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# PET Imaging of CXCR4 Receptors in Cancer by a New Optimized Ligand

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Nowadays, personalized medicine is considered to be of utmost importance to target the different causes of identical phenotypes.[1–5] For example, cancer of the same type can significantly differ in its biochemical phenotypes and thus its molecular profile between patients. The disease-specific characterization of malignant cells at the molecular level is a prerequisite for targeted therapy and personalized treatment. Positron emission tomography (PET) and its combination with computer tomography (PET/CT) and magnetic resonance tomography (PET/MRT) in modern hybrid systems offer the possibility to localize and quantify biochemical function by means of PET with anatomical (CT) and morphological (MRT) information. For this purpose, radiolabeled probes are used that target, for example, enzyme activities, transport systems, and surface receptors with high affinity and specificity.[6–8] We describe the development of the first gallium-68 ($t_{1/2}$ = 68 min) ligand for the G-protein-coupled receptor CXCR4 and preliminary demonstrate its potential for in vivo imaging of CXCR4 expression using a mouse model with a human small-cell lung cancer xenograft. This ligand offers the possibility to be used as an initial tool for diagnosis in an approach of personalized medicine for treating CXCR4-related cancer.

The chemokine receptor subtype CXCR4 is an attractive target for cancer diagnosis and treatment as it is overexpressed on more than 70% of human solid tumors, including mammary cancer, prostate cancer, B-cell lymphoma, neuroblastoma, melanoma, cervical adenocarcinoma, and glioma, among others.[9] Moreover, it is involved in three fundamental aspects of cancer: primary tumor growth, cancer cell migration, and establishment of metastatic sites; and therefore, it can be considered an ideal target. Being also a coreceptor for the cellular entry of the HIV, many peptidic and nonpeptidic ligands with different modes of antagonistic activity have been developed.[10–18] These highly CXCR4-specific agents can serve for the introduction of PET-active prosthetic groups. This approach is often complicated by loss of binding affinity, undesired alteration of biodistribution and instability in vivo.[19,20] A careful optimization of many molecular parameters is necessary to develop a suitable tracer for diagnostic application.

As a starting point for the development of the first $^{68}$Ga-labeled, CXCR4-directed PET probe, we used cyclic pentapeptide **1a** (Figure 1) developed by Fujii et al. and the later published

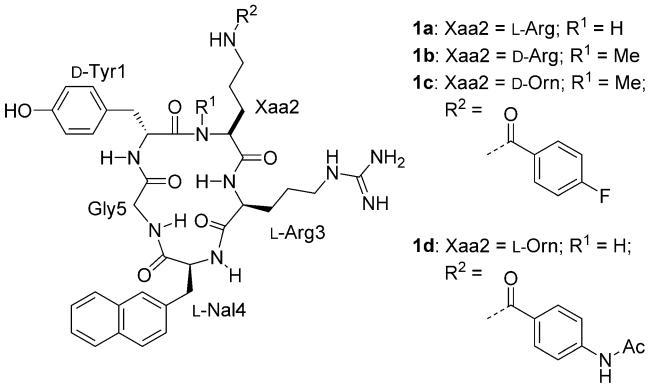


**Figure 1.** CXCR4 ligands modified to introduce radioisotopes.

analogue **1b**, as it is an inverse agonist of CXCR4.[21–23] Small, cyclic peptides such as these should exhibit high in vivo stability towards enzymatic degradation, especially as they contain D-amino acids and N-methylated peptide bonds.[6] Although allowing first positive imaging experiments, radioiodination of the tyrosine residue increased the lipophilicity and turned out to be unsuccessful for our purpose. Consequently, we investigated the introduction of more hydrophilic groups and focused on the (radio)metal chelator 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) because it can be used in combination with the corresponding radiometals for different imaging techniques like PET (e.g., $^{68}Ga^{3+}$), single photon emission tomography (SPECT; e.g., $^{111}In^{3+}$), or magnetic resonance imaging (MRI; e.g., $Gd^{3+}$, $Fe^{3+}$) and also for radionuclide therapy (e.g., $^{177}Lu^{3+}$, $^{90}Y^{3+}$).

Previous studies of our group and others have shown that all side chains of peptides **1a** and **1b** contribute to binding affinity. An attempt to remove the side chain of Arg 3 to introduce anchoring functions in this position resulted in a total loss of activity, whereas substitution of Arg 2 by ornithine (Orn) and its acylated derivatives gave a reduction of only one order of magnitude. Unfortunately, introduction of larger acyl or alkyl substituents on Orn 2 also strongly reduced the affinity (for details see Supporting Information).[24] Unexpectedly, ligands with benzoic acids attached to the Orn 2 side chain retained most

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