *33*, 2258−2267.
(10) Heppeler, A.; Froidevaux, S.; Eberle, A. N.; Maecke, H. R. *Curr. Med. Chem.* **2000**, *7*, 971−994.
(11) Prasanphanich, A. F.; Lane, S. R.; Figueroa, S. D.; Ma, L. X.; Rold, T. L.; Sieckman, G. L.; Hoffman, T. J.; McCrate, J. M.; Smith, C. J. *In Vivo* **2007**, *21*, 1−16.
(12) Kung, M. P.; Stevenson, D. A.; Plossl, K.; Meegalla, S. K.; Beckwith, A.; Essman, W. D.; Mu, M.; Lucki, I.; Kung, H. F. *Eur. J. Nucl. Med.* **1997**, *24*, 372−380.
(13) Kung, H. F.; Kung, M. P.; Wey, S. P.; Lin, K. J.; Yen, T. C. *Nucl. Med. Biol.* **2007**, *34*, 787−789.
(14) Petrig, J.; Schibli, R.; Dumas, C.; Alberto, R.; Schubiger, P. A. *Chem.—Eur. J.* **2001**, *7*, 1868−1873.
(15) Schibli, R.; Dumas, C.; Petrig, J.; Spadola, L.; Scapozza, L.; Garcia-Garayoa, E.; Schubiger, P. A. *Bioconjugate Chem.* **2005**, *16*, 105−112.
(16) Mikata, Y.; Takahashi, K.; Noguchi, Y.; Naemura, M.; Ugai, A.; Itami, S.; Yasuda, K.; Tamotsu, S.; Matsuo, T.; Storr, T. *Eur. J. Inorg. Chem.* **2012**, 217−225.
(17) Storr, T.; Obata, M.; Fisher, C. L.; Bayly, S. R.; Green, D. E.; Brudzinska, I.; Mikata, Y.; Patrick, B. O.; Adam, M. J.; Yano, S.; Orvig, C. *Chem.—Eur. J.* **2005**, *11*, 195−203.
(18) Bowen, M. L.; Orvig, C. *Chem. Commun.* **2008**, 5077−5091.
(19) Ferreira, C. L.; Ewart, C. B.; Bayly, S. R.; Patrick, B. O.; Steele, J.; Adam, M. J.; Orvig, C. *Inorg. Chem.* **2006**, *45*, 6979−6987.
(20) Liu, Y.; Pak, J. K.; Schmutz, P.; Bauwens, M.; Mertens, J.; Knight, H.; Alberto, R. *J. Am. Chem. Soc.* **2006**, *128*, 15996−15997.
(21) Banerjee, S. R.; Maresca, K. P.; Francesconi, L.; Valliant, J.; Babich, J. W.; Zubieta, J. *Nucl. Med. Biol.* **2005**, *32*, 1−20.
(22) Liu, S. *Chem. Soc. Rev.* **2004**, *33*, 445−461.
(23) Jaouen, G.; Top, S.; Vessières, A.; Pigeon, P.; Leclercq, G.; Laios, I. *Chem. Commun.* **2001**, 383−384.
(24) Bernard, J.; Ortner, K.; Spingler, B.; Pietzsch, H. J.; Alberto, R. *Inorg. Chem.* **2003**, *42*, 1014−1022.
(25) N'Dongo, H. W. P.; Raposinho, P. D.; Fernandes, C.; Santos, I.; Can, D.; Schmutz, P.; Spingler, B.; Alberto, R. *Nucl. Med. Biol.* **2010**, *37*, 255−264.
(26) Top, S.; El Hafa, H.; Vessieres, A.; Quivy, J.; Vaissermann, J.; Hughes, D. W.; McGlinchey, M. J.; Mornon, J.-P.; Thoreau, E.; Jaouen, G. *J. Am. Chem. Soc.* **1995**, *117*, 8372−8380.
(27) Yanagida, O.; Kanai, Y.; Chairoungdua, A.; Kim, D. K.; Segawa, H.; Nii, T.; Cha, S. H.; Matsuo, H.; Fukushima, J.; Fukasawa, Y.; Tani, Y.; Taketani, Y.; Uchino, H.; Kim, J. Y.; Inatomi, J.; Okayasu, I.; Miyamoto, K.; Takeda, E.; Goya, T.; Endou, H. *Biochim. Biophys. Acta, Biomembr.* **2001**, *1514*, 291−302.
(28) Wang, H. E.; Wu, S. Y.; Chang, C. W.; Liu, R. S.; Lee, D.; Chen, J. C.; Chen, F. D. *J. Nucl. Med.* **2003**, *44*, 167P−167P.
(29) Kramer, S. D.; Mu, L. J.; Muller, A.; Keller, C.; Kuznetsova, O. F.; Schweinsberg, C.; Franck, D.; Muller, C.; Ross, T. L.; Schibli, R.; Ametamey, S. M. *J. Nucl. Med.* **2012**, *53*, 434−442.
(30) Kersemans, V.; Cornelissen, B.; Kersemans, K.; Bauwens, M.; Achten, E.; Dierckx, R. A.; Mertens, J.; Slegers, G. *J. Nucl. Med.* **2005**, *46*, 532−539.

(31) Kersemans, V.; Cornelissen, B.; Kersemans, K.; Bauwens, M.; Dierckx, R. A.; De Spiegeleer, B.; Mertens, J.; Slegers, G. *Eur. J. Nucl. Med. Mol. Imaging* **2006**, *33*, 919−927.
(32) Meder, H.-J.; Beck, W. *Z. Naturforsch.* **1986**, *41b*, 1247−1254.
(33) Severin, K.; Bergs, R.; Beck, W. *Angew. Chem., Int. Ed.* **1998**, *37*, 1637−1654.
(34) Krämer, R.; Maurus, M.; Bergs, R.; Polborn, K.; Sünkel, K.; Wagner, B.; Beck, W. *Chem. Ber.* **1993**, *126*, 1969−1980.
(35) Dialer, H.; Polborn, K.; Ponikwar, W.; Sunkel, K.; Beck, W. *Chem.—Eur. J.* **2002**, *8*, 691−699.
(36) Dialer, H.; Steglich, W.; Beck, W. *Tetrahedron* **2001**, *57*, 4855−4861.
(37) Osgerby, J. M.; Pauson, P. L. *J. Chem. Soc.* **1958**, 656−660.
(38) Ozdemir, V.; Williams-Jones, B. *Nat. Biotechnol.* **2006**, *24*, 1324−1326.
(39) Pene, F.; Courtine, E.; Cariou, A.; Mira, J. P. *Crit. Care Med.* **2009**, *37*, S50−S58.
(40) Dilworth, J. R.; Parrott, S. J. *Chem. Soc. Rev.* **1998**, *27*, 43−55.
(41) Alberto, R. *J. Organomet. Chem.* **2007**, *692*, 1179−1186.
(42) Alberto, R. In *Bioinorganic Medicinal Chemistry*; Alessio, E., Ed.; Wiley-VCH: Weinheim, 2011; pp 253−282.
(43) Heldt, J.-M.; Fischer-Durand, N.; Salmain, M.; Vessières, A.; Jaouen, G. *J. Organomet. Chem.* **2004**, *689*, 4775−4782.
(44) Brunet, J. C.; Cuingnet, E.; Gras, H.; Marcincal, P.; Mocz, A.; Sergheraert, C.; Tartar, A. *J. Organomet. Chem.* **1981**, *216*, 73−77.
(45) Hromadoa, M.; Salmain, M.; Sokolova, R.; Pospisil, L.; Jaouen, G. *J. Organomet. Chem.* **2003**, *668*, 17−24.
(46) Kira, M.; Matsubara, T.; Shinohara, H.; Sisido, M. *Chem. Lett.* **1997**, *26*, 89−90.
(47) Armstrong, A.; Edmonds, I. D.; Swarbrick, M. E.; Treweeke, N. R. *Tetrahedron* **2005**, *61*, 8423−8442.
(48) *Xcalibur CCD System*, 171.32 ed.; Oxford Diffraction Ltd.: Oxford, UK, 2007.
(49) Altomare, A.; Burla, M. C.; Camalli, M.; Cascarano, G. L.; Giacovazzo, C.; Guagliardi, A.; Moliterni, A. G. G.; Polidori, G.; Spagna, R. *J. Appl. Crystallogr.* **1999**, *32*, 115−119.
(50) Sheldrick, G. M. *Acta Crystallogr.* **2008**, *A64*, 112−122.