The Renaissance of the ^{68}Ge/^{68}Ga Radionuclide Generator Initiates New Developments in ^{68}Ga Radiopharmaceutical Chemistry

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Abstract: ^{68}Ge/^{68}Ga radionuclide generators have been investigated for almost fifty years now, since the cyclotron-independent availability of positron emitting ^{68}Ga *via* the ^{68}Ge/^{68}Ga system had always attracted researches working in basic nuclear chemistry as well as radiopharmaceutical chemistry. However, it took decades and generations of research (and researchers) to finally approach a reliable level of ^{68}Ge/^{68}Ga generator designs, adequate to the modern requirements of radiometal labeling chemistry. ^{68}Ga radiopharmacy now is awaking from a sort of hibernation. The exciting perspective for the ^{68}Ge/^{68}Ga generator, now – more than ever, asks for systematic chemical, radiochemical, technological and radiopharmaceutical efforts, to guarantee reliable, highly-efficient and medically approved ^{68}Ge/^{68}Ga generator systems. The expected future broad clinical impact of ^{68}Ga-labelled radiopharmaceuticals – beyond the ^{68}Ga-DOTA-octreotide derivatives – for imaging tumors and many organs, on the other hand, identifies the development of sophisticated Ga^{III} chelating structures to be a key factor. Today, open chain complexing agents have almost completely been displaced by macrocyclic DOTA and NOTA-derived conjugates. Structures of chelating moieties are being optimized in terms of thermodynamic stability and kinetic inertness, in terms of labeling efficacies at different, even acidic pH, and in terms of synthetic options towards bifunctionality, directed to sophisticated covalent coupling strategies to a variety of biologically relevant targeting vectors. Today, one may expect that the ^{68}Ge/^{68}Ga radionuclide generator systems could contribute to and facilitate the clinical impact of nuclear medicine diagnoses for PET in a dimension comparable to the established ^{99}Mo/^{99m}Tc generator system for SPECT.

Keywords: ^{68}Ge, ^{68}Ga, radionuclide generators, post-processing, chelators, co-ordination chemistry, labeling, radiopharmaceuticals, molecular imaging, PET.

1. INTRODUCTION

^{68}Ge/^{68}Ga radionuclide generators have been investigated for almost fifty years, since the cyclotron-independent availability of positron emitting ^{68}Ga *via* the ^{68}Ge/^{68}Ga system had always attracted researches working in basic nuclear chemistry as well as radiopharmaceutical chemistry. However, it took decades and generations of research (and researchers) to finally reach a level of ^{68}Ge/^{68}Ga radionuclide generator designs adequate to the modern requirements of radiometal labeling chemistry. This landmark can directly be associated to the radiochemical concept of providing cationic $^{68}Ga^{3+}$ rather than ^{68}Ga-fluoride, oxalate or EDTA complexes as eluates. With the beginning of this century such systems with ^{68}Ge absorbed on "modified TiO_2" columns and ^{68}Ga eluted using 0.1 N HCl became commercially available. This type of radionuclide generator was immediately adopted for labeling DOTA-conjugated peptides.

Interestingly, the same time the first PET/CT systems became operational, and this coincidence of a new PET tracer and a superior imaging technology was part of the success of molecular imaging neuroendocrine tumors using ^{68}Ga-DOTA-octreotide(s). This success in turn became a starting point of a renaissance of a broader ^{68}Ga radiopharmaceutical chemistry.

The exciting perspective for the ^{68}Ge/^{68}Ga radionuclide generator system, however, asks for systematic chemical, radiochemical, technological and radiopharmaceutical efforts, to guarantee reliable, highly-efficient and medically approved ^{68}Ge/^{68}Ga generator systems. The production of the radionuclide generator parent, its separation from the target material, and the chemical and technical concept of the separation of the daughter radionuclide are factors to result in an efficient and easy application of the generator. The expected future broad clinical application of ^{68}Ga labeled radiopharmaceuticals – beyond the ^{68}Ga-DOTA-octreotide derivatives – for imaging various tumors and other organs, on the other hand, identifies the development of sophisticated Ga^{III} chelating structures to be a key factor.

These individual challenges are becoming more and more accepted by the nuclear chemical, radiopharmaceutical and nuclear medicine communities. The IAEA has recently initiated comprehensive review on the production of several generator mother nuclides including a chapter on ^{68}Ge [1]. The radiochemistry of ^{68}Ge itself was covered comprehensively

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in 1996 [2]. A recent compilation on radionuclide generators systems including ^{68}Ge/^{68}Ga can be found in [3]. Finally, ^{68}Ga coordination compounds and radiopharmaceuticals were reviewed in 2003 and 2008 [4,5].

The present article aims to comprehensively cover the various aspects, namely the production of ^{68}Ge and the chemistry of ^{68}Ge/^{68}Ga generators, as well as the organic and co-ordination chemistry of chelating ligands and radioactive Ga-ligand complexes. In addition, some of the current medical applications of specific types of ^{68}Ga radiopharmaceuticals are highlighted, whereby this later aspect of medical application of ^{68}Ga tracers is beyond the scope of this paper.

2. NUCLEAR DECAY CHARACTERISTICS

The parent radionuclide ^{68}Ge with a half-life of $T_{½}$ = 270.95 d decays *via* electron capture to ^{68}Ga. This daughter subsequently decays ($T_{½}$ = 67.71 min) to stable ^{68}Zn. ^{68}Ga is a positron emitter with 89% positron branching accompanied by low-abundant photon emission (1077 keV, 3.22%) [6,7]. ^{68}Ge itself does not emit significant photon radiation, Fig. (**1**).

3. PRODUCTION OF ^{68}Ge

Nuclear Reactions

Most relevant processes are categorized according to the type of particle utilized and listed in Table **1**. All of the ^{68}Ge production pathways described use charged particle-induced reactions as schematically illustrated in Figs. (**2**). Deuterium or Helium-3 induced processes on gallium (reaction no. 5) and zinc (reaction no. 7) targets are not shown as they represent analogues to the proton (reaction no. 1) and Helium-4 (reaction no. 6) induced processes.

The investigation of precise nuclear data is mandatory for optimum production parameters providing the corresponding thick target yields and the desired radionuclidic purity. In fact, a number of data are available on either an individual nuclear reaction and / or on systematic comparisons both in terms of experiments and theory [8-17]. The IAEA recommended cross section data for the Ga(p,2n) processes [16], cf. Fig. (**3**). Horiguchi *et al.* [18] provided excitation functions and thick target yields for Ge(p,pxn)^{68}Ge reactions. The Ge(p,pxn) reaction cross sections increase even at higher proton energy. This is in particular relevant as natural germanium contains many stable isotopes from ^{70}Ge to ^{76}Ge, cf. Fig. (**2b**). Consequently, a large yield of ^{68}Ge is expected for the Ge(p,pxn) reactions. The specific activity of ^{68}Ge produced in these processes, however, is significantly lower than for the proton induced reactions on gallium targets. In parallel, radioarsenic isotopes are also formed according to Ge(p,xn) processes.

Thick-Target Yields

Integral ^{68}Ge thick targets yields have been calculated [18] for natural metallic germanium and zinc, and Ga_2O_3 targets. The yield for the proton-induced reactions on Ga is larger than that on Ge in the region lower than 60 MeV, although the latter becomes larger above 60 MeV, Fig. (**4**). The yield for the Zn(α,2n) reaction is more than one order of magnitude smaller than for the others.

The Ga(p,2n) process thus appears to be most relevant. Following its excitation function shown in Fig. (**3**), thick target yields per μA were calculated for a 1 h irradiation as well as for saturation, Fig. (**5**). Experimental thick-target yields reach values of 1 and 2 MBq μA^{-1} h^{-1} already at 25 or 45 MeV proton energy, respectively.

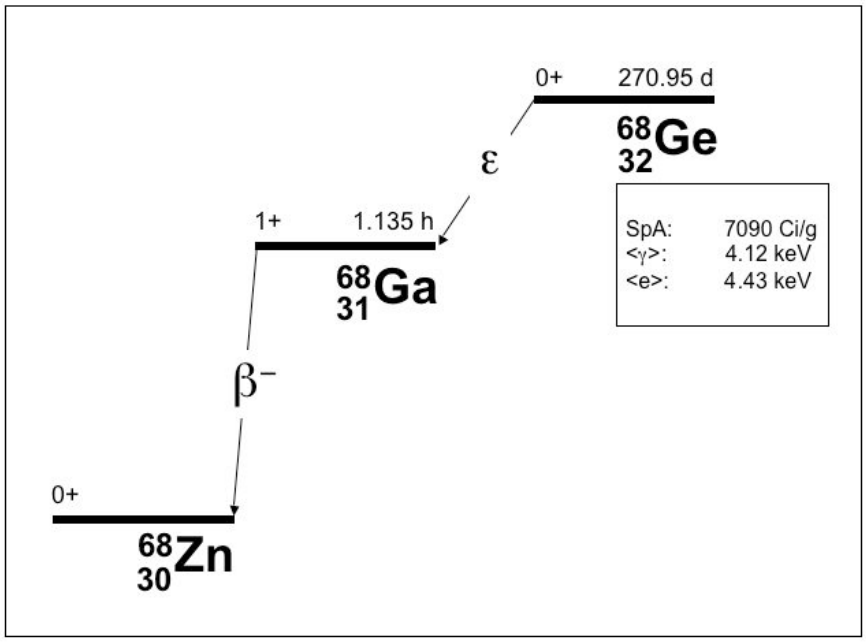
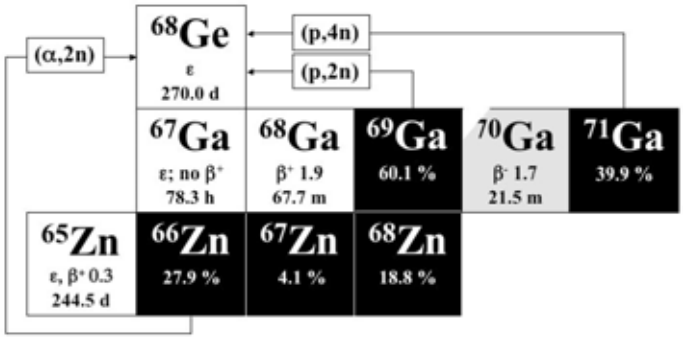


Fig. (1). Principal decay scheme of ^{68}Ge. SpA = theoretical maximum specific activity; <γ> = average electromagnetic radiation energy per disintegration; <e> = average atomic electrons energy per disintegration [6].

Table 1. Most Relevant Nuclear Reactions Yielding ^{68}Ge

Particle	Nuclear Reaction	Target Nuclei	Reaction No.
Proton	(p,2n)	^{69}Ga	[1]
Proton	(p,xn), x = 2, 4	^{nat}Ga ($^{69,71}Ga$)	[2]
Proton	(p,pxn)	^{nat}Ge	[3]
Proton	(p,xnyp), y = 2, 4, 6,	^{75}As, $^{79,81}Br$, $^{85,87}Rb$	[4]
Deuteron	(d,3n)	^{69}Ga	[5]
Helium-4	(α,2n)	^{66}Zn	[6]
Helium-3	(^{3}He,xn), x = 1,2,3	$^{66,67,68}Zn$	[7]



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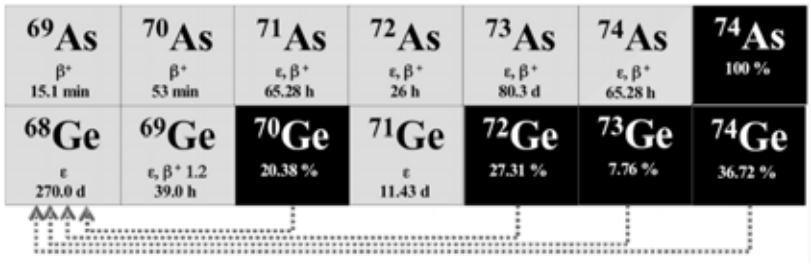


2b

Fig. (2). Indication of the processes $^{69}Ga(p,2n)$, $^{nat}Ga(p,xn)$ (represented by $^{69}Ga(p,2n) + ^{71}Ga(p,4n)$ reactions), **$^{66}Zn(a,2n)$ (2a) and the proton induced processes of type (p,pxn) on germanium targets (2b).** Excerpts of the Karslruhe chart of nuclei.

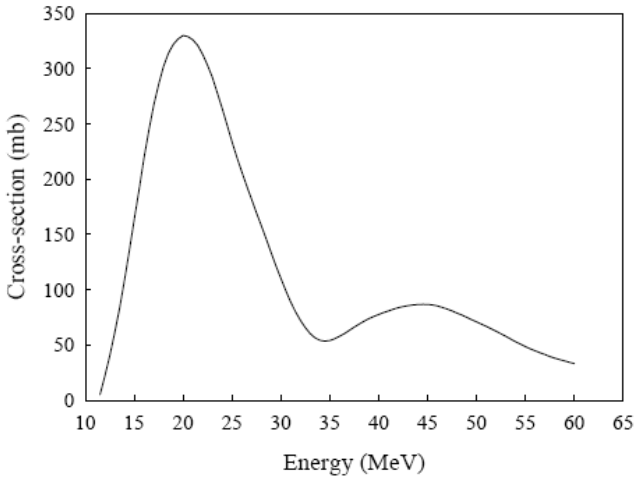


Fig. (3). IAEA recommended excitation function for $^{nat}Ga(p,xn)^{68}Ge$ reactions [16].

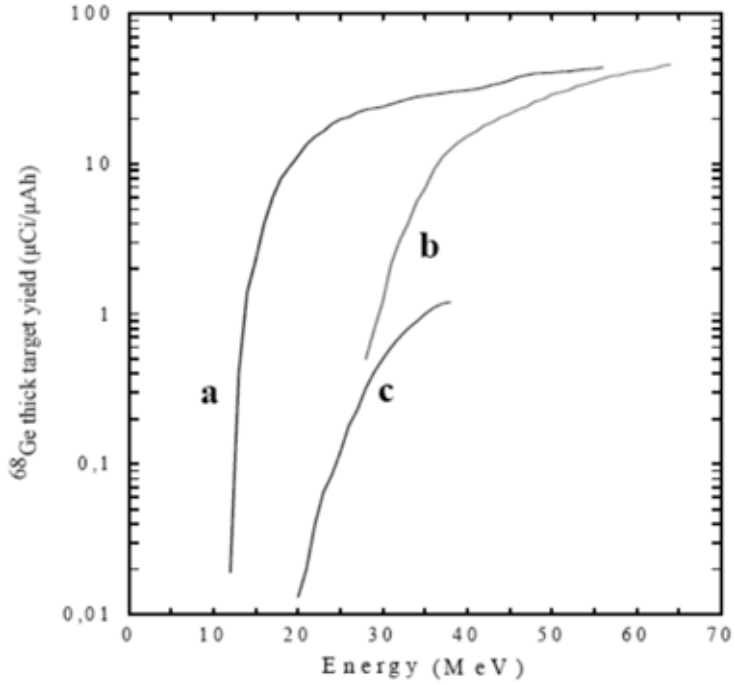


Fig. (4). Comparison of reaction yields for the Ga(p,xn)^{68}Ge (a), Ge(p,pxn)^{68}Ge (b) and Zn(a,xn)^{68}Ge (c) reactions [18].

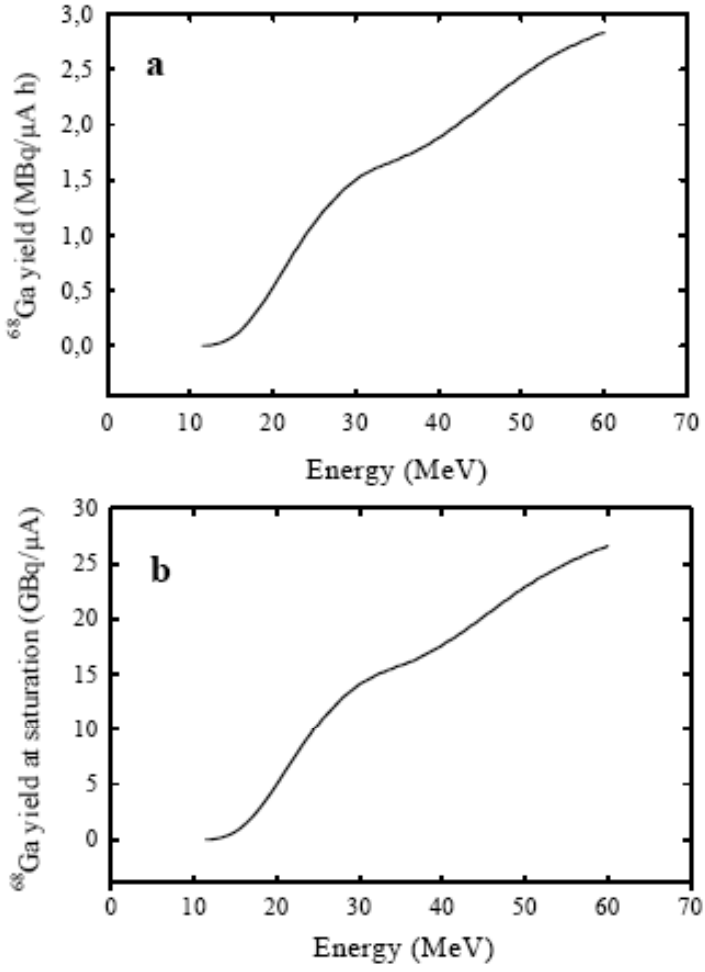


Fig. (5). Thick-target yields for ^{nat}Ga(p,2n) reactions [16]: calculated for a 1 h irradiation (**a**) as well as for saturation (**b**).

Comparison of Large-Scale Productions of ^{68}Ge

Proton-induced nuclear reactions with on-gallium-target energies of at least 23 MeV are the choice of nuclear processes to produce no-carrier-added ^{68}Ge. Due to the long half-life of ^{68}Ge, however, high-current accelerators with on-target beam intensities of 40 to more than 100 μA and ideally approaching the mA level are required for sufficient batch yields such as e.g. about 37 GBq. In addition, long term irradiation periods of several days may be mandatory. Consequently, the number of adequate accelerators available is limited. Routine production is mainly known for Brookhaven National (BNL) and Los Alamos National Laboratories (LANL), USA, iThemba Laboratories/NAC, Faure, Republic of South Africa, and Obninsk, Cyclotron Ltd., Russian Federation. These centers report on production capacities of about 18.5 to 74 GBq (0.5 to 2 Ci) ^{68}Ge per batch.

At LALN, 100 MeV protons are delivered into a stack system of various targets, being irradiated with individual optimum proton energies of approximately 90, 65 or 40 MeV [19]. About 4 g of ^{nat}Ga metal inside a ~5 g Nb encapsulation is irradiated for 20.2 or 16.5 days with 125 μA proton beam intensity. At BNL, ^{nat}Ga targets are irradiated with 30±2 MeV at the Brookhaven Linac Isotope Producer (BLIP). 81 g of ^{nat}Ga metal are encapsulated in a Niobium container of 6.98 cm diameter, 5.08 mm thick. For a one-month irradiation, overall batch yields are 33.3 - 51.8 GBq. The production rate is 1.18 MBq (0.032 mCi)/μAh. At iThemba LABs, Ga/Ga_2O_3 targets are irradiated with initially 66 MeV protons, the on-Ga-target energy being 34→0 or 34→2.4 MeV. For Ga, the mass is about 5 g with a thickness of 4 mm [20]. At Obninsk, Cyclotron Co., Ltd., gallium-nickel alloys are used as target material, prepared on copper backings. Irradiations are performed at rather high proton beam intensity of several hundred μA at 23 MeV proton energy [21].

Targetry

The chemical and mechanical design of the target are crucial issues mainly due to the thermal aspects of high-current irradiations as the power dissipated in the targets reaches values of about 300-1000 W and more. It is mandatory to provide sufficient cooling for the thermal stability of a given target. Main criteria thus are adequate thermal properties such as melting and/or boiling points, heat transfer coefficients etc. of the target materials and its cooling systems. Others are corrosion and radiation resistance. For Ga(p,xn) production routes, potentially useful target compounds include Ga_2O_3 (melting point: 1900°C) and Ga_4Ni alloy (melting point: 900°C). Mixtures of Ga metal and Ga_2O_3 have been used as well as Ga_2O [20]. However, even Ga metal (melting point: 39°C) itself is used as target. In this case, appropriate containments are required. Usually, Ga is used encapsulated in Nb containers [19, 20, 22] with corrosion-resistant Nb allowing effective water cooling of the target. Irradiation of gallium as oxide (Ga_2O_3) prohibits the use of high particle currents since absorbed energy dissipation is inadequate and such targets deteriorate quickly. Naidoo *et al*. [23] thus developed Ga_2O targets as discs of 20 mm diameter and 1 mm thickness, which is encapsulated in aluminum canister and sealed by cold welding process.

Loc'h *et al*. prepared 15 g Ga_4Ni with a thickness of 3 mm between a copper backing and a titanium foil in direction of the proton beam [24]. Ga_4Ni is in routine use at Obninsk [21]. During irradiation the target is cooled at the back side of the Cu body by water and the Ti foil front by helium gas flow.

4. CHEMICAL ASPECTS

Chemical Separations

Principal techniques to isolate nca ^{68}Ge from irradiated massive gallium targets include distillation of ^{68}Ge as tetrachloride, ion exchange chromatography or liquid-liquid extraction. The most often applied separation technique today is liquid-liquid extraction using CCl_4 [19, 21, 22, 25]. For Ga/Nb systems, ^{68}Ge is extracted into CCl_4 after dissolution of the target in 12 M H_2SO_4 (with the aid of HCl and H_2O_2). ^{68}Ge is back-extracted into 0.05 N HCl and evaporated to the appropriate volume [20]. For Ga_4Ni targets, initial processing consists of electrochemical target dissolution. Following extraction of ^{68}Ge from 9.0-9.5 M HCl into CCl_4 it is back extracted into water [21]. Special attention has to be paid to the extreme volatility of ^{68}Ge tetrachloride.

Purity of ^{68}Ge as Produced in Ga(p,xn) Reactions

Specific activities: A central issue is the specific activity of ^{68}Ge. Two facilities provide the corresponding parameters, namely 58 GBq/mg [22] and >74 GBq/mg [21].

Radionuclidic impurities: Radionuclidic impurities may be divided into short-lived and long-lived radionuclides, co-produced mainly within the materials of the target encapsulates (in the case of Nb containments) or backing (i.e. Ni or Cu in the case of Ga_4Ni alloys with Cu backings) used as target materials. Typical examples of long-lived contaminations are ^{88}Y ($T_{½}$ = 106.6 d), ^{88}Zr ($T_{½}$ = 83.4 d). When irradiations are done at proton energies above 40 MeV, ^{65}Zn ($T_{½}$ = 244.3 d), ^{57}Co ($T_{½}$ = 271.8 d) and ^{58}Co ($T_{½}$ = 170.9 d) are formed in addition to short-lived radionuclides such as ^{69}Ge ($T_{½}$ = 39 h) and ^{67}Ga ($T_{½}$ = 78.3 h). Most of the short-lived radionuclides decay to insignificant percentages within cooling periods after EOB of up to two weeks [22]. Long-lived radionuclidic impurities such as ^{88}Zr and ^{88}Y are removed using alumina column chromatography [19]. Overall radionuclidic purities of >99.9% [20] or 99.8% [21] related to ^{68}Ge are described. However, the above numbers do not take into account the presence of ^{71}Ge ($T_{½}$ = 11.43 d) as a co-product of ^{68}Ge in (p,xn) reactions on gallium targets of natural isotopic composition (^{69}Ga = 60.108%, ^{71}Ga = 39.892%). Thus, ^{71}Ge activities at EOB may be higher than those of ^{68}Ge by a factor of 10 to 20.

Non-radioactive metallic impurities: Following irradiations, ^{68}Ge needs to be separated from macroscopic quantities of ^{nat}Ga (several g) as presented in the target. The finally separated ^{68}Ge contains amounts of Ga < 1 μg [19] as less as 0.085 μg per 37 MBq of ^{68}Ge [22]. The traces of Ga remaining will be separated during the $^{68}Ge/^{68}Ga$ generator production as sophisticated radiochemical separation strategies are employed to absorb ^{68}Ge effectively and to elute ^{68}Ga selectively from the generator. Other chemical non-radioactive impurities arise from the remaining traces of non-Ga target

components, i.e. Nb, Ni or Cu. Chemical separation employed reduces their amounts to ppm levels such as e.g. Zn (2 ppm), Nb (< 7 ppm) and Cu, Pb, Co, Cr, Cd, Ni, Fe, Mn and Al (all < 1 ppm) [22].

5. RADIONUCLIDE GENERATORS

Interestingly, the positron emitter Gallium-68 was one of the first radionuclides introduced to non-invasive molecular imaging in its very early phase. In the 1950s and 1960s, it was investigated in comparison to the positron emitters Arsenic-74 (T ½ = 17.77 d, 34% β^+) and Copper-64 ($T_{½}$ = 12.7 h, 18% β^+), as well as in comparison to Mercury-203 ($T_{½}$ = 46.61 d, β^-, 279.2 keV photon emission) for "radioisotope brain scanning", cf. for example [26, 27]. Compared to these radionuclides, ^{68}Ga primarily appeared to be of interest because of the much easier preparation of the tracers needed and its adequate half-life (for brain imaging), low toxicity and superior patient radiation safety / dosimetry. One of the other reasons to include ^{68}Ga-tracers was a much earlier report on the value of ^{72}Ga as a possible therapeutic agent for bone tumors [28].

Shealy *et al.* [29] prepared [^{68}Ga]Ga(EDTA) from buffered ^{68}Ge solutions *via* a solvent extraction. In order to obtain satisfactory separation of ^{68}Ga, one milligram of $GaCl_3$ carrier was added. In detail, a 35% fresh acetyl-acetonate / cyclohexane solution was added to the ^{68}Ge stock solution and extracted (by a two minutes vigorous mixing) 70-80% of the ^{68}Ga available. Subsequently, ^{68}Ga was re-extracted into 0.1 N HCl solution and neutralized with sodium hydroxide. Finally, a solution containing one mg or less of gallium of 2 – 3 mCi activity was obtained. The content of ^{68}Ge is described in terms of: "No Germanium-68 is carried over under these circumstances" [29]. Shearly *et al*. [29] from theses batches synthesized a number of ^{68}Ga-compounds, such as [^{68}Ga]Ga(EDTA) (gallium versenate), ^{68}Ga-protoporphyrin, ^{68}Ga-phtalocyanate, ^{68}Ga-sodium gallate and ^{68}Ga-sodium gallo-arsonate, which were evaluated in tumor bearing mice and in patients. ^{68}Ga was involved clinically in several hundred patients. Many of these patients were diagnosed in parallel with the other tracers available these times, such as [^{64}Cu]Cu(EDTA) or [^{74}As]AsO_4^-.

From these studies several conclusions have been derived. One was of more technical character, namely the improvement of the scanners to achieve better count (= imaging) statistics, generating the breakthrough of Angers camera. In fact, Anger's and colleague's developments towards a Positron Scintillation Camera have been performed to a significant amount with ^{68}Ga-EDTA [30-33]. The other message was of radiochemical character addressing the fundamental advantages of radionuclide generators, namely that: "The relative ease of obtaining gallium makes further work with it worthwhile" [29], or "With the positron cow, the ^{68}Ga-EDTA is readily available and is inexpensive, because hundreds of doses of the isotope can be obtained before the parent isotope has decayed. Since the ^{68}Ga has such a short half-life, an examination can be repeated or other isotope studies done the following day if desired, and only minimal precautions are necessary in handling the isotope" [30]. Compared to the other state-of-the-art radionuclides of these years, it was "readily obtainable" (as generated from the ^{68}Ge parent) and "incorporable into a chemical form of high specific uptake.", cf. [29].

Anger and coworkers utilized the ^{68}Ge/^{68}Ga radionuclide generator concept described by Gleason [34] (in 1960) which was modified by Green and Tucker [35] (1961), passing 0.005 N EDTA solutions through a ^{68}Ge-alumina column-based generator, instantaneously providing [^{68}Ga]Ga(EDTA) as the initial tracer directly as the generator eluate. As the pH of the solution was 9.8, the pH was adjusted to 7.0 by means of hydrochloric acid. Hundreds of patients were investigated again.

Early Generator Developments

Concerning the generator chemistry, early attempts to adopt liquid-liquid extraction chemistry for the routine generator use were not sustainable [29, 36]. The early column-based generator systems separated ^{68}Ga with 0.005 N EDTA solution from ^{68}Ge, absorbed on alumina or zirconium oxides [35, 37] providing neutral [^{68}Ga]Ga(EDTA) eluates. Elution efficacies have been almost quantitative, and [^{68}Ga]Ga (EDTA) became a popular brain imaging tracer. However, the thermodynamically very stable GaEDTA complex (cf. Table **2**) prevented the direct use of this eluate for versatile ^{68}Ga labeling reactions. The clinical use thus was rather limited to measure the increased blood flow of brain tumors, in particular.

Analogously, ^{68}Ge was retained on antimony oxide Sb_2O_5 and ^{68}Ga was eluted with oxalate solutions [38]. Anion exchange resins using dilute hydrofluoric acid solutions as eluent allowed high-purity separations due to the significant differences of distribution coefficients of the elements [39]. The breakthrough of ^{68}Ge was $< 10^{-4}$ for up to 600 elutions, and the ^{68}Ga yield was > 90%. In all these cases, further application of the generator eluate for ^{68}Ga labeling reactions was not possible or difficult.

Organic Resins

Ge^{IV} is known to form very stable complexes with phenolic groups [40], and its adsorption on a 1,2,3-trihydroxybenzene (pyrogallol)-formaldehyde resin was utilized [41,42] to elute $^{68}GaCl_4^-$ in strong (4.5 M) hydrochloric acid. Average yields of ^{68}Ga of 75% during a period of 250 days were reported [41]. The Ge breakthrough was < 0.5 ppm with no detectable radiolytic by-products for a 370 MBq generator. The pyrogallol-formaldehyde resin was found to be resistant to dissociation from radiation. An organic macroporous styrene-divinylbenzene co-polymer containing N-methyl-glucamine groups was developed to provide ^{68}Ga with a solution of a low-affinity gallium chelating ligand such as citric or phosphoric acid. The ^{68}Ge breakthrough was less than 0.0004% of the ^{68}Ge adsorbed on the resin [42].

"Ionic" Generators

Consequently, ^{68}Ge/^{68}Ga generators were developed avoiding the formation of stable ^{68}Ga-ligand complexes in the eluate system. This strategy is achieved for both $^{68}Ga(OH)_4^-$ or $^{68}Ga^{3+}$ providing eluates. Indeed, ^{68}Ga has been eluted in 0.1 M NaOH solution as gallate anion from alumina columns [43]. A comparison of performances of

alumina/EDTA, alumina/NaOH and tin oxide/HCl generators [44], however, indicated superior characteristics for the latter system in terms of ^{68}Ge breakthrough (10^{-6}-10^{-5}% per bolus) and $^{68}Ga^{3+}$ elution (70-80%) in 1 M hydrochloric acid solution [45]. To design other 'ionic' $^{68}Ge/^{68}Ga$ generators based on inorganic matrices *and* providing $^{68}Ga^{3+}$ eluates, ^{68}Ge was absorbed on alumina, $Al(OH)_3$ or $Fe(OH)_3$ and iron oxide [46-48], SnO_2, ZrO_2, TiO_2 [49-52] and CeO_2 [53]. Fig. (**6**) gives a sketch of the main directions towards the radiochemical design of $^{68}Ge/^{68}Ga$ radionuclide generators for routine (medical) application.

For commercial generator productions, a modified TiO_2 phase was used by the Cyclotron Ltd., Obninsk, Russian Federation, since about 2000 [21]. These generators are eluted with 0.1 N HCl and show initial ^{68}Ga elution yields of about 80% with ^{68}Ge breakthrough of about $1{\cdot}10^{-3}$%. For ^{68}Ga, these values decrease over time (e.g. after about one year) or with increasing number of elutions (e.g. 200), while for ^{68}Ge these values increase, approaching values of about 50% of ^{68}Ga elution and about 10^{-2}% of ^{68}Ge breakthrough. A similar generator is available as 'IGG 100' providing improved elution characteristics [54]. Another system is produced at iThemba, Republic South Africa, using a SnO_2-based solid phase [52]. Optimum ^{68}Ge elution efficacy is reported at 0.6 N HCl, being somewhat higher at 1.0 N HCl, but decreasing significantly at lower HCl concentration. Fig. (**7**) represents elution parameters for various concentrations of hydrochloric acid [55]. For these SnO_2 based generators, a more dramatic drop of ^{68}Ga elution efficacy over time is reported, indicating that elution efficacy is down to half of the initial value already after 100 elutions [56]. This decrease in ^{68}Ga elution efficacy maybe explained by the in-growth of $^{68}Ge^{IV}$ into the Me^{IV} oxide-based particles representing the column material (Me = Sn, Ti, Zr, …) at higher concentration of HCl. While primary adsorption of $^{68}Ge^{IV}$ occurs on the surface of these freshly prepared particles, diffusion and crystal growth processes may result in an increasing incorporation of ^{68}Ge inside the particles. The ^{68}Ga generated than "inside" the particles need to diffuse / migrate to the particles´ surface to be available for elution.

Impact of Impurities for Handling ^{68}Ga Eluates

Obviously, all commercially available generators need further improvement towards medically approved systems [57]. The presence of metallic impurities in the ^{68}Ga eluate is highly relevant and can affect the utility of the product. The $^{68}Ge/^{68}Ga$ radionuclide generator systems available today are not necessarily optimally designed for direct application for making a diagnostic product for clinical and routine use in humans. The eluate from the commercial generator still contains measurable activities of long-lived ^{68}Ge. In addition, the rather large volume and the relatively high concentration of hydrochloric acid in many cases prevent the direct use for labeling reactions. Furthermore, labeling yields and specific

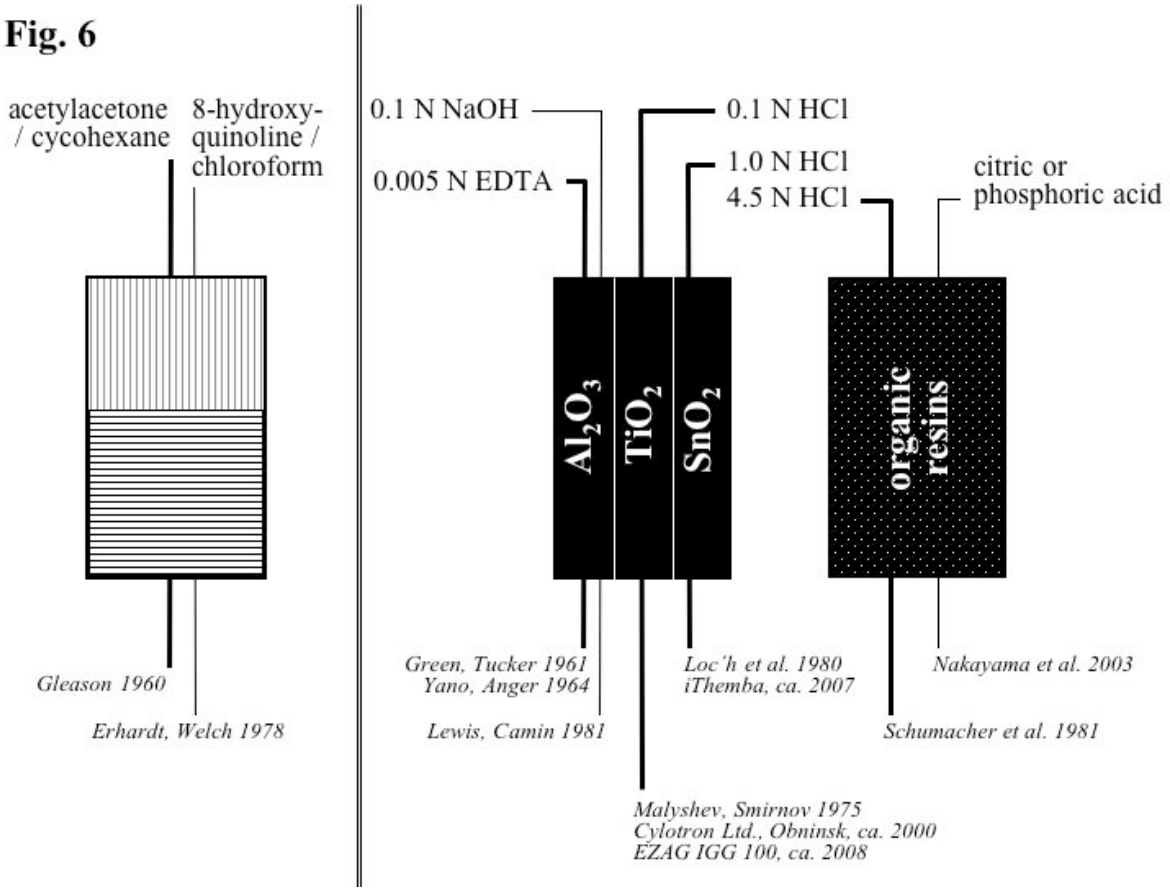


Fig. (6). Liquid-liquid extraction (left) and column-based (right) $^{68}Ge/^{68}Ga$ separation strategies towards routine $^{68}Ge/^{68}Ga$ radionuclide generators: Thick lines of separation routes illustrate concepts adopted for application of ^{68}Ga in human studies.

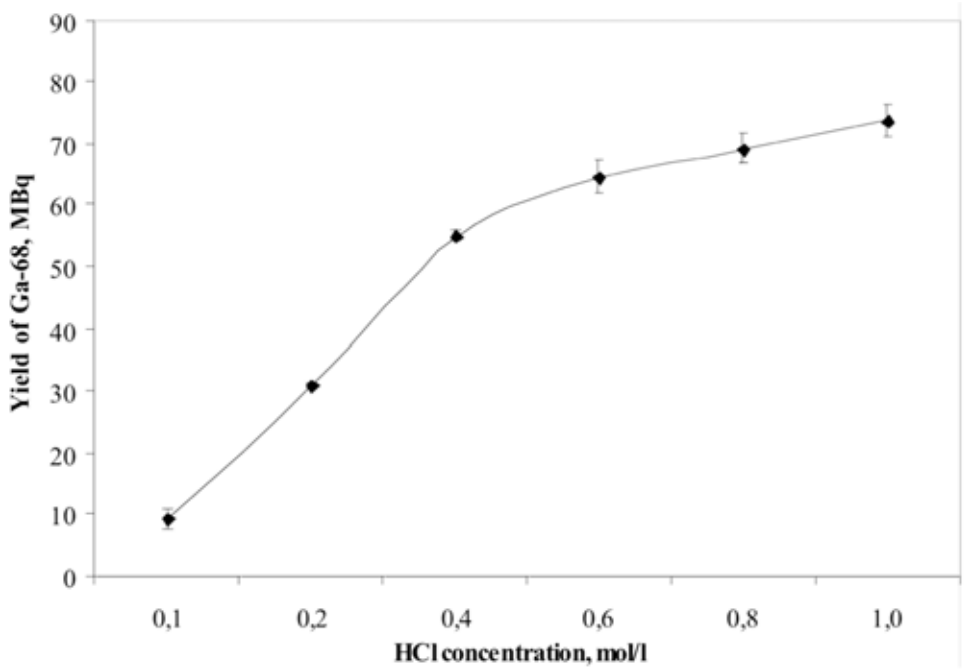


Fig. (7). ^{68}Ga elution efficacy of SnO_2 based generators for 10 ml elute volume of various concentrations of hydrochloric acid [55].

activities might not reach maximum values due to the presence of metallic impurities. For example, significant amounts of Zn^{II} are generated from the decay of ^{68}Ga Fig. **1**. For a 'fresh' 1110 MBq ^{68}Ge/^{68}Ga generator, the number of stable ^{68}Zn atoms generated within one day after an elution amounts to $8.93 \cdot 10^{13}$ atoms (i.e. 10 ng of Zn^{II}) as compared to $4.69 \cdot 10^{12}$ atoms of ^{68}Ga in 800 MBq of the ^{68}Ga eluted. In the case of 'fresh' generators, amounts of stable ^{71}Ga as generated from the ^{71}Ge decay may be even one order of magnitude higher than those of stable ^{68}Zn.

In addition, Ti^{IV} or other residuals from the generator column material and Fe^{III} are present in the eluate. All these metallic impurities may adversely affect the ^{68}Ga-labeling yields as well as specific activity of the labeled product. ["Specific activity" in this context is less related to the presence of stable gallium isotopes, but to the co-formation of e.g. iron complexes with the same ligands.] Thus, dedicated procedures to process the eluate from the radionuclide generator and including labeling and purification of ^{68}Ga radiopharmaceuticals need to be developed. Several approaches for processing generator derived $^{68}Ga^{III}$ were described recently. Fig. (**8**) gives a schematic flow sheet of the individual procedures.

Anion exchange chromatography: Processing of ^{68}Ga eluate from a TiO_2 based ^{68}Ge/^{68}Ga generator - adopting an earlier concept [41] - was introduced first [58]. The initial volume of 10 mL of the 0.1 N HCl eluate is transferred into a vial containing 15 mL of 9.5 N HCl to obtain a final hydrochloric acid concentration of 5.5 M. Under these conditions, ^{68}Ga is effectively adsorbed on a strong anion exchanger as the anionic chloro complex of $^{68}Ga^{III}$, i.e. $^{68}GaCl_4^-$. Following a washing step with 1 mL of 5.5 N HCl, the resin is flushed with a stream of nitrogen and then eluted with H_2O in small volumes. This strategy separates ^{68}Ge, but does not allow *direct* loading of $^{68}Ga^{III}$ on the anion exchange resin from 0.1 N HCl and either it does not provide purification of Ga^{III} from e.g. Zn^{II} and Fe^{III}. The time needed to process the generator eluate, to synthesize and purify the labeled product (e.g. ^{68}Ga-DOTA-conjugated peptides) etc. reduces the overall yields of the product. A final yield of 46±5% for a ^{68}Ga labeled DOTA-conjugated octreotide is reported to be obtained [59,60].

Fractionation: Another approach to overcome problems like eluate volume, acidic pH and content of ^{68}Ge and chemical impurities is to fractionate the initial generator eluate [61]. The concept utilizes the fact that the eluted ^{68}Ga activity peaks within ~ 1– 2 mL, representing about 2/3 of the total activity. In the context of synthesizing ^{68}Ga labeled compounds, decay corrected yields of ^{68}Ga radiopharmaceuticals thus cannot exceed 60-70%. Due to processing times and labeling efficacies, in practice, effective yields of labeled DOTA-conjugated peptides like ^{68}Ga-DOTA-TOC amount to about 50% [62]. Contents of ^{68}Ge and metallic impurities are minimized because of the lower eluate volume used, but principally not chemically removed prior to the ^{68}Ga labeling steps.

Cation exchange chromatography: The key step in this procedure consists of the direct transfer of the initial 0.1 N HCl ^{68}Ga eluate to a cation exchanger [63]. Due to high distribution coefficients, ^{68}Ga is quantitatively adsorbed on only about 50 mg of the resin directly from the generator eluate. A small volume of 1.0 ml of an 80% acetone / 0.15 N HCl mixture is applied to purify ^{68}Ga from Ge^{IV}, Ti^{IV}, Zn^{II} and Fe^{III}. The 0.4 ml of a 98% acetone / 0.05 N HCl mixture are sufficient to completely desorb ^{68}Ga from the resin and to transfer this purified fraction online to a labeling vial. The post-processing takes 4 minutes with overall ^{68}Ga recovery yields of 97±2%. This efficient and simplified system for

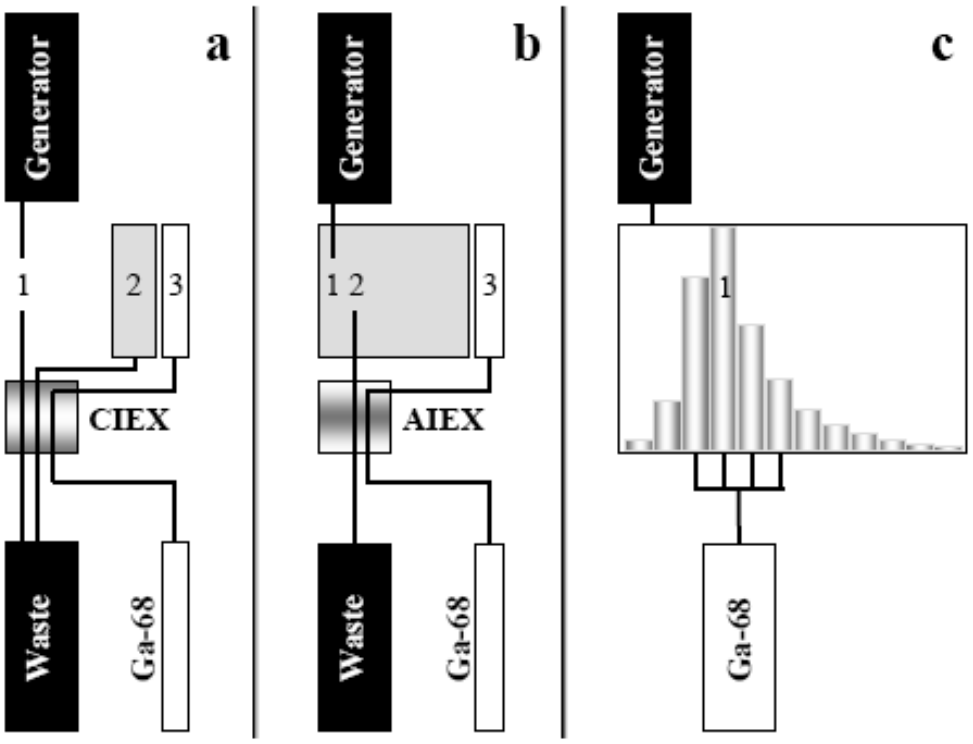


Fig. (8). On-line generator eluate post-processing utilizing cation-exchange resins CIEX (a), anion exchange resins AIEX (b) and fractionation (c): (**a**) 1- elution of 10 ml of 0.1 N HCl through CIEX into the waste with ^{68}Ga adsorbed on the CIEX; 2- purification of ^{68}Ga using 1 ml of a HCl/acetone mixture; 3- desorption of ^{68}Ga by means of 400 µl of an 0.05 N HCl / 98% acetone mixture. (**b**) 1- elution of 10 ml of 0.1 N HCl into a reservoir of 9.5 N HCl to achieve a total concentration of 5.5 N HCl; 2 adsorption of ^{68}Ga onto AIEC; 3- desorption of ^{68}Ga by means of small fractions of water. (**c**) 1- fractionated elution of the 0.1 N HCl eluate and identification of those fractions representing about ⅔ of the eluted activity.

processing ^{68}Ga eluates results in volume reduction along with chemical and radiochemical purification. This procedure leads to almost complete removal of metallic impurities including ^{68}Ge breakthrough, thus providing the purified ^{68}Ga in a form useful for direct labeling with acceptable pH, volume and purity [63,64].

For subsequent and online radiopharmaceutical syntheses, the 400 µl fraction of the acetone / 0.05 N HCl mixture containing the processed ^{68}Ga is transferred to 5 ml of water, containing appropriate amounts of e.g. DOTA-TOC. The resulting mixture is of pH 2.3. Due to the high purity of the ^{68}Ga fraction and the renouncement of buffer systems (which may introduce a load of metallic impurities), high labeling yields of >95% are achieved within 5 minutes heating at ~95°C. The initial amount of about 400 µl of acetone as used to desorb ^{68}Ga from the cation exchange resin neither is toxic (due to the same toxicology of e.g ethanol and acetone) nor does it prevent labeling reactions, in particular for the synthesis of ^{68}Ga labeled DOTA or NOTA derivatised peptidic tracers. Furthermore, acetone evaporates while the labeling mixture is kept at ~95°C in the open vial. After 10 minutes, about 4.5 µg acetone remained in the reaction mixture. Those numbers are far below the i.v. toxicity of acetone in rats or mice (LD_{50} > 1 g / kg [65, 66]). In the case the minor content of acetone is of some concern, purified ^{68}Ga is eluted from the cation exchange resin with 1 ml of 5 N HCl and transferred to a small anion exchange cartridge in high yields, from which it is eluted in pure water [67].

Similarly, the purified ^{68}Ga adsorbed on the small cation exchange resin can be used as a source of turning ^{68}Ga into a labeling synthon applicable to work in anhydrous or organic media. From this resin ^{68}Ga was eluted with different acetone-based, non-aqueous solvent systems. More than 95% of the generator-eluted ^{68}Ga was obtained from the cation exchange resin with 600 µl of a 98% acetone / 2% acetylacetone mixture providing nca $[^{68}Ga]Ga(acac)_3$ as labeling agent. Water-insoluble macrocyclic polypyrrole derivatives were chosen as model compounds for a proof-of-principle labeling of lipophilic compounds. Labeling was performed in chloroform in a focused microwave synthesis system in yields of up to 97% within one minute. Based on these results, the novel procedure providing nca $[^{68}Ga]Ga(acac)_3$ offers a wide scope of applications for this labeling agent [68].

Due to the highly efficient purification and concentration performance of the post-processing protocol and due to the low hydrodynamic resistance of the generator system, it is straight forward to connect several generators in a cascade scheme [63]. Utilization of several generators in a cascade scheme thus optimizes the shelf-life of the generators and reduces costs.

Automated Syntheses Modules

Several automated modules are commercially available to combine generator elution, post-processing (adopting all versions of the above mentioned approaches) and ^{68}Ga labeling reactions. The modules can be used in a clinical environment for the preparation of ^{68}Ga labeled radiopharmaceuticals. Recently, a GMP-based module using cassettes to both elute a generator including cation-exchange post-processing and radiopharmaceutical synthesis and product purification became available [54].

Cyclic Elutions

Radionuclide generations are distinguished according to the half-lives of the parent and daughter radionuclides. Depending on which of the two radionuclides has the longer half-life, two principal cases occur: (i) parent 1 is longer-lived, but not more than by a factor of about 100, i.e. $T_{½,1} < 100\ T_{½,2}$, *transient equilibrium*, (ii) parent is much longer-lived than the daughter 2 ($T_{½,1} » T_{½,2}$, i.e. $\lambda_1 « \lambda_2$), *secular equilibrium*. In a *secular equilibrium*, the parent activity does not decrease measurably during many daughter half-lives. Expressed in terms of real time and multiples of the half-life of ^{68}Ga.

Following the growths of ^{68}Ga activity on the generator column, 50% of the theoretical maximum is generated already within one half-life. A period of three half-lives, which is about 3.4 hours, provides already 88% of the maximum value. Consequently, the generator may be eluted, for example, each 3.5 hours to give almost complete (90%) radioactivity. This perfectly allows 3 individual elutions per day. Interestingly, this period exactly reflects the handling regime of a batch of a ^{68}Ga-radiopharmaceutical for 2 to 4 patients per batch – at least for the imaging of e.g. neuroendocrine tumors using ^{68}Ga-DOTA-conjugated octreotide derivatives. Fig. (**10**) illustrates, that for this indication clinically relevant images are obtained already within less than 1 hour post injection.

6. THE CHEMISTRY AND COMPLEX CHEMISTRY OF GALLIUM

Gallium is element number 31 in the periodic table of the elements. Belonging to group 13, it has the electron configuration [Ar] $3d^{10}4s^24p^1$. Apart from the low melting metallic form, ionic gallium can exist in two oxidation states, Ga^I and Ga^{III}. Only the latter is stable under aqueous conditions and thus, only the Ga^{III} is of relevance for *in vivo* applications [4, 70,71]. The Ga^{3+}-ion displays a van der Waals radius of 62 pm, which is quite similar to Fe^{3+} (65 pm) and Mn^{3+} (64 pm) [69-71]. The standard potential for the oxidation of Ga^0 to Ga^{III} is -530 mV, clearly indicating that unlike Mn^{III} and Fe^{III} redox chemistry does not apply for Ga^{III} *in vivo* [71,72], yet eliminating one potential source of radioactive metabolites. According to its small size and the high charge density of the threefold positive ion, it is characterized as a hard acid according to the Pearson concept. Due to its small size, the Ga^{III} cation prefers five-membered chelate rings, e.g. in ethylene-1,2-diamine or glycine-like chelators. As such, in first approximation it forms stable complexes with hard donors such as oxy and amino functions. Its main coordination number is six corresponding to more or less distorted octahedral geometry [75]. However, several lower coordinated

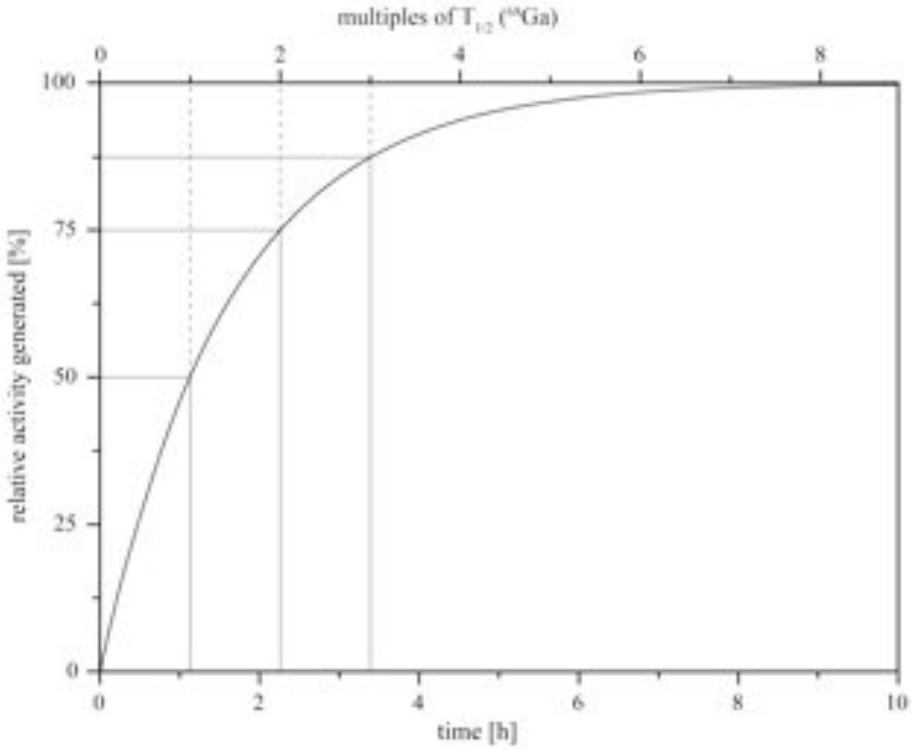


Fig. (9). Generation kinetics of ^{68}Ga on the generator column following an initial elution expressed in terms of real time and multiples of the half-life of ^{68}Ga.

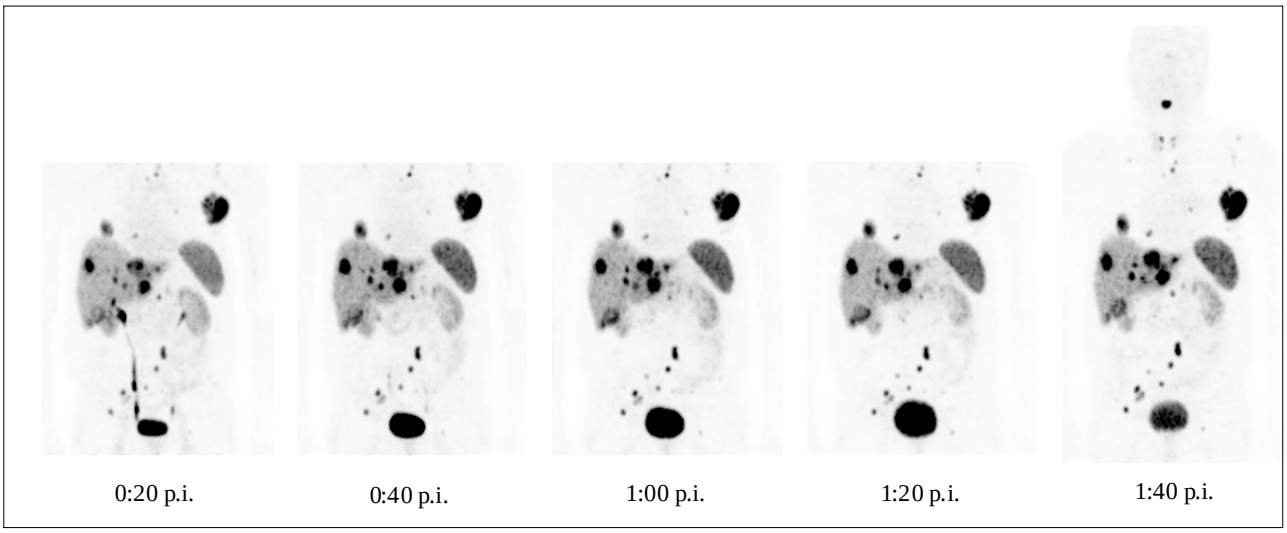


Fig. (10). [^{68}Ga]DOTA-DPhe^{1}-Tyr^{3}-octreotide PET imaging of neuroendocrine tumors at different time points p.i. [69].

complexes of sufficient stability have been reported, mostly bearing a smaller coordination sphere. Four, five and six coordinate $N_xO_yS_z$ donor sets as well as tripodal four or six-coordinate chelates are known.

Some of these are stabilized by strong binding interactions between sulfhydryl donor functions and the metal core. This is due to the presence of the completed d-shell and electronic interactions between these electrons and the empty d-shell of sulphur. Ga^{III} can form extraordinarily stable complexes with soft thiophenol donors.

In many cases, a set of N or N *and* O/S donors form the equatorial plane with two axial ligands, sometimes with slightly higher bond distances between axial donors and the core. Rarer, O_n donor sets have also been investigated as potential imaging agents.

Another main characteristic of the Ga^{III} is its high susceptibility to aqueous hydrolysis at moderately acidic to basic pH-values. At pH-values higher than 3, the cation rapidly forms oxide and hydroxide species. A variety of different species of low solubility can be found between pH 3.5 and 7.5. The above mentioned species however, tend to form colloidal or pseudo-colliadal precipitates which cannot be subjected to complexation any more.

Consecutively, the highly stable gallate anion $Ga(OH)_4^-$ is formed at pH-values higher than 7.4 (to 9), cf. [73]. Its properties include a high solubility and very slow ligand exchange with trans-chelation to other complexing agents. Therefore the metal core has to be shielded from attack by H_2O or OH^- nucleophiles, necessary for basic hydrolysis. In most cases these complexes can only be opened under highly acidic conditions and protonation.

Due to the inherent similarity between high-spin Fe^{III} and Ga^{III}, the iron transporter protein *apo*-transferrin (*apo*-TF) has a significant affinity to Ga^{3+} as well. It provides two binding sites for threefold positively charged cations like Fe^{III} and Ga^{III} with equilibrium constants K_{ML} (for gallium) of 20.3 and 19.3, respectively [74]. *In vitro* experiments have shown that there is only a slow trans-chelation from the gallate-Ga which is the by far dominating species (49:1; $Ga(OH)_4^-$: $Ga(OH)_3$) formed by non-chelated Ga^{III} at physiological pH.

Trans-chelation to *apo*-TF in blood serum is mainly due to the high abundance of transferrin (0.25 g/l) which thereby is able to compete with the strong ligand OH^-. In contrast, weakly coordinated Ga^{III} which is stable to hydrolysis will be trans-chelated to transferrin [74]. *In vivo* trans-chelation to transferrin occurs with [^{67,68}Ga]Ga(citrate) (K_{ML} = 10, pM = 18.4) but not with [^{67,68}Ga]Ga(EDTA) (K_{ML} = 18.9, pM = 19.9) which somewhat defines the lower exclusion level for biomedical applications of Ga–complex conjugates. These findings illustrate that any weakly coordinated trace amount of Ga^{III} that is incorporated to human blood will ultimately end up bound to one of the binding sites in *apo*-TF. For detailed information on the molar dependence of the distribution of gallium in the blood see [75]. Otherwise, the obtained radioactivity distribution corresponds to the distribution (and action) of transferrin throughout the body [77-79]. As a result for targeted molecular imaging, an appropriate chelator is required to avoid trans-chelation to the relatively high-concentrated transferrin.

In terms of Ga^{III} complex chemistry related to the design of ^{68}Ga radiopharmaceuticals, this has resulted in a couple of basic requirements:

a) Any chelator used for this purpose does either have to be a targeting moiety itself or has to be functionalized in a manner that allows conjugation of the complex to a separate targeting moiety. The basic concept for the latter complex ligand is referred to as *bifunctional chelators.*

b) Ga-ligand complexes have to provide sufficient thermodynamic stability, resulting in high equilibrium constants K_{ML}, expressed as ratios between the chemical activities of complexed cation and free cation in solution. This property is referred to as *thermodynamic stability*.

$$K_{ML} = \frac{[ML]}{([M]\cdot[L])}$$

with [ML] – the chemical activity of the octahedral (tetrahedral complex), [M] – the chemical activity of the free Ga^{III}, [L] – the chemical activity of the free complexing agent. pM values (pM = -log [M]) are used to compare the actual quality of different chelators rather than K_{ML} values. The pM value takes the influence of protonation, hydrolysis, ligand basicity, dilution and stoichiometry into account. Increasing pM correlates with increasing stability [73].

c) The corresponding complexes have to provide sufficient kinetic inertness under physiological conditions, which means, that the rate constants of Ga^{III} exchange between the bound and the unbound Ga^{III} in the complex have to be reasonably low. Core-exchange to other cationic metals present in the blood (e.g. Mg^{II}) does also contribute to the displacement of bound Ga^{III}. These characteristics are usually referred to as *kinetic inertness*.

7. COMPLEX FORMATION

Ga^{III} in acidic solution rapidly forms complexes upon exposure to chelating agents such as EDTA, DTPA, NOTA and similar at moderate temperature. This is mainly due to the fact that these multidentate chelating agents quickly form stable octahedral chelate-complexes with Ga^{3+}. More wide, twelve-membered macrocycles basically form similar complexes at moderate temperature, which however can be transformed to stable inclusion compounds applying sufficient activation energy, i.e. by heating, Fig. (**13**). Until recently, the fact that a variety of these chelating agents can form multiple isomers when complexing Ga^{III} has not attracted reasonable attention [76].

Most commonly, $^{67,68}Ga^{3+}$ is handled under mildly acidic conditions such as pH 2.8 to 3.8 in buffered solution where it is present as $[Ga(H_2O)_6]^{3+}$. Most common buffers are acetate, citrate and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid HEPES. Such weakly coordinating compounds will rapidly form semi-stable complexes with Ga^{III}, shielding the Ga^{III} from immediate hydrolysis to $Ga(OH)_3$. Trans-chelation to the more stable desired complex, employing the labeling precursor and sometimes heat (to speed up the trans-chelation kinetics when the formed complex showed slow kinetics) can then be performed at significantly higher pH values, up to neutral pH. In other cases, where no stabilizing weakly coordinating agent has been added, once the pH-value exceeds 3, macroscopic Ga^{III} begins to form oxide and hydroxide species with limited solubility, sometimes resulting in insoluble gel-like colloids. Desiring high $^{68}Ga^{III}$ incorporation into a suitable chelator, resulting in high specific activity, extreme precautions have to be taken concerning pH-values and precursor concentration. An appropriate balance between a) protonation of the required basic donor functions under acidic pH, which leads to inhibition of complex formation on the one hand, and b) hydrolysis of $^{68}Ga^{3+}$ at moderately acidic pH values, which results in the irreversible formation of colloidal ^{68}Ga-oxide-hydroxide species on the other hand, has to be maintained.

Given the fact that many standard buffer substances itself contain hard donor functions, precautions also include an appropriately low concentration of buffer salts, to avoid kinetic competition with the complex precursor. Ideally, the latter should be present in nanomolar concentration, to maintain a reasonably low specific activity.

The corresponding gallium chelate should possess a reasonably low molecular weight, a low tendency to form intermolecular hydrogen-bonds, kinetic inertness and sufficient thermodynamic stability, low non-specific binding and no relevant metabolism to facilitate PET-imaging.

Gallium is commonly complexed in five-membered or six-membered chelate rings, using combinations of the set of standard donors shown above, Fig. (**11**). Several of these covalently bridged chelate rings then form the stable Ga^{III} complex.

8. CHELATORS

8.1. β-Aminocarboxylates

Linear ('open chain') aminocarboxylates: Fig. (**12**) shows the basic complexing agents which have been used from the early stage of ^{68}Ga-radiopharmaceutical chemistry up to the present years. [^{67,68}Ga]Citrate itself has been classified as imaging agent, though its actual role is to 'label' transferring *in vivo*. The citrate-bound Ga^{III} is transferred to transferrin under physiological conditions and thereby used for tumor detection and blood flow imaging [79-82]. While in these cases typically ^{67}Ga was used, only recently similar approaches have been reported for ^{68}Ga – comparing injections of ^{68}Ga citrate and injections of pre-formed ^{68}Ga-transferrin itself [84].

Though fiercely limiting the potential application of the generator eluate, EDTA was used as eluate in early genera-

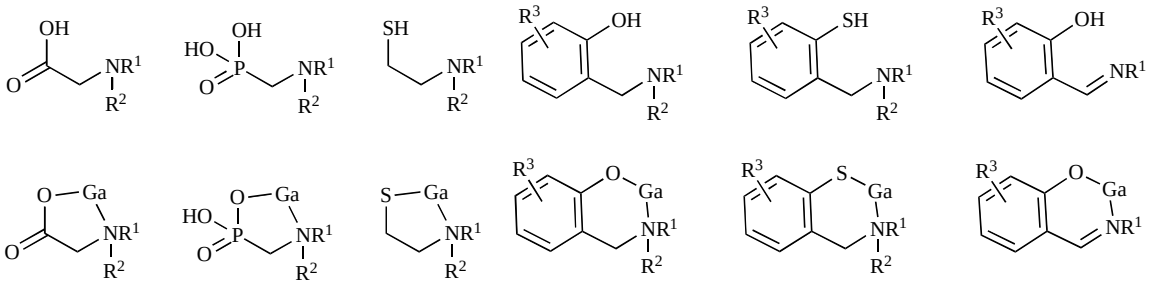
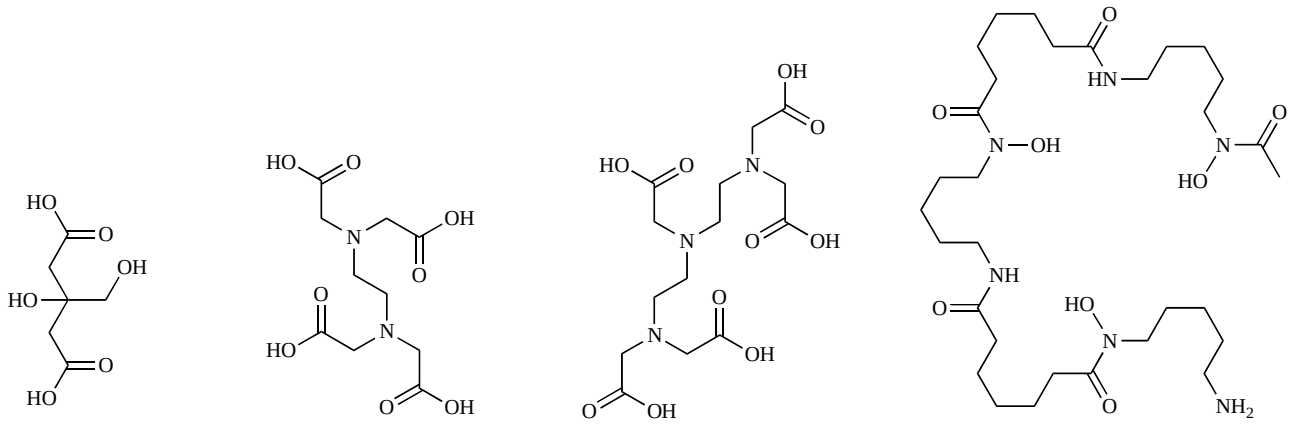


Fig (11). Most abundant modes of Ga^{III} complex formation in five to six membered chelate rings.



A **B** **C** **D**

Fig. (12). Basic linear chelators: (**A**) citric acid, (**B**) ethylenediamine-N,N,N',N'-tetraacetic acid (EDTA), (**C**) diethylenetriamine-N,N,N',N'',N''-pentaacetic acid (DTPA), (**D**) desferrioxamine-B (DFO).

tors [37]. Examples of the use of EDTA and DTPA include direct injection as well as imaging of myocardial, renal, cerebral and pulmonal blood flow and function. Labeling of serum albumin and albumin-derived microspheres for imaging of vasculature and function of liver, kidney, heart and lung has been performed using these chelators, as well as using Ga^{III} directly [85-95]. Both chelators have been modified in their polyamine backbone, to represent some of the earliest examples of bifunctional chelators for medical imaging. Experiences and designs derived from these examples have later been the scaffold for bifunctional chelators (BFC)s of all kinds [94-101].

Desferrioxamine (DFO) was one of the early 'naturally' bifunctional chelators, e.g. it has been conjugated to e.g. folic acid, antibodies, human serum albumin aggregates and the somatostatin analogue octapeptide octreotide [102-117]. It forms complexes with Ga^{III} under deprotonation of the three included hydroxamate oxygens, leaving the terminal primary amine unaffected. This function has been employed for either direct conjugation [118-123], or conversion into a carboxylic acid using a succinic acid linker. However, DFO shows some disturbing characteristics, e.g. it only forms stable Ga^{III} complexes when present in high concentration. In addition, using the DFO moiety would add more than 600 g/mol of mass to the obtained biomolecule-chelator conjugate [102-117]. It appeared thus to be preferentially an appropriate chelator for antibody imaging. However, due to the slow pharmacokinetics of (labeled) antibodies or other larger targeting moieties, the short-lived ^{68}Ga seems to be less relevant within the DFO strategies.

Macrocyclic aminocarboxylates: More recent studies have devised macrocyclic polyamino polycarboxylate such as 9-14 membered tri- or tetraazacycloalkanes. These provide outstanding characteristics as thermodynamically stable and kinetically inert chelators, thus shielding the Ga-core effectively under physiological conditions. The 12-14 membered derivatives furthermore possess an impressive history in the successful application for imaging with Lanthanide derived MRI relaxation enhancing agents and heavy nucleus based X-ray contrast agents. Comparative stability data of the Ga^{III} complexes of the ligands mentioned are listed in Table **2**.

The results for macrocyclic chelators of earlier studies published by Martell have been questioned recently [130]. It has been stated, that the inherent stability constants of these chelators are higher due to the contribution of kinetics, which has been omitted in Martell's calculations [130].

Considerable effort has been spent on the development and optimization of their bifunctional derivatives as well as of the smaller 9-membered analogues, perfectly suited for smaller trivalent metal ions such as Al^{III}, Ga^{III}, Fe^{III} and Mn^{III}. Today, DOTA and its derivatives are readily obtained from straightforward and convenient synthetic routes and available from commercial suppliers [131-136]. DOTA forms two different octahedral coordination geometries, with either an N_2O_2 plane (A) or an N_4-plane (B) and two corresponding axial donors, Fig. (**13**). It is evident from the complex structure that DOTA itself possesses two redundant carboxylic acid groups when complexing Ga^{III}. These may therefore be used for conjugation or even omitted to leave one (in the case of DO3A) or two (in the case of DO2A) secondary nitrogen functions free for further substitution.

Amide bonds from excess carboxylate donor functions in DOTA-like chelators, as well as direct alkylation of free secondary nitrogen functions have been shown to be stable *in vivo* and are therefore very well suited for conjugation to biomolecules. This is furthermore convenient due to the easy access to these compounds when starting a total synthesis from 1,4,7,10-tetraazacyclododecane (cyclen).

Complexes obtained from DOTA, DO3A and its derivatives and DO2A and its derivatives have been shown to be sufficiently stable to avoid the loss of the Ga^{III} core under physiological conditions. Multiple applications of DOTA and its congeners have been reported in literature, cf. Figs. (**13**) and (**17**). Though readily available it is not the most appropriate chelator when it comes to Ga^{III} as its K_{ML} and pM values suggest.

Table 2. Relative Thermodynamic Stability of Ga-Ligand Complexes Indexed from Log K_{ML} and pM(-log M) [124-129].

#		log K_{ML}	pM	Ref.
1	Citrate[a]	10.0	18.4	[126]
2	EDTA[b]	18.9	19.9	[126]
3	DTPA[b]	23.3	22.8	[127]
4	DFO[a]	23.5	24.4	[126]
5	DOTA[c]	21.3	15.2	[125]
6	NOTA[c]	31	26.4	[128]
7	TETA[c]	19.7	14.1	[125]
	Transferrin (K1)[b]	20.3	21.3	[124]
	Transferrin (K2)[b]	19.3	20.3	[124]
	HO^{-}[b]	39.4	19.01	[124]

[a]at pH 7.4, [b]for 1 μM Ga, 10 μM Ligand, 27 mM Carbonate; [c]for K_{ML}: μ= 0.10 M (KNO_3), 25 ºC;for pM: pH 7.4, 100% Excess Ligand

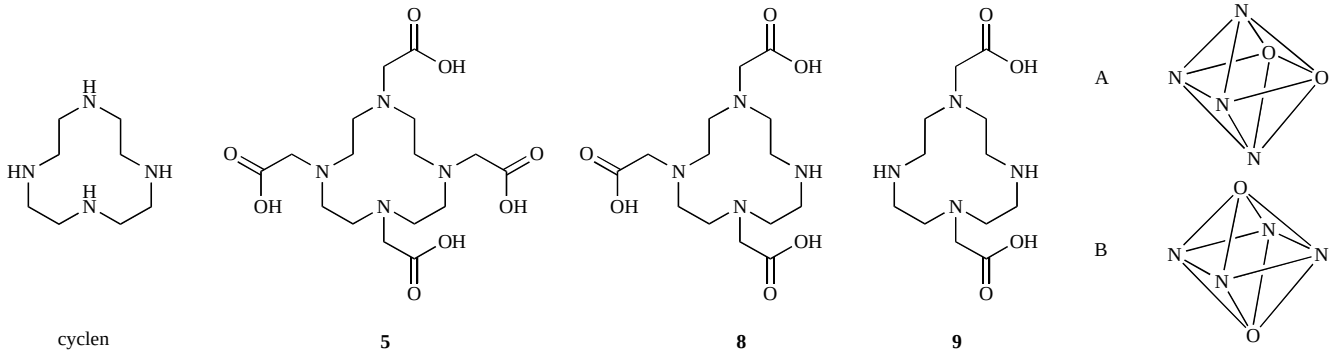


Fig. (13). The molecular structures of DOTA (5), DO3A (8) and DO2A (9) and the facial (A) and inclusion type (B) complexing mode of DOTA like chelators.

It provides the potential of being used as chelator for Ga^{III}, In^{III} as well as for trivalent rare earth elements with beta or alpha emitting therapeutic nuclides. However, the redundant carboxy groups which emerge from octahedral coordination with Ga will not be present with higher coordinating metals and contribute to differences in behavior of the different chelates. Therefore comparability of the final complexes will not necessarily be given although the same basic chelator is used. However, the betaine like structure of the [^{68}Ga]DOTA moiety present in the clinically proven ^{68}Ga-imaging agents such as DOTA-TOC, DOTA-TATE and DOTA-NOC seems to improve the liver clearance of the imaging agent, thus yielding an improvement towards other metals.

DO2A has been used in several approaches to include two targeting moieties in one imaging agent. Thereby the local concentration in the vicinity of the metal core is much higher than with twice the concentration of a monomeric imaging agent, due to hydratisation of the molecule. This may lead to an increase in receptor binding, due to the high inherent concentration when the imaging agent gets close to biological membranes and receptors. It has been proposed, that the imaging agent might bind to two binding sites. This claim, however, has to be questioned when taking the short distance between the two targeting moieties and the dimensions of membrane receptor proteins and their binding sites into account [137-139]. Apart from using single, redundant carboxylate functions or unsubstituted secondary amines within the macrocycle for conjugation to biomolecules a number of modifications of the macrocyclic backbone as well as pendant arms, analogously to the designs applied with EDTA and DTPA, have been reported (Table **3**).

In contrast to DOTA, the smaller congener NOTA is much more suited for Ga, as its smaller 1,4,7-triazacyclononane (TACN) ring apparently allows the formation of multiple five-membered chelate rings with one central Ga^{III} core, without the intramolecular strain accompanied by the Ga-DOTA complex. This is reflected by the higher K_{ML} and pM values of NOTA and other 1,4,7-triazacyclononane derivatives. Apart from variations in the donor functions to phos-

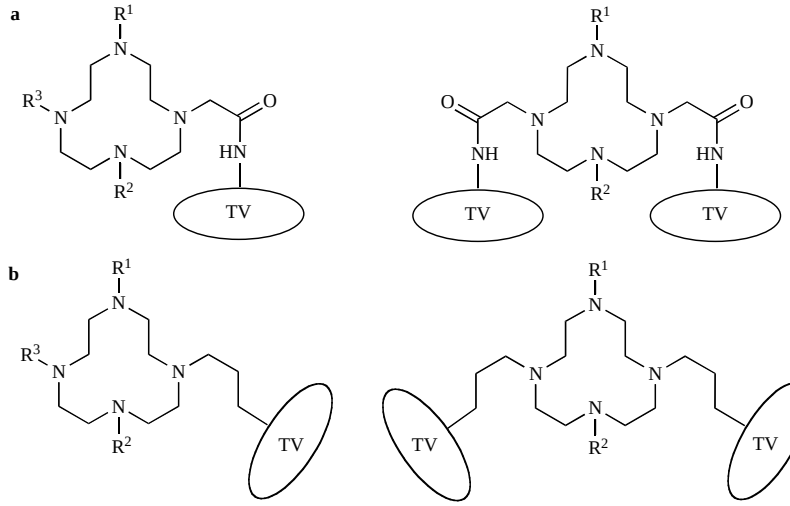
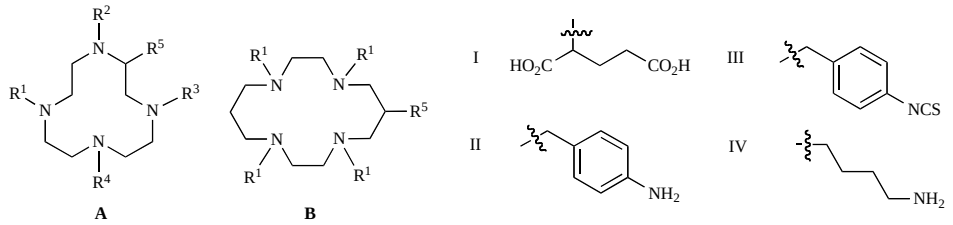


Fig. (14). Exemplified mono- and di-valent conjugation of biomolecules to DOTA-like structures. R^1, R^2 and R^3 = CO_2H, TV = targeting vector. **a**) exploiting surplus carboxylate functions; **b**) *via* alkylation of secondary amines.

Table 3. Bifunctional DOTA Derivatives. 1,4,7,10-Tetraazacyclododecanes (A) and 1,4,8,11-Tetraazacyclotetradecane (B), I to IV Illustrate Side Chain Structures (R^5).



#	R^1	R^2	R^3	R^4	R^5	log K_{ML}[a]	pM[a]	Ref.
5 (DOTA)[a]	CH_2CO_2H	CH_2CO_2H	CH_2CO_2H	CH_2CO_2H	H	21.3	15.2	[131,135]
8 (DO3A)	CH_2CO_2H	CH_2CO_2H	CH_2CO_2H	H	H			[134-137, 142]
9 (DO2A)	CH_2CO_2H	H	CH_2CO_2H	H	H			[133, 138]
10	CH_2CO_2H	CH_2CO_2H	CH_2CO_2H	CR^5HCO_2H	III			[136]
11	CH_2CO_2H	$CH_2P\ CH_3O_2H$	$CH_2P\ CH_3O_2H$	$CH_2P\ CH_3O_2H$	H			[169]
12	CH_2CO_2H	CH_2CO_2H	CH_2CO_2H	CH_2CO_2H	II			[140,141]
13	CH_2CO_2H	CH_2CO_2H	CH_2CO_2H	CH_2CO_2H	III			[140,141]
14	CH_2CO_2H	CH_2CO_2H	CH_2CO_2H	CH_2CO_2H	IV			[132]
15	CH_2CO_2H	I	CH_2CO_2H	I	H			[139]
16	CH_2CO_2H	CH_2CO_2H	CH_2CO_2H	I	H			[137, 139]
7 (TETA)[a]	CH_2COOH	CH_2COOH	CH_2COOH	CH_2COOH	H	19.7	14.1	[125]

[a]for K_{ML}: μ= 0.10 M (KNO_3), 25 ºC; for pM: pH 7.4, 100% Excess Ligand.

phonates or thiols, which still form 5 membered chelates, there are quite a few highly stable 2-hydroxy or 2-mercaptobenzyl substituted TACN derivatives which provide exceptional K_{ML} values.

NOTA forms slightly distorted octahedral complexes with Ga^{III} in geometry as shown in Fig. (**15**). Due to the facial arrangement of donors, the energetic barrier for complexation is significantly lower than with DOTA. Therefore, NOTA readily forms stable complexes with Ga^{III} already at

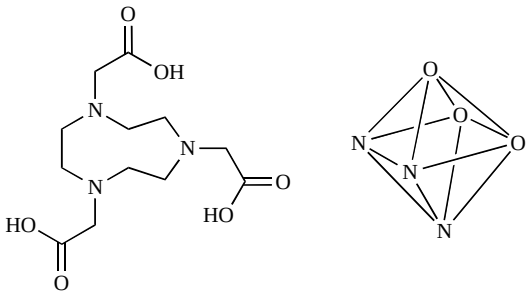


Fig. (15). 1,4,7-triazacyclononane-1,4,7-triacetic acid (6) NOTA, and its corresponding mode of octahedral chelation [143].

moderate temperature. Bifunctional derivatives of NOTA or NOTA-biomolecule conjugates provide high potential for the development of ^{99m}Tc-kit-like formulations. These can be used to provide labeled imaging agents in injectable formulation using no more than generator eluate and prepacked vials.

Bifunctional derivatives of NOTA: With respect to functionalization of the NOTA structure, it becomes clear, that NOTA does not offer the same convenience of using a spare secondary amine or a redundant carboxylate function for conjugation. Instead elaborate chemical modifications have to be undertaken to obtain bifunctional derivatives. Motivated by the potential of Ga^{III} for medical application, several bifunctional derivatives of NOTA have been reported during the last decades (Table **4**).

However, in comparison to the rather "convenient" DOTA chemistry some approaches were limited in efficiency (e.g. for **22** and **23**) due to the low overall yields obtained. Therefore only a few applications have been reported so far, although these BFCs offer all desired properties.

Functions for conjugation to biomolecules: Apart from orthogonally protected carboxylate functions which will allow conversion to amide bonds with primary and secondary nitrogen nucleophiles, NCS and maleimide functions are the most frequently employed electrophilic linker functions for conjugation of most chelators to biomolecules. Conversely, amino functions, thiols and hydroxyl functions have been proposed as nucleophilic linker functions (Table **5**). These are often chemoselective, as in the case of NCS-functions which readily convert amines into thioureas (Fig. **16**, **B**) or the Michael acceptors maleimides and vinylsulfones which can be used for site specific labeling of large proteins (Fig. **16**, **C** and **E**), chemoselectively addressing thiol groups. For conjugation to proteins, a variety of derivatization reagents exist that can be used to non-selectively introduce certain anchoring functions on large proteins, such as e.g. the 2-iminothiolane reagent, which introduces a thiol function together with a so called spacer moiety (Fig. **16**, **F**). A spacer is a chemical moiety which increases the distance between a targeting vector and a chelate. This will be discussed later on. These spacer functions have been shown to be crucial to obtain reasonable labeling yields on large proteins in some cases [159,160]. A variety of reactive functions and corresponding coupling moieties have been successfully employed for conjugation (Table **5**).

Table 4. Bifunctional Derivatives of NOTA

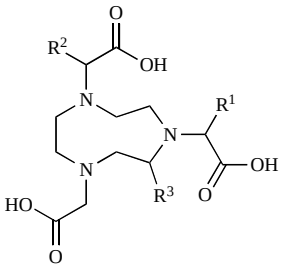
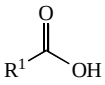
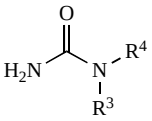
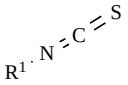
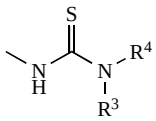
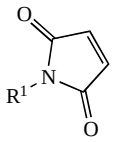
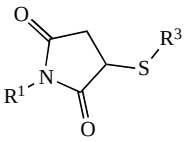
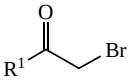
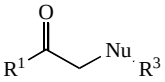
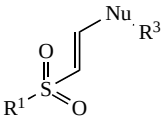
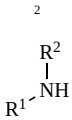
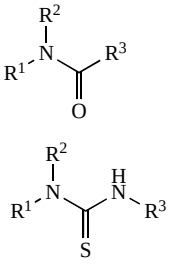
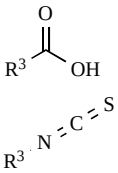
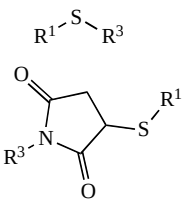
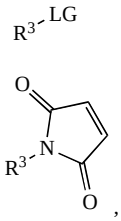
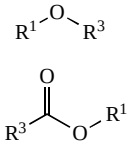
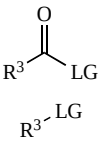
			**R^1**	**R^2**	**R^3**	**Ref.**
16	NODASA		CH_2COOH	H	H	[148]
17	NODAGA		CH_2CH_2COOH	H	H	[145]
18			CH_2(p-Ph-NCS)	H	H	[146]
19	NODAPA-NCS		p-Ph-NCS	H	H	[149]
20	NODAPA-$(NCS)_2$		p-Ph-NCS	p-Ph-NCS	H	[149]
21	NODAPA-OH		p-Ph-OH	H	H	[149]
22	*p*-NCS-Bn-NOTA		H	H	p-Bn-NCS	[144]
23	H		H	H	$(CH_2)_4NH$	[132]

Table 5. Common Conjugation Functionalities for Bifunctional Chelates.

#	Linker	Formed Bond	Corresponding Conjugation Moiety	Ref.
1	carboxylates 	amide 	HNR^1R^2 	[132,139, 142,146, 148]
2	isothiocyanatesNCS 	Thiourea 	HNR^1R^2 	[132,136, 140,144, 147,149, 153]
3	maleimides 	2-thio succinimide 	RSH 	[150-152, 155,157]
4	2-bromoacetyl functions 	CH_2Nu^a 	NuH^a 	[144,154]
5	Vinyl sulfones 	$SO_2(CH_2)_2Nu^a$ 	NuH^a 	[153]
6	amines 	amides, thioureas 		[132-138]
7	mercapto functions SH 	2-thio succinimide 		[142]
8	hydroxyl functions OH	esters, ether bonds 		[149]

[a](Nu – Nucleophile)

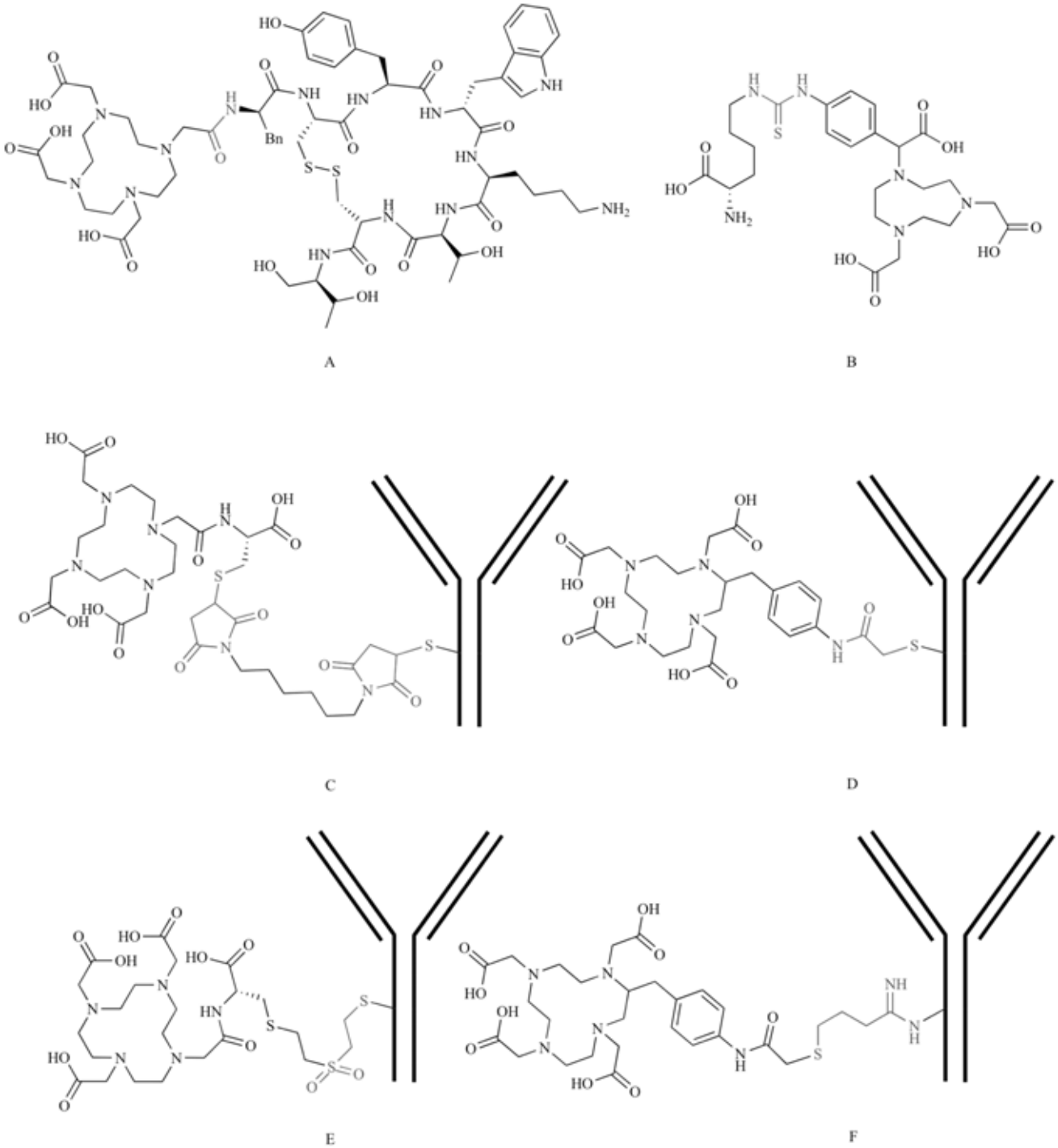
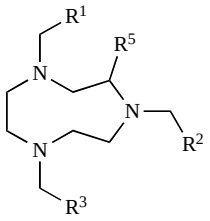


Fig. (16). Examples for chelator-conjugated biomolecules. A) amide bond: somatostatin antagonist DOTA-D-Phe-Tyr^3-octreotide (DOTATOC), cf. entry 1 in Table 5; **B**) thiourea: amino acid conjugate NODAPA-NCS-L-Lys, cf. entry 2; **C**) 2-thiosuccinimide: A DOTA-antibody conjugate, cf. entries 3 and 7; **D**) 2-thioacetamide: DOTA-p-Bn-acetamide conjugated antibody, cf. entries 4 and 7; **E**) 2-thioethylsulfone: a DOTA antibody conjugate, cf. entry 5; **F**) the spacer-linker introducing reagent 2-iminothiolane: 4-mercaptobutaneimidamide (cf. entries 4 and 7).

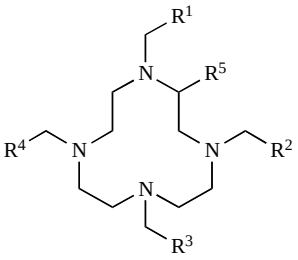
8.2. Macrocycles with Non-Carboxylate Donor Functions

Phosphonate and phosphinate donor functions have been elucidated as parts of open chain chelators, where they were used to replace carboxylate donors to obtain the phosphonate analogues, e.g. EDTMP from EDTA [161-163]. They were employed in macrocycles such as TACN and cyclen as well. It has been shown that phosphonates can be labeled under acidic conditions, forming stable complexes already at mild temperatures [161,169,171]. These complexes have been evaluated with profound success as bone targeting agents [165], as well for the use as alternative donors to common carboxylates [161-173]. Bifunctional analogues have been studied as a marker for tissue hypoxia (Table **6**) [168].

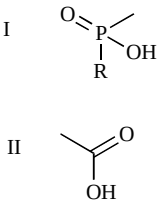
Table 6. Macrocyclic Posphonates and Phosphinates used for ^{68}Ga-Labeling

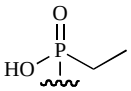
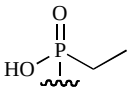
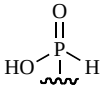
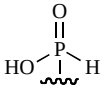
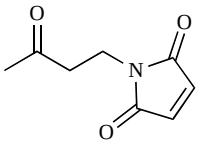
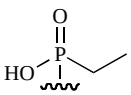
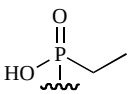
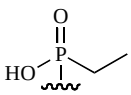


A

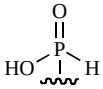
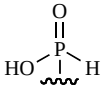
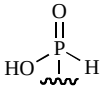
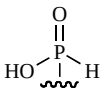
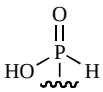
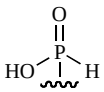


B



#		R^1	R^2	R^3	R^4	R^5	R^6	R^7	Ref.
23	A	II	II	I	-	H	OH		[165]
24	A	II	I	I	-	H	OH		[165]
25	A	I	I	I	-	H	OH		[165-167]
26	A	I	I	I	-	H	OCH_2CH_3		[164-167]
28	A	I	I	I	-	H	CH_3		[168, 171]
29	A	I	I	I	-	H	C_6H_5		[171]
30	A	I	I	I	-	$(CH_2)_4NHR^7$	CH_3	$CO(CH_2)_2CO_2$-$C_6H_4NO_2$	[168, 171]
31	A	I	I	I	-	$(CH_2)_4NHR^7$	CH_3	H	[168, 171]
32	A	I	I	I	-	$(CH_2)_4NHR^7$	CH_3	COPh	[168]
33	A			I		H	$CH_2C_6H_4N^7H$	COPh	[171]
34	A			I		H	$CH_2C_6H_4N^7H$	H	[171]
35	A	I	I	I		H	$CH_2CH_2CO_2H$	H	[161]
36	B	I	I	I	I	H	CH_3		[171]
37	B	I	I	I	I	H	C_6H_5		[171]
38	B	I	I	I	I	H	$(CH_2)_3CH_3$		[171]
39	B	I	I	I	I	$(CH_2)_4NHR^7$	CH_3	$CO(CH_2)_2CO_2$-$C_6H_4NO_2$	[171]
40	B	I	I	I	I	$(CH_2)_4NHR^7$	CH_3	H	[171
41	B	I	I	I	I	$(CH_2)_4NHR^7$	CH_3		[171]
42	B	I	I	I	I	H	OH		[165]
43	B	I	II	II	II	H	$CH_2C_6H_4NH_2$		[169]
44	B				I	H	$CH_2C_6H_4N^7H$	COPh	[171]

(Table 6) Contd….

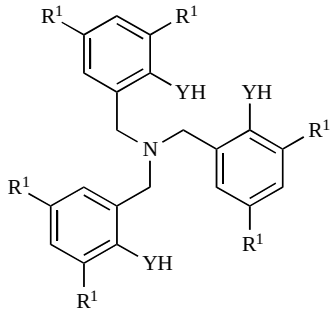
#		R^1	R^2	R^3	R^4	R^5	R^6	R^7	Ref.
45	B				I	H	$CH_2C_6H_4N^7H$	H	[171]
46	B				I	H	$CH_2C_6H_4N^7H$	$CO(CH_2)_2CO_2$-$C_6H_4NO_2$	[171]

Compound **35**, the most recent example of macrocyclic ligands bearing methylenephosphinic acid donors, is equipped with three symmetrical *P*-propion-3-yl substituted pendant arms. The carboxylic acid functions do not participate in the stable octahedral Ga^{III} complex which is formed by **35** and can be used for conjugation [161].

8.3. Phenolate and Thiolate Donor Functions

The idea of thiolate donor metal-chelates, dedicated for gallium has been elucidated originally to specifically target rather lipophilic complex precursors, to be used in the formation of lipophilic Ga-chelates. Tripodal, tetradentate NY_3-type chelators, wherein Y is a substitute for O or S, were developed (Table **7**). The stability constants of various metal complexes formed by the former have also been accessed. These investigations yielded several approaches with potential for successful application in imaging.

Table 7. Tripodal, Monofunctional Tetradentate NY_3-type Chelators for ^{68}Ga-Labelling.



#	Y	R	K_{ML}[a]	pM[a]	Ref.
47	O	H			[174,175]
48	O	CH_3	44.2	25.2	[128]
49	S	H	20.5		[175,176]

[a]for K_{ML}: μ= 0.10 M (KNO_3), 25 ºC; for pM: pH 7.4, 100% Excess Ligand.

These chelators yield highly stable complexes with trivalent metals, however, crystal structures of the non-radioactive complexes show bulky structures, that easily excel many bioactive small molecules by both, size and molecular weight [174-176]. Tripodal phenolate and thiolate bearing complexing agents were extensively studied with various intentions. It was found that the corresponding Ga-complexes of these chelators were highly lipophilic, neutral compounds of exceptional stability, which were readily formed from $^{68}Ga^{III}$ at room temperature [174,175].

Compound **49** formed a stable (>95% intact tracer in rat serum after 2h) four coordinate complex with Ga^{III}, which was found to exhibit considerable brain and myocardial uptake, paired with fast blood clearance *via* the liver in rats and primates. The brain/blood ratio was 3.8 after 60 min, indicating high potential as brain imaging agent for PET. The heart/blood ratio was 11 at the same time point. In macaque imaging studies the high potential of the compound was verified. Recent progress includes a bifunctional derivative based on compound **49**, cf. Fig. (**17**) [177].

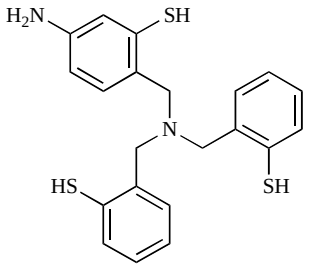
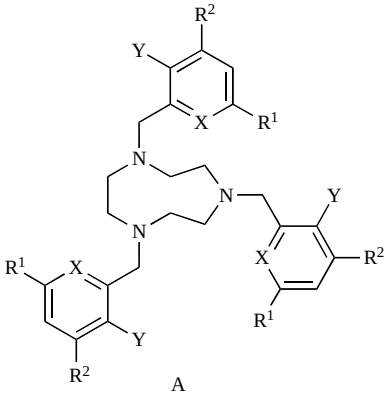


Fig. (17). Tripodal, bifunctional NS_3-type Chelator for ^{68}Ga-labeling [177].

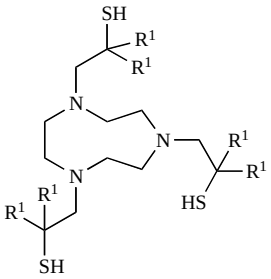
The extraordinarily high potential of the chelate motivated the synthesis of a bifunctional derivative bearing an aniline nitrogen for conjugation. Successful conjugation to an amino acid proved suitability as a bifunctional chelator for peptide and protein labeling. Several phenolate analogues have also been prepared and the Ga^{III} complex structures and stability constants were investigated. However, it was shown, that these analogues surprisingly formed pentacoordinate complexes, with water occupying the free coordination site.

Hexacoordinate N_3Y_3 chelators were derived from the macrocyclic polyamine 1,4,7-triazacyclononane (TACN). These formstable complexes with Ga^{III} as shown in Table **8**. Unfortunately, some of these i.e. compounds **50** and **51** are even more sterically demanding and exhibit a higher molecular weight than the tripodal chelators mentioned earlier. Moore and coworkers [180] have introduced compound **53**, and a more lipophilic analogue was later on studied in rats

Table 8. Hexadentate N_3O_3 and N_3S_3-Type Chelators Based on 1,4,7-Triazacyclononane



A



B

#	Structure	X	Y	R^1	R^2	K_{ML}[a]	pM[a]	Ref.
50	A	N	OH	CH_3	H	45.6	34.9	[128, 178]
51	A	CH	OH	CH_3	CH_3	44.2	25.6	[128, 179]
52	A	CH	SH	H	H			[182]
53	B	-	-	H	-	34.2	23.87	[128,180-183]
54	B	-	-	CH_3	-			[184]

[a]for K_{ML}: μ= 0.10 M (KNO_3), 25 °C; for pM: pH 7.4, 100% Excess Ligand

with regard to a potential application as radio-gallium ^{67}Ga labeled tracer for hepatobiliary imaging by John *et al.* [184]. The ^{67}Ga-chelate displays rather lipophilic properties, is mainly excreted *via* the liver and remained stable versus trans-chelation to the iron binding sites of transferrin.

Nevertheless, the compound did not show any uptake into the brain. Also compound **52** has been labelled with ^{67}Ga and studied for its biological properties. The complex showed some degradation only in rat plasma at 37°C. Biodistribution studies in rats showed moderate clearances *via* the liver over 60 min with high blood uptake and only slow washout from the blood. Unfortunately, combining the low heart and brain uptake with the high blood uptake somewhat compromises its use [178-184]. Probing the high stability of the Ga-S bond in thiolate precursors, several research groups investigated open chain chelators, commonly used for ^{99m}Tc-labelling for their suitability with ^{68}Ga (Table **9**). Among these, compound **63**, also referred to as BAT-TECH, has been evaluated as myocardial imaging agent with success.

Ethylene dicysteine (EC, **56**) has been elucidated as bifunctional chelating agent bearing two carboxylate functions for conjugation. Yang *et al.* have demonstrated the versatility of this approach using ^{99m}Tc and ^{68}Ga, Fig. (**18**) [191]. It has been shown that the chelator forms stable complexes with Ga^{III} and In^{III} in a tetracoordinate manner (for EC) and hexacoordinate complexes with EC [185-186,191-194].

8.4. Bissalicylaldimine Complexing Agents

Tetraazaalkyl bis(salicylideneimines) were devised as complexing agents suitable for the formation of cationic

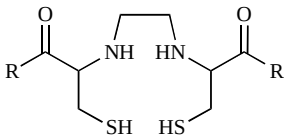
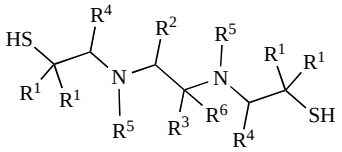


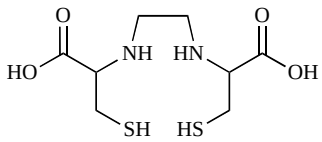
Fig. (18). Ethylene dicysteine (EC) as a bifunctional chelating agent for Ga^{III}. (R = 2-amino-desoxyglucose, guanine).

$^{67,68}Ga^{III}$ complexes as potential myocardial imaging agents (Table **10**). These complexes provided the general requirements needed for such an imaging agent: one-fold positive charge, somewhat spherical shape and lipophilic properties. The complexing agents summarized in Table **8** complexed Ga^{III} in a pseudo-octahedral geometry. The organ distribution in rats revealed prospectively high and rapid uptake into the heart and retention in the myocardium over a long period of time. The ratios between heart and blood were very high at 2 h p.i.. Together with the overall *in vivo* behavior of these compounds, the authors were let to the conclusion, that compound **78** even showed a behavior superior to ^{99m}Tc-sestamibi [197].

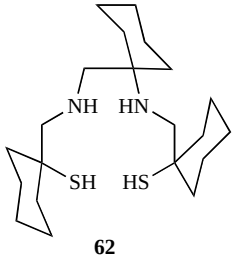
Bissalicylaldimines have also been extensively studied as potential imaging agents for Multi Drug Resistance 1 P-glycoprotein (MDR1 Pgp), an efflux transporter highly involved with the failure of certain forms of chemotherapy. The Pgp membrane transporter is responsible for the outward transport of many structurally unrelated, cationic forms of a variety of drugs and poisons. Notably, it is present on vascular glia cells which form an important part of the blood brain barrier. Thereby it contributes to the pharmacokinetic prop-

Table 9. Open Chain $N_xO_yS_z$ Chelators for ^{68}Ga Labeling





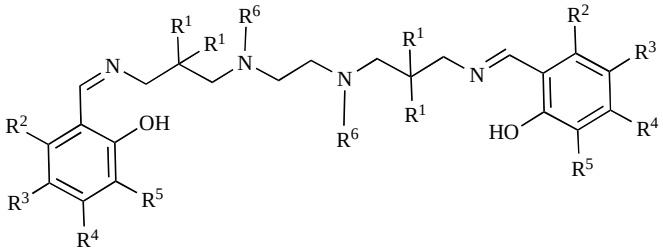
55



62

#	R^1	R^2	R^3	R^4	R^5	R^6	K_{ML}	pM	Ref.
55	H	H	H	H	CH_2COOH	H	35.6	29.0	[185]
56	H	H	H	COOH	H	H	31.5	24.7	[185,186]
57	CH_3	CH_3	H	H	H	H	24.7		[185,186]
58	CH_3	CH_3	COOH	H	H	H	27.4		[185]
59	CH_3	CH_3	H	H	CH_2COOH	H	41		[185]
60	CH_3	CH_3	H	H	CH_3	CH_3			[186]
61	CH_2CH_3	CH_2CH_3	H	H	H	H			[185]
62	CH_2CH_3	CH_2CH_3	H	H	C_5H_{10}				[186-190]
63	C_5H_{10}		H	H	C_5H_{10}				[188]

Table 10. Bissalicylaldimine Chelators Useful for ^{67,68}Ga Labeling



#	R^1	R^2	R^3	R^4	R^5	R^6	Log P	Ref.
64	H	H	H	H	H	H	0.84	[195]
65	H	H	OCH_3	H	H	H	0.75	[195]
66	H	H	H	OCH_3	H	H	1.1	[195]
67	H	CH_3	H	OCH_3	H	H	1.60	[195]
68	H	OCH_3	H	OCH_3	H	H	1.96 [194] 1.68 [195]	[195-197]
69	H	H	CH_3	OCH_3	H	H	2.12	[195]
70	H	CH_3	H	O CH_2CH_3	H	H	2.01	[195]

(Table 10) Contd….

#	R^1	R^2	R^3	R^4	R^5	R^6	Log P	Ref.
71	H	H	$CH(CH_3)_2$	H	H	H	2.96	[195]
72	H	H	H	$N(CH_2CH_3)$	H	H	2.59	[195]
73	CH_3	OCH_3	H	OCH_3	H	H	2.88	[195]
74	CH_3	H	H	$N(CH_2CH_3)$	H	H	3.00	[197]
75	H	H	H	H	H	CH_3	1.59	[197]
76	H	H	H	OCH_3	H	CH_3	1.92	[197]
77	H	OCH_3	H	OCH_3	H	CH_3 CH_3	2.97	[197]
78	H	H	H	H	OCH_3	CH_3	1.39	[197]
79	H	OCH_3	H	H	H	CH_3	2.56	[197]
80	CH_3	H	H	H	OCH_2CH_3	H	1.7	[198-200]

erties of multiple drugs and reduces intracellular concentration of those, thereby leading to drug resistance *in vivo*. The main benefit when employing bissalicylaldimines is their apparent insensitivity to metabolism, which renders a highly advantageous property compared to ^{11}C or ^{18}F labeled tracer candidates. Compliant to the high stability reported for the earlier examples and summarized in Table **10**, the complex remained stable in human serum for more than 24 h. Rodent studies did not detect any metabolized tracer in heart, liver and kidney. Finally, it was found that the Ga complex of **80** is a highly sensitive substrate for Pgp, potentially superior to established ^{99m}Tc tracers. It furthermore might prove to be useful as myocardial imaging agent [195-200].

Recent reports focused on the individual biodistribution and pharmacokinetic properties of the two different rotamers obtained from bis(3-aminopropyl)ethylendiamine (BAPEN) bissalicylidene-like chelators, which showed an remarkable difference in Pgp-KO mice and wildtype controls. Obviously, both different isomers of **78** showed differences in plasma protein binding as well as cellular excretion pathways [201-202].

8.5. 2-Hydroxybenzyl-Ethylene-1,2-Diamine-N,N'-Diacetate Chelators

After the non-radioactive, octahedral complex of 2-hydroxybenzyl-ethylene-1,2-diamine-N,N'-diacetate (HBED) **81** showed a complex formation constant of 35.5 with the Ga^{III} core, bifunctional derivatives of HBED were studied (Table **11**). HBED has been functionalized with propionic acid functions to obtain **82**, one of which was furthermore converted into a reactive trifluorophenyl ester (**83**), for direct, non-selective incorporation into antibodies. These provide the potential of synthesizing chelator conjugated sensitive large proteins such as antibodies suitable for Ga-labeling. This is due to the fact, that **81-83** form Ga^{III} complexes at mild conditions, i.e at ambient temperature, comparable to **6** and compounds **48-49**. Due to their main feature, complexes of these chelators appear particularly well suited for protein and antibody labeling, where harsh conditions may cause protein degradation and loss of the desired biological activity [203-210]. Apart from this work antibodies have been studied with respect to medical imaging throughout the last 3 decades, a variety of chelators and radionuclides, including Ga have been used. For reviews see [211-215].

8.6. Trisaminomethylene Ethane (TAME) Based Tripodal Chelators

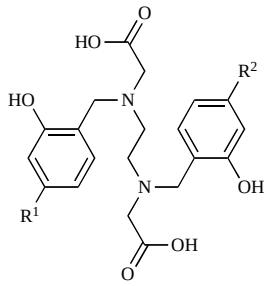
Trimethylol ethane-derived trisaminomethylene ethane (TAME) based tripodal chelators with 2-hydroxy aldimine N_3O_3 donor sets were functionalized with various alkyl ethers to tune the complex lipophilicity (Table **12**). These were elucidated in view of potential myocardial imaging agents. The chelators were obtained in a manner comparable to the BAPEN-based bissalicylaldimine complexes discussed earlier. Biodistribution and PET imaging studies of **85-88** highlighted compound **87** as it revealed high quality images of the myocardium in dogs [216-218].

Compound **88** showed rapid blood clearance *via* the liver and the kidneys and high heart to blood ratios. The compound was moderately taken up into the brain. However, the compound had been rendered potentially suited as a perfusion agent, though it proved to be less suitable than $H_2[^{15}O]O$, [^{11}C]butanol and ^{99m}Tc-microspheres [216].

Recently, the 2-hydroxy-TAME chelate was glycated with β-D-glucose, galactose and xylose to obtain radiogallium labeled sugar derivatives for imaging purposes. However, this work did not envisage the uptake mechanism of the glucose transporter systems (GluT) which utilize the hydroxy group at the hemi-acetalic position one during the uptake mechanism [219].

In this case the TAME trissalicylideneimine chelator is used as a bifunctional chelating agent for Ga-mediated imaging. The ^{68}Ga-complex of **91** has been formed, however, rather harsh conditions had to be employed and the complex

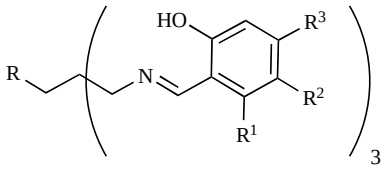
Table 11. 2-Hydroxybenzyl-Ethylenediamine Diacetate Chelators Used for ^{67,68}Ga-Labeling.



#	R^1	R^2	K_{ML}[a]	pM[a]	Ref.
81[a]	H	H	35.57	30.9	[73, 208]
82	CH_2COOH	CH_2COOH			[203-208]
83	CH_2COOH	$CH_2COOTFP$			[205]
84	Br	Br			[210]

[a]for 1 μM Ga, 10 μM Ligand, 27 mM Carbonate

Table 12. TAME Based Chelators for ^{67,68}Ga-Labeling



#	R	R^1	R^2	R^3	R^4	Log P	Ref.
84	$OCH_2CH_2CH_2CH_3$	H	H	H	H	3.1	[217]
85	$OCH_2CH(CH_3)$	H	H	H	H	3.1	[217]
86	$OCH(CH_3)CH_2CH_3$	H	H	H	H	2.58	[217]
87	$OCH_2CH_2CH_3$	H	OCH_3	H	H	2.54	[217]
88	CH_3	H	OCH_3	H	H	1.43	[216]
89	β-D-xylose	H	H	H	H		[218]
90	β-D-xylose	H	CH_3	H	H		[218]
91	β-D-glucose	H	H	H	H		[218]
92	β-D-galactose	H	H	H	H		[218]
93	β-D-galactose	H	$C(CH_3)$	H	$C(CH_3)$		[218]

precursors showed sensitivity even towards mildly acidic labeling conditions. Nevertheless, the sugar conjugation apparently did not affect ^{68}Ga complexation, and the derivatives obtained remained stable to 90% in a transferrin challenge over two hours (10% loss of intact complex) [218].

8.7. Cis,cis-Triaminocyclohexane Chelators

Derivatives of cis,cis-1,3,5-triaminocyclohexane (TACH) based "face-capping" ligands have been elucidated as thermodynamically stable and kinetically inert Ga^{III} chelators for PET imaging agents, Fig. (**19**). Investigations of **95-97** with

^{67}Ga have shown that the corresponding chelates of neutral charge remained stable in serum for up to 5 h (TACHTA, **95**). Log D measurement did not reveal significant lipophilicity. Supposedly more lipophilic analogues **96** and **97** suffered from lower stability and may therefore be rated as not suited for ^{68}Ga imaging applications [220].

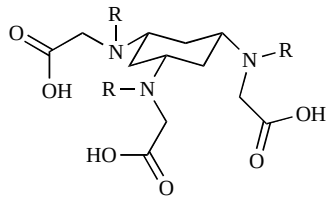


Fig. (19). Cis,cis-triaminocyclohexane based chelators for ^{68}Ga. R = H (**95**), Me (**96**), Et (**97**).

The biodistribution of the $^{67}Ga^{III}$ chelate of **95** showed fast renal clearance, indicating suitability as a scaffold for the development of bifunctional chelators for conjugation to biomolecules and application in molecular imaging [220-224].

8.8. N-alkyl-3-Hydroxy-4-Pyridinones

N-alkyl-3-hydroxy-4-pyridinones have been studied as bidentate Ga^{III} ligands *in vivo*, with 3 ligands forming the common octahedral geometry around one gallium core (Table **13**). The complexes showed high stability constants and high stability *in vivo*, with an increased clearance *via* the liver, compared to non-chelated Ga^{III}. One complex, **99**, exhibited significant bone uptake, and was therefore hypothesized to be a potential bone imaging agent. Compounds **104**-**107** exhibited slower blood clearance and increased uptake into bone, compared to the more hydrophilic analogues **98**-**103** [225-231].

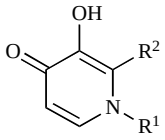
8.9. Macrocyclic Tetrapyrroles

Gallium-labeled porphyrins and chlorins have been reported as potential imaging agents for arteriosclerotic plaques in blood vessels, as well as prospective tumor targeting probes. These planar chelators form complexes with Ga^{III} in a square pyramidal manner and leave the axial coordination site free for being occupied by a co-ligand. It has been shown, that Ga^{III}-labeled polypyrroles show promising *in vitro* behavior, as well as long term stability towards transchelation. Their *in vitro* behavior though, does not seem to be largely affected by the metal [67,232].

9. LABELING CONSIDERATIONS

Effect of pH: For two main reasons labeling with ^{68}Ga is mainly pH-dependant, cf. also chapters 6 and 7. The Ga^{III} ion is heavily hydrolyzed at pH levels ascending already at pH 3.5, forming an insoluble colloid precipitate. Upon further increasing of the pH the formation of gallate anions, $Ga(OH)_4^-$, leads to redissolution of the metal. However due to the extremely high stability of Ga-OH species, only the dissolved Ga^{III} is available for complexation. Therefore particular care has to be taken to ensure a low pH-value. Conversely, the kinetic complex formation with carboxylate and amino functions is sensitive to protonation. As a result, the complex formation is inhibited at very low pH values. High H^+ concentration in solution usually tends to hydrolyze even very stable complexes.

Table 13. N-alkyl-3-hydroxy-4-pyridinones as Bidentate Chelators for Ga^{III}

	**R^1**	**R^2**	**$K_{ML}(pM)^a$**	**Ref**
98	$CH_2CH_2CH_2NH_2$	CH_3	60.15	[230]
99	CH_2CH_2COOH	CH_3	36.84	[230]
100	CH_2CH_2COOH	H	-	[230]
101	CH_3	CH_3	38.76	[230]
102	$CH_2CH_2CH_2COOH$	CH_3	36.19	[229]
103	$CH_2\ CH_2CH_2CH_2COOH$	CH_3	36.41	[229]
104	$CH_2CH_2CH_2NHCH_2C_6H_5$	CH_3	33.88 (15.8)	[231]
105	$CH_2CH_2CH_2NHCH_2C_6H_4$-pNO_2	CH_3	32.03 (15.2)	[231]
106	$CH_2CH_2CH_2N(C_2H_4)_2NC_6H_5$	CH_3	31.57 (14.6)	[231]
107	$CH_2CH_2CH_2N(C_2H_4)_2NC_6H_4$-pCl	CH_3	30.19 (14.1)	[231]

[a]K_{ML}: 0.1 M KNO_3, 25 ºC: for pM: pH = 7.4, tenfold Excess of Ligand

Effect of buffers: Several approaches have been devised in the past to overcome these problems. Different buffer systems such as acetate, phosphate and N-(2-hydroxyethyl) piperazine-N9-(2-ethanesulfonic acid) (HEPES) have been used to overcome the pH issues. Other approaches include the use of defined amounts of water to dilute the preprocessed generator eluent, as well as the use of weakly coordinating co-ligands. The latter were used for complex formation at low pH, prior to trans-chelation to the more stable desired complex at elevated pH and temperature.

A major drawback in using buffer substances as well as weakly coordinating chelators in the labeling solution generally results in a competition between the buffer substance and the labeling precursor, particularly when taking the difference in concentration into account. For example, a concentration of approximately 1.4 micromoles per liter is sufficient for labeling of the peptide DOTA-TOC, whereas the concentration of HEPES buffer in this case is no lower than 1 M. Furthermore, the buffer has to be removed prior to application of the labeled compound to living systems. In terms of more elegant solutions, the use of lower buffer concentrations or pre-basified reaction media has been considered [62, 67,138,153].

Effect of ^{68}Ga precursors: In most cases, $^{68}Ga^{III}$ is obtained as $[^{68}Ga]GaCl_3$ from the generator system, or, as in the case of ^{67}Ga, from the dissolved cyclotron target following removal of the zinc target material and other metal contaminants. In the gross chemical purification, $GaCl_3$ plays a major role as it is readily extractable from HCl-solutions using organic solvents. However, several groups have reported the use of $^{68}Ga(acac)_3$ as a highly soluble labeling agent which can be employed for labeling of chelator of low polarity, even under anhydrous conditions. Apart from that, Ga-citrate has been proposed and successfully used as a carrier chelator to transfer weekly coordinated but hydrolytically shielded Ga^{III} from acidic to neutral media.

Effects of temperature: Most approaches for the gallium labeling of water soluble chelators involve the addition of a nanomolar amount of chelator or chelator-biomolecule conjugate to a small amount of water or buffer (usually in 0.2 to 5 ml volumes), addition of the pre-purified, pre-concentrated or fractionated radioactivity in solution (cf. Chapter 5) and subsequent heating for 1 - 30 min in an oil bath. More recent approaches have elucidated the use of microwave irradiation as a source of activation energy, combined with very small amounts of reaction media (200 μL). Thereby remarkably increased specific activities were obtained, which might be particularly beneficial in some cases [58]. These authors have also claimed increased specific activities *via* the efficient labeling of smaller precursor amounts. However, when comparing the precursor concentrations, these are similar to those reported in [62] and [135], only for the lowest amounts applied in 200 μl, otherwise the concentrations are twice to three times as high [233-235]. The increase in specific activity is evidently increased due to the lower total volume of labeling mixture employed in these studies. Apart from increasing the amount of activation energy that is transferred to the chelator and the metal ion, which clearly possess the highest tan δ value in the labeling solution, microwave heating does not affect the specific activity.

Decent chelators such as NOTA and its analogues can be labeled at room temperature within 10 minutes when higher amounts of precursor (>10 μM) are employed. These conditions, though lacking reference from successful further application, may prove as extraordinarily valuable for labeling of sensitive biomolecules. In addition, these conditions might facilitate the design and application of kit-like preparation of ^{68}Ga-radiopharmaceuticals [236].

10. ^{68}Ga-LABELED COMPOUNDS FOR MEDICAL A APPLICATION

The clinical application of ^{68}Ga-labelled PET-tracers is beyond the intention of the present review. However, principal applications of ^{68}Ga-labelled compounds may concern a broad variety of clinical indications, covering ideally all the directions known today from ^{99m}Tc-imaging options: blood flow, myocardial function, renal function, pulmonal blood flow, tumor detection etc.. Many of these directions have already been covered in small animal studies as surveyed in Table **14**. For a detailed summary cf. [239].

In principal, the complex ligands characteristics alone may provide interesting imaging potential, such as the "early" [^{68}Ga]Ga(EDTA) to measure brain tumor perfusion [240], [^{68}Ga]Ga(EDTA) to access renal function [239], amorphous and spherical HSA aggregates for pulmonal and renal perfusion, general blood flow and quantification of blood proteins [86-92, 240], [^{68}Ga]Ga(citrate) for labeling of transferrin and quantification of the blood protein pool [239], [^{68}Ga]Ga(EDTMP) for assessment of bone structure abnormalities [161-162,166], ^{68}Ga-labelled BAT-TECH for myocardial perfusion imaging [189,192]. Salicylaldimine ^{68}Ga-complexes to monitor myocardial function, cerebral perfusion and P-glycoprotein status [199-202, 218-219].

Human studies, however, have been most impressive in the context of utilizing the binding of ^{68}Ga-labelled peptidic targeting vectors to G-protein-coupled transmembrane tumor receptors. The synthetic concept for the design of the appropriate molecular structures is illustrated in Fig. (**20**), cf. [241] for a review. It illustrates the combination of a chelating ligand, a linking group and sometimes a spacer function and finally the biological targeting vector. The spacer is mainly employed to increase the distance between the receptor binding moiety and the non-biogenic metal chelate. Thereby the adverse effect of such chelates to biochemical interactions should be minimized. Peptide based imaging probes have been reviewed recently [237,238]. Medical application of ^{68}Ga-DOTA-conjugated octreotide derivatives (DOTA-TOC, DOTA-TATE, DOTA-NOC etc.) has become the golden standard of PET/CT imaging neuroendocrine tumors today, cf. Figs. (**21**, **22**).

The similar approach holds true for substituting the peptidic tumor targeting vectors by other molecular units, such as 2-methyl-5-nitro-imidazol-1-yl moieties for tissue hypoxia imaging [242], peptide sequences such as the $\alpha_V\beta_3$ binding Arg-Gly-Asp (RGD) motif to measure tumor angiogenesis [243], amino acids to measure protein synthesis rates or amino acid transporter activity, i.e. tumor metabolism [138], folate to image folate receptor positive tumors [244], sulfonylureas for analyzing the progress of diabetic diseases,

Table 14. Survey of *in vivo* Animal Studies Concerning Applications of Potential ^{68}Ga Labeled Imaging Agents

Compound	Application	Ref.
[^{68}Ga]Ga(EDTA)	brain tumor imaging	[239]
[^{68}Ga]Ga(EDTA)	renal function	[239]
[^{68}Ga]Ga(HSA microspheres)	pulmonal blood flow	[86-92, 240]
[^{68}Ga]Ga(HSA aggregates)	blood flow, cerebral and myocardial perfusion	
[^{68}Ga]Ga(citrate)	blood protein pool, blood flow	[239]
[^{68}Ga]Ga(EDTMP)	bone abnormalities, bone trauma	[161-162]
[^{68}Ga]Ga(49)	cerebral perfusion	[176]
[^{68}Ga]Ga(62)	myocardial perfusion	[189, 192]
[^{68}Ga]Ga(78)	myocardial perfusion	[199]
[^{68}Ga]Ga(80)	MDR1 P-glycoprotein status	[200-202]
[^{68}Ga]Ga(86)	myocardial perfusion	[219]
[^{68}Ga]Ga(88)	myocardial perfusion	[218]

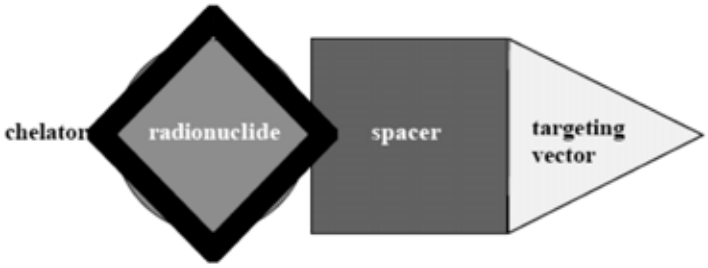


Fig. (20). The Bauhaus Representation of Common Radio-Chelate / Spacer / Peptide Conjugates.

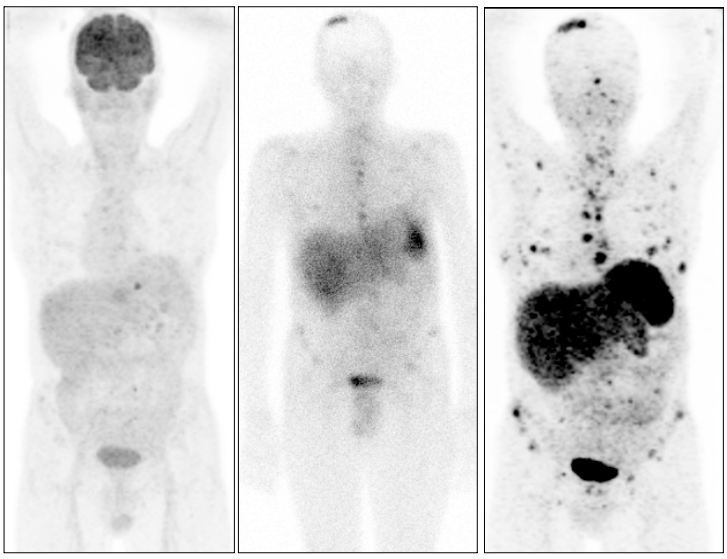


Fig. (21). Imaging of Neuroendocrine Tumors by ^{68}Ga-DOTA-TOC: Enhanced visualization of disseminated bone metastases of primary neuroendocrine tumors; Comparison of ^{18}F-FDG (PET), ^{111}In-OctreoScan (SPECT) and ^{68}Ga-DOTATOC (PET); Patient: male, *1939, neuroendocrine tumor with unknown primary, multiple liver and bone metastases. With permission [69].

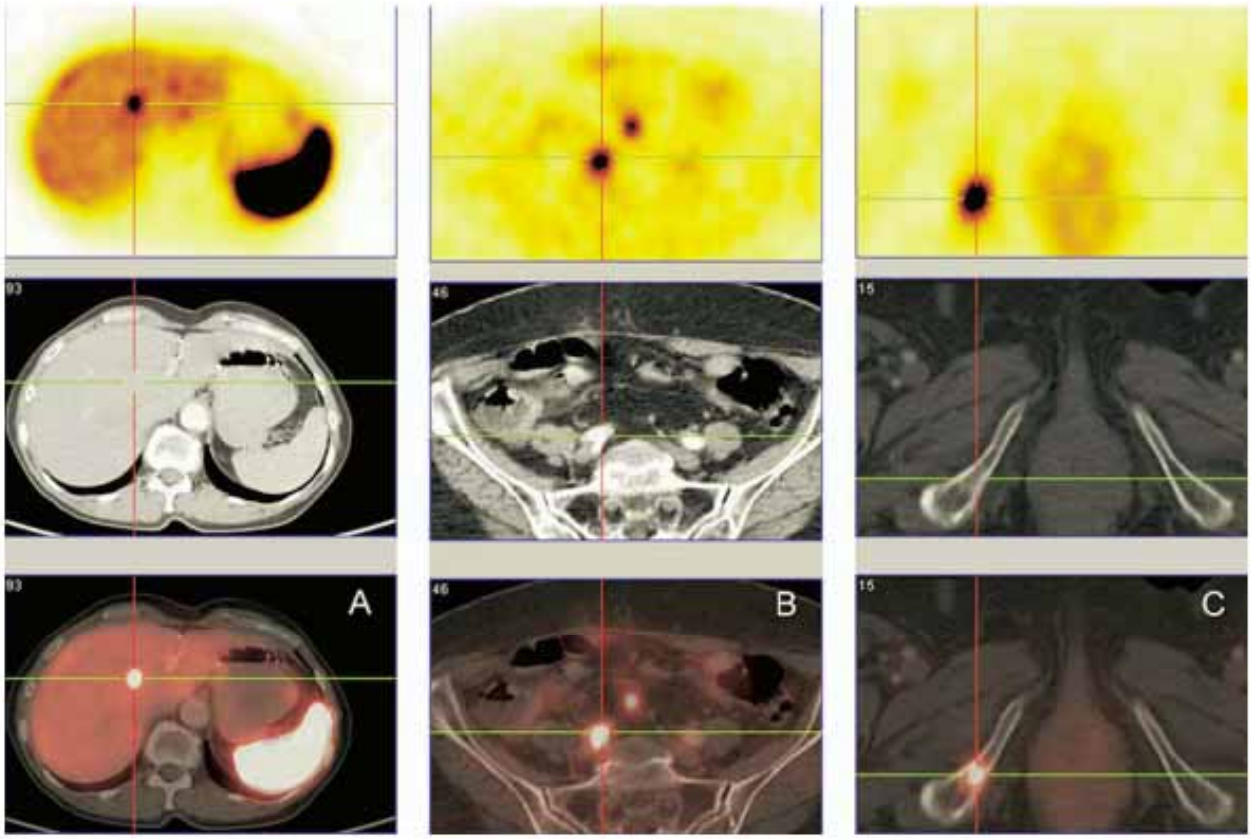


Fig. (22). Imaging of Neuroendocrine Tumors by ^{68}Ga-DOTA-NOC PET/CT: Small liver metastasis (**A**), not seen on contrast-enhanced (portal-venous phase) CT scan as well as small lymph node (**B**) and bone (**C**) metastases. With permission [246].

bisphosphonates to analyze osteoblastic activity [245], glucosamine to visualize inflammative processes *in vivo* [191]. In addition to the molecular concept illustrated in Fig. (**20**), representing mono-functionalized chelate complexes of $^{67,68}Ga^{III}$, di- and multi-functionalized chelators may be used, e.g. 1,7-dialkylated derivatives of DO2A (**9**) as shown in Fig (**23**).

11. CONCLUSIONS

Availability of Germanium-68: The most effective route to ^{68}Ge appears to be proton-irradiation of gallium targets. However, high beam intensities in the range of 100 μA or more are required to produce Curie-batches of ^{68}Ge activities. As the number of accelerators with the above features is limited worldwide, the ^{68}Ge production sites limited too. Los Alamos, Brookhaven, Obninsk and Faure report successful productions. As some of these sites have demonstrated sufficient production capacities to cover the world-wide need of ^{68}Ge calibrations sources for PET scanners, the capacities are still adequate for the world-wide need of ^{68}Ge for the preparation of ^{68}Ge/^{68}Ga generators. Nevertheless, a drastically increasing demand of those generators for routine medical application – supposed a relevant number of kit-based ^{68}Ga-radiopharmaceuticals show superior imaging qualities compared to existing tracers used in SPECT and PET diagnoses today – might ask for the installation of cyclotrons dedicated to large-scale ^{68}Ge productions.

^{68}Ge/^{68}Ga Radionuclide Generators: Commercial generators available today use Me^{IV} oxide-based column materials to adsorb ^{68}Ge. ^{68}Ga is eluted by 0.1 N HCl or 0.6-1.0 N HCl solutions. Due to the volume of the hydrochloric acid, the content of metallic impurities and the breakthrough of ^{68}Ge, various post-processing methodologies have been developed. Among them, the cation exchange-based method to online process ^{68}Ga eluates simultaneously purifies this generator-derived positron emitter from metallic impurities such as zinc, iron and titanium, significantly improving the yield of subsequent labeling reactions. It eliminates the initial breakthrough of ^{68}Ge by more than 4 orders of magnitude down to negligible levels thus minimizing concerns of radiopharmaceutical contaminations by this long-lived radionuclide, reduces the total volume of the purified ^{68}Ga fraction to 0.4 ml, and removes excess of hydrochloric acid originating from the generator eluate. Recent improvements, in addition, demonstrate that the intermediate adsorption of purified ^{68}Ga on the small cation exchange cartridge opens directions towards the synthesis of lipophilic ^{68}Ga- compounds in non-aqueous solvents. Moreover, the removal of ^{68}Ge represents an inherent safety procedure, as it automatically eliminates an unexpected leakage of the generator. Such a sudden dramatic breakthrough, eventually induced by defects and incorrect handling of the generator, is neither directly `seen´ nor avoided by using fractionation as an approach of eluate post-processing. Both handling of generators including the different post-processing approaches and radiopharmaceutical synthesis and product purification modules are available and in routine (clinical) use. Future developments may be desir-

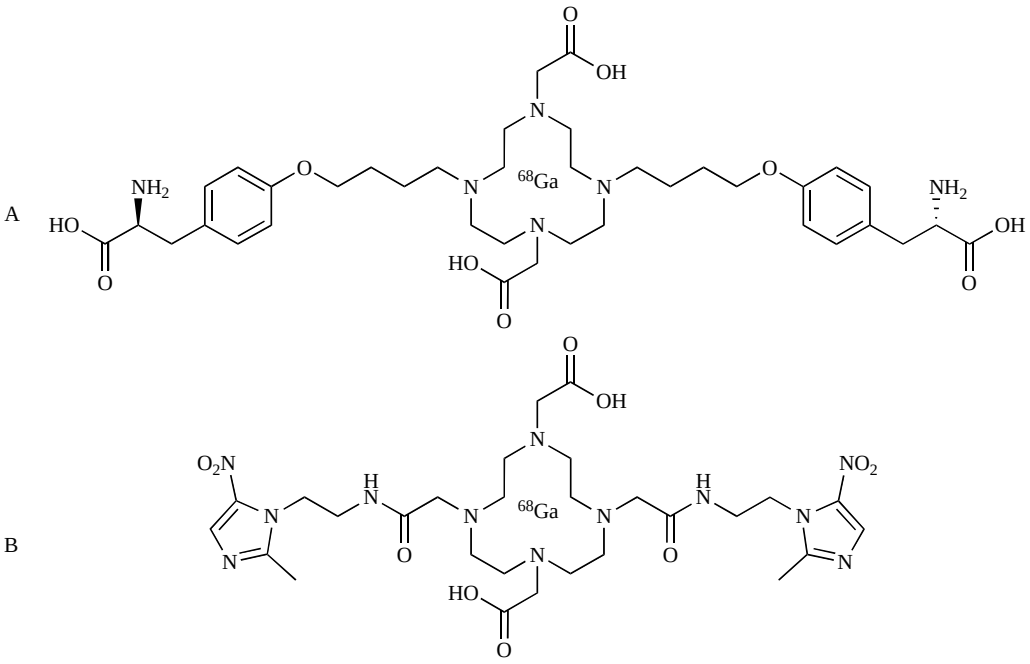


Fig. (23). Divalent Imaging Agents Based on DO2A (9): **A**) An L-tyrosine DO2A conjugate for imaging the amino acid transporter expression, **B**) a 2-methyl-5-nitro-imidazol conjugated probe for tissue hypoxia [138, 242].

able towards new generator concepts, trying (a) to avoid "metal"-based matrices for ^{68}Ge adsorption and (b) to elute ^{68}Ga in hydrochloric acid solutions of concentrations less than 0.1 N (approaching 0.01 N concentration) in order to achieve optimum conditions for instant and high-yield radiolabeling.

Radiopharmaceutical Chemistry of Trivalent Gallium: The idea of using generator-derived $^{68}Ga^{III}$ as a highly available PET-nuclide, combined with the presence of two additional Ga isotopes (^{66}Ga and ^{67}Ga) with potential in molecular imaging applications, has motivated considerable effort in the development of chemical methods for the incorporation of $^{67,68}Ga^{III}$ into biologically or physiologically relevant molecules of various kinds. Early approaches have included the versatile complexing agents DFO, EDTA and DTPA, which readily form Ga^{III} complexes with a thermodynamic stability in the range of *apo*-TF, but lack the superior kinetic inertness present with macrocyclic Ga^{III}-complexes. Even weaker complexes, such as the Ga^{III} citrate complex, have been used for intentional *in vivo* labeling of *apo*-TF and subsequent imaging of the [^{68}Ga]Ga(TF) distribution in living subjects. Bifunctional derivatives have been successfully conjugated to targeting vectors and employed in molecular imaging.

Today, open chain complexing agents have almost completely been displaced by DOTA and NOTA-derived BFCs and their conjugates. In this regard, NOTA and its bifunctional derivatives provide promising chelating agents. Combining mild labeling conditions, stable and inert complexation and a variety of available bifunctional chelating agents based on the NOTA scaffold, this macrocyclic structure is - to date - the first choice for all designs and applications of $^{67,68}Ga^{III}$-based medical imaging agents.

Though all of the presently used concepts and multidentate ligands used for today's $^{67,68}Ga^{III}$ chemistry originate in the early 1990's, recent publications in all of these areas illuminate the ongoing research interest. Furthermore, many of the former approaches, which were based on ^{67}Ga, are now driven to perfection in the light of the first commercially available "ionic" generators with potential in routine application, particularly when seeing the fact, that $^{67,68}Ga^{III}$ - analogues for some of the relevant routine applications of ^{99m}Tc- and ^{111}In-based SPECT imaging agents or ^{18}F-based PET racers exist or are close to clinical applications. Cerebral and myocardial perfusion, pulmonal blood flow, bone imaging, even quantitative assessment of MDR1 Pgp or membrane receptor status can be expected to be observable with sophisticated ^{68}Ga-PET analogues. Still there is room for improvement, which is reflected by the continuous increase in research papers from all areas of $^{67,68}Ga^{III}$-based radiopharmaceutical chemistry. The variety of useful methods adapting the various ways of generator eluate handling to labeling reagents and ligand concepts paves the way for almost unlimited application of $^{67,68}Ga^{III}$ in all fields of non-invasive molecular imaging with radioactive probes. Is it just a vision to expect that the $^{68}Ge/^{68}Ga$ radionuclide generator systems could contribute to the clinical impact of nuclear medicine diagnoses for PET similar to the established $^{99}Mo/^{99m}Tc$ generator system for SPECT?

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