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Seminars in
NUCLEAR
MEDICINE

Bifunctional Gallium-68 Chelators: Past, Present, and Future

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This article reviews the development of bifunctional chelates for synthesising ^{68}Ga radiopharmaceuticals. It structures the chelates into groups of macrocycles, nonmacrocycles, and chimeric derivatives. The most relevant bifunctional chelates are discussed in chelate structure, parameters of ^{68}Ga-labeling, and stability of the ^{68}Ga-chelate complexes. Furthermore those derivatives are included, where ^{67}Ga was applied instead of ^{68}Ga. A particular feature discussed is the ability of certain bifunctional chelate structures to function in kit-type preparation of the ^{68}Ga radiopharmaceuticals. Currently, nonmacrocyclic and chimeric derivates attract particular attention such as THP-derivates and DATA-derivates.
Semin Nucl Med 46:373-394

Introduction

^{68}Ga-radiopharmaceutical chemistry in itself is an interdisciplinary field. It involves (1) the production of ^{68}Ga, either via ^{68}Ge or ^{68}Ga radionuclide generators or via direct production at cyclotrons, (2) the design of adequate chelate ligands (CL) with appropriate thermodynamic and kinetic characteristics for Ga(III) ligand complex formation, (3) the development of versatile bifunctional (bf) derivatives thereof, (4) their covalent coupling to relevant targeting vectors, and finally, (5) the radiochemistry of ^{68}Ga-labeling itself, including optimisation of yields in short period of time as well as the stability of the ^{68}Ga-label under physiologic conditions. This review covers the aspect of bifunctional chelates, which has, however, always to be seen in the context of the other aspects mentioned.

Bifunctional CL (bfCL) for ^{68}Ga rely on the CL itself. Ga(III) ligand complex formation was systematically studied in the past century, but essentially not in the context of ^{68}Ga radiopharmacy. Yet, both Ga(III) hydrolysis and Ga(III) ligand complex formation equilibria for many of the conventional "inorganic" and "organic" ligands have been investigated in detail, and thermodynamic and stoichiometric complex formation constants have been obtained (for a compilation of these parameters[1-3]).

Historically (which refers to the 1960s and 1970s), the first clinically relevant ^{68}Ga CL complex was derived from a ligands, well established for trivalent metal coordination chemistry—EDTA. ^{68}Ga-EDTA was synthesized in situ by eluting the first generation of ^{68}Ge/^{68}Ga radionuclide generators with an aqueous solution containing EDTA. The generator eluate, thus represented the ^{68}Ga-EDTA complex, which was directly injected intravenously. The intention was to monitor perfusion characteristics in various organs, particularly in the brain. The next CL used for ^{68}Ga complex formation appeared only several decades later, namely DOTA. This time, the pure ^{68}Ga-DOTA complex was not of any medical interest. In contrast, the task was on labeling a small peptide (octreotide) with ^{68}Ga (instead of radioiodine) to visualize the enhanced uptake of the somatostatin analogue on neuroendocrine tumour cells, overexpressing transmembrane G-protein coupled somatostatin receptors. Consequently, the sole CL had to be transformed into a bifunctional derivative bfCL. This goal was achieved by spending one COOH functionality to form a peptide bond with the N-terminus of the octreotide.

Interestingly, neither EDTA nor DOTA are CL specifically designed for ^{68}Ga(III) coordination chemistry. They simply appeared to be of effect because of the performance of other M(III) complexes, such as the trivalent radioisotopes for EDTA or the trivalent stable lanthanides, in particular Gd, well established in MR imaging. Nevertheless, both CL significantly contributed to the success of ^{68}Ga-based molecular imaging: The search for indeed ^{68}Ga(III) specific new CL structures.

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http://dx.doi.org/10.1053/j.semnuclmed.2016.04.003
0001-2998/

This review would discuss both the rather conventional approach of M(III) CL to become ^{68}Ga-specific (eg, DOTA toward NOTA and AAZTA toward DATA) and the modification of CL known for Fe(III) toward ^{68}Ga(III) specificity. However, it is not the aim to cover all CL developed for ^{68}Ga in detail. For that purpose, there are comprehensive reviews on those topics such as Price and Orvig,[4] Roesch and Riss,[5] and Liu.[6] All relevant CL should provide high thermodynamic and kinetic stability of the ^{68}Ga-bfCL complexes concerned. Candidates, who successfully passed the selection processes mentioned, are grouped into three categories: macrocyclic, nonmacrocyclic, and chimeric. Their (nonbifunctional) CL structures are given in Table 1 together with their thermodynamic complex formation stability constant (log *K*) and typical parameters and radiochemical yields (RCY) of ^{68}Ga complex formation.

The following tables list the main bifunctionalisation pathways of the individual CL structure. There are three principal routes to bifunctionalisation and they are as follows:

1. One initial function of the CL (which is basically in all cases the carboxy group) is preserved for coupling (typically for amide bond formation), whereas the others remaining still satisfy the demand of ^{68}Ga(III) coordination (cf DOTA to tris-protected DOTA).
2. This COOH group is substituted by another coupling functionality (to NH_2, to NCS, etc).
3. Introducing of an extra functional group on one carbon atom of the CL (cf NOTA to NODAGA).

Finally, the relevance of the various groups of bfCL is discussed in (1) their potency to effectively form complexes with ^{68}Ga and to (2) guarantee in vitro and in vivo stability of the label.

Nonmacrocyclic Chelates

DTPA

As one of the most often applied acyclic chelators used in radiochemistry, DTPA can be radiolabeled with many different radio metal ions like ^{64}Cu, $^{67/68}$Ga, $^{44/47}$Sc, ^{111}In, ^{177}Lu, and many more under mild conditions.[4] Accordingly, there is an impressive number of bf derivatives of DTPA, cf Table 2.

DTPA is, for example, used in the Food and Drug Administration-approved SPECT agent Octreoscan (^{111}In-DTPA-octreotide) for imaging the somatostatin receptor of neuroendocrine tumors.[28] To couple the DTPA structure to the peptide, the dianhydride DTPA-CA was used.[29] Further work with the DTPA-CA was the coupling with folate to use as a tumor targeting radiopharmaceutical.[30] To image insulin receptors, DTPA-CA was coupled to human insulin and radiolabeled with ^{67}Ga in 0.1 M phosphate buffer (pH = 8.0) for 30 minutes at ambient temperature. Testing the in vitro stability in saline and human serum, no evidence of large-scale release of ^{67}Ga was found after 2 hours.[23] Furthermore, the DTPA-CA was conjugated to the monoclonal antibody (mab) 103A for the imaging of erythroleukemic mice. Labeling with ^{67}Ga was performed starting from a citrate complex and adding the antibody conjugate in water or MES buffer.[31] With DTPA-CA, the radiolabeling of lectins was performed by conjugating the anhydride to the lectin and labeling in 0.1 M glycine HCl buffer (pH = 3.5) for 30 minutes at room temperature (RT) with ^{67}Ga. Comparison with DFO-labeled lectins showed, that the agglitung ability of the DTPA-lectins is significantly lowered compared with the DFO-lectins.[24] Because of the disadvantage, that one ligand carboxylate metal binding site is occupied forming an amide bond in the coupling of DTPA-CA, another approach is the synthesis of DTPA monoamides using EDC or DCC.[32]

Alternatively, *p*-SCN-DTPA has been synthesized.[33] After conjugation of *p*-SCN-DTPA to an anti-CD45 mab, ^{68}Ga labeling was performed in 1 M sodium acetate (pH = 5.0) for 10 minutes at RT to guarantee radiochemical purities >95%. The serum stability was found to be very high after 4 hours.[25] The nontargeted *p*-SCN-DTPA-gallium complex was formed in 0.5 M ammonium acetate buffer (pH = 5.0) to challenge the complex formation (with Fe, Cu, Zn, Al, Sn, and Ti) showing only small effects on the ^{68}Ga complexation ability (except in the presence of Fe) with a ppm concentration of the metal ions. Challenging the stability of the formed complex with ions present in human serum (Cu, Fe, Ca, and Zn) showed a large influence on the complex stability with a 1000 times molar excess of the metal ions.[26]

Within the synthesis of the *p*-SCN-DTPA, *p*-NH_2-DTPA was one of the steps inbetween[33] and could be coupled to HPPH, a tumor-avid photosensitizer (currently undergoing phase I and II human clinical trials).[34] The compound only was labeled with Gd^{3+} in this case. For labeling with ^{111}In, *p*-NH_2-DTPA was conjugated to a peptide for liver fibrosis (pPB-HAS).[35]

The CHX-DTPA-structure was originally designed to improve stability in the chelation of ^{90}Y.[36] The steric rigidity in the complex because of that fusion of the *trans*-cyclohexyl moiety into DTPA improves the orientation of the chelating groups. The *trans*-structure results in two different stereoisomers called A and B.[37] The CHX-B-DTPA diastereomer is more unstable in serum than CHX-A-DTPA for ^{88}Y.[38]

p-SCN-Bn-CHX-A"-DTPA was coupled to a peptide (DUPA-Pep). Radiolabeling was performed in DMSO and 0.25 M HEPES buffer (pH = 7.4) at RT for 60 minutes. Precursor amounts of >18 nM gave high RCY > 96% after 30 minutes.[27] A mab (YAML568) was conjugated to the *p*-SCN-Bn-CHX-A″-DTPA and radiolabeled in 1 M sodium acetate (pH = 5.0) 10 minutes at RT yielding radiochemical purities >95%.[25]

The mx-DTPA or 1B4M-DTPA structure with an additional methyl-group attached to the backbone was coupled to YAML568 and labeled in 1 M sodium acetate (pH = 5.0) 10 minutes at RT yielding radiochemical purities >95%.[25] For the radiolabeling of mab B3, 1B4M-DTPA was used.[39] Syntheses proceeded, for example, in four steps with good yields via the amine like the *p*-SCN-DTPA structure too.[40]

Nevertheless, ^{68}Ga-bfDTPA derivatives are supposed to suffer from stability in vivo,[36] making its use less common in recent years.

Table 1 Overview on Structures of the Sole Chelate Chelators (CL), Their Thermodynamic Complex Formation Stability Constant (log *K*) and Typical Reaction Parameters to Achieve the High-Radiochemical Yields (RCY) Mentioned of the ^{68}Ga Ligand Complexes. Also, Those Derivatives Are Included, Where ^{67}Ga Was Applied Instead of ^{68}Ga.

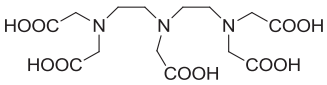
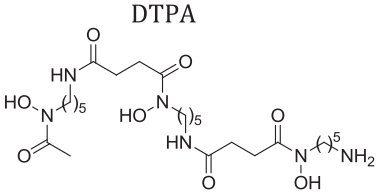
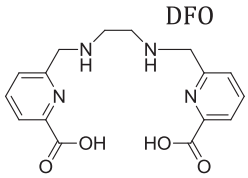
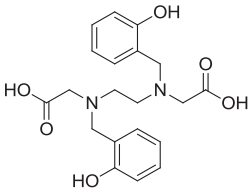
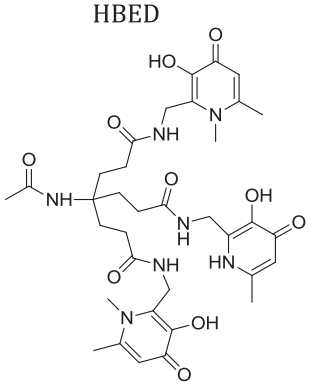
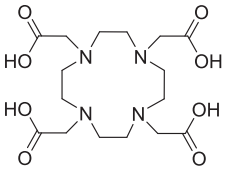
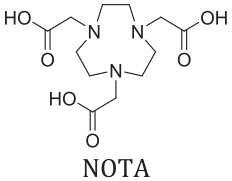
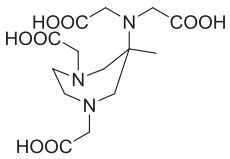
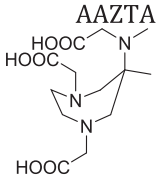
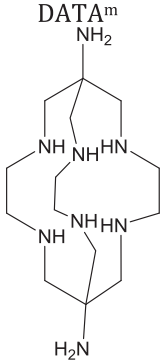
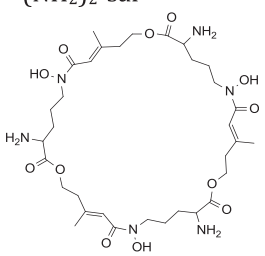
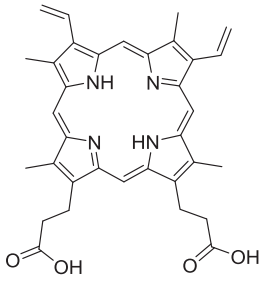
CL	log K_{GaL}	Typical Radiolabeling (Buffer, pH, Reaction Time, and Reaction Temperature)	RCY (%)	Ref
	24.3			7
DTPA 	28.6	0.1 M ammonium acetate (pH = 4.5), 5 min, RT	96	8,9
DFO 	28.1	0.1 M sodium acetate, 10 min, RT	97	10,11
H_2dedpa 	38.5	2.1 M HEPES buffer (pH = 4.2), 4 min, ≈95°C/RT	99	12,13
HBED 	–	1 M ammonium acetate, 5 min, RT	99	14
H_3THP-Ac 	21.3	1 M HEPES buffer (pH = 4.8), 5 min, ≈95°C	>90	2,15
DOTA  NOTA	31.0	1 M HEPES (pH = 3.5), 10 min, ≈95°C	>95	1,16

Table 1 (*continued*)

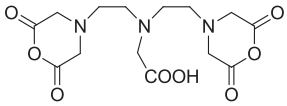
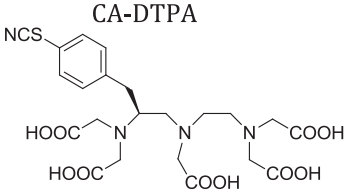
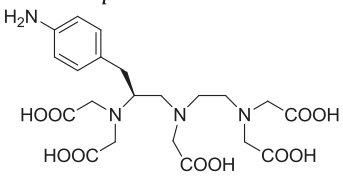
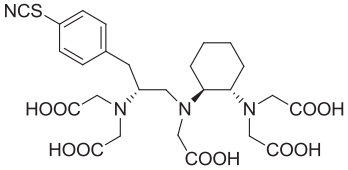
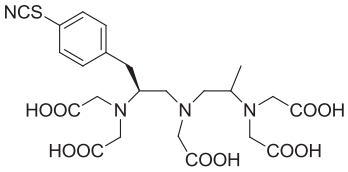
CL	log K_{GaL}	Typical Radiolabeling (Buffer, pH, Reaction Time, and Reaction Temperature)	RCY (%)	Ref
	22.2	1 M sodium acetate (pH = 4.5), 10 min, RT	>95	17,18
AAZTA 	21.7	0.2 M sodium acetate, 1 min, RT	>95	19
DATA^m 	–	0.1 M sodium acetate, 35 min, ≈85°C	98	20
$(NH_2)_2$-sar 	–	2 M sodium acetate (pH = 5), 5 min, RT	96	21
Fusarinine C  Porphyrins	–	sodium acetate (pH = 4.5), 45 min, ≈120°C(MW)	33	22

MW, microwave; RT, room temperature.

DFO

DFO is a well-known chelator for Fe(III).[41] For ^{89}Z, DFO is the only competent chelator for in vivo use.[42] The stability constant (log K_{GaL} = 28.6[8]) of the Ga(III)-DFO complex is higher than the one with most other acyclic chelators. Pure DFO itself can be considered already as a bifunctional chelator. With its free amine, it was bound to one of the carboxylic acids of the folic acid for tumour imaging via formation of an amide bond using DCC as a coupling reagent. ^{67}Ga labeling was performed in TRIS-buffered saline (pH = 7.4) in 24 hours with ambient temperature.[43,44] Starting with the mesylate DFO salt, DFO-octreotide was made by forming an amide bond between DFOs free amine and the peptides carboxylic acid to yield

Table 2 Overview on bfCL Based on DTPA (For Remarks cf Table 1)

bfCL	Radiolabeling	RCY (%)	Ref
	0.1 M phosphate buffer (pH = 8.0), 30 min, RT	96	23
	0.1 M glycine HCl buffer (pH = 3.5), 30 min, RT	98	24
CA-DTPA 	1 M sodium acetate (pH = 5), 10 min, RT	95	25
	0.5 M ammonium acetate (pH = 5.0), 10 min, RT	98	26
p-SCN-DTPA 	No $^{67/68}$Ga labeling performed		
p-NH_2Bn-DTPA 	0.25 M HEPES buffer (pH = 7.4), 60 min, RT	96	27
	1 M sodium acetate (pH = 5), 10 min, RT	95	25
p-SCN-Bn-CHX-A"-DTPA 	1 M sodium acetate (pH = 5), 10 min, RT	95	25
1B4M-DTPA			

DFO-octreotide. Labeling was performed with both ^{67}Ga and ^{68}Ga. Labeling conditions for ^{68}Ga were 0.1 M ammonium acetate buffer (pH = 4.5) for 5 minutes at ambient temperature[9,45] (Table 3).

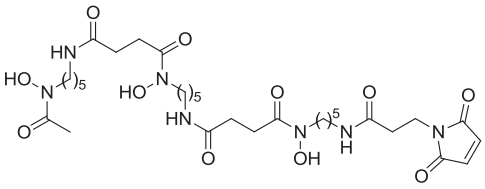
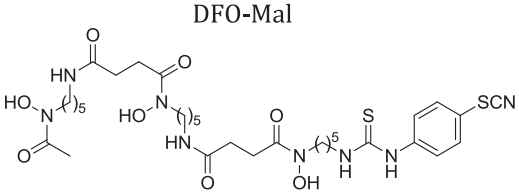
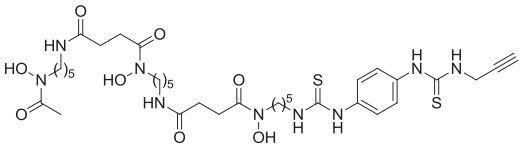
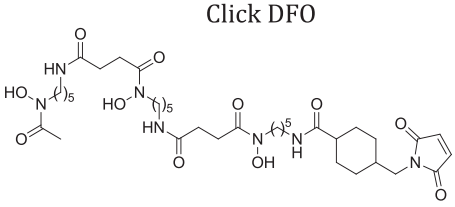
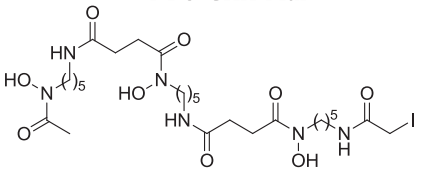
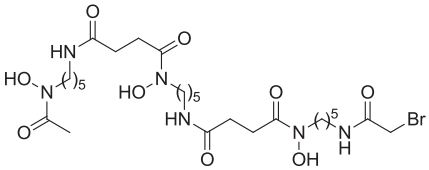
Starting with DFO, a thiol (with SATA [Pierce]) was generated of the thioester and then coupled to a mab(anit-CD74). The same mab was modified starting with the DFO mesylate (in DMSO with SMCC). Both conjugates were labeled with ^{67}Ga in 0.5 M NH_4OAc (pH = 5.3) for 1 hour at RT.[50] Comparison of three different coupling methods of DFO to a mab was performed using Mal-DFO, the pyridyl disulphide method (linking DFO by an intramolecular disulfide bridge) and the glutaraldehyde (linking DFO with glutaraldehyde) method. All antibody conjugates were labeled with ^{67}Ga in 0.05 M Phosphate-buffered saline (pH = 7.5) at RT for 30 minutes.[9,46]

p-SCN-DFO or DFO-Bz-NCS was synthesized starting with DFO in good yields in one step.[51,52] A Nanobody (heavy-chain-only antibody) 7D12 and U36 were conjugated to *p*-SCN-DFO and labeled in 3 M ammonium acetate buffer (pH = 7.2) in 5 minutes with ambient temperature in good yields. In vitro stability in ammonium acetate buffer and human serum showed only low $^{67/68}$Ga dissociation (6%-8% after 24 hours).[47] Other antibodies (H6-11) were conjugated to *p*-SCN-DFO with good results too.[53]

A "clickable" DFO with an alkine was developed for the conjugation of the chelator to peptides starting with *p*-SCN-DFO in a one-step synthesis. It was clicked to a model peptide known to target gastrin-releasing peptide receptors and was radiolabeled with ^{64}Cu.[48]

For the reaction with thiols, three different types of bifunctional DFO were synthesized. Starting with the Mal-CHX-DFO, a maleimid ester derivative, BAC-DFO and IAC-DFO, with bromine and iodine as leaving groups attached. All three were made in only one step starting with the free amine of the DFO reacting with SMCC, bromoacteylbromide or

Table 3 Overview on bfCL Based on DFO (For Remarks cf Table 1)

bfCL	Radiolabeling	RCY	Ref
 DFO-Mal	0.05 M PBS (pH = 7.5), 30 min, RT	>90%	9,46
 *p*-SCN-Bn-DFO	3 M ammonium acetate (pH = 7.2), 5 min, RT	>90%	47
 Click DFO	Not applied to ^{68}Ga radiolabeling	–	48
 DFO-CHX-Mal	Not applied to ^{68}Ga radiolabeling	–	49
 DFO-Iac			
 DFO-Bac			

PBS, phosphate-buffered saline.

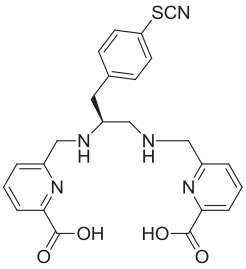
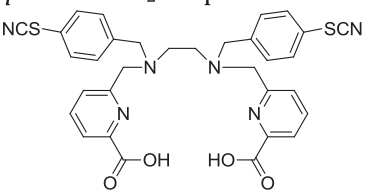
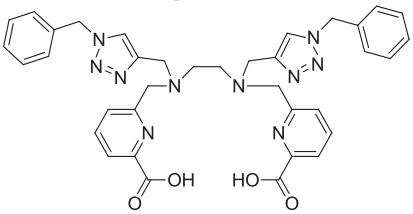
N-hydroxysuccinimidyl iodoacetate. All three resulting bfCL were coupled with trastuzumab, but none of the conjugates was labeled with ^{68}Ga.[49]

Dedpa

Dedpa is an acyclic chelator based on an ethylendiamin backbone with two pyridine rings attached and a carboxyl-group on the pyridine ring to fulfil the coordination chemistry demands of Ga(III). The general labeling procedure initially applied was 0.1 M NaOAc (pH = 4.5) for 10 minutes at RT. Transchelation studies with *apo*-transferrin indicated no decomposition after 2 hours. Completion for chelation with NOTA was performed by adding H_2dedpa and NOTA to $^{67}Ga^{3+}$ for 10 minutes at ambient temperature. More than 98% of the ^{67}Ga-dedpa complex was detected. Potentiometric titration with EDTA gave a log K_{GaL} = 28.11[10] (Table 4).

Attempts to further improve the stability of the dedpa-complex by using a preorganised backbone gave

Table 4 Overview on bfCL Based on dedpa (For Remarks cf Table 1)

Chelator	Radiolabeling	RCY (%)	Ref
 H_2-dp-bb-NCS	0.1 M sodium acetate, 0.2 10 min, RT	97	11
p-SCN-BnH_2dedpa 	0.1 M sodium acetate, 0.2 10 min, RT	99	11
H_2-dp-NCS  H_2azapa	0.1 M sodium acetate, 10 min, RT	96	54

H_2CHXdedpa, but chelation abilities with Ga(III) have not been reported yet.[55] A benzyl-NCS dedpa, H_2dp-bb-NCS, or *p*-SCN-Bn-H_2dedpa,[56] was conjugated to an RGD peptide. Radiolabeling of dedpa-RGD was performed in 0.1 M sodium acetate for 10 minutes at ambient temperature with 97% RCY. In the presence of an excess of *apo*-transferrin, 92% of the ^{68}Ga-dedep-RGD-complex were intact after 2 hours.[11] Within the synthesis of the H_2dp-bb-NCS, the diamine compound H_2dp-N-NH_2 was synthesized, but not used for further work.[11]

A chelator for the formation of dimeric compounds, H_2dp-N-NCS, was synthesized for coupling to an RGD peptide. The dimeric compound was radiolabeled in 0.1 M sodium acetate for 10 minutes at ambient temperature with 99% RCY and 73% of the complex remainded stable against *apo*-transferrin after 2 hours.[11]

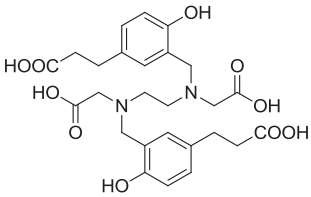
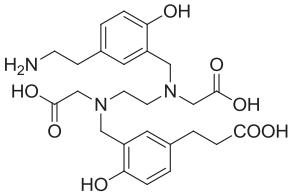
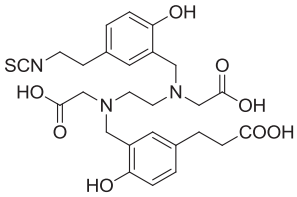
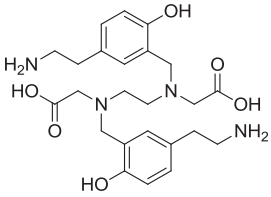
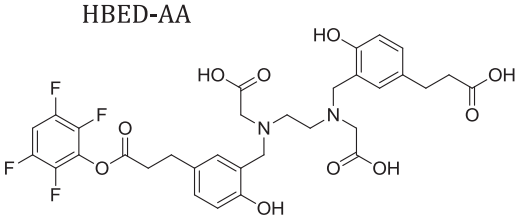
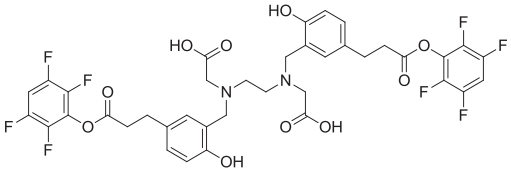
H_2dedpa-propyl$_{pyr}$-NH_2 was coupled with FITC for radio imaging and fluorescence imaging. The bfCL with the free amines could be radiolabeled with ^{67}Ga under standard procedure (10 minutes, ambient temperature).

H_2azapa was developed as a bifunctional triazole-containing acyclic chelator. The bfCL contains two alkine parts, and a click reaction to the triazole was performed with benzyl azide on both sides to the dimeric H_2azapa-chelator. To yield dimeric compounds, targeting vectors with azides could be used for the click reaction. Radiolabeling with ^{67}Ga was performed in 0.1 M sodium acetate (pH = 4.0) at ambient temperature for 10 minutes with good labeling yields. Blood serum stability for the complex seems to be not to very high in first challenging studies.[54]

HBED

HBED (N′N-bis(2-hydroxybenzyl)ethylendiamin-N,N′-diacetic acid) is an acyclic chelator based on an EDTA-type structure with two additional phenol coordination sides for Ga(III). With its high stability constant for the Ga^{3+} complex (log K_{GaL} = 38.51[12]) and its acyclic structure, HBED is well known for the rapid and efficient radiolabeling at ambient temperature and its high in vivo stability.[57] Closely related to the HBED, the SHBED structure (log K_{GaL} = 37.47[12]) was developed to alter the complexation ability of phenol groups of the HBED, the charge of the Ga-ligand complex and the solubility in water. The introduction of the *para*-sulfonate groups decreases the pK_a of the phenolic protons, raises the solubility in water and increases the negative charge (formal charge of the fully deprotonated ligand is −6 compared with −4 for the HBED). The greater pK_a, and therefore acidity of the

Table 5 Overview on bfCL Based on HBED (For Remarks cf Table 1)

bfCL	Radiolabeling	RCY (%)	Ref
	In 2.1 M HEPES buffer (pH = 4.2), 4 min, RT	99	13
	0.1 M MES buffer (pH = 4.8), 10 min, RT	96	59
HBED-CC 	Not radiolabeled with $^{67/68}$Ga		
HBED-CA 			
HBED-CI 			
HBED-AA 	0.1 M phosphate buffer (pH = 7) at RT		60-62
	In 0.1 M HEPES buffer (pH = 7.5)		60-62
HBED-CC-TPF 	In 0.1 M HEPES buffer (pH = 7.5)		60-62
HBED-CC-TPF$_2$			

phenolic protons, result in an effective radiolabeling at lower pH-values for the SHBED[58] (Table 5).

Single chain vascular endothelial growth factor proteins for the imaging of the vascular endothelial growth factorreceptor were synthesized as HBED and NOTA conjugates, starting with the [Fe(HBED-CC)]$^-$ complex and converting it to the activated N-hydroxysuccinimid. [^{68}Ga]Ga^{3+} labeling of the HBED-conjugate (and the NOTA conjugate as well) was performed in 2.1 M HEPES buffer with a pH of 4.2 and ambient temperature. The HBED-conjugate labeled nearly

quantitative (98.7 ± 0.7%) within 4 minutes, whereas the NOTA-conjugate required times >10 minutes to reach comparable high labeling yields. Both the HBED and NOTA-conjugate showed similar high stabilities in NaCl, phosphate buffer and human serum, cell studies gave compared binding abilities with almost the same K_D values and biodistribution studies and PET imaging showed similar results (with a notably lower liver uptake of the HBED-CC conjugate and a lower kidney uptake of the NOTA-conjugate).[13]

Labeling of antibodies via HBED-CC (AntiMUC1/anti-Ga antibody and 1.1ASML antibody) was performed in acetate buffer (pH = 4.8) in 15 minutes with a temperature of 95°C[63] and 0.1 M MES buffer (pH = 4.8) resulting in an pH of 3.0 in 10 minutes at ambient temperature.[59]

Peptide conjugation of HBED-CC to prostate specific membrane antigen (PSMA) targeting vector gives a powerful diagnostic tool for prostate cancer. Radiolabeling was performed with 0.1-1.0 nmol of the precursor in 0.1 M HEPES buffer (pH = 7.5) adjusting the pH value of the solution to 4.2 using NaOH.[61] Interestingly, most of the routine labeling protocols prefer to synthesize ^{68}Ga-HBED-CC-PSMA at elevated temperatures (eg ca. 95°C), although high RCY are reported for labeling at ambient temperature.

Within the synthesis of the HBED-CC chelator, three more bifunctional derivatives were synthesized. The HBED-CA, HBED-AA, or the HBED-CI chelator are not commonly used. For example, the HBED-CI chelator was used to prepare a HBED-transferrin complex to investigated blocking abilities in the evaluation of a 1.1ASML F(ab)$_2$ antibody fragment.[59]

With the TFP-ester, a versatile conjugation method for HBED-CC was developed. Starting with [Fe(HBED-CC)]$^-$, the HBED-CC(TFP) was synthesized in one step with purification by high performance liquid chromatography. Even after 3 months, no deterioration was observed. After coupling to an anti–epidermal growth factor antibody (mAb425), radiolabeling was performed in 0.1 M phosphate buffer (pH = 7.0) at ambient temperature.[57] HBED-CC(TFP) peptide conjugation was also performed. Radiolabeling of the HBED-CC(TFP)-peptides was performed in 0.1 M HEPES buffer (pH = 7.5).[60-62] To design dimeric compounds with two targeting vectors attached, the HBED-CC(TFP)$_2$ was synthesized. After coupling of two peptide structures to yield the dimeric compound by the same method performed for monocoupling, radiolabeling was performed by the same procedure.[62]

Deferiprone (HPO-Ligands)

Deferiprone, a HPO-type (3-hydroxy-4-pyridinone) ligand, is the only orally active iron-chelating drug to be used therapeutically in conditions of transfusional iron overload.[64] The hydroxypyridinone-type ligands are known to interact with the group 13 ions, especially Fe(III). 3-Hydroxy-pyridinones are most effective in neutral pH.[65-67] Multidentate ligands using HPO as a chelating part have recently received increased attention for use in ^{68}Ga radiochemistry[68] (Table 6).

CP256 (H_3THP-Ac) is a hexadentate HPO-based Fe^{3+} chelator shows remarkable labeling abilities, rapidly chelating ^{68}Ga at RT at almost neutral pH (6.5). Stability studies with the ^{67}Ga-labeled compound showed no evidence of protein binding or ^{67}Ga release in human serum in 4 hours at 37°C.[14]

With YM103 (H_3THP-Mal), a bifunctional maleimide derivative was developed, for example, to couple to free cysteine residue on proteins.[14] YM103 was conjugated to the mab trastuzumab, but only labeled with Zr.[69]

H_3THP-NCS was made starting with a literature known amine derivative of CP256[72] to yield the NCS-ester in good overall yields and conjugated to a cyclic (RGDfK) peptide. Radiolabeling in 1 M ammonium acetate (pH = 6.5) gave RCY > 99% within 2-5 minutes at RT[70].The synthesis of H_3THP-PhNCS started with the same amine-containing starting material such as the one for H_3THP-NCS. The H_3THP-PhNCS was conjugated to the cyclic (RGDfK) peptide too and gave RCY > 99% under the same conditions than the H_3THP-NCS.[70] Serum stability studies for both H_3THP-NCS and H_3THP-PhNCS cyclic (RGDfK) peptide peptide conjugates indicated no transchelation of $^{68}Ga^{3+}$ in human serum.[70] With H_3THP-NCS, the octreotide derivative TATE was conjugated to attend a kit-type labeling approach for ^{68}Ga. Radiochemical yields > 99% at RT in NH_4OAc buffer (pH: 5-6) and high serum stabilities were reported recently.[71]

Newer deferiprone based chelator types designed for Ga-chelation chemistry are NTA(BuHP)$_3$ and NTP(PrHP)$_3$, but no bifunctional derivatives are know so far. Labeling conditions show promising results for the usage in radiopharmaceutical chemistry. ^{67}Ga labeling was performed in HEPES buffer (pH = 5) with RCY > 98% at RT.[73,74]

Macrocyclic Chelates

DOTA

DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) is a cyclen-based chelator, and the most frequently used macrocyclic chelator for PET applications. [^{68}Ga]DOTA-TOC is the only ^{68}Ga radiopharmaceutical that is approved as orphan drug.[75] It was first synthesized 1976 by Stetter and Frank,[76] but it needed 12 years to get the first bfCL derivate by Moi et al.[77] DOTA has a relatively low stability constant for gallium (log K_{GaL} = 21.3) because the metal is too small to perfect fit in the cavity of DOTA.[2,78,79] Rapid and efficient radiolabeling needs ≈ 95°C temperature and at least 5 minutes heating periods.[15] Recent publications show the wide application of DOTA as prominent gallium chelator[80-90] (Table 7).

DOTA has a wide diversity in bf derivates for conjugation to target vectors. Because DOTA only needs four nitrogen atoms of the cyclen ring and two oxygen atoms of the carboxylic acid groups to coordinate Ga(III), it can be used as a bifunctional chelator without further modification (if disregarding the protection groups of remaining carboxylic acids).[96] One possibility is DOTA-tris(*t*-Bu)ester (2-(4,7,10-tris(2-(tert-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl)acetic acid) that can be coupled to free amine groups with coupling agents.[91,97] Another way is the use of DO3A*t*Bu where the free amine reacts with a bromine.[98] Both reactions need a deprotection of the carboxylic acid groups after coupling.

Table 6 Overview on bfCL Based on Deferiprone (For Remarks cf Table 1)

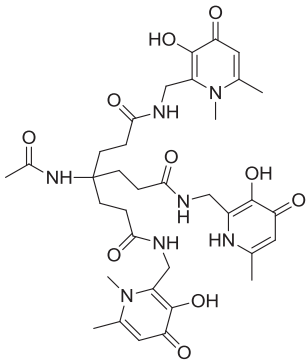
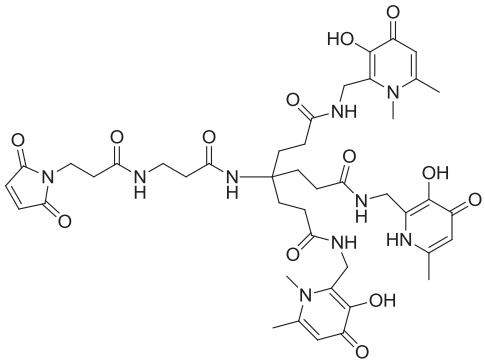
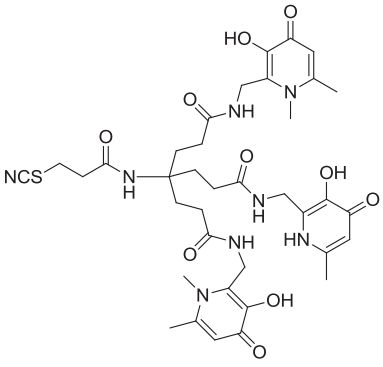
bfCL	Radiolabeling	RCY (%)	Ref
 CP256	1 M ammonium acetate 5 min, RT	95	14
H_3THP-Ac  YM103	Zr-labeling only	–	69
H_3THP-Mal  H_3THP-NCS	1 M ammonium acetate, 5 min, RT 1 M ammonium acetate, 5 min, RT	99 99	70 71

Table 6 (*continued*)

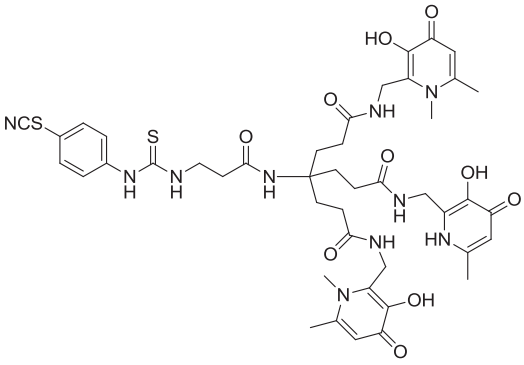
bfCL	Radiolabeling	RCY (%)	Ref
 H_3THP-PhNCS	1 M ammonium acetate, 5 min, RT	99	70

Table 7 Overview on bfCL Based on DOTA (For Remarks cf Table 1)

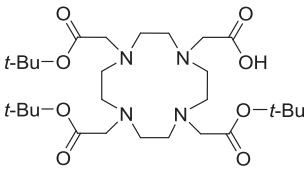
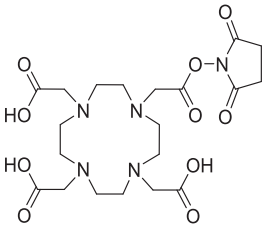
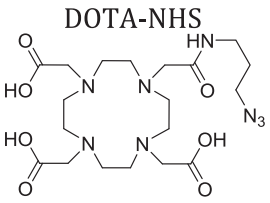
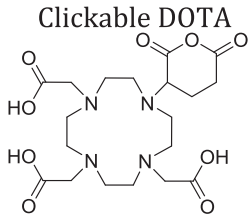
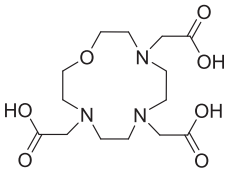
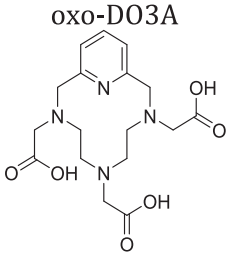
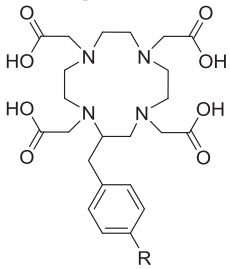
bfCL	Radiolabeling	RCY (%)	Ref
 DOTA-tris (*t*-Bu)	1 M sodium acetate, 7 min, 80°C	95	91
	1 M HEPES buffer (pH = 4.8), 5 min, 90°C	95	15
 DOTA-NHS	No gallium radiolabeling	–	92
 Clickable DOTA	2.7 M HEPES (pH = 3.5), 5 min, 95°C	95	93,94
DOTAGA-anhydride			

Table 7 (*continued*)

bfCL	Radiolabeling	RCY (%)	Ref
	0.01 M sodium acetate, 5 min, RT	98	95
oxo-DO3A 	0.01 M sodium acetate, 5 min, RT	98	95
PCTA  R = -NH_2, -NCS	0.01 M sodium acetate, 5 min, 80°C	95	95
p-SCN/NH_2-Bn-DOTA			

It is possible to conjugate unprotected DOTA to the target vector by using active esters. Usually the pure or in situ produced DOTA-NHS ester is used.[15,92,99-104] A typical protocol with pure DOTA-NHS ester needs a polar solvent such as DMF or DMSO and a base such as triethylamine for coupling to an amine group.[15] Further active esters are mentioned besides the NHS esters.[105-107]

A new approach to conjugate DOTA to target vectors is the use of clickable compounds.[48,104,112] DOTA-NHS ester reacts with clickable compounds such as aminoalkylazid or the DO3*t*buA with ω-alkynhalogenids. Both clickable fragments react with the target vector counterpart. The advantage is a fast and easy conjugation to the target vector.

By using a carboxylic acid arm of DOTA some stability of the bfCL gets lost (a problem more for heavier lanthanides than for gallium), hence there is a derivate that contents a glutamic acid arm DOTAGA.[108-110] For a faster reaction with less side compounds DOTAGA-anhydride is superior to activated DOTAGA.[93] There are some examples for the use of DOTAGA and gallium.[94,111,112] The negative charge of ^{68}Ga-DOTAGA can be a problem for the affinity and the tumor to nontumor ratio.[112]

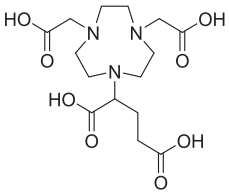
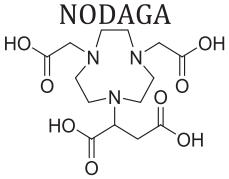
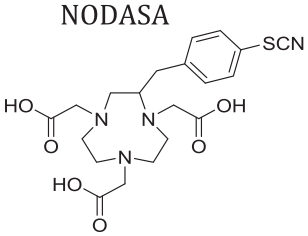
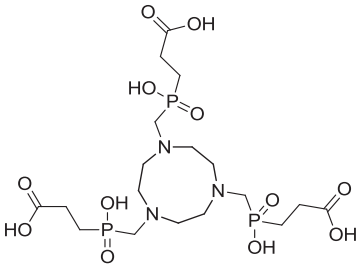
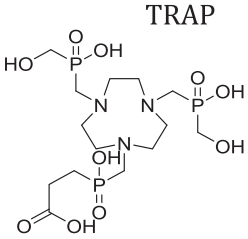
PCTA[95,113] and oxo-DO3A[114] with changed ring geometry were developed to avoid the slow labeling at high temperature. In PCTA one nitrogen is substituted by a pyridine fragment. With this modification ^{68}Ga was labeled within 5 minutes at RT and with a radiochemical yield >95%.[95] The PCTA was coupled to a cyclo-RGDyK, successfully labeled and it shows a behavior similar to NOTA-RGD.[113] In oxo-DO3A one nitrogen is replaced by an oxygen atom and it can chelate ^{68}Ga completely at ambient temperature within 5 minutes.[95]

Besides the conjugation of one pendant arm of DOTA a conjugation can directly take place on the cyclen scaffold. For this propose p-Bn-NH_2 or p-Bn-NCS units are used.[95,115] DOTA-p-Bn-NCS shows the same labeling conditions in comparison with DOTA.[115]

NOTA

NOTA (N,N′,N″-(1,4,7-triazacyclononane-1,4,7-triyl)triacetic acid) is the current gold standard for the complexation and bioconjugation of Ga(III). Because of the smaller 1,4,7-triazacyclononane ring structure, gallium fits better in the

Table 8 Overview on bfCL Based on NOTA (For Remarks cf Table 1)

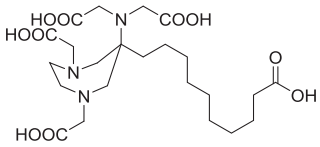
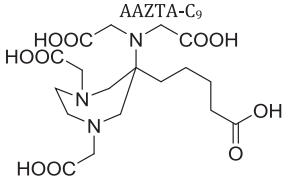
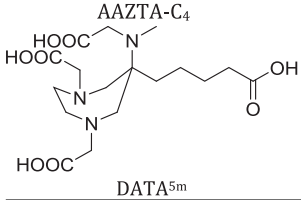
bfCL	Radiolabeling	RCY (%)	Ref
	Different buffers, 25 min, 95°C	95	116
NODAGA 	0.5 M ammonium acetate, 25 min, 90°C	No yield given	117
NODASA 	7% sodium bicarbonate sol. (pH = 6.0), 10 min, RT	89	118,119
p-SCN-benzyl NOTA 	HEPES (pH = 3), 3 min, 40°C	95	120
TRAP  NOPO	2.7 M HEPES, 5 min, 25°C	99	121,122

cavity and has a high stability constant (log K_{GaL} = 31.0).[1,3] It is a N_3O_3 hexadentate chelator with good labeling conditions for gallium at ambient temperature within 15-60 minutes.[4] Interestingly, most of the routine labeling protocols for ^{68}Ga-bf(NOTA)-conjugated compounds apply higher temperatures (eg ca. 95°C), although high RCY are reported in some studies for RT (Table 8).

There are several known and applied bifunctional derivates.[4,6,123,124] The easiest way to conjugate NOTA to a target is the use of one carboxylic acid arm, which is coupled to an amine with coupling agents (such as HBTU, HATU, etc.)[84,125-131] or with active esters.[99,132] Recently a new solid phase synthesis has been introduced.[127,133,134] Besides those methods, there are less used ones such as clickable compounds,[99] coupling with thiols,[135] or the use of maleimidoethylmonoamide NOTA.[136] The disadvantage of this conjugation is the loss of one carboxylic acid arm, which becomes a less coordinating amide bond.[126]

NODAGA is one of the most important bifunctional chelators (besides p-SCN-Bn-NOTA) for gallium and it has

Table 9 Overview on bfCL Based on AAZTA and DATA (For Remarks cf Table 1)

bfCL	Radiolabeling	RCY (%)	Ref
 AAZTA-C_9	1.1 M sodium acetate, 10 min, RT	>95	18
 AAZTA-C_4	Sodium acetate, 10 min, RT	93	83
 $DATA^{5m}$	1 M ammonium acetate, 10 min, RT	>98	172,173

been synthesized in 2002 for the first time by Eisenwiener et al.[125] Using glutaric acid as conjugation arm instead of acetic acid has the following two advantages: on the one hand the hexadentate N_3O_3 structure does not get destroyed as in direct conjugated NOTA. On the contrary there is some space between the target and the chelator. There are recently a lot of publications that use NODAGA to coordinate gallium.[84,87,116,126,128,131,137-143] The conjugation applies via coupling reagents or the use of NHS-esters. NODASA is the homologue between NOTA and NODAGA with its one carbon atom shorter side arm. In 1998, Maecke et al. published an article about this topic.[117]

NOTA-p-Bn-SCN is the most important scaffold for bifunctional chelators of NOTA. The first scaffold-modified chelator was mentioned 1989 by Craig et al.[144] In the subsequent years many synthesis have been published[145-148] NOTA-p-Bn-NCS can easily be conjugated to an amine function without touching the ring geometry of gallium. There are many publications with several target vectors.[113,118,127,136,143,149-157]

A really new and promising TACN derivate is TRAP (3,3′,3″-(((1,4,7-triazonane-1,4,7-triyl)tris(methylene))tris(hydroxyphosphoryl))tripropanoic acid). It has been mentioned 2010 by Notni et al.[120] and many articles have been published about this topic during the past 5 years.[140,158-163] TRAP has good labeling conditions (RT, 10 minutes), a good stability with gallium (log K_{GaL} = 26.2)[120] and it is very robust against impurities of the gallium generator.[159,164] TRAP has been applied to the following target vectors: bisphosphonates,[165] cyclo-RGDfk,[160] and nitroimidazoles.[162] Because trimerization was not always the best option, NOPO had been developed.[122] NOPO has a smaller stability constant (log K_{GaL} = 25.0)[121] than TRAP, but the stability is still high enough. NOPO was conjugated to NOC[121,122] and c(RGDfk).[122,166] Radiolabeling needs RT and 5 minutes.[121] MA-NOTMP is the only carbon modified derivate of the phosphinic-TACN chelators.[167] There are some other known bifunctional TACN derivates without further significance in the past few years.[168-171]

AAZTA and DATA

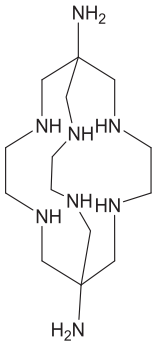
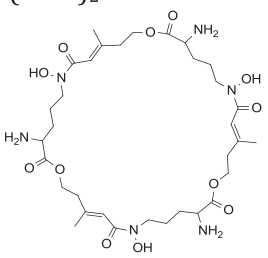
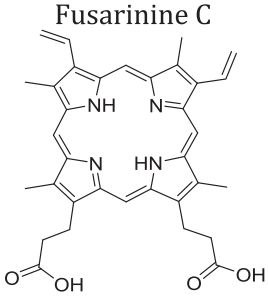
AAZTA was developed in 2004 as a MRI contrast agent for gadolinium,[174] but it forms stable complexes with gallium (log K_{GaL} = 22.18) as well.[17] AAZTA and DATA are chimeric chelators, because they combine a macrocyclic 1,4-diazapam ring with a free acyclic arm. Bifunctional derivates of AAZTA have been applied in small animal ^{68}Ga-PET studies, using minigastrin[18]—or RGD peptidomimetic D58[83] target vectors. Both studies required mild conditions for labeling (10 minutes, RT), but evaluations also indicate that AAZTA is not completely stable against human serum and chelators such as EDTA and DTPA.[18]

An auspiciously derivate of AAZTA is DATA.[175] It was successfully labeled under mild conditions and had shown no loss of gallium in human serum or DTPA solution.[19,172] It resembles the strategy of turning from DOTA to NOTA (Table 9).

Other Macrocyclic bfCL

Sacrophagines: The macrobicyclic hexaamine sarcophagine (3,6,10,13,16,19-hexaazabicyclo[6.6.6]icosane) chelator is well known for the chelation of different ions,[176,177] but its main application is the complexation of copper and its bioconjugation to target vectors.[4] There is only one publication of the use of sacrophagine for ^{68}Ga-PET.[20] It was conjugated to a cyclic-(RGDFfK) peptide via an active ester. Radiolabeling was performed at 85°C within 35 minutes and the gallium complex is stable against *apo*-transferrin. PET imaging showed an uptake in the tumor (with tumor-to-kidney and tumor-to-blood ratios of 0.32, respectively 26.37) without bone uptake.

Table 10 Overview on bfCL Based on Different Macrocyclic Types (For Remarks cf Table 1)

bfCL	Radiolabeling	RCY (%)	Ref
	0.1 M sodium acetate, 35 min, 85°C	98	20
$(NHR)_2$-sar 	2 M sodium acetate (pH = 5), 5 min, RT	96	21
Fusarinine C 	Sodium acetate (pH = 4.5), 45 min (MW), 120°C	33	22
Phorphyrins			

MW, microwave.

Siderophores: Siderophores are known for the chelation of ferric ions. They are produced by bacteria, mushrooms, and plants as iron storage.[178] Triacetylfusarinine was successfully radiolabeled with ^{68}Ga (RT, 15 minutes), showed a good stability and a high uptake in infected lung tissues.[179-182] It also has been used for the diagnosis of invasive pulmonary aspergillosis in rats[179] and identified as a potential radiopharmaceutical for the imaging of *aspergillus* infection.[181] Recently, Fusarine C has been used as a bifunctional chelator and conjugated trimeric to cyclic-(RGDfK).[21,183] Radiolabeling requires mild conditions (RT, 15 minutes, 3.6 mmol chelator) and the chelator is stable against phosphate-buffered saline, $FeCl_3$, DTPA, and fresh human serum. The in vitro biodistribution data show that [^{68}Ga]FSC-c(RGDfK) is comparable to [^{68}Ga]NODAGA-RGD.

Poryphyrins: Porphyrin based bifunctional chelators are rare and there are only few publications on ^{68}Ga labeling.[22,184] Radiolabeling needs harsh conditions (120°C, 45 minutes, microwave) and still results in low RCY (33%). Only in vitro experiments, but no in vivo ones were performed with the chelator attached to a target vector (Table 10).

Discussion

If one compares the number of CL used for ^{68}Ga radiopharmaceutical preparations in the period from ca. 1960-2000 (basically EDTA only), between ca. 2000 and 2010 (basically DOTA) , there is for the period after 2010 a dramatic increase in the number and availability and clinical use of CL and their corresponding bifunctional derivatives.

DOTA is still one of the major CL used for ^{68}Ga, and there is an impressive number of bifunctional derivatives. Most of them use one of the 4 carboxy groups for covalent attachment in

targeting vectors—either as it is or via substitution modes. Most established and commercially available bfCL for DOTA are DO3A, p-SCN-DOTA and DOTA-NHS-ester, but there are many more commercially available DOTA-based structures. DOTAGA, where derivatization is arranged at a methyl group of the side arm structure, adding a fifth arm with a COOH terminus, was recently applied for a PSMA derivative.[92] In all cases, ^{68}Ga-labeling requires temperatures close to the boiling point of water, which is no problem for many of the relevant targeting vectors (small peptides, bisphosphonates, and nitroimidazole derivatives[115]). Interestingly, bifunctionalisation using click chemistry moieties (such as alkynes and azides) for covalent attachment to a targeting vector modified via the complementary click moiety are not in routine use. However, the approach might consist in first labeling the DOTA-click component at high temperature, and subsequently couple the ^{68}Ga-DOTA-click bfCL to the targeting vector under mild conditions.

The next group of bfCL relies on the triaza structure. NOTA satisfy the requirements for ^{68}Ga coordination, and indeed labeling protocols hint on improved labeling under milder conditions. NOTA actually is the best example to reflect the new route of chelate structures functionalization: Occupying on carboxy group for covalent attachment in targeting vectors is not an option for the six donor NOTA; thus adding a new moiety coupled to a methylene ring carbon or an side arm carbon atom is the method of choice. A number of coupling functionalities have been substituted to that extra-arm of the triaza cycle. In addition, the triaza macrocyclic core was modified toward the group a TRAP molecules, which emphasizes the advantages of the triaza- vs tetraaza-ring strategy for ^{68}Ga labeling: ^{68}Ga labeling is working well at temperatures well below 95°C. With optimum labeling conditions, high RCY have been reported for TRAP derivatives.

Among the many nonmacrocyclic CL known to coordinate ^{68}Ga, only a few are described within the bfCL approach. The most straightforward example appears to be DTPA with its many bifunctional versions used for example ^{111}In radiopharmaceutical chemistry. Surprisingly, only very seldom articles report their use for ^{68}Ga. Another example is DFO, where several modifications are known, many of them commercially available. Common to these nonmacrocyclic bfCL is the ease of ^{68}Ga labeling at temperatures at or close to RT. The principal concern, on the contrary, is the in vivo stability of the corresponding radiopharmaceuticals. Unfortunately, there is a lack of systematic stability studies under in vitro (which is basically steady-state) and in vivo (which is "dynamic") conditions.

However, the use of HBED in molecular imaging of prostate cancer using the HBED-CC-PSMA derivative from Heidelberg demonstrated, that ^{68}Ga radiopharmaceuticals are able to combine the comfort of straightforward labeling and in vivo stability. Interestingly, the early believe of macrocyclic chelates as the ultimate need to guarantee for kinetic inertness of ^{68}Ga radiopharmaceuticals is changing. Following this strategy, a number of new nonmacrocyclic CL have been designed for ^{68}Ga coordination chemistry. The most important examples are dedpa[79,185] and THP-based structures.[22]

Also relatively new is the hybrid structure of DATA,[19] as derived from AAZTA. Similar to the relationship between DOTA and NOTA, DATA seems to perfect hybrid-chelate for ^{68}Ga(III) compared to AAZTA

^{68}Ga-Labeling

The relevance of the various groups of bfCL is discussed in (1) their potency to quantitatively form complexes with ^{68}Ga and to (2) guarantee in vitro and in vivo stability of the label. For the first aspect, most promising bfCL should complex ^{68}Ga

1. within short time (eg, <7 minutes, which is 10% of the physical half-life of the radionuclide),
2. ideally under mild conditions (pH and temperature),
3. in quantitative yields of $>95\%$ (to make product purification obsolete and to approach the kit-type radiolabeling protocols for the other radionuclide generator-derived isotope ^{99m}Tc, which made ^{99m}Tc radiopharmaceuticals such a success),
4. already at low concentration of the bfCL (note that ^{68}Ga-labeling is coordination chemistry and coordination chemistry is equilibrium chemistry, and thus a huge excess of precursor concentrations may easily shift labeling to high yields. However, utilization of large amounts of bfCL is contra-productive in terms of costs and specific activities), and
5. tolerating traces of competing metals such as Fe(III), Zn (II), and metals present in typical aqueous media.

The ultimate goal of ^{68}Ga-radiolabeling would be a kit-type characteristic to mirror the state-of-the-art of preparing ^{99m}Tc-radiopharmaceuticals. Several bfCL are reported to allow almost quantitative ^{68}Ga-labeling already at RT: almost all nonmacrocyclic structures (DTPA, DFO, and HBED), the macrocyclic NOTA-based, dedpa-based, THP-based structures, and the chimeric AAZTA-based derivative DATA. A critical aspect, however, is the amount of labeling precursor needed for those RCY. The widespread acceptance of ^{68}Ga-PET depends on radiopharmaceuticals that can be prepared in a simple, quick, and convenient manner. A kit-type labeling protocol would provide such characteristics and widen the portfolio of pliable biomolecules, but requires chelators that can be radiolabeled under exceptionally mild conditions and remains stable in vitro and in vivo. Recently the DATA chelators have been introduced that fulfill these requirements. The DATA chelators represent a novel approach to chelator design in that they are hybrids: possessing significant cyclic and acyclic character. It is believed that flexibility of the acyclic portion facilitates rapid complexation, whereas the preorganised cyclic portion minimizes the energy barrier to complexation and inhibits decomplexation processes. A recent article described its kit-labeling at RT at physiological pH, in short time and with quantitative yield.[19] Subsequently, the synthesis of first bfDATA derivatives and conjugation to [Tyr^3]-octreotide, a targeting vector for

neuroendocrine tumors and metastases, was investigated. DATA-TOC can be radiolabeled with ^{68}Ga to >95% in less than 5 minutes at ambient temperature using a range of commonly used technology, and for the first time in a kit-type manner from a lyophilized solid. The speed, reliability, flexibility, and simplicity with which ^{68}Ga-DATA-TOC can be prepared makes it a very attractive alternative to introduce kit-type labeling to ^{68}Ga-PET. The product solution only requires dilution with saline, sterile filtration and is ready for injection.[173] The final proof of the value of that DATA-derivative was to demonstrate, that ^{68}Ga-DATA-TOC is identical in its hsstr-binding profile, its pharmacology in animals and its performance in human neuroendocrine tumor imaging. Indeed, all those criteria are true for ^{68}Ga-DATA-TOC and have been reported recently.[173]

In a similar manner, the THP-Tyr^3-TATE was studied.[71] It demonstrated fast ^{68}Ga radiolabeling under mild conditions at 25 μg of the precursor. In vitro cell SSTR2-binding was comparable with that of ^{68}Ga-DOTA-TATE and in vivo PET imaging in AR4J tumor bearing mice showed comparable accumulation of the two tracers.

Stability of the ^{68}Ga-Label In Vivo

A measure of these rather physico-chemical parameters is the stability of the ^{68}Ga-complexes formed in aqueous solution in vitro (which is: the solution where complex formation was induced or the purified complex transferred to saline or to human serum the purified complex transferred to aqueous physiologic solutions containing certain concentrations of challenging substrates, such as *apo*-transferrin or "strong" CL such as DTPA for ligand exchange processes or stable metal cations such as Fe, Zn, Cu, Ca, etc. for metal exchange processes. (For protocols on how to perform those "challenge-type" stability studies see Riss et al.[186])

However, in vitro studies are typically performed under steady-state conditions, that is, in closed vials of small volume. Under those batch-type circumstances, the ^{68}Ga released from the bfCL may again form the ^{68}Ga-bfCL complex. Consequently, in vivo stability may differ, because the ^{68}Ga released diffuses away from the initial ^{68}Ga-bfCL-radiopharmaceutical, is excreted or bound at different targets. Thus, systematic studies are needed to fully understand the in vivo stability of a ^{68}Ga-bfCL complex.

Conclusion

Today, there is an impressive library of versatile chelate structures with each of them owing one or more bifunctional modifications. Many of the bfCL derivatives are commercially available. This allows to create an even broader library of ^{68}Ga radiopharmaceuticals. The philosophy would consist in synthesising a systematic number of derivatives of one and the same targeting vector (eg, a certain peptide) owing different kinds of bfCL. Next, in vitro evaluation screening should reveal a correlation between pharmacologic parameters of the final Ga compound (in binding affinities, lipophilicity, charge, etc). It appears obvious, that the kind of bfCL may affect those parameters. For the most relevant combination of bfCL + target vector, the radiochemistry part has to be added: What bfCL allows to label the compound under the appropriate experimental conditions in optimum radiochemical yield. Ideally, this should meet the requirements of instant kit-labeling. Finally, in vivo stability should be measured depending on the selection of a certain bfCL. This all together parallels ^{68}Ga radiopharmacy with medicinal chemistry. Overall, this is would be the fundament of new era of potent ^{68}Ga radiopharmaceuticals to come. Currently, those strategies are realized for a certain number of potent bfCL for ^{68}Ga. Among the most interesting candidates are THP, DATA, and TRAP. Indeed these BfCL have been applied to clinically relevant targeting vectors, such as THP-RGD,[70] THP-TATE,[71] DATA-TOC,[173] and TRAP-(RGD)$_3$.[160]

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