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*Review*

# Synthesis of Peptide Radiopharmaceuticals for the Therapy and Diagnosis of Tumor Diseases

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**Abstract:** Despite the advances in molecular biology and biochemistry, the prognosis of patients suffering from tumor diseases remains poor. The limited therapeutic success can be explained by the insufficient performance of the common chemotherapeutic drugs that lack the ability to specifically target tumor tissues. Recently peptide radiopharmaceuticals have been developed that enable the concurrent imaging and therapy of tumors expressing a specific target. Here, with a special emphasis on the synthesis of the building blocks required for the complexation of metallic radioisotopes, the requirements to the design and synthesis of radiolabeled peptides for clinical applications are described.

**Keywords:** radionuclides; chelator; prosthetic groups; carrier molecules; peptides; medicinal application; radiopharmaceutical; diagnostic imaging; radiotherapeutics

## 1. Introduction

The incidence of human malignant tumor diseases is still increasing worldwide. Generally, cancer treatment can be performed using one or a combination of the following methods: surgery, chemotherapy and radiation therapy. Their side effects limit the efficiency of chemo- and radiotherapeutic agents, but can be avoided and a much more effective therapy is possible if the drugs used have tumor selectivity. This involves the determination of biochemical processes that distinguish tumor tissue samples from healthy tissue (Table 1). As a result, tumor-specific biomarkers are used in oncology. Several types of agents have been developed for specific accumulation in the malignant cells

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to reduce the cytotoxic effect on the normal cells. These agents can be labeled with radionuclides that accumulate in the tissue of interest. Depending on the purpose, gamma or positron emitters are used for diagnosis and beta, alpha or Auger electron emitters are used for therapeutic applications in cancer treatment. The higher the specific activity of a drug, the better the imaging and the lower the cytotoxic side-effects in therapeutic applications [1].

Modern imaging methods include computer tomography (CT), magnetic resonance tomography (MRI), ultrasound, single-photon emission computed tomography (SPECT) and positron emission tomography (PET). They provide information about the phenotypic functional changes associated with the development of the disease. New treatment modalities based on the biological properties of tissues have been developed, where important progress has been achieved using antibodies and peptides [2]. When labeled with therapeutic radioisotopes, these agents are suitable for endoradiotherapy and exploit their high specificity. This has been realized for antibodies against the tumor associated epitope CD20 [3] or peptides binding to the somatostatin receptors [4].

**Table 1.** Biomarkers used in clinical routine for tumor-diagnosis [5].

| | |
|---|---|
| Perfusion | [$^{15}$O]$H_2O$ |
| Glucose metabolism | [$^{18}$F]FDG |
| Bone metabolism | [$^{18}$F]Fluoride |
| Choline metabolism | [$^{18}$F]Choline |
| DNA synthesis | [$^{18}$F]FLT |
| Amino acid transport and protein synthesis | [$^{18}$F]FET, [$^{11}$C]MET, [$^{18}$F]FDOPA |
| Receptor binding | [$^{68}$Ga]-DOTA-TOC |
| Antigen binding | [$^{111}$In]-anti-CD20 mAb |
| PSMA | [$^{68}$Ga]-PSMA |
| Angiogenesis | [$^{18}$F]Galacto-RGD |
| Lipid synthesis | [$^{11}$C]AcOH |
| Hypoxia | [$^{18}$F]FAZA, [$^{18}$F]MISO |
| Apoptosis | [$^{124}$I]Annexin V |
| Gene expression | [$^{18}$F]FHBG |

Many specific radiopharmaceuticals have been developed in various preclinical and clinical stages for imaging and therapy of tumor diseases and some of them are currently in routine clinical use. They can be classified into three major categories according to the molecular weight of the carrier: (a) radiolabeled monoclonal antibodies (b) receptor specific small proteins and peptides and (c) small molecules.

## 2. Carrier Molecules

Many tumors overexpress specific targets on the surface of their cells. The target ligands are used with radiolabels in cancer diagnosis and therapy in accordance with the key-lock principle (Figure 1). As the number of receptors on the surface of tumor cells compared with that in normal tissues often is higher, the effect on the tumor cells is stronger than that on the normal cells resulting in a wide therapeutic window [6].

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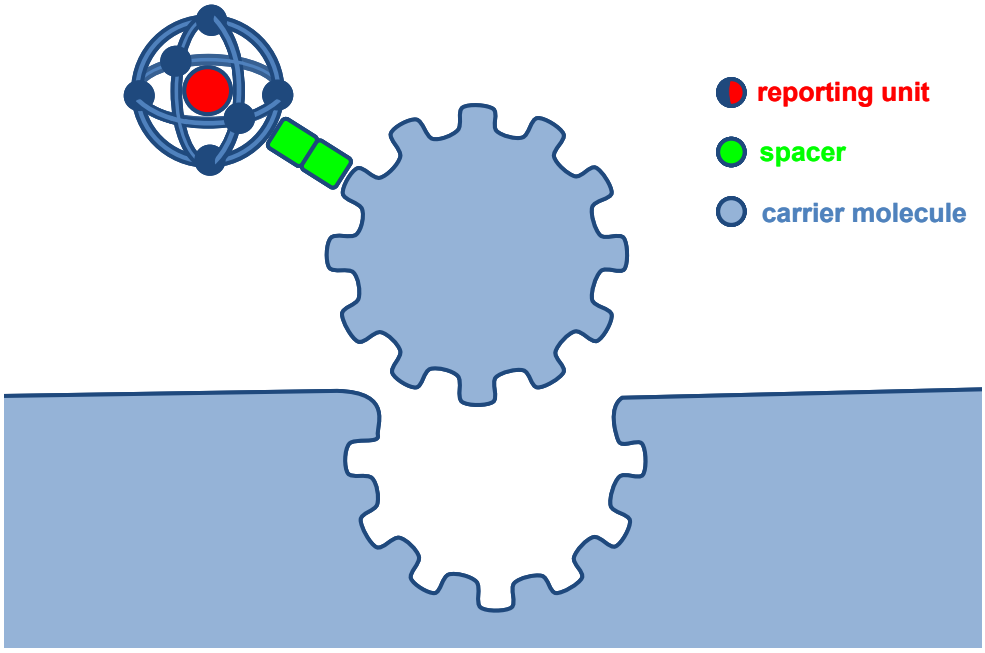
**Figure 1.** Binding of ligand to target like a peptide-receptor has been visualized by a "lock and key" arrangement, where the peptide fits into a binding pocket of the receptor on the surface of tumor cells in a similar manner to a key fitting into a lock.




*2.1. Small Molecules*

A variety of molecular and functional alterations has been shown to change the morphology and functional status of tumor tissue. Molecular imaging has been established as a tool to measure biomarkers or indicators of disease or therapeutic effects [7]. There are numerous different carriers that have been designed and developed for the targeting of tumors. Several radiolabeled small molecules have been applied *in vivo* for PET imaging [5]. PET radiopharmaceuticals have a significant potential for routine clinical imaging studies. The efficiency of these radiotracers is based on their ability to accumulate in the tumor cells (Table 1).

*2.2. Antibodies*

Antibodies with a very high specificity for their target antigen overexpressed in tumors can display a direct therapeutic effect and must therefore not necessarily be combined with a drug for application as anticancer drugs. However, as many antibodies are not sufficiently cytotoxic, radionuclides have been shown to significantly enhance the therapeutic effects of monoclonal antibodies (mAb). Radiolabeled antibodies exert a certain cytotoxic effect on surrounding cells, depending on the emitted energy of radionuclide radiation over its reach in the tissue decides. In contrast, the unlabeled antibodies interaction is limited on the targeted cells [8]. Zevalin®, a $^{90}$Y-anti-CD20 mAb and Bexxar®, a $^{131}$I-anti-CD20 mAb have been shown to ideally fulfill this task by selectively transporting radionuclides to tumors [9,10].

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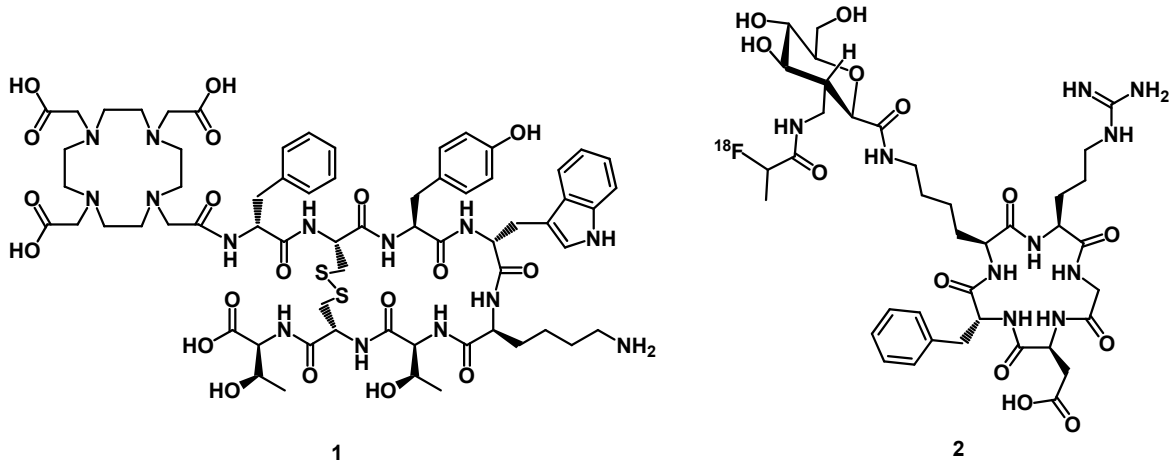
*2.3. Peptides*

Several receptors with small regulatory peptide ligands are overexpressed in certain human cancers, offering the possibility to target these tumors with radiopeptides. The somatostatin analogs DOTA-TOC and DOTA-TATE (**1**) can be labeled with $^{111}$In or $^{68}$Ga for imaging, or with $^{90}$Y, $^{177}$Lu for radiotherapy of somatostatin receptor (SSTR)-positive tumors (Figure 2). The excellent results obtained led to the development of analogs of other peptide families, such as bombesin, neurotensin, cholecystokinin/gastrin, exendin, RGD (Arg-Gly-Asp) and substance P. Numerous radiolabeled peptides are currently under preclinical research or clinical evaluation for both diagnostic imaging of peptide receptor expression [11,12] and peptide receptor mediated therapy (PRRT) [13–15].

**Figure 2.** Chemical structures of DOTA-TATE and [$^{18}$F]Galacto-RGD, two typical radiolabeled peptide tracers.



**1** **2**

2.3.1. Peptides and Radiopeptides as Targeting Agents

The overexpression of peptide receptors in human tumors led to the development of peptide radio-pharmaceuticals for specific diagnostic imaging and/or therapy of cancers. Table 2 summarizes the receptors-binding peptides and their specificity of overexpression in tumors. Neuroendrocrine tumors (NETs), including primaries and metastases, overexpress somatostatin receptor types (sst1-sst5) [6], particularly sst2 [16]. These receptors present the molecular basis for peptide-based probes for cancer imaging and therapy. The somatostatin analogs DOTA-TOC and DOTA-TATE (**1**) can be labeled with $^{111}$In, $^{64}$Cu and $^{67/68}$Ga for *in vivo* imaging of SST receptor-expressing tumors [17] or with β-emitters ($^{90}$Y or $^{177}$Lu) or α-emitters ($^{213}$Bi or $^{225}$Ac), these labeled analogs can be utilized for peptide receptor mediated therapy (PRRT) [14]. For bombesin receptors family, four subtypes are known (BB1-BB4). Gastrin-releasing peptide receptor (GRPR/BB2) has been found to be overexpressed in a variety of tumors, including prostate, breast, pancreas, gastrointestinal and small cell lung cancer [6]. Several radiolabeled bombesin-like peptides, which bind to BN/GRP receptors with high affinity, have been developed in order to be used for diagnostic and/or therapeutic purposes. Bracco has developed the first radiolabeled BN analog [$^{177}$Lu]-AMBA for imaging and PRRT [18,19]. Bombesin antagonists with favorable tumor-to-normal tissue ratios have been by developed Manci *et al.* [20–22]. The preliminary clinical study shows that [$^{64}$Cu]-CB-TE2A-AR-06 is a

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promising ligand for imaging GRP-Receptor-positive tumors in humans [23]. An other application of peptide-ligands as attractive agents is radiolabeled peptides based on the lead structure cyclo(Arg-Gly-Asp-D-Phe-Val) as the integrin $\alpha_v\beta_3$-targeted radiotracers. Many radiolabeled cyclic RGD peptide antagonists have been evaluated for imaging integrin $\alpha_v\beta_3$-positive tumors by SPECT or PET [24,25]. Among the radiotracers evaluated in preclinical tumor-bearing models, [$^{18}$F]Galacto-RGD (**2**) is currently under clinical studies in patients suffering from malignant melanomas, sarcomas, head and neck cancer, glioblastomas, and breast cancer [5]. Cholecystokinin (CCK) receptors have been identified in numerous human cancers, like medullary thyroid carcinomas, small cell lung cancers, stromal ovarian cancers and astrocytomas [6]. Radiolabelled CCK/gastrin analogues have been synthesized and characterized for imaging using positron emission tomography and single photon emission computed tomography imaging. All peptides are mostly based on the C-terminal CCK receptor-binding tetrapeptide sequence Trp-Met-Asp-Phe-$NH_2$. $^{99m}$Tc-demogastrin 2 has been evaluated and compared with [$^{111}$In]-DOTA-CCK8 and [$^{111}$In]-DOTA-MG11 in patients with medullary thyroid cancers (MTC) [26]. The results obtained show that [$^{99m}$Tc]-demogastrin 2 showed the best visualization, which may be due to better imaging properties of $^{99m}$Tc as compared to $^{111}$In. The glucagon-like peptide-1 receptor (GLP-1R) is one of the most frequently studied peptide receptors. The high density of glucagon-like peptide-1 receptors (GLP-1R) in human insulinomas provides an attractive target for molecular imaging and internal radiotherapy [6]. For this purpose DTPA- and DOTA-conjugate of exendin-4 were synthesized. The peptide [Lys$^{40}$(Ahx-DOTA)-$NH_2$]-Exendin-4 radiolabeled with $^{111}$In shows success in the detection of tumors in patients with insulinomas [27–29]. Using the Auger electrons of $^{111}$In, [Lys$^{40}$(Ahx-DOTA)-$NH_2$]-Exendin-4 was evaluated as a radiotherapeutic for glucagon-like peptide-1 receptor-targeted therapy for insulinoma [30]. The peptide receptors, melanocortin receptors exist in five subtypes. The melanocortin 1 receptor (MC1R) is overexpressed in most murine and human melanoma metastases [6], and hence is an attractive target for the detection and treatment of these cancers. Radiolabeled α-MSH analogs, contain the sequence His-Phe-Arg-Trp. They have been developed for MC1R targeting. Recently data demonstrates that radiolabeled α-MSH analogs DOTA-Nle-CycMSH$_{hex}$ and DOTA-Re-CCMSH(Arg$^{11}$) are potential candidates for diagnostic imaging or radiotherapy of melanoma tumors [31,32]. The overexpression of neurotensin receptor NTR1 has been found in several human cancers including Ewing sarcomas, meningiomas, astrocytomas, medulloblastomas and pancreatic carcinomas [6], and several NT analogs have been synthesized and conjugated with a chelator, like DTPA or DOTA. Among all the radiopeptides, DOTA-NT-20.3 is a promising candidate for $^{68}$Ga-PET imaging of neurotensin receptor-positive tumors [33]. Human adenocarcinomas of the gastroenteropancreatic system overexpress vasoactive intestinal peptide (VIP) receptors [6] and therefore represent logical diagnostic targets for receptor scintigraphy. $^{99m}$Tc labeled VIP analog (TP3654) is a promising agent for imaging colorectal cancer [34]. Neurokinin type 1 (NK-1) receptors are overexpressed in malignant gliomas. The radiopeptide [$^{111}$In]/[$^{90}$Y]-DOTAGA-substance P binds to these receptors and can be used for treatment of brain tumors [35]. Neuropeptide Y receptors involve Y1R and/or Y2R have been found to be expressed in neuroblastoma, breast carcinomas, ovarian cancers [6]. The chemokine receptors CXCR4 are highly expressed in breast and prostate cancer. These receptors (NPY1R and CXXR4) are promising additional candidates in the oncology field and their advanced status is under preclinical studies.

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