J|A|C|S
COMMUNICATIONS

Published on Web 12/01/2006

Amino Acids Labeled with $[^{99m}Tc(CO)_3]^+$ and Recognized by the L-type Amino Acid Transporter LAT1

Yu Liu,[†] Jae Kyoung Pak,[†] Paul Schmutz,[†] Matthias Bauwens,[‡] John Mertens,[‡] Hector Knight,[§] and Roger Alberto*,[†]

Institute of Inorganic Chemistry, University of Zürich, Winterthurerstr. 190, 8057 Zürich, Switzerland, Radiopharmaceutical Chemistry, Vrije Universiteit Brussels, Laarbeeklaan103, 1090 Brussels, Belgium, and Mallinckrodt Medical B.V., NL-1755 Petten, The Netherlands

Received August 18, 2006; E-mail: ariel@aci.unizh.ch

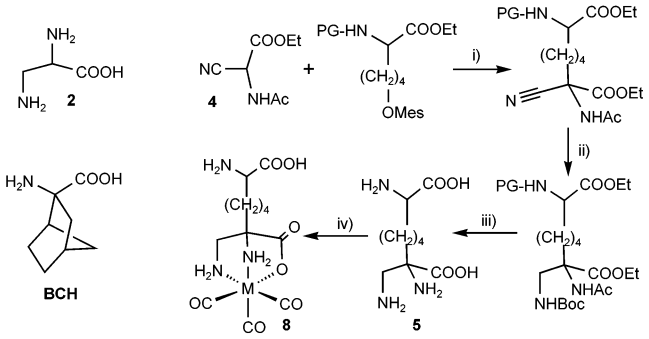
The labeling of small biomolecules for PET (positron emission tomography) and SPECT (single photon emission tomography) imaging represents the incentive for radiopharmaceutical chemistry. The success of ^{18}FDG (^{18}F-deoxyglucose) in molecular PET imaging in oncology, neurology, and cardiology emphasizes the importance of radioactive metabolic tracers.[1] Since ^{99m}Tc is cheap, permanently available in many clinics, and burdens a low dose to patients, it would be desirable to have comparable molecular imaging agents with ^{99m}Tc instead of cyclotron-produced ^{18}F or ^{11}C. The preparation of ^{99m}Tc-labeled glucose as ^{18}FDG analogue has been attempted, though without breakthrough so far.[2−4] The failure of the metal-labeled small biomolecules is not surprising since a complex will affect the molecular recognition by transporters, receptors, or enzymes. No small biomolecules such as glucose or amino acids with pendent metal complexes retaining recognition and specific transportation through the cell membrane have been reported so far.

Lipophilic and neutral amino acids are taken up by the L-type amino acid transporter LAT1 (a Na^+ independent antiport transporter) which is highly overexpressed in some tumor cell lines.[5−7] Yanagida et al. stated that the LAT systems show a relatively broad and symmetrical selectivity but strongly asymmetrical substrate affinity. Intracellular amino acids control their antiport activity. A variety of natural and artificial amino acids such as iodinated phenylalanine and tyrosine analogues, the anticancer drug melphalan, and even BCH (Scheme 1) are efficiently transported by the LAT system.[8−12] Kersemans et al. showed that the D isomer of 2-I-phenylalanine was taken up in different types of tumor cells expressing the LAT1.[13] Insights into substrate recognition reveal binding sites for the zwitterionic amino acid group, while a pocket interacts with the neutral side chains.[11] We present in this study the first example of a metal-labeled amino acid which is accepted by the LAT1 transmembrane transporter.

The complexes $[M(OH_2)_3(CO)_3]^+$ (M = Re (**1**), ^{99m}Tc (**1a**)) bind to a variety of ligands.[14] Active transport of labeled small molecules through protein channels requires complexes of low molecular weight and "slim" shape. Compound **2** (Scheme 1) has rarely been used as a tripod ligand. It has a low molecular weight (103.1 vs 65 for $[C_5H_5]^-$ or 129 for 9-ane-N3) and forms strong complexes with **1** or **1a**. Reaction of **2** with **1** gives [Re(**2**)$(CO)_3$] (**3**) and with **1a** at high dilution [^{99m}Tc(**2**)$(CO)_3$] in quantitative yield. An X-ray structure of **3** is shown in Figure 1 together with a sketched van der Waals radii based size comparison with [CpRe$(CO)_3$] from the top view, along a hypothetical amino acid side chain.

To afford a symmetric amino acid−metal complex conjugate, **3** must be attached at its α-carbon to the terminus of an amino acid

Scheme 1. Synthesis of **8** (PG = Protecting Group, Cbz) [(i) NaOEt, EtOH; (ii) $NaBH_4$, $NiCl_2$, Boc_2O, MeOH; (iii) NaOH, then HCl; (iv) $[ReBr_3(CO)_3]^{2-}$, MeOH, rt]



alkyl side chain. One of the pathways to amino acid−tripod compounds is exemplified in Scheme 1 for a butyl linker (**5**). A similar approach yields the corresponding conjugates with pentyl (**6**) and hexyl (**7**) spacers (Supporting Information).

The α-C−H in **4** is deprotonated with NaOEt. Reaction with protected ϵ-mes-lysine (i) followed by reduction and deprotection (ii and iii, respectively) gave the desired amino acid−tripod conjugate **5**. The α-C in the tripod is chiral and present in its D- and L-configuration after the synthesis. Coupling with an enantiomerically pure amino acid results in two diastereomers (e.g., **5** from L-lysine). Starting from D,L-amino acids, racemic mixtures of two diastereomers are received (**6** and **7**). Due to the low specificity of the pocket interacting with the side chain, the selection of one single diastereomer is not yet decisive for the proof of concept.

Compounds **5**−**7** react in water with **1** or **1a** to form the complexes [Re(**5**)$(CO)_3$] (**8**), [Re(**6**)$(CO)_3$] (**9**), [Re(**7**)$(CO)_3$] (**10**), and the corresponding ^{99m}Tc analogues. The amino acid part and the tripod provide similar sets of donors, but binding to the tripod was found preferentially (>95%). The complexes **8**−**10** are hydrophilic and the ^{99m}Tc complexes serum stable for at least 24 h.

Within a structure−activity relationship (sar) study comprising a series of natural and custom synthesized amino acid analogues (S-alkylated derivatives of cysteine), the rhenium complexes **8**−**10** have been subjected to competitive uptake studies with ^{3}H-L-Phe in R1M rhabdomyosarcoma cells. These cells overexpress LAT1 and allow one to calculate K_i (affinity constant) and to measure stimulated efflux (Figure 2). The bioassays followed literature procedures and are described in the Supporting Information.[15]

The LAT transport system works by a 1:1 exchange of amino acids.[12] This allows one to investigate if nonradioactive compounds enter the cell by measuring the efflux of an already taken up radiolabeled amino acid such as ^{3}H-L-Phe.[5] Hence, it is possible to

[†] University of Zürich.
[‡] Vrije Universiteit Brussels.
[§] Mallinckrodt Medical B.V.

10.1021/ja066002m CCC: $33.50

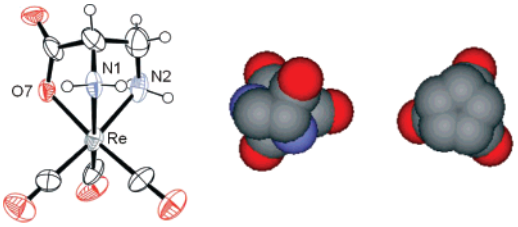


Figure 1. ORTEP presentation of **3** and size comparison with [CpRe(CO)$_3$] based on van der Waals radii.

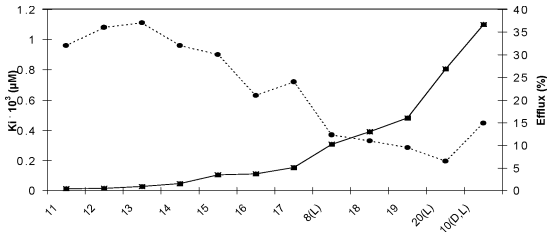
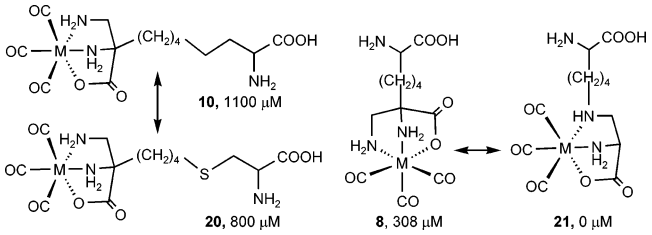


Figure 2. K_i values and stimulated efflux of the rhenium amino acids and reference compounds; K_i values of inhibitor with ^{3}H-L-Phe/L-Phe as substrate; efflux of ^{3}H-L-Phe out of R1M cells after 1 min.

Scheme 2. Structure−Activity Relationships between **10** and **20** and **8** and **21** (**8** and **21** have the same stoichiometric composition)



differentiate between compounds which only bind to the exterior part of the transport system and actual substrate compounds that are taken up.

Figure 2 shows the standard BCH substrate (**17**) (K_i = 153 μM vs L-leucine **13** or L-phe, **14**). Whereas L-cysteine (**18**) is not a good substrate, its S-alkylated derivatives L-cys-R might have higher (**11**, R = benzyl and **12**, R = butyl, R = methyl **16**) but also lower affinity (R = neopentyl, **19**) than, for example, L-methionine (**15**). The deduction of sar's is difficult, but LAT1 indeed tolerates steric changes in all spatial dimensions to different extents.

The data for **8**−**10** show that they all exhibit affinity for LAT1 and cause efflux. Complex **8** has the highest (K_i = 308 μM) and **9** the lowest affinity (K_i = 5300 μM). Compound **10** is in between (K_i = 1100 μM). Replacing one CH_2 group in **10** by a thioether sulfur (**20**, Scheme 2) slightly increases K_i to 800 μM. Due to the antiport LAT1 system, all compounds causing efflux of ^{3}H-L-Phe are also taken up into these cells. Competitive inhibition of the uptake together with the coupled stimulated efflux proves that **8**−**10** are transported by the same LAT system as L-Phe. The example of **8** shows for the first time that a small biological moiety, such as an α-amino acid, can be combined with a complex while retaining affinity and transport.

We emphasize that conjugation of many other ligands such as histidine or methionine to an α-amino acid, regardless of spacer type, resulted in complete loss of affinity. This implied that those conjugates were too bulky. However, the spatial orientation of the complex relative to the receptor binding part is at least as important. Changing the anchoring atom in **8** from the α-C to the 3-NH_2 group yields **21** (Scheme 2). The number of atoms in **21** is not changed with respect to **8**, but affinity for LAT1 is lost. Consequently, for the successful labeling of small molecules, the ligand sizes have to be considered but the orientation of the complex relative to the receptor binding site is equally important.

In conclusion, we have synthesized new α-amino acid derivatives conjugated to small tripod ligands and their *fac*-$[Re(CO)_3]^+$ based complexes. They represent the first examples of small molecule−metal complex conjugates that are actively internalized into cells by a transporter (LAT1). In vivo studies will show if the ^{99m}Tc analogues are sufficiently accumulated for imaging of cancer tissue.

Acknowledgment. This research was in part supported by an Eureka project and Mallinckrodt Medical B.V.

Supporting Information Available: Synthetic details of K_i's, efflux determination, and crystallographic data for **4** in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Marx, V. *C&EN* **2005**, *83*, 25−36.
(2) Banerjee, S. R.; Babich, J. W.; Zubieta, J. *Inorg. Chim. Acta* **2006**, *359*, 1603−1612.
(3) Schibli, R.; Dumas, C.; Petrig, J.; Spadola, L.; Scapozza, L.; Garcia-Garayoa, E.; Schubiger, P. A. *Bioconjugate Chem.* **2005**, *16*, 105−112.
(4) 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.* **2004**, *11*, 195−203.
(5) Meier, C.; Ristic, Z.; Klauser, S.; Verrey, F. *EMBO J.* **2002**, *21*, 580−589.
(6) Ohkame, H.; Masuda, H.; Ishii, Y.; Kanai, Y. *J. Surg. Oncol.* **2001**, *78*, 265−271.
(7) 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* **2001**, *1514*, 291−302.
(8) Kanai, Y.; Segawa, H.; Miyamoto, K.; Uchino, H.; Takeda, E.; Endou, H. *J. Biol. Chem.* **1998**, *273*, 23629−23632.
(9) Shikano, N.; Kanai, Y.; Kawai, K.; Ishikawa, N.; Endou, H. *Nucl. Med. Biol.* **2003**, *30*, 31−37.
(10) Lahoutte, T.; Caveliers, V.; Camargo, S. M. R.; Franca, R.; Ramadan, T.; Veljkovic, E.; Mertens, J.; Bossuyt, A.; Verrey, F. *J. Nucl. Med.* **2004**, *45*, 1591−1596.
(11) Uchino, H.; Kanai, Y.; Kim, D. K.; Wempe, M. F.; Chairoungdua, A.; Morimoto, E.; Anders, M. W.; Endou, H. *Mol. Pharmacol.* **2002**, *61*, 729−737.
(12) Fuchs, B. C.; Bode, B. P. *Semin. Cancer Biol.* **2005**, *15*, 254−266.
(13) Kersemans, V.; Cornelissen, B.; Kersemans, K.; Bauwens, M.; Dierckx, R. A.; De Spiegeleer, B.; Mertens, J.; Slegers, G. *Eur. J. Nucl. Med. Mol. I* **2006**, *33*, 919−927.
(14) Banerjee, S. R.; Maresca, K. P.; Francesconi, L.; Valliant, J.; Babich, J. W.; Zubieta, J. *Nucl. Med. Biol.* **2005**, *32*, 1−20.
(15) Mertens, J.; Kersemans, V.; Bauwens, M.; Joos, C.; Lahoutte, T.; Bossuyt, A.; Slegers, G. *Nucl. Med. Biol.* **2004**, *31*, 739−746.

JA066002M