Design of Molecular Ligands for (Nuclear) Imaging

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Abstract

There is a keen interest in methods which allow non-invasive monitoring of molecular targets involved in several pathological processes such as cancer. The most important one is certainly nuclear imaging. A long time only usual contrast agents were available which only accumulate in the region they were injected to. A more specific agent is demonstrated by radiolabeled molecular ligands which exhibit a high affinity for their receptor. Receptors are mostly molecules which occur only in the pathological process or which are more abundant in case of disease. This review will describe the design of molecular ligands considering as example of tumor angiogenesis.

Abbreviations:
TGF-α: transforming growth factor α, bFGF: basic fibroblast growth factor, aFGF: asic fibroblast growth factor, PDGF: platelet-derived endothelial cell growth factor, MMP: matrix metalloproteinase, RGD: Arg-Gly-Asp, FBA: 4-[^{18}F]-fluorobenzoate (FBA), AcOF: [^{18}F]-acetylhypofluoride, SAA: sugar amino acid, NFP: 4-nitrophenyl-2-[^{18}F]fluoropropionate, GABA: γ-amino butyric acid, DTPA: diethylenetriamine-pentaacetic acid, DOTA: 1,4,7,10-tetra-azacyclododecane-N,N',N'',N'''-tetra acetic acid, HYNIC: hydrazinonicotinamide, GBHO: guanidinobenzoyl hydrazino oxopentanoic acid, sst: somatostain

I. Introduction to Radiopharmaceuticals

Radiopharmaceuticals are radioactive agents and used in the field of nuclear medicine as tracers in diagnosis and treatment of many diseases. However, this summary will focus on the use of tracers in diagnosis only.
Nuclear medical methods can measure either the accumulation of a radiopharmaceutical in an organ or its expulsion from an organ. Thus, physiological parameters can be measured and the function of the specific organ, its regional blood circulation or metabolic rate can be determined. Furthermore, pathological aberrances can be detected which plays an important role in tumor diagnosis.
Radiopharmaceuticals may be given to the patient in several different ways, e.g. oral, by injection or placed into the eye or into the bladder.
When they are used to help diagnose medical problems, only small amounts are given to the patient and thus the radiation the body receives from them is very low and is considered safe. The radiopharmaceutical then passes through, or is taken up by an organ of the body (which organ depends on what radiopharmaceutical is used and how it has been given). Then the radioactivity is detected, and pictures are produced, by special imaging equipment.

In nuclear medicine diagnosis mainly gamma emitters are used. These should have an optimal acceleration energy between 100 and 250 keV which is applied to the following nuclides:

Technetium : ^{99m}Tc
Iod : ^{123}I
Indium : ^{111}In
Gadolinium : ^{67}Ga
Thallium : ^{201}Tl
Krypton : ^{81m}Kr

But also other radioactive nuclides like Iod ^{125}I, Fluor ^{18}F, Rhenium ^{188}Re, Yttrium ^{90}Y and Lutetium ^{177}Lu. The dosage of radiopharmaceuticals that are used to diagnose medical problems depend on each patient and on the type of test. The amount of radioactivity of a radiopharmaceutical is expressed in units called becquerels or curies. Radiopharmaceutical dosages given may be as small as 0.185 megabecquerels (5 microcuries) or as high as 1295 megabecquerels (35 millicuries). The radiation received from these dosages may be about the same as, or even less than, the radiation received from an x-ray study of the same organ.
Some aspects need to be discussed before using radiopharmaceuticals: allergies; pregnancy, breast-feeding, age (children/older people), intake of other medicine and any other medical problems.

II. Ligands as Radiopharmaceuticals

There are two different radiopharmaceuticals which increase contrast in nuclear imaging: **contrast agents** are non-specific and accumulate in the bloodstream or in organs to which they are injected; **radiopharmaceutical ligands** are highly specific. They do not accumulate in whole organs or in the bloodstream, as they only bind to specific molecules. To turn ligands into radiopharmaceutical ligands, they need to be labeled with radionuclides, which are mentioned above.

Structure of Radiopharmaceutical Ligands

A radiopharmaceutical ligand usually consists of a bioactive molecule (the ligand itself) and is labeled with a radionuclide. The bioactive side exhibits a very high affinity to its specific receptor molecule which is connected to the target (e.g. tumor cells). (Figure 1)

Characterisctics of Radiopharmaceutical Ligands

There is a number of things considered essential for the ideal radiopharmaceutical ligand. To distinguish between health and disease, molecular ligands should bind exclusively to pathologically occurring molecules or molecules which are expressed in a much higher extent due to disease. Thus, the non-target organs and tissues should have no or low uptake of the agent. The target-molecules should also preferably be accessible from the outside of the cell. A high percentage of the radiopharmaceutical agent should be localizing at the target very rapidly and it should reside long enough at the target to allow optimal imaging. The ligand should be cleared from blood-pool rapidly to improve the target to blood ratio. Its uptake should be proportional to the degree of the disease (e.g. by infection or inflammation imaging). The ligand itself should be suitable for radionuclide labelling and ideally in a kit formulation. Labelling should be simple and uncomplicated. The cold-kit of the agent should have a long shelf-life and it should not be expensive. Furthermore, the radiation dosimetry should be reasonable and immunological response should be minimal (e.g. anaphylactic shock).

Molecules Used as Radiopharmaceutical Ligands

Nowadays an increased number of peptide-based ligands are used for radiolabeling as these exhibit high affinities to their specific receptors. Antibodies are the most widely used type of targeting agent today. Their large size can, however, lead to poor solid tumor penetration and slow elimination from the circulation. The letter can in unfortunate cases lead to a high level of exposure of normal tissues such as bone marrow. To avoid these problems, smaller antibodies constructs, such as Fab-fragments, minibodies and scFv-fragments have successfully been made. Affibodies, much smaller fragments can also be used as the other fragments are often difficult and expensive to produce and as they are sometimes not very stable.

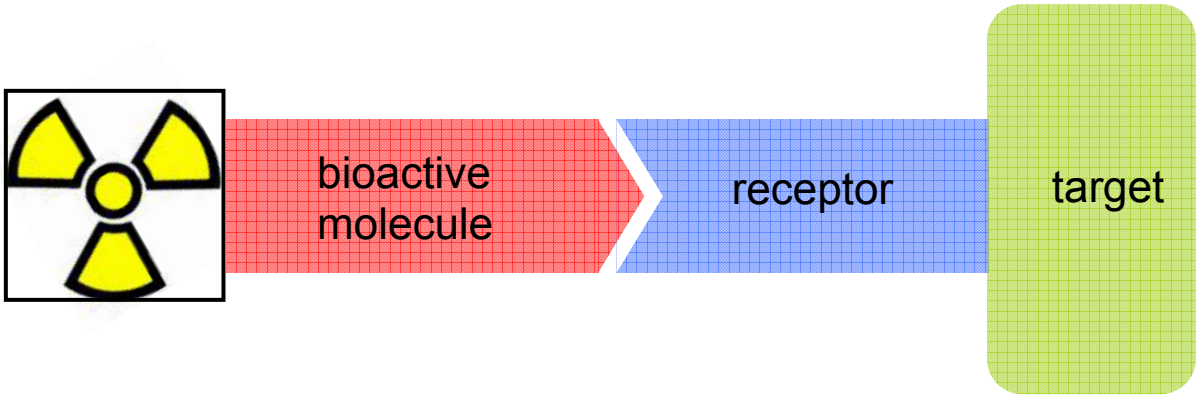

Figure 1: Structure and binding of a radiopharmaceutical ligand. The ligand, a bioactive molecule, (red) is labeled with a radionuclide (yellow) and binds in a high affine manner to its specific receptor (blue) which is connected with the target-tissue (green).

Furthermore, also non-peptidic low molecular mass molecules (peptidomimetics) can serve as ligands. Also whole white blood cells can be used, e.g. for direct targeting of microorganisms for infection imaging.

Diseases Detected by Radiopharmaceutical Ligands

Numerous pathological processes can be detected by radiolabeled ligands used in nuclear imaging. In doing so, the most known one is the detection of tumors in various tissues and organs. Therefore a lot of different approaches are possible. But also many other diseases can be determined like infections, inflammations, various lesions (e.g. osteomyelitis, cellulites, diabetic foot, Crohn's disease, inflammatory bowel disease (IBD), fever of unknown origin), neurological diseases, blood vessel diseases, bone and bone marrow diseases, diseases of several organs (e.g. heart, kidney, liver, lung), red blood cell diseases and many more.

III. Design of Molecular Ligands Considering as Example

In general: *When designing a molecular ligand for nuclear imaging, lead structures are needed. First the diseases which should be detected must be investigated to find proteins like membrane proteins, receptors, enzymes, etc. which are specific for the pathogenic process. Then naturally occurring ligands for these proteins need to be found. These are the lead structures which can be radiolabeld and further modified.*

Detection of Tumor-Induced Angiogenesis

Angiogenesis is not only important for physiological processes like embryogenesis and wound healing, but also for pathological processes like tumor growth, rheumatoid arthritis and many more. Tumor induced angiogenesis is a multistep process involving a variety of different and many more. Tumor induced angiogenesis is a multistep process involving a variety of different pro- and antiangiogenic factors. The angiogenic switch is often triggered by insufficient nutrient supply, metabolic and mechanical stress, immune/inflammatory response and genetic mutations. Thus, the production of angiogenic factors (e.g. transforming growth factor α (TGF-α), basic and acid fibroblast growth factor (bFGF, aFGF) and platelet-derived endothelial cell growth factor (PDGF)) is activated. Various cells like cancer cells, stromal cells and endothelial cells are enabled to secret these factors. Nevertheless, they can also emanate from blood and the extracellular matrix. After activation, endothelial cells produce proteolytic enzymes like matrix metalloproteinases (MMPs) which degrade

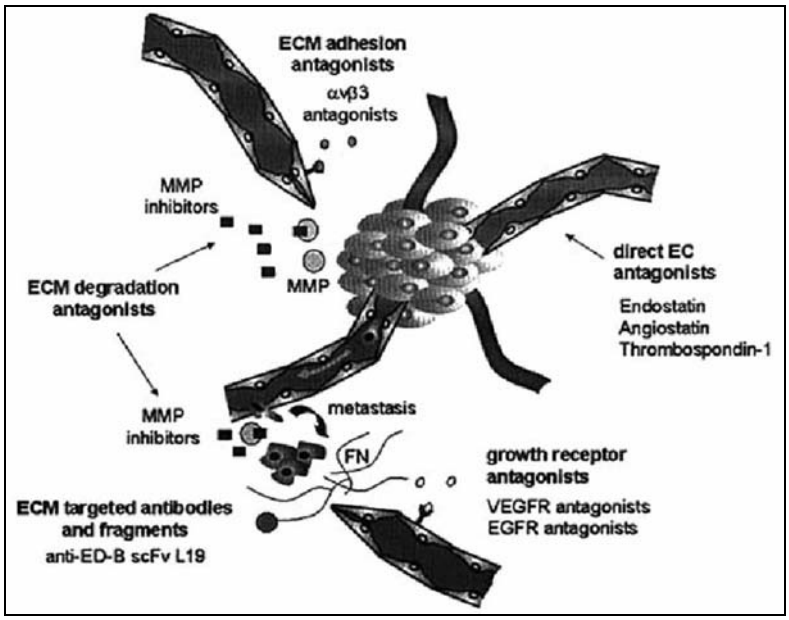

Figure 2: Schematic presentation of angiogenic processes during tumor growth. Potential targets for *in vivo* imaging purposes are: a) inhibition of endothelial cell (EC) migration through the extracellular matrix (ECM) by blocking of cell adhesion receptors (e.g. αvβ3 integrins), b) antagonists blocking endothelial cell function (.e.g. ATP synthase inhibitor angiostatin), c) growth receptor antagonists for inhibition of endothelial cell activation (e.g. VEGF-R2 inhibitor SU 5416), d) binding of antibodies and fragments to the ED-B domain of a fibronectin (FN) isoform (scFV L19), e) inhibition of the degradation of the extracellular matrix by matrix metalloproteinase inhibitors (e.g. Marimastat).

the extracellular matrix allowing migration of endothelial cells through the basement membrane. However, MMPs can also play an anti-angiogenic role by cleaving matrix components into anti-angiogenic factors. Furthermore, integrins like αvβ1, αvβ3 and αvβ5 play an important role in angiogenesis. They can be both, pro-angiogenic by mediating cell-matrix interactions and anti- angiogenic or negative regulators of angiogenesis by inducing apoptosis.

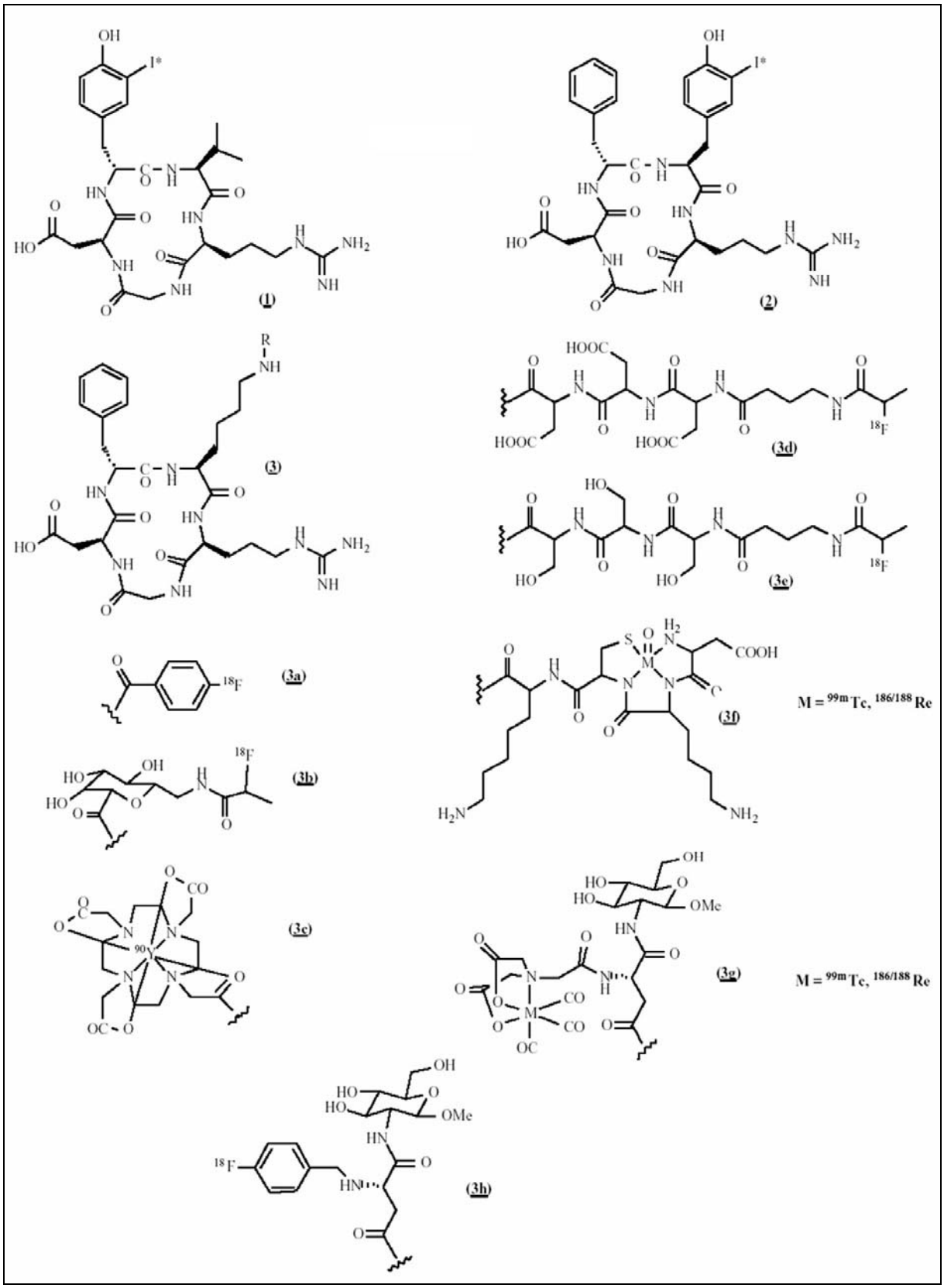

Figure 3: Structures of radiolabeled RGD (ARG-Gly-Asp) containing antagonists. (A)

Moreover, extracellular matrix proteins such as laminin, tenascin, or collagen type IV are produced to provide new basement membrane components. Mesenchymal cells release angiopoietin-1, which interacts with Tie-2 receptor tyrosine kinase mediating capillary organization and stabilization. The newly built endothelial cells reorganize by forming tight junctions with each other leading to tube formation which connect with the microcirculation resulting in an operational new vasculature. Regarding all the physiological processes in angiogenesis, there are many targets for *in vivo* imaging of tumor-induced angiogenesis (Figure 2).

αvβ3 INTEGRIN ANTAGONISTS

The design of molecular ligands for nuclear imaging will be described in the following only for αvβ3 integrin antagonists. Here RGD-peptides play an important role. They are called RGD-peptides as they contain a arginine-glycine-aspartate sequence motif. For monitoring αvβ3 integrin expression the pentapeptide cyclo(-Arg^1-Gly^2-Asp^3-DPhe^4-Val^5-) is used as lead structure.

1. "Direct" Labeled Cyclic RGD-Peptides

In general: *As the simplest way to label a lead structure is direct labeling this way is usually carried out first in designing a radipharmaceutical ligand. But before labeling can be conducted, it is necessary to find out which parts of the lead structure are essential for high affinity to the receptor.*

To investigate the structure-activity of the cyclo-RGD-pentapeptide, amino acids 4 and 5 were replaced by a variety of hydrophobic and hydrophilic amino acids. It was found that a hydrophobic amino acid in position 4 is important for high αvβ3 affinity. In contrast, the amino acid in position 5 could be modified without loss of αvβ3 affinity. Radiohalogenated peptides were obtained by introduction of an iodinated tyrosine at position 4 as well as at position 5 resulting in 3-[*I]iodo-DTyr^4-cyclo(-Arg^1-Gly^2-Asp^3-DTyr^4-Val^5-) (**1**) and 3-[*I]iodo-Tyr^5-cyclo(-Arg^1-Gly^2-Asp^3-DPhe^4-Tyr^5-) (**2**) (Figure 3).

Another way for direct labeling the cyclo-RGD-pentapeptide is the introduction of ^{18}F. This can be done in a no-carrier-added and in a carrier-added manner. **No-carrier-added ^{18}F-labeling** can be carried out via prosthetic groups which can easily be introduced to a lysine. Therefore, the valine at position 5 was replaced by a lysine resulting in cyclo(-Arg^1-Gly^2-Asp^3-DPhe^4-Lys^5-) (**3**). Is then N$^\varepsilon$-Lys^5 labeled with N-succinimidyl 4-[^{18}F]-fluorobenzoate (FBA) the new structure is cyclo(-Arg^1-Gly^2-Asp^3-DTyr^4-Lys([^{18}F] FBA)-) (**3a**). **Carrier-added ^{18}F-labeling** can be done using ^{18}F-acetylhypofluoride ([^{18}F]AcOF) and direct electrophilic radiofluorination of the phenylalanine residue at position 4 resulting in [*F]fluoro-DPhe-cyclo(-Arg^1-Gly^2-Asp^3-DPhe^4-MeVal^5-) **(5)**. However, this resulted in a product mixture with different mono- and difluorinated isomers (Figure 3, Figure 5)

It was proven by an integrine binding assay that all structures obtained by direct labeling show high αvβ3 affinities, but due to their lipophilic character the highest activity is found in liver and intestine.

2. Cyclic RGD-Peptides Modified with Carbohydrates

In general: *Modification of lead structures with carbohydrates may is useful as these can improve different properties of biologically active peptides including bioavailability, solubility under physiological conditions or resistance against proteases.*

The introduction of sugar moieties reduces liver uptake and increases tumor accumulation of RGD-peptides. To improve the pharmacokinetics of the directly labeled RGD-peptides, sugar amino acids (SAA) were introduced. A glucose based sugar amino acid (SAA1) was conjugated to the ε-amino function of the lysine in the pentapeptide 3-[*I]iodo-DTyr^4-cyclo(-Arg^1-Gly^2-Asp^3-DTyr^4-Lys^5-) **(4)** resulting in 3-[*I]iodo-DTyr^4-cyclo(-Arg^1-Gly^2-Asp^3-DTyr^4-Lys^5(SAA1)-) **(4a)**. This sugar added structure shows reduced accumulation in liver, increased concentration in blood and an increased uptake in tumor tissue (Figure 5).

Based on these data a galactose-based sugar amino acid (SAA2) was conjugated with cyclo(-Arg^1-Gly^2-Asp^3-DPhe^4-Lys^5-) (**3**) resulting in [*F]fluoro-SAA2-cyclo(-Arg^1-Gly^2-Asp^3-DTyr^4-Lys^5(SAA2)-) (**3b**) and allowing prosthetic group labeling. Due to the low lipophilicity of the small propionyl moiety, 4-nitrophenyl-2-[^{18}F]fluoropropionate ([^{18}F]NFP) was used for ^{18}F-labeling. [^{18}F]Galacto-RGD (**3b**) shows receptor specific visualization of αvβ3-positive tumors using positron emission tomography (PET) (Figure 3, Figure 4).

Cyclo(-Arg^1-Gly^2-Asp^3-DPhe^4-Lys^5-) (**3**) can also be conjugated via a D-aspartic acid linker with a glucosamine derivative. The free N$^\alpha$-amino function of the aspartic acid allows then coupling with chelator systems for metallation (**3g**) as well as with prosthetic groups for ^{18}F-labeling (**3h**) (Figure 3).

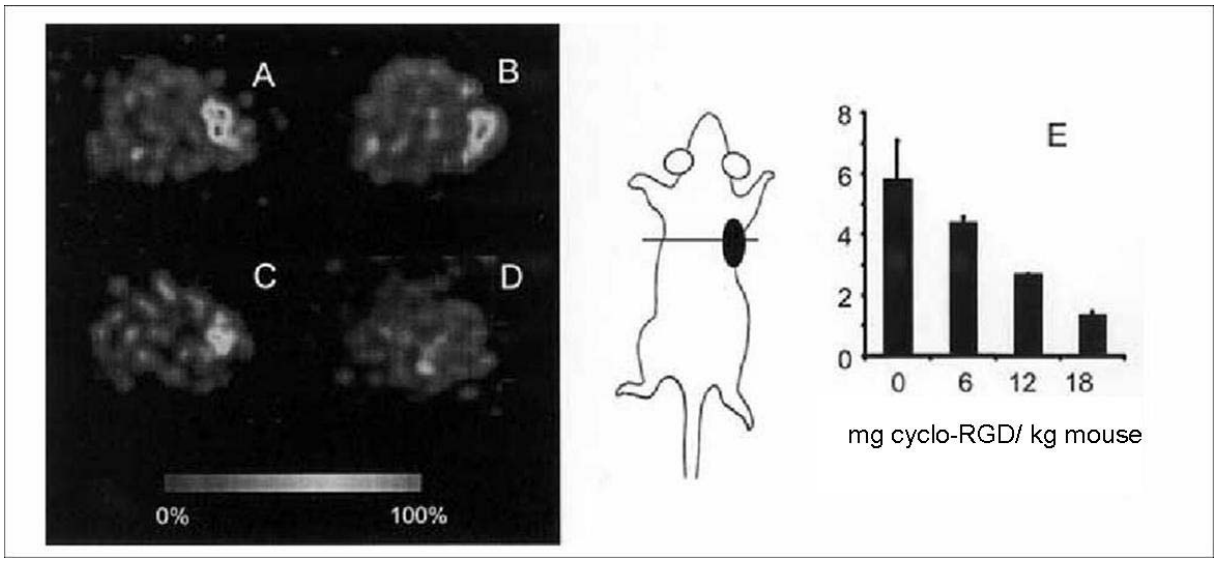

Figure 4: Transaxial images of the tumor region of mice bearing a αvβ3-positive tumor on the right flank. Images were acquired 90 min after injection of [^{18}F]Galacto-RGD (3b) on a small animal scanner. Blocking experiments (injecting increasing amounts of the αvβ3 selective pentapeptide cyclo(Arg-Gly-Asp-DPhe-Val) 10 min prior to tracer injection) showed a concentration-dependent inhibition of tracer uptake in the tumor. These results demonstrate receptor-specific tracer accumulation in αvβ3-positive tumors: non-carrier added (A), 6 mg/kg (B), 12 mg/kg (C) and 18mg/kg (D) cyclo(Arg-Gly-Asp-DPhe-Val). E) tumor-to-background ratios resulting from substracting the intensity of the same size at the contralateral side from the intensity of the tumor region.

3. Cyclic RGD-Peptides Modified with Hydrophilic Tetrapeptides

In general: *An alternative approach to improve pharmacokinetics uses tetrapeptides containing hydrophilic D-amino acids. In addition to the general effects of an increased hydrophilicity on the pharmacokinetics of a peptide tracer, D-amino acids are known to increase proteolytic stability and enable a molecular "fine tuning" of the desired in vivo characteristics by variation of the amino acids used.*

For labeling the RGB-pentapeptide, two tetrapeptides containing γ-amino butyric acid (GABA) and either three D-aspartic acids or three D-serines were synthesized and conjugated with Lys^5 of the pentapeptide cyclo(-Arg^1-Gly^2-Asp^3-DPhe^4-Lys^5-) (**3**) resulting in [*F]fluoroAsp$_3$GABA-cyclo(-Arg^1-Gly^2-Asp^3-DPhe^4-Lys^5(Asp$_3$GABA)-) (**3d**) and [*F]fluoroSer$_3$GABA-cyclo(-Arg^1-Gly^2-Asp^3-DPhe^4-Lys^5(Ser$_3$GABA)-) (**3e**) (Figure 3).
These radiolabeled and with hydrophilic tetrapeptides labeled RGD-structures show comparable in vivo behavior as the sugar added ones.

4. Cyclic RGD-Peptides Labeled with Radiometals

In general: *Lead structures can also be labeled with radiometals. These can improve metabolic stability and pharmacokinetics.*

For the labeling of cyclo-RGD-pentapeptides with radiometals, the ε-amino function of the lysine in the lead structure cyclo(-Arg^1-Gly^2-Asp^3-DXaa^4-Lys^5-) (Xaa: Phe or Tyr) can also be conjugated with several chelating systems. Labeling of the pentapeptide cyclo(-Arg^1-Gly^2-Asp^3-DPhe^4-Lys^5-) (**3**) with diethylenetriamine-pentaacetic acid (DTPA) results in [*In]indiumDTPA-cyclo(-Arg^1-Gly^2-Asp^3-DPhe^4-Lys^5(DTPA)-) (**4b**). Beside ^{111}In-indium-labeling of DTPA, it can also be iodinated with ^{125}I.
^{99m}Tc-, ^{188}Re- and ^{90}Y-labeled analogues can be obtained by conjugation of the cysteine-containing tetrapeptide sequence H-Asp-Lys-Cys-Lys-OH as well as conjugation of 1,4,7,10-tetra-azacyclododecane-N,N',N",N'"-tetra acetic acid (DOTA) with Lys^5 of the pentapeptide cyclo(-Arg^1-Gly^2-Asp^3-DPhe^4-Lys^5-) (**3**) resulting in [*T/R]technetium/rheniumH-Asp-Lys-Cys-Lys-OH-cyclo(-Arg^1-Gly^2-Asp^3-DPhe^4-Lys^5(H-Asp-Lys-Cys-Lys)-) (**3f**) and [*Y]yttriumDOTA-cyclo(-Arg^1-Gly^2-Asp^3-DPhe^4-Lys^5(DOTA)-) (**3c**) (Figure 3, Figure 5).
A contrasting tumor can be received with these radiometal-labeled RGD-peptides, but there is a high accumulation in kidneys. In these case, pharmacokinetic and biological stability of the lead structure is declined due to radiometal-lead structure is declined due to radiometal-labeling.

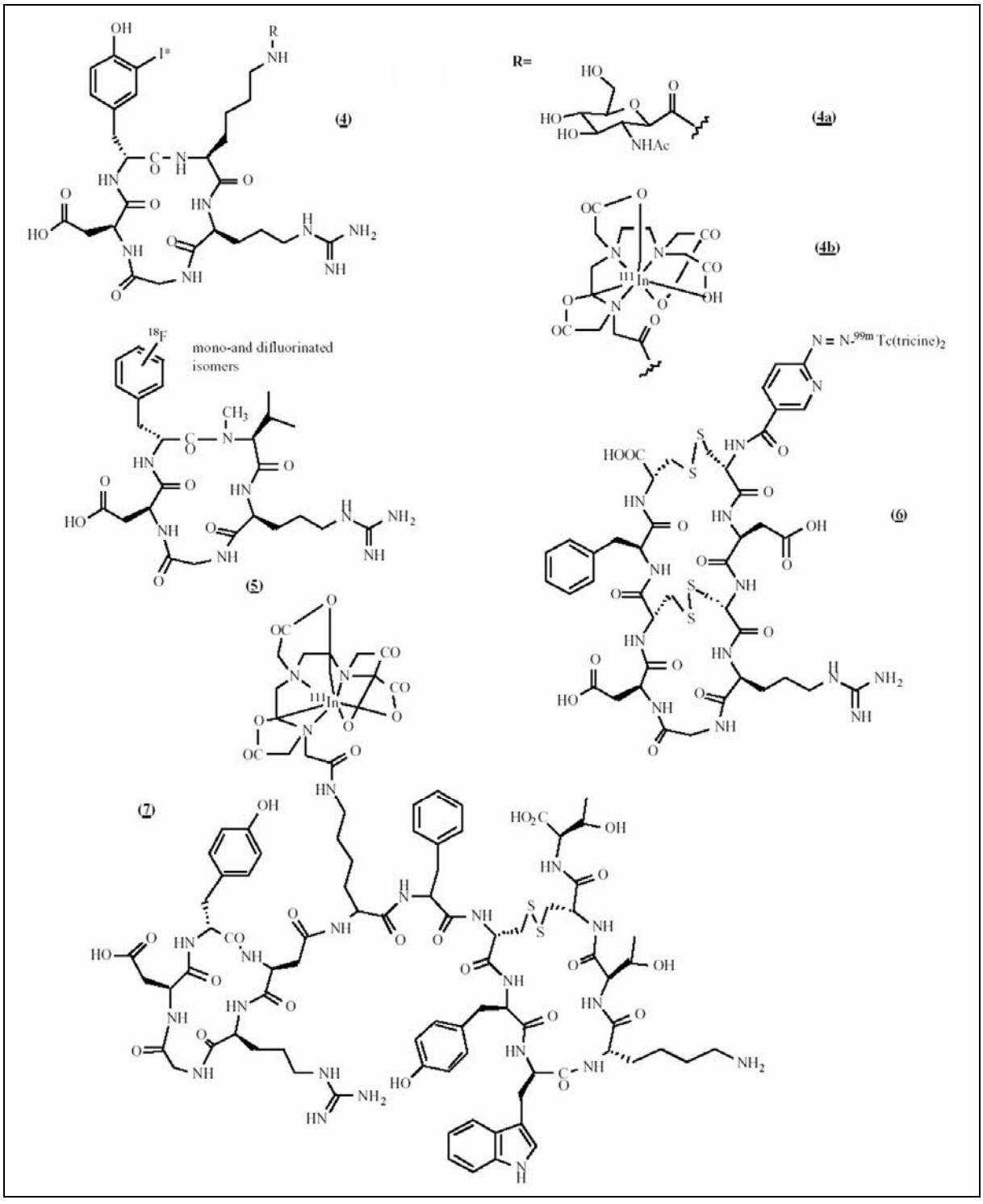

Figure 5: Structures of radiolabeled RGD (ARG-Gly-Asp) containing antagonists. (B)

5. Phage Display Derived RGD-Peptides

In general: *Page display derived peptides can also be used as lead structures. It is important to investigate if the radiolabeling and modification of this structure influence the biological activity and specifity.*

RGD-4C ((Cys2-Cys10,Cys4-Cys8)H-Ala-Cys-Asp-Cys-Arg-Gly-Asp-Cys-Phe-Cys-Gly-OH) is an αvβ3-binding peptide which results from a phage display library. It contains two disulfide bridges and binds with moderate affinity (KD ~ 100nM) to both, the αvβ3 and αvβ5 integrin. A shortened derivative of RGD-4C ((Cys1-Cys9,Cys3-Cys7)H-Cys-Asp-Cys-Arg-Gly-Asp-Cys-Phe-Cys-OH) can be coupled with hydrazinonicotinamide with hydrazinonicotinamide

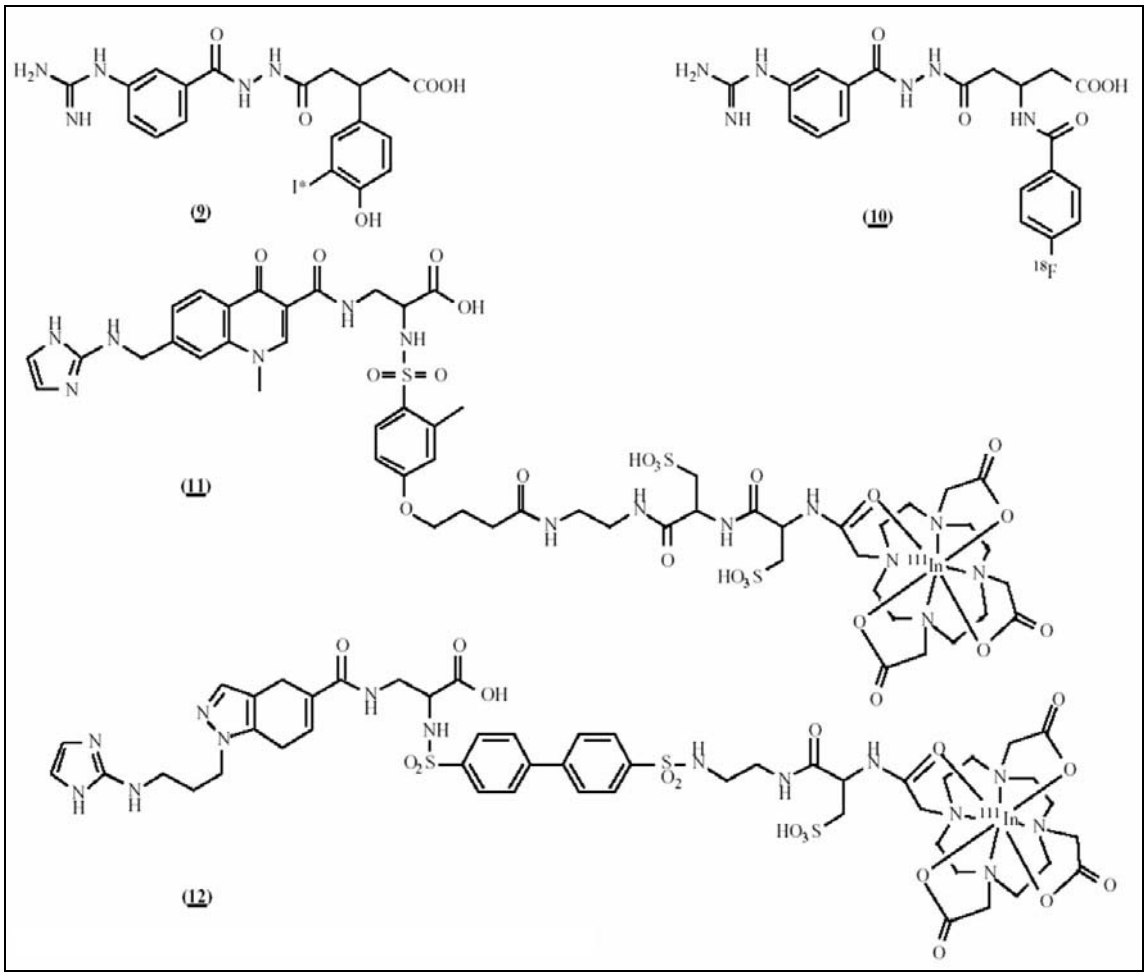

Figure 6: Structures of radiolabeled RGD (ARG-Gly-Asp) containing antagonists. (C)

and labeled with ^{99m}Tc resulting in [*Tc]technicium(HYNIC)-(H-Cys^{1}-Asp^{2}-Cys^{3}-Arg^{4}-Gly^{5}-Asp^{6}-Cys^{7}-Phe^{8}-Cys^{9}(HYNIC)) (**6**).
Tumor uptake of this radiolabeled RGD-peptide was declined. Thus, either the conjugation with HYNIC, the deletion of the terminal amino acids and/or the labeling with ^{99m}Tc-technetium impairs the affinity to the αvβ3 integrin resulting in a peptide that appears not suitable for *in vivo* imaging of αvβ3 expression (Figure 5).

6. Linear RGD-Peptides

In general: *If the lead structure is cyclic also linear peptides can be investigated. They may increase tumor accumulation, but they can also decline the wanted characteristics.*
Apart from cyclic RGD-structures, linear peptides containing either one (H-Lys-Pro-Gln-Val-Thr-Arg-Gly-Asp-Val-Phe-Phe-Glu-Gly-NH2) or two RGD sequences (H-Arg-Gly-Asp-Ser-Cys-Arg-Gly-Asp-Ser-Tyr-OH) can be investigated. The first can be labeled performing a new strategy allowing ^{18}F-labeling of peptides on a solid support. The resulting 4-[^{18}F]fluorobenzoyl-Lys-Pro-Gln-Val-Thr-Arg-Gly-Asp-Val-Phe-Phe-Glu-Gly-NH2 shows only low activity accumulation in tumor. HPLC analysis of blood and urine samples reveal no intact peptides to be present as soon as 5 min after tracer injection demonstrating the low metabolic stability of the linear peptide.
The linear decapeptide containing two RGD-motifs can be labeled with ^{99m}Tc exploiting the included Cys^{5}. This tracer can image tumors with positive contrast with high background activity in lung and abdomen.
Small linear peptides show often low selectivity for distinct integrin subtypes recognizing the RGD-sequence. Thus, the affinity is increased by having two RGD-sites within the small linear peptide.

7. Non-Peptidic αvβ3-Antagonists (Peptidomimetics)

In general: *To overcome the general low oral bioavailability of peptide based radiopharmaceuticals, non-peptidic low molecular mass compounds (peptidomimentics) are usually designed. Combinatorial chemistry approaches are essential to generate promising mimetics. Sometimes additional information from x-ray or NMR investigations on the involved receptor or*

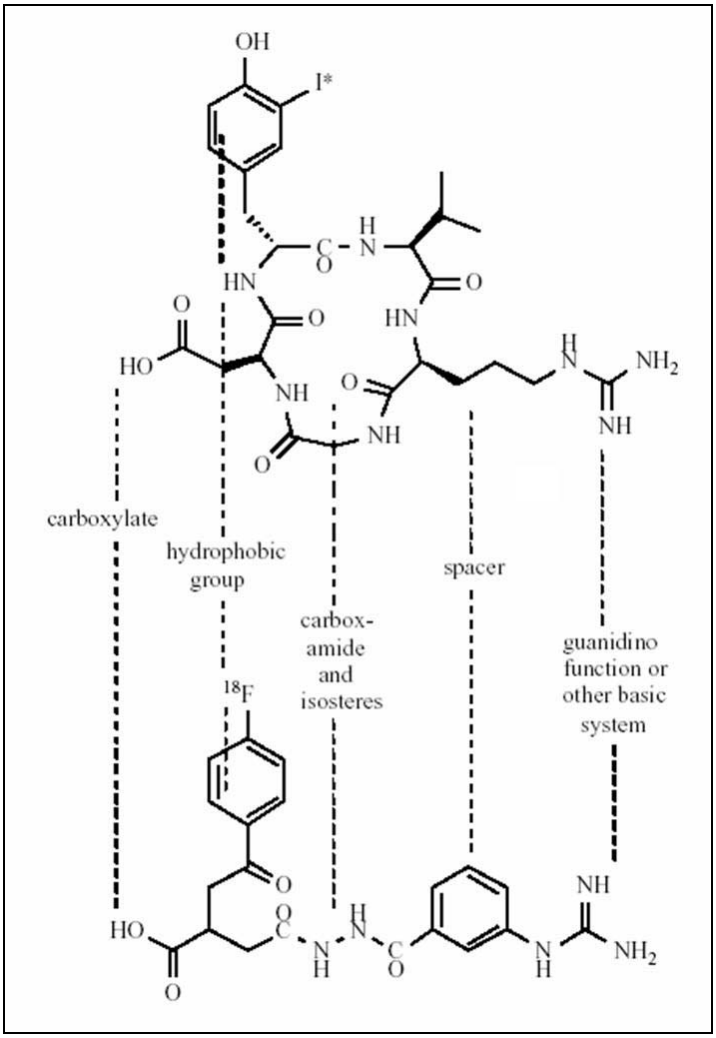

Figure 7: Schematic presentation of the homologies between peptidic and non-peptidic αvβ3-antagonists.

receptor/ligand complex is available.

A great variety of small molecular mass αvβ3 antagonists is available. They include isoxazolines, indazoles, 2-benzazepine Gly-Asp mimics and nonpeptidic azacarba-derivatives. A schematic presentation of essential homologies between peptidic and non-peptidic αvβ3 antagonists can be found in Figure 7. Derivatives from the guanidinobenzoyl hydrazino oxopentanoic acid scaffold (GBHO) can be used for preparation of radioiodine- and ^{18}F-labeled analogues by direct iodination and 4-[^{18}F]fluorobenzoylation. The resulting iodinated structure is called [^{123}I]GBHO-1 or [^{123}I]Aza-RGD-1 (**9**). Comparison of this structure with [^{125}I]Gluco-RGD (**4a**) results in a 3-fold lower activity accumulation for the peptidomimetic. However, due to the rapid renal and hepatic elimination of [^{123}I]GBHO-1 (**9**), tumor-to-background ratios are comparable with those obtained with [^{125}I]Gluco-RGD (**4a**). However, the peptidomimetic shows specific binding to the receptor which was investigated using control experiments with mice having a non-αvβ3 expressing tumor.

Even higher tumor-to-background ratios can be found for the iodinated structure, 4-[^{18}F]fluorobenzoylated derivative [^{18}F]GBHO-2 (**10**), which is also known as 5-[N'-(3-guanidinobenzoyl)-hydrazino]-3-(4-[18F]fluorobenzoyl)-amino-5-oxopentanoic acid. *In vivo* studies in mice and rats indicate that [^{18}F]GBHO-2 (10) is an alternative to the [^{18}F]-Galacto-RGD (**3b**) for the *in vivo* imaging of αvβ3 expression.

Indazole (TA120) (**12**) and quinolone (TA138) (**11**) core based peptidomimetics can also be synthesized. In TA120 and TA138, the guanidino function and the carboxyl group of the RGD-sequence is mimiced by aminoimidazole and diaminopropionate. Both compounds used cysteic acid as a pharmacokinetic modifier. However, both show high activity accumulation in tumor *in vivo*, whereas T138 gives a better tumor-to-background ratio than TA120.

It is very reasonable to use peptidomimetics

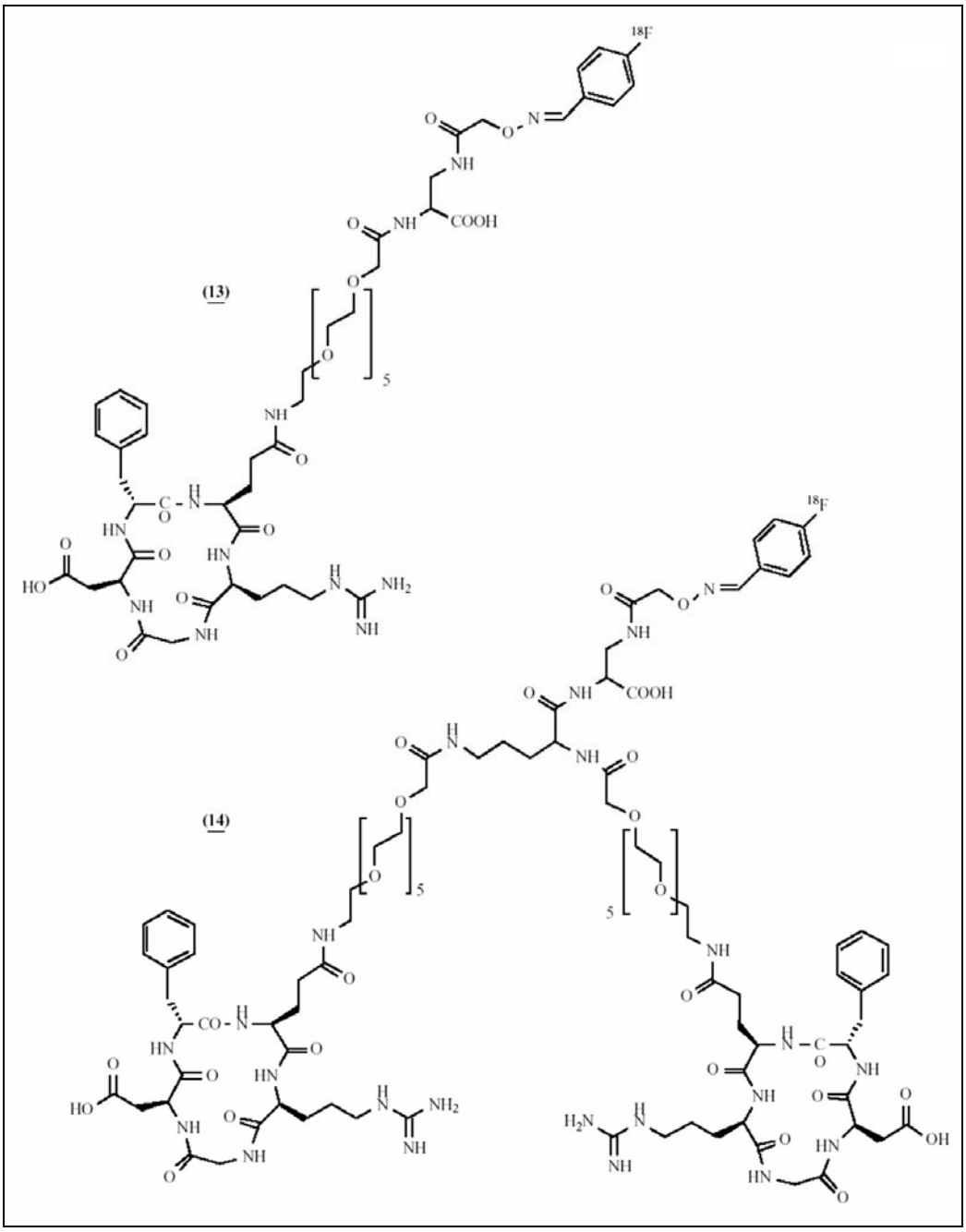

Figure 8: Structures of radiolabeled RGD (ARG-Gly-Asp) containing antagonists. (D)

to detect αvβ3 expressing tumors, as these increase the oral bioavailability of the ligand and they show good tumor-to-background ratios (Figure 5, Figure 6, Figure 7).

8. Heterodimeric and Homomultimeric αvβ3-Ligand Systems

In general: *To improve efficacy and selectivity in peptide receptor targeting, radiolabeled homogeneous and heterogeneous dimeric, oligomeric and multimeric ligand systems are important. Homogeneous ligand constructs with two or more receptor ligands coupled to linker systems or to a common backbone are expected to improve targeting by cooperative receptor-ligand interactions and receptor shielding to endogenous competition. Heterogeneous systems are investigated to enhance tracer accumulation by taking advantage of the heterogeneity of receptor expression on target cells (targeting of the cumulative receptor density). Furthermore, heterodimers offer the possibility of addressing successive biochemical processes within the cell, e.g. using the first bioactive structure as a vehicle and addressing unit and the second one as effector.*

These strategies are also used for evaluation of RGD-sequence based ligands. A heterodimer can be received due to conjugation of cyclo(-Arg^1-Gly^2-Asp^3-DTyr^4-Lys^5-) via a DTPA moiety with octreotate (**7**). This heterodimer shows comparable high affinity and selectivity for somatostatin 2 (sst2) and αvβ3 as the monomer. This can be explained by high affinity of octreotate to sst2.

A homodimer can be created by linking two cyclo(-Arg^1-Gly^2-Asp^3-DPhe^4-Lys^5-) via a glutamic acid (**8**). The free δ-carboxylate function of the glutamic acid linker can be used to conjugated the peptide with DOTA (**8a**) or HYNIC (**8b**), which enables labeling with ^{111}In, ^{90}Y, ^{177}Lu and ^{99m}Tc.

The compounds show high affinity and selectivity for the αvβ3 integrin *in vitro* and high tumor uptake *in vivo*. The HYNIC (**8a**) conjugated structure causes an *in vivo* tumor growth delay. Thus, radiolabeled homodimer RGD-peptides show also potential for peptide receptor radiotherapy.

Form a RGD-monomer (**13**) RGD-dimers (**14**), -tetramers (**15**) and octamers can be developed. Therefore, two, three or eight cyclo(-Arg^1-Gly^2-Asp^3-DPhe^4-Lys^5-) moieties were conjugated to a modified hexaethylene glycol linker and branched via lysines. *In vitro* studied reveal increasing affinity to αvβ3 in the series monomer<dimmer<tetramer<octamer with an improvement in binding of about two orders of magnitude from the monomer to the tetramer.

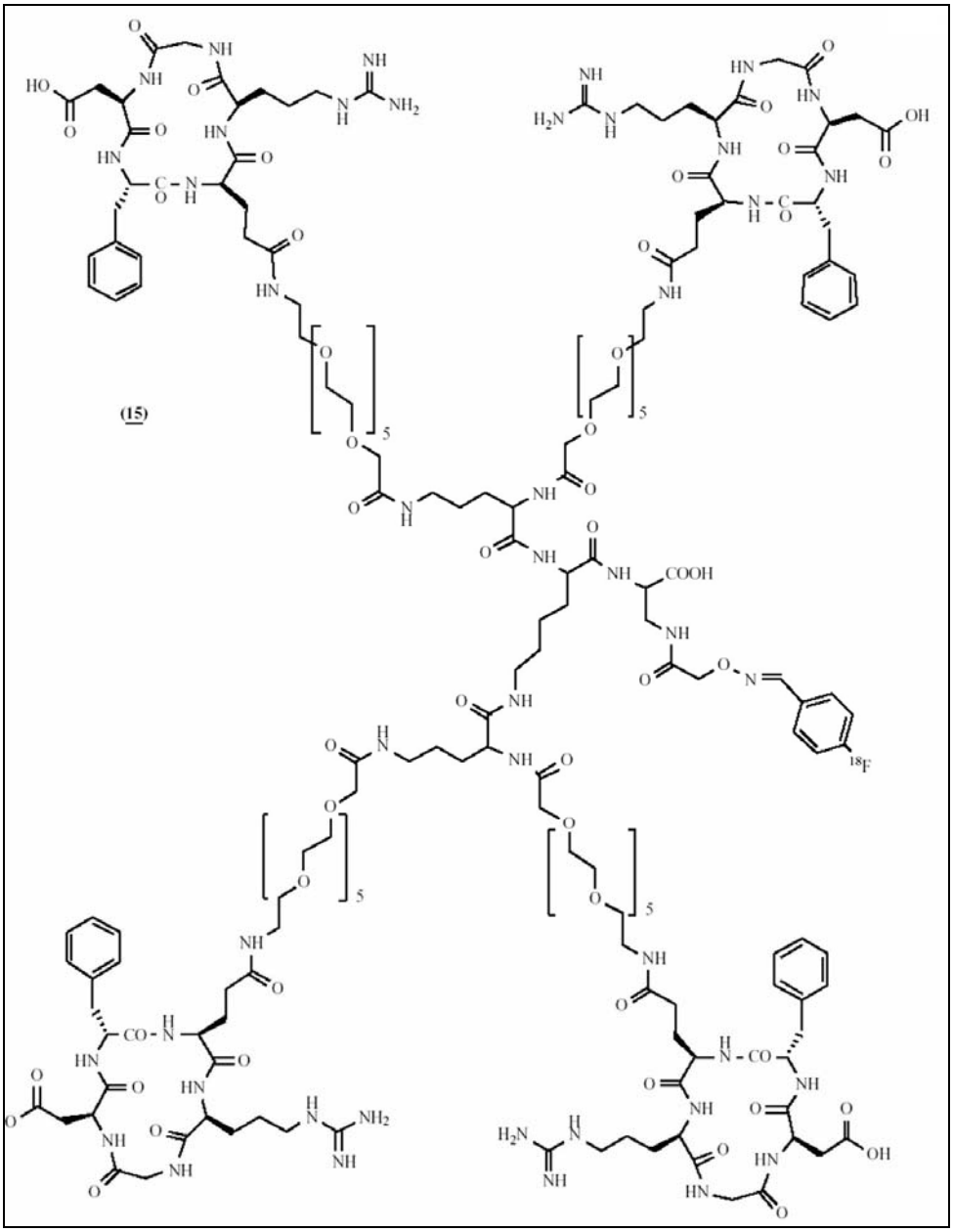

Figure 9: Structures of radiolabeled RGD (ARG-Gly-Asp) containing antagonists. (E)

IV. Conclusion

While designing a molecular ligand for nuclear imaging, a lot of aspects need to be considered. At first a lead structure is required. *Direct labeling* of this lead structure is the most easiest method to get a radiopharmaceutical ligand, but if the lead structure has a strong lipophilic character, the tracer will probably be taken up by liver and intestine. Therefore, a lot of other ways for labeling are present, like *modification with carbohydrates* (which can increase bioavailability, solubility and resistance against proteases), *modification with hydrophilic tetrapeptides* (which improve pharmacokinetics and if D-amino acids are present also the proteolytic stability of the ligand) and *radiometal-labeling* (which increases pharmacokinetics and metabolic stability). It is also important to prove if cyclic or linear ligands are more specific. There are also other alternatives for lead structures such as peptides from *phage display* or *non-peptidic lead structures* (peptidomimetics). It is hardly to say which source of lead structure and which labeling is the most efficient one. Thus, it is important to try a lot and to compare them for each single case.

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