RSC Advances

RSC Publishing

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Advances*, 2013, **3**, 3249

''Click'' chemistry toward *bis*(DOTA-derived) heterometallic complexes: potential bimodal MRI/PET(SPECT) molecular imaging probes†

Mojmír Suchý,[ab] Robert Bartha[b] and Robert H. E. Hudson*[a]

Received 10th December 2012,
Accepted 1st January 2013

DOI: 10.1039/c3ra23260c

www.rsc.org/advances

A general synthetic methodology has been developed for the preparation of DOTA-derived heterometallic omplexes as potential bimodal MRI/PET(SPECT) molecular probes. Both alkyne- and azide substituted DOTA-derived chelators have been synthesized and metallated with Gd^{3+} (MRI reporter) or ''cold'' isotopes of Cu^{2+}, Ga^{3+} and In^{3+} (potential PET or SPECT reporters). The Cu^{+}-catalyzed Huisgen [3 + 2] cycloaddition has been utilized as a key step in the synthesis of potential bimodal MRI/PET(SPECT) molecular probes. A preliminary optimization of the reaction conditions has been carried out to make the reaction conditions compatible with the radioactive isotopes of Cu^{2+}, Ga^{3+} and In^{3+} possessing short life times. The MRI sensitivity of the potential bimodal MRI/PET(SPECT) molecular probes has been determined and compared to that associated with a clinically used MRI contrast agent.

Introduction

Magnetic resonance imaging (MRI) and positron emission tomography (PET) are widely used complementary medical imaging techniques. While MRI provides high-resolution, (submillimeter) anatomical information it is recognized to be limited by low sensitivity. Conversely, PET imaging is an exceptionally sensitive technique which is limited to some extent by its poor spatial resolution. It is now widely believed, that integrated MRI/PET molecular imaging possesses a tremendous potential for future clinical application. It has been estimated that full human body scanning will be possible within the next few years.[1]

Although a wide variety of molecular probes have been developed for both MRI[2] and PET,[3] the synthesis of dual probes capable of enhancing the contrast in bimodal MRI/PET imaging is still in its infancy.[4] It has been recently proposed, that a simultaneous MRI/PET acquisition will have great advantages, should chemists be able to develop the bimodal molecular probes, capable of exploiting the complementarities between the two imaging modalities.[2c]

The first example of a discrete[5] bimodal MRI/PET molecular probe appeared in 2010, in a landmark publication by Caravan, Sherry, Catana and coworkers.[6] A Gd^{3+} derived complex was subjected to Cu^{+}-catalyzed Huisgen [3 + 2] click cycloaddition (CuAAC) with 2-fluoroethylazide labeled with a radioactive fluorine isotope (^{18}F, $t_{1/2}$ 110 min) to form the bimodal MRI/PET molecular probe **1** (Fig. 1), capable of quantitative pH imaging.[6] In more recent work, a DOTA-derived chelator was coupled with a porphyrin moiety *via* a peptide bond, followed by the metallation of the DOTA chelation cage with Gd^{3+} and porphyrin subunit with Cu^{2+} (structure **2**, Fig. 1).[7] Although the authors did not use a radioactive isotope of copper (^{64}Cu, $t_{1/2}$ 12.7 h), they suggested that their synthetic methodology would be fully compatible with prospective radiolabeling chemistry. In addition, the fluorescence response resulting from the presence of the porphyrin moiety can be utilized for optical imaging.[7]

A slightly different approach to bimodal MRI/SPECT (single photon emission computer tomography) molecular probe has been developed by Aime and coworkers.[8] A pH sensitive sulfonamide-modified DO3A chelator has been metallated with Gd^{3+} (MRI reporter) and with a radioactive isotope of Ho^{3+} (^{166}Ho, $t_{1/2}$ 26.6 h). Assuming that both lanthanide(III) complexes (structures **3**, Fig. 1) will have identical pharmacokinetic properties; for their further use the authors proposed a simultaneuos injection of the Gd^{3+} complex **3a** along with a small amount of the radiotracer labeled complex **3b**.[8] In a very recent work, a conjugation of two L-histidine (His) residues to central DTPA substructure resulted in the formation of a

[a]*Department of Chemistry, The University of Western Ontario, London, Ontario, Canada. E-mail: robert.hudson@uwo.ca*
[b]*Robarts Research Institute, The University of Western Ontario, London, Ontario, Canada*

† Electronic supplementary information (ESI) available: ^{1}H NMR spectra of compounds **6a**, unmetallated version of **6b**, **7a**, ester deprotected version of **7a**, **12** and **15**. ^{13}C NMR spectra of compounds **12** and **15**. HR-ESI-MS spectra of compounds **5a**, **5b**, **6a**, **6b**, unmetallated version of **6b**, **7a**, ester deprotected version of **7a**, **7b–7d**, **13**, DO3A-O*t*Bu-Ac 2-chloroethylamine. Semi-preparative HPLC chromatograms of crude reaction mixtures containing compounds **5a–5c**. NMRD profiles associated with compounds **5a–5c**, **6b** and Dotarem (**16**) acquired at 25 °C and 37 °C. See DOI: 10.1039/c3ra23260c

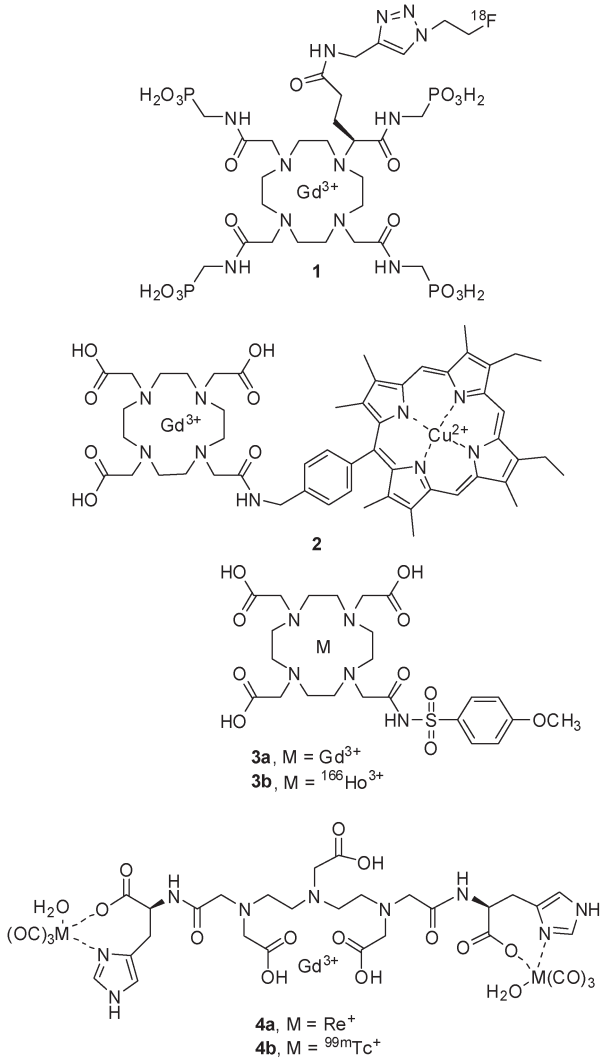


Fig. 1 Structures of bimodal MRI/PET(SPECT) molecular probes **1–4**.

trifunctional ligand.[9] The DTPA substructure was labelled with Gd^{3+}, while the His residues have been labelled with ''cold'' $[Re(CO)_3(H_2O)]^+$ (structure **4a**, Fig. 1) or with ''hot'' $[^{99m}Tc(CO)_3(H_2O)]^+$ (^{99m}Tc, $t_{1/2}$ 6.02 h, structure **4b**, Fig. 1). To account for the differences in sensitivity between MRI and SPECT, a ''cocktail mixture'' containing a large amount of the ''cold'' molecular probe **4a** and a tiny amount of the ''hot'' molecular probe **4b** has been injected and subsequently detected in mouse liver and kidneys.[9]

The evaluation of the different approaches to bimodal MRI/PET(SPECT) molecular probes described above indicates, that no general methodology for their preparation is currently available and existing syntheses can be labour intense and require the preparation of the variety of diverse synthetic intermediates. It is easy to envision that a more general approach should allow for preparation of bimodal MRI/PET(SPECT) molecular probe libraries (with the possibility of fine tuning their properties) from relatively few building blocks.

Herein, we have investigated the possibility of using ''click'' CuAAC-based chemistry to assemble the dual MRI/PET(SPECT) molecular probes. Previously, we reported the development of a library of alkyne modified DOTAM-derived complexes for use in PARACEST (PARAmagnetic Chemical Exchange Saturation Transfer) MRI[10] and ^1H MRS (magnetic resonance spectroscopy) thermometry.[11] The present work describes the synthesis of alkyne- and azide-modified complexes based on the DOTA (1,4,7,10-tetraazadodecane-1,4,7,10-tetraacetic acid) framework.[12] The alkyne-derived building block was metallated with Gd^{3+} (MRI reporter), while the azide-derived subunit was used for the chelation of ''cold'' non-radiocative Cu^{2+}, Ga^{3+} and In^{3+} cations. The ''click'' CuAAC reaction was utilized[13] to couple the two building blocks and a preliminary optimization was carried out in order to obtain the reaction conditions compatible with the limited life time of the radioactive isotopes (^{64}Cu, $t_{1/2}$ 12.7 h; ^{68}Ga, $t_{1/2}$ 68 min; ^{111}In, $t_{1/2}$ 67.9 h).[3b] The MRI sensitivity of the potential bimodal MRI/PET(SPECT) molecular probes was evaluated and compared to that associated with the commercially available MRI contrast agent Dotarem.

Results and discussion

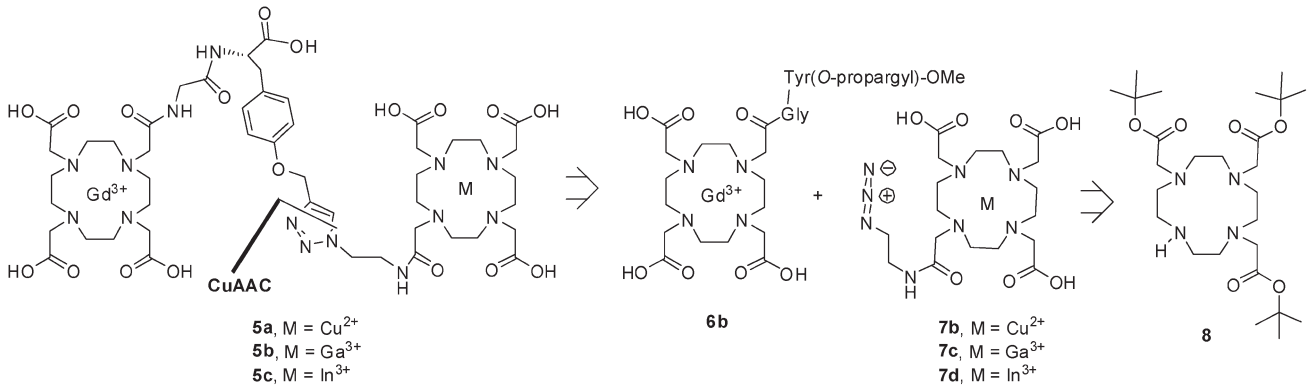
Retrosynthetic analysis of potential bimodal MRI/PET(SPECT) molecular probes

The structural design and retrosynthetic analysis of potential bimodal MRI/PET(SPECT) molecular probes **5** is depicted in Scheme 1. Based on our previous experience,[10] we decided to use ''click'' CuAAC-based chemistry for the preparation of probes **5**. For this purpose we were required to prepare the corresponding alkyne- and azide-functionalized intermediates.

The alkyne-modified building block **6b** (Scheme 1) consists of a DOTA framework metallated with Gd^{3+} and decorated with a short dipeptide sequence containing *O*-propargyl-L-tyrosine (Tyr). We have previously developed the synthesis of the corresponding dipeptide linker and utilized it to form a library of PARACEST MRI contrast agents.[10a] Another advantage associated with the linker is the presence of the Tyr moiety, which provides a UV chromophore that is useful during semi-preparative HPLC purification of the molecular probes **5**.

Taking into account the long term goal of these efforts to prepare bimodal MRI/PET(SPECT) molecular probes containing ''hot'' radioactive isotopes, we decided to keep the structure of the azide-modified subunit as simple as possible to allow for a facile and rapid complexation. An azide-modified building block (structures **7**, Scheme 1) is represented by a DOTA-derived complexes (Cu^{2+}, Ga^{3+} and In^{3+}) decorated with a short alkyl azide appendage.

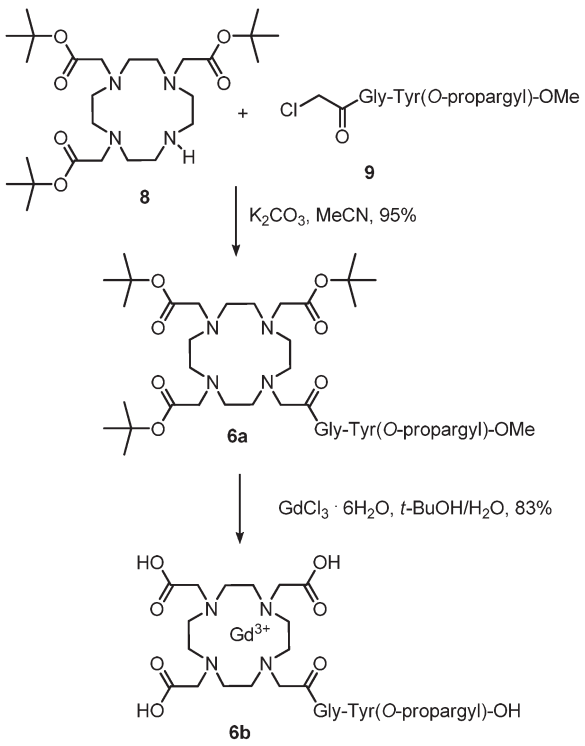
Both, the alkyne- and the azide-modified subunits can be obtained by a simple alkylation of a common starting material, commercially available DO3A-O*t*Bu (**8**, Scheme 1). Moreover, the DOTA framework is known to form sufficiently stable complexes with the metal ions used in this study.[2b,3b]



Scheme 1 Retrosynthetic analysis of potential bimodal MRI/PET(SPECT) molecular probes **5a–5c**.

Synthesis of the alkyne-modified building block 6c

The preparation of the *N*-chloroacetyl-Gly-L-Tyr(*O*-propargyl)OMe (**9**) has been described previously.[10a] Alkylation of DO3A-O*t*Bu (**8**, Scheme 1) with electrophile **9** afforded the ligand **6a** in excellent yield (95%, Scheme 2).[14] Refluxing the ligand **6a** in *t*-BuOH/H_2O mixture in the presence of $GdCl_3 \cdot 6H_2O$ resulted in the removal of protecting ester functionalities along with the metallation of the DOTA framework to furnish the alkyne building block **6b**. Interestingly, if the same reaction is carried out in the absence of $GdCl_3 \cdot 6H_2O$ using a catalytic amount of HCl, an unmetallated version of the alkyne building block **6b** can be obtained as described in the Experimental.



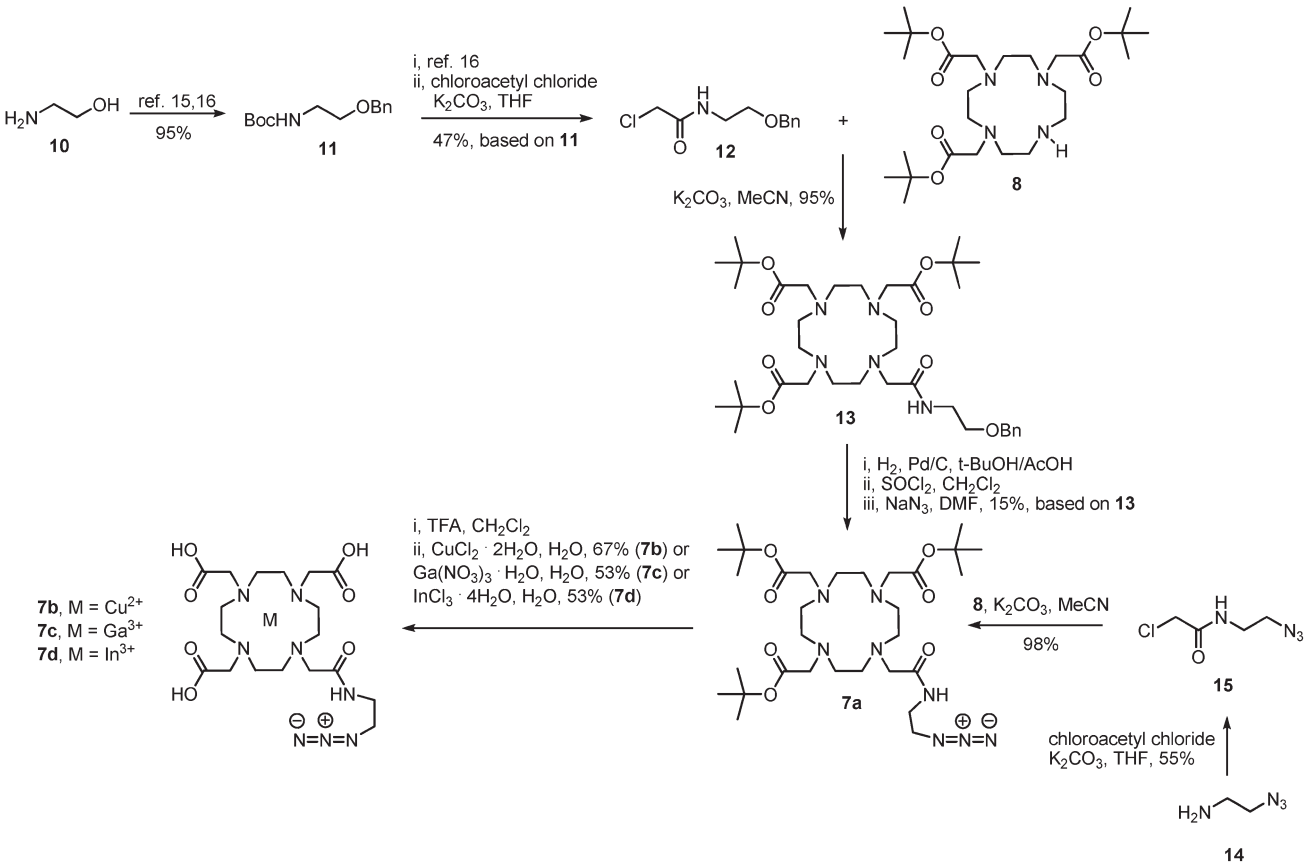
Scheme 2 Synthesis of the alkyne building block **6b**.

The alkyne-modified building block **6b** (83% yield, Scheme 2) was purified by size exclusion chromatography (SEC) as described in the Experimental, its identity was confirmed by high resolution mass spectrometry (HR-MS) and infra red (IR) spectrometry. The proper charged state envelope was observed in the mass spectrum of **6b** along with the typical isotope pattern due to the presence of Gd^{3+} [see ESI† for more details]. In the IR spectrum, the shift of the absorbtion band due to the presence of the peripheral carbonyl groups (1617 cm^{-1}) confirms their involvement in the Gd^{3+} chelation (1727 cm^{-1} for non-metallated ligand **6a**).

Synthesis of the azide-modified building blocks 7b–7d

The synthesis of ligand **7a** was found to be less straightforward. It was first decided to alkylate DO3A-O*t*Bu (**8**) with an electrophile possessing a protected terminal OH group, which was expected to be converted to the azide functionality at the end of the synthetic sequence. The primary amino group of 2-ethanolamine (**10**) was protected with a Boc group,[15] while the OH was benzylated.[16] Literature procedures were used,[15,16] the protected 2-ethanolamine derivative **11** was obtained in 95% overall yield (Scheme 3). The Boc group was removed,[16] followed by the acylation with chloroacetyl chloride[17] to give electrophile **12** (47% yield based on **11**, Scheme 3) after purification by flash column chromatography (FCC). For spectral characterization of electrophile **12** see ESI.†

Alkylation of DO3A-O*t*Bu (**8**) with electrophile **12** proceeded smoothly,[14] affording the OBn protected ligand **13** of sufficient purity for the subsequent reaction (Scheme 3). The hydrogenolytic removal (10% Pd/C) of the benzyl group proved to be troublesome. Atmospheric pressure hydrogenation was found to be sluggish (*ca.* 50% conversion after 7 days) while higher pressure hydrogenation (*ca.* 40 psi) resulted in notable decomposition. The rate of the hydrogenation at ambient pressure was increased by the addition of AcOH; unfortunately this lead to the formation of some inseparable impurities (structures were not determined) in the reaction mixture along with the desired alcohol. The isolated, crude residue was subjected to treatment with $SOCl_2$ (it was found to give better results compared to MsCl), to effect hydroxyl-to-chloro functional group exchange. The crude mixture containing the desired chloro derivative was isolated and subjected to

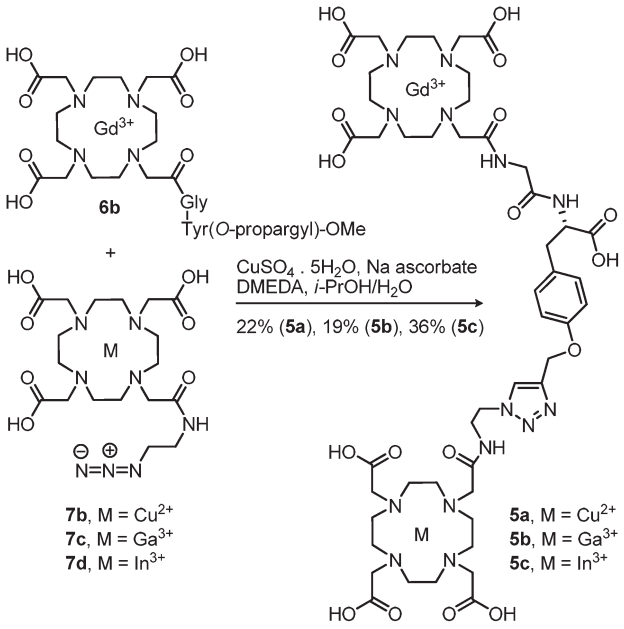


Scheme 3 Synthesis of the azide building blocks **7b–7d**.

reaction with NaN_3 in DMF. The ligand **7a** was obtained after the purification by FCC in an overall yield 15%, based on **13**. We felt that this was not a satisfactory yield and the reaction sequence was too laborious. In order to improve the chemical route, we used a chlorine-based electrophile containing an azido group. Thusly, 2-azidoethylamine (**14**) was prepared from the HI salt of 2-iodoethylamine using the literature procedure.[18] Acylation of **14** with chloroacetyl chloride[17] afforded *N*-chloroacetyl 2-azidoethylamine (**15**, Scheme 3) as a slightly yellow oil in 55% yield after the FCC purification. It is worth noting that synthesis of electrophile **15** has been reported very recently using slightly different synthetic procedure by Borbas and coworkers.[19] Full spectroscopic characterization of electrophile **15** is provided in the the Experimental and ESI,† the presence of an intact azido group was confirmed by infra red (IR) spectrometry (strong absorbtion band at 2096 cm^{-1}). With **15** in hand we performed the alkylation of the DO3A-O*t*Bu (**8**, Scheme 3).[14] This step was found to proceed smoothly, providing the crude ligand **7a** of sufficient purity for the next steps in 98% yield. Analytical samples of **7a** were obtained upon purification by FCC, the ligand **7a** was characterized by ^1H NMR spectroscopy and IR spectrometry (strong absorbtion band at 2098 cm^{-1} confirmed the presence of an intact azido group) and by HR-MS. The azide **7a** has been prepared only very recently by a slightly different synthetic route,[19] a related azide possessing one carbon longer side chain is also known, although no synthetic details are available.[20]

Having synthesized the desired azido-modified ligand **7a**, we investigated the possibilities of its metallation with transition metal cations relevant to PET(SPECT) (Cu^{2+}, Ga^{3+}, In^{3+}). The O*t*Bu ester functionalities were removed by treatment with trifluoroacetic acid (33% TFA) in CH_2Cl_2. It was found, that 1 h reaction time was sufficient to achieve the complete deprotection (confirmed by ^1H NMR and HR-MS). The volatiles were removed by evaporation and the residues were subjected to treatment with $CuCl_2 \cdot 6H_2O$, $Ga(NO_3)_3 \cdot H_2O$ and $InCl_3 \cdot 4H_2O$ in water (Scheme 3). The pH of the reaction mixtures was adjusted to achieve the fast complex formation[3b] (see the details in the Experimental). Optimization of the reaction conditions was performed to achieve the full complexation within 30 min (confirmed by HR-MS, see the Experimental for the details). Complexes **7b** (Cu^{2+}), **7c** (Ga^{3+}) and **7d** (In^{3+}) were purified by dialysis (see General experimental procedures), the presence of an intact azido group was confirmed by IR spectrometry, strong absorbtion band at *ca.* 2100 cm^{-1} was observed for each complex.

Importantly, the reaction times for complex formation (30 min) are compatible with the short life times of radioactive isotopes used in this study. The complexes **7b–7d** were prepared in larger amounts (*ca.* 30–40 mg) to allow for the detailed investigation of the subsequent ''click'' reaction with



Scheme 4 ''Click'' CuAAC reaction between the alkyne building block **6b** and the azide building blocks **7b–7d**.

an alkyne precursor **6b**. Yet more optimization will be required when using complexes **7b–7d** in the ''real life'' radiolabeling, wherein it is expected, that no purification of **7b–7d** will be carried out. Subsequent ''click'' reaction of **7b–7d** with an alkyne precursor **6b** is expected to be carried out in ''one-pot'' fashion, followed by the high pressure liquid chromatography (HPLC) purification of the desired bimodal MRI/PET(SPECT) molecular probes.

''Click'' reaction between an alkyne precursor 6b and azide precursors 7b–7d

The methodology for the ''click'' CuAAC chemistry of DOTA-derived alkynes has been recently developed in our laboratory.[10] The reactions were carried out in a mixture of *i*-PrOH/H_2O, using excess of the azide (1.5–8 equivalents) in the presence of $CuSO_4{\cdot}5H_2O$ (0.25 equivalents) and sodium ascorbate (1.2 equivalents). We decided to use the identical conditions to carry out the first reaction between an alkyne building block **6b** and a Cu^{2+} derived azide building block **7b** (1.2 equivalents have been used, Scheme 4). Very slow progress in the reaction was observed (HR-MS spectrometry) even after extended period of heating to 70 °C (48 h). Neither addition of $CuSO_4{\cdot}5H_2O$ and sodium ascorbate, nor increasing the temperature to 80 °C appeared to speed up the reaction.

It is well documented that addition of agents capable of the formation of transient Cu chelates can be used to increase the rate of the ''click'' CuAAC reactions.[13] We turned our attention to *N*,*N*′-dimethyl ethylenediamine (DMEDA) an inexpensive and widely available reagent often used for this purpose.[21] Addition of 1.2 equivalents (based on the alkyne **6b**) of DMEDA was found to increase the rate of the reaction dramatically, resulting in the completion of the reaction in 18 h at 60 °C as indicated by HR-MS (complete consumption of alkyne **6b**). Of course this is still an undesirably long reaction time and optimization is required, *vide infra*.

The Gd^{3+}/Cu^{2+} heterometallic complex **5a** (Scheme 4) was isolated by semi-preparative HPLC (taking advantage of the presence of the UV active Tyr scaffold), followed by the removal of salts by dialysis (22% yield, see Experimental for details). The reactions between the alkyne **6b** and azides **7c** and **7d** (Scheme 4) were carried out in similar fashion, heterometallic complexes **5b** (Gd^{3+}/Ga^{3+}, 19% yield) and **5c** (Gd^{3+}/In^{3+}, 36% yield) were purified in an analogous manner. Complexes were produced in amounts (*ca.* 20 mg) sufficient for the characterization of their sensitivities in MRI.

The identity of the heterometallic complexes **5a–5c** was confirmed by HR-MS spectrometry. Proper charge state envelopes along with isotope patterns due to the presence of heavy metals have been observed in the mass spectra associated with **5a–5c** (see Fig. 2 and ESI† for details). Also worthy of note, no absorption band which can be attributed to the presence of the azide group (*ca.* 2100 cm^{-1}) was present in the IR spectra associated with complexes **5a–5c**.

Optimization of the reaction conditions for the ''click'' CuAAC reaction between an alkyne 6b and azides 7b–7d

Bearing in mind that the long term goal of this work is the preparation of bimodal MRI/PET(SPECT) molecular probes containing ''hot'' radioactive isotopes, we decided to perform an optimization of the reaction conditions used to perform the ''click'' CuAAC chemistry. Thus a solution (in 1 ml of water) containing 10^{-5} mol of the alkyne building block **6b**; 10^{-5} mol of the azide precursors **7b**, **7c** or **7d**; 2.5×10^{-6} mol $CuSO_4{\cdot}5H_2O$; 1.25×10^{-5} mol of sodium ascorbate and 10^{-5} mol of DMEDA was incubated for 1 h at 80 °C. Samples were withdrawn prior to the placement of the reaction vial to a heating block, then after 15 min and 1 h, followed by an HPLC analysis. In the case of Gd^{3+}/Cu^{2+} heterometallic complex **5a**, a complete conversion was observed in the sample taken prior to the placement of the vial in the heating block. Compared to other results discussed below, this observation indicates, that the Cu^{2+} cation present in the structure of azide **7b** might be involved in catalyzing the ''click'' CuAAC reaction between the alkyne **6b** and the azide **7b**.

Surprisingly, quite different results were obtained when alkyne **6b** was incubated with azide **7c** which contains Ga^{3+}. The formation of Gd^{3+}/Ga^{3+} heterometallic complex **5b** was found to proceed rather sluggishly with only *ca.* 10% conversion to **5b** was observed after 1 h at 80 °C (Fig. 3). Taking into consideration the short life time of the widely available radioactive isotope of gallium (^{68}Ga, $t_{1/2}$ 68 min), it appears clear that further optimization of the reaction conditions (e. g. microwave irradiation[22]) is required to make this methodology feasible for the preparation of the radioalabelled complex **5b**. It may also be possible to accelerate the reaction by use of internal alkynes in the so-called strain-promoted Cu-free cycloaddition reaction.[23]

On the other hand, incubation of the alkyne **6b** with an In^{3+} containing azide **7d** revealed *ca.* 70% conversion to the Gd^{3+}/In^{3+} heterometallic complex **5c** after incubation for 15 min at 80 °C (Fig. 3), full conversion was observed in the sample withdrawn after 1 h.

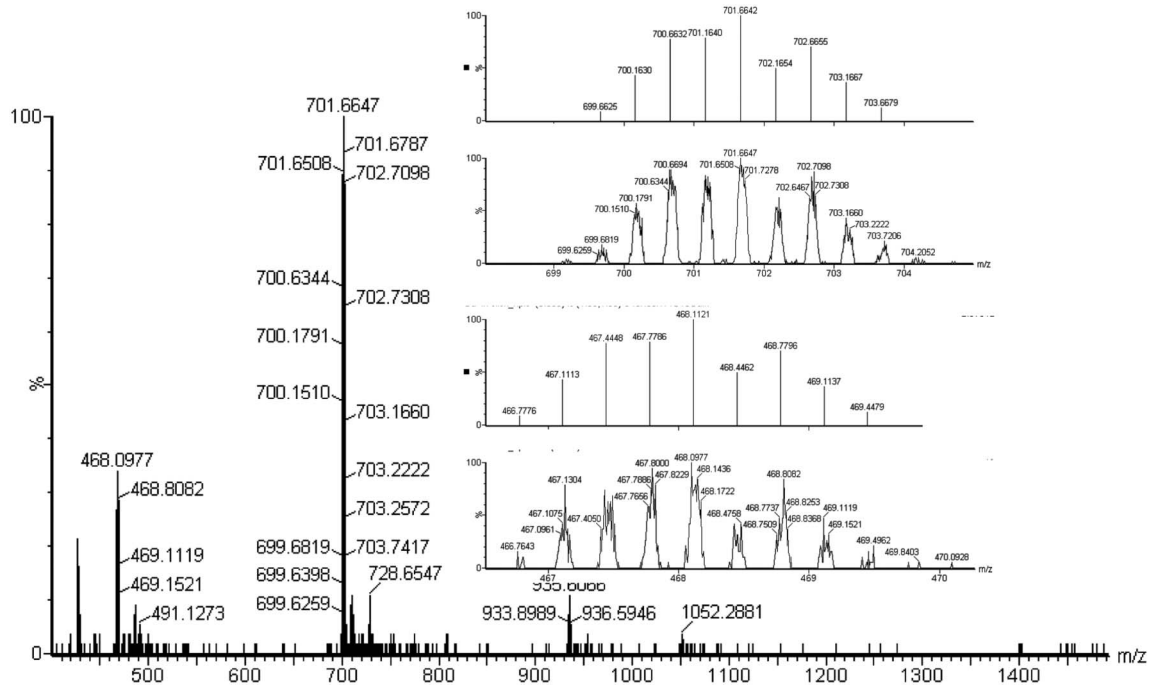


Fig. 2 HR-ESI-MS spectrum of Gd^{3+}/In^{3+} heterometallic complex **5c** showing a proper charge state envelope (701.7, M^{2+} charge state; 468.1, M^{3+} charge state) and isotope pattern. Insets show the agreement between the observed (bottom) and simulated (top) charge states. HR-ESI-MS spectra of associated with complexes **5a** and **5b** can be found in the ESI.†

To summarize the results from this study, our methodology is suitable for the preparation of radiolabeled heterometallic complexes related to Gd^{3+}/Cu^{2+} complex **5a** and Gd^{3+}/In^{3+} complex **5c**, while more work is warranted to make the presented methodology compatible with the preparation of radiolabeled heterometallic complexes related to Gd^{3+}/Ga^{3+} complex **5b**.

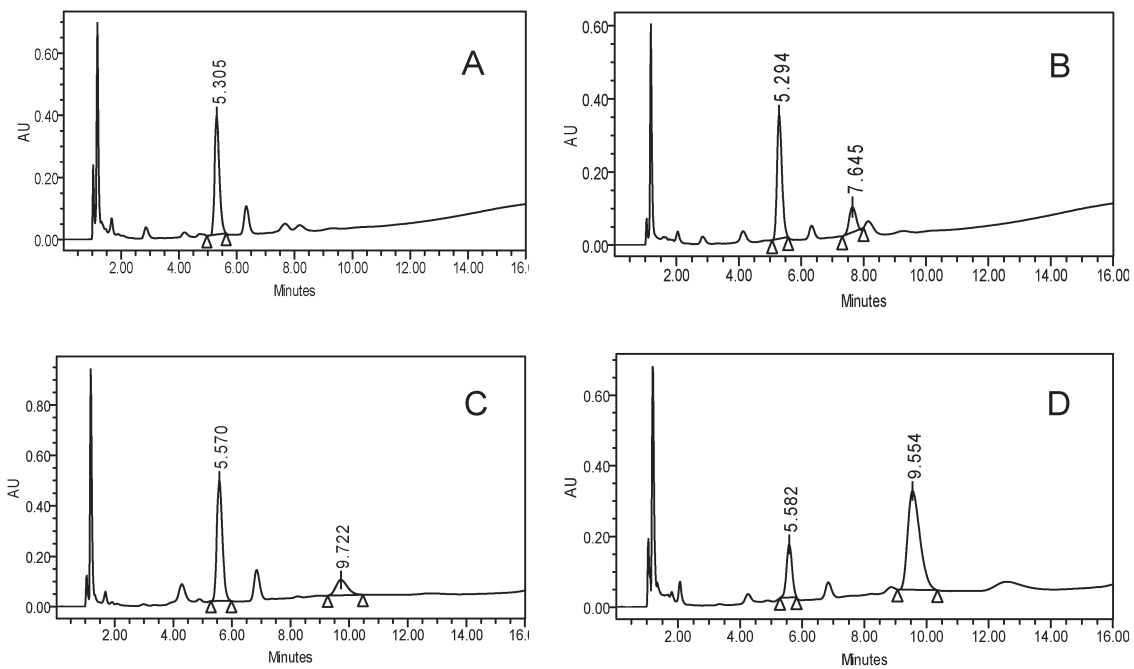
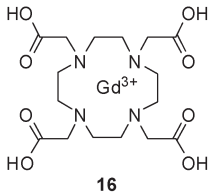


Fig. 3 HPLC chromatograms of reaction mixtures before the placement in a heating block (panels A, C) and after being incubated for 15 min at 80 °C (panels B, D). The slow formation of Gd^{3+}/Ga^{3+} heterometallic complex **5a** (t_R 7.6 min) from the alkyne building block **6b** (t_R 5.3 min, panels A, B) is shown at the top, while the rapid formation (*ca.* 70% conversion after 15 min at 80 °C) of Gd^{3+}/In^{3+} heterometallic complex **5a** (t_R 9.6 min) from the alkyne building block **6b** (t_R 5.6 min, panels C, D) is shown at the bottom.



16

Fig. 4 Chemical structure of Dotarem (Gd^{3+} DOTA, **16**).

MRI sensitivity associated with the potential bimodal MRI/PET(SPECT) molecular probes 5a–5c

Since the detection sensitivity associated with MRI is several orders of magnitude lower than imaging modalities involving radioisotopes (PET, SPECT), the ability to detect the bimodal MRI/PET(SPECT) molecular probes by MRI therefore represents a limiting factor. To obtain more information on the MRI sensitivity associated with the molecular probes **5a–5c** we measured their relaxivities and compared them to those associated with a clinically used MRI contrast agent Dotarem[2b] (Gd^{3+} DOTA, **16**, Fig. 4).

NMRD profiles associated with heterometallic complexes **5a–5c** were acquired using fast field cycling NMR in water at 25 °C and 37 °C (see Experimental for details). The dependence of the relaxivity associated with **5a–5c** on the strength of the magnetic field (Fig. 5, ESI†) correlates well with the same measurement carried out for Dotarem (**16**, Fig. 5). The shape of the curves confirms the presence of one molecule of water exchanging with Gd^{3+} centre for all of the complexes **5a–5c** (Fig. 5, ESI†).[24]

The relaxivity of the complexes **5a–5c** at 20 MHz (*ca.* 5.0–5.5 $mmol^{-1}$ s^{-1}) is slightly higher, compared to the relaxivity of Dotarem (**16**, *ca.* 4.5 $mmol^{-1}$ s^{-1}).[2b] This is likely attributable to the increase in molecular size of the probes **5a–5c**, resulting in a somewhat slower rate of molecular tumbling in solution.[25] The remaining NMRD profiles associated with complexes **5a–5c** acquired at 25 °C and 37 °C can be found in the ESI.†

Overall, the MRI sensitivity of the potential bimodal MRI/PET(SPECT) molecular probes **5a–5c** compares well with the MRI sensitivity of clinically used MRI contrast agent Dotarem (**16**). The presence the second metal ion does not affect the MRI sensitivity of complexes **5a–5c**.

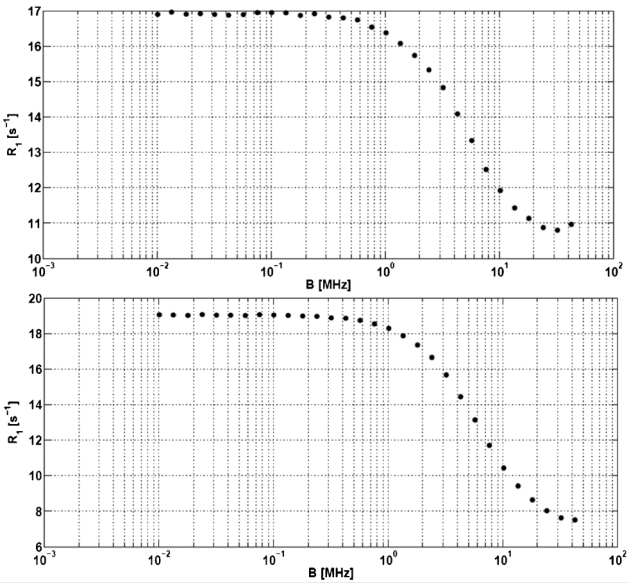


Fig. 5 NMRD profiles in water at a concentration of 2.0 mM and at 37 °C. Top: Dotarem, **16**. Bottom: Gd^{3+}/In^{3+} heterometallic complex **5c**.

Experimental

General experimental procedures

All reagents were commercially available unless otherwise stated. All solvents were HPLC grade and used as recieved. Water was deionized (18.2 MΩ cm^{-1} Millipore water) and CH_2Cl_2 and THF were dried over Al_2O_3 in a solvent purification system. Organic extracts were dried with Na_2SO_4 and solvents were removed under reduced pressure using a rotary evaporator. Aqueous solutions were lyophilized. Flash column chromatography (FCC) was carried out using silica gel, mesh size 230–400 Å. Thin layer chromatography (TLC) was carried out on Al backed silica gel plates, compounds were visualized by UV light, phosphomolybdic acid staining (compound **7a**) or anisaldehyde staining (compound **15**). Size exclusion chromatography (SEC) was peformed using BIO-GEL P2, 45–90 μm mesh resin (15 g, per 0.23 mmol of compound). Ten fractions (10 ml each) were collected and identified by UV and I_2 vapours, fractions containing complex **6b** were combined and lyophilized. The absence of free Gd^{3+} in **6b** was verified by a xylenol orange test.[26] Dialysis was performed for 5 days against water using cellulose ester membrane with molecular weight cut off 500 Da. HPLC analysis and purification was performed using a Resolve CN column (particle size 10 μm; 8 × 100 mm Radial-Pak cartridge). Mobile phase: Method A: 90% H_2O/10% MeCN – 31% H_2O/69% MeCN over 13 min, linear gradient and flow rate 3 ml min^{-1}. Ultra performance liquid chromatography (UPLC) was performed using a BEH C18 column (particle size 1.7 μm; 1.0 id × 100 mm) and HR-ESI-MS detector. Mobile phase: Method B: 100% H_2O – 100% MeCN over 6 min, linear gradient, flow rate 0.25 ml min^{-1}. NMR spectra were recorded on 400 MHz spectrometer; for ^1H (400 MHz), δ values were referenced as follows: $CDCl_3$ (7.27 ppm), D_2O (4.75 ppm), for ^{13}C (100 MHz) $CDCl_3$ (77.0 ppm). Mass spectra (MS) were obtained using an electron spray ionization (ESI†) Time-of-Flight (TOF) instrument, or chemical ionization (CI) (compound **15**). FTIR spectra (in KBr) were acquired using an IR spectrometer. Longitudinal relaxation rates in the range from 0.01–42 MHz were acquired using a Stelar fast field-cycling nuclear magnetic resonance relaxometer at 25 °C and 37 °C. The samples [complexes **5a–5c**, Dotarem (**16**)] were dissolved in water to achieve the final concentration 2.0 mM.

Alkylation of DO3A-O*t*Bu (8) with *N*-chloroacetyl-Gly-L-Tyr(*O*-propargyl)-OMe (9)

DO3A-O*t*Bu (**8**, 257 mg, 0.5 mmol) was dissolved in MeCN (10 ml), followed by the addition of K_2CO_3 (180 mg, 1.3 mmol) and *N*-chloroacetyl-Gly-L-Tyr(*O*-propargyl)-OMe (**9**, prepared

according to the literature,[10a] 183 mg, 0.5 mmol). The mixture was stirred for 18 h at 60 °C. The solvent was evaporated, the residue was partitioned between water (30 ml) and EtOAc (2 × 30 + 20 ml). The combined organic extract was dried and was concentrated. The residue was triturated with hexanes, affording DO3A-O*t*Bu-Gly-L-Tyr(*O*-propargyl)-OMe (**6a**, 400 mg, 95%), colorless solid. ^{1}H NMR ($CDCl_3$) δ 9.45 (m, D_2O exch., 2H), 7.18–6.77 (br m, 4H), 4.63 (m, 3H), 3.84–2.17 (br m, 32H), 1.42 (m, 27H). HRMS (ESI†) *m*/*z*; found 845.5052 $[M + H]^+$ (calcd 845.5024 for $C_{43}H_{69}N_6O_{11}$). FTIR: *ν* 3244, 2974, 2821, 1727, 1674, 1510, 1368, 1306, 1227, 1162, 1107, 584 cm^{-1}.

Metallation of ligand 6a with $GdCl_3 \cdot 6H_2O$

DO3A-O*t*Bu-Gly-L-Tyr(*O*-propargyl)-OMe (**6a**, 192 mg, 0.23 mmol) was dissolved in a mixture of *t*-BuOH (6 ml) and H_2O (3.5 ml). $GdCl_3 \cdot 6H_2O$ (85 mg, 0.23 mmol) was added and the mixture was refluxed with stirring for 48 h. *t*-BuOH was evaporated and the aqueous solution was subjected to size exclusion chromatography (SEC) as described in General experimental procedures. The fractions containing the product were combined and lyophilized to afford Gd^{3+} DO3A-OH-Gly-L-Tyr(*O*-propargyl)-OH (**6b**, 155 mg, 83%), colorless solid. HRMS (ESI†) *m*/*z*; found 816.1987 $[M + H]^+$ (calcd 816.1976 for $C_{30}H_{40}N_6O_{11}Gd$). FTIR: *ν* 3392, 3256, 3066, 2870, 1614, 1511, 1398, 1323, 1242, 1085, 1022, 980, 904, 721, 591 cm^{-1}.

An unmetallated version of the complex **6b** can be obtained, if the reaction is carried out under the same experimental conditions replacing $GdCl_3 \cdot 6H_2O$ with diluted HCl (1 M solution, 250 μl), followed by the removal of *t*-BuOH by evaporation and lyophilizing the aqueus phase. DO3A-OH-Gly-L-Tyr(*O*-propargyl)-OH (150 mg, 98%) is obtained as pale brown solid. Analytical samples can be obtained upon purification by SEC as described above. ^{1}H NMR (D_2O) δ 7.12 (m, 2H), 6.92 (m, 2H), 4.69 (m, 2H), 4.54 (m, 1H), 3.77–2.87 (br m, 29H). HRMS (ESI†) *m*/*z*; found 663.2985 $[M+ H]^+$ (calcd 663.2990 for $C_{30}H_{43}N_6O_{11}$).

N-Chloroacetyl-*O*-benzyl 2-ethanolamine (12)

N-Boc-*O*-benzyl 2-ethanolamine (**11**) was prepared from 2-ethanolamine (**10**) using a literature protocol.[15,16] Removal of the Boc protecting group from **11** was carried out as described in the literature.[16] A crude *O*-benzyl 2-ethanolamine (3.59 g, 23.7 mmol) was dissolved in dry THF (70 ml), the solution was cooled to 0 °C, followed by the addition of K_2CO_3 (6.56 g, 47.5 mmol). A solution of chloroacetyl chloride (2.83 ml, 35.6 mmol) in dry THF (10 ml) was added dropwise (over *ca.* 10 min period) to a stirred reaction mixture, while the temperature was maintained at 0 °C. The stirring continued for 1 h at 0 °C and for 3 h at room temperature (rt). The reaction was quenched by slow addition of water (5 ml), the solvent was evaporated and the residue was partitioned between saturated $NaHCO_3$ solution (50 ml) and EtOAc (50 + 30 ml). The combined organic extract was dried and was concentrated, the residue was subjected to FCC on 50 g SiO_2, hexanes-acetone (2 : 1). Evaporation of the eluate afforded *N*-chloroacetyl-*O*-benzyl 2-ethanolamine (**12**, 2.54 g, 47%) as slightly yellow oil. ^{1}H NMR ($CDCl_3$) δ 7.33 (m, 5H), 6.95 (br s, D_2O exch., 1H), 4.53 (s, 2H), 4.03 (s, 2H), 3.58 (m, 2H), 3.52 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 165.9, 137.7, 128.5, 127.9, 127.8, 73.2, 68.2, 42.6, 39.7. HRMS (ESI†) *m*/*z*; found 228.0793 $[M + H]^+$ (calcd 228.0791 for $C_{11}H_{15}ClNO_2$). FTIR: *ν* 3299, 3066, 2950, 1720, 1660, 1536, 1452, 1272, 1106, 1028, 713 cm^{-1}.

Alkylation of DO3A-O*t*Bu (8) with *N*-chloroacetyl-*O*-benzyl 2-ethanolamine (12), formation of azide modified ligand 7a

DO3A-O*t*Bu (**8**, 515 mg, 1 mmol) was dissolved in MeCN (15 ml), followed by the addition of K_2CO_3 (359 mg, 2.6 mmol) and *N*-chloroacetyl-*O*-benzyl 2-ethanolamine (**12**, 228 mg, 1 mmol). The mixture was stirred for 18 h at 60 °C. The solvent was evaporated, the residue was partitioned between water (40 ml) and EtOAc (40 + 30 ml). The combined organic extract was dried and was concentrated. The residue was triturated with hexanes, providing DO3A-O*t*Bu-Ac-*O*-benzyl 2-ethanolamine (**13**, 670 mg, 95%) of sufficient purity for the subsequent reaction. HRMS (ESI†) *m*/*z*; found 706.4772 $[M + H]^+$ (calcd 706.4755 for $C_{37}H_{64}N_5O_8$).

DO3A-O*t*Bu-Ac-*O*-benzyl 2-ethanolamine (**13**, 653 mg, 0.93 mmol) was dissolved in *t*-BuOH (10 ml) and AcOH (1 ml) and 10% Pd/C (1 g) was added. The mixture was stirred vigorously for 48 h at rt under an atmosphere of H_2. The catalyst was filtered off using a small plug of CELITE, the filter was washed with MeOH and the filtrate was concentrated to leave 625 mg of an oily residue containing inseparable mixture of desired DO3A-O*t*Bu-Ac 2-ethanolamine and some other unknown impurity. The crude mixture was dissolved in CH_2Cl_2 (10 ml), followed by cooling the reaction mixture to 0 °C. $SOCl_2$ (220 μl, 3.04 mmol) was added dropwise (over *ca.* 1 min period), the cooling bath was removed and the mixture was stirred for 18 h at rt. The mixture was diluted with CH_2Cl_2 (10 ml) and was washed with saturated $NaHCO_3$ solution (20 ml). The aqueous phase was extracted with CH_2Cl_2 (2 × 10 ml), combined organic extract was dried and was concentrated to leave a brown solid residue (610 mg), containing the mixture of the desired DO3A-O*t*Bu-Ac 2-chloroethylamine along with other unknown impurity. The residue was used for the next step without further purification, HRMS (ESI†) *m*/*z*; found 634.3972 $[M + H]^+$ (calcd 634.3947 for $C_{30}H_{57}ClN_5O_7$).

The residue obtained in the previous step was dissolved in DMF (3 ml), followed by the addition of NaN_3 (195 mg, 3 mmol). The mixture was stirred for 4 h at 80 °C, was cooled to rt and was diluted with saturated $NaHCO_3$ solution (40 ml), followed by the extraction with EtOAc (2 × 30 ml). Combined organic extract was washed with brine (2 × 60 ml), was dried and was concentrated. The residue was subjected to FCC on 60 g SiO_2, CH_2Cl_2–MeOH (9 : 1), the fractions containing the product were identified by phosphomolybdic acid staining. Evaporation of the eluate afforded the azide modified ligand **7a** (96 mg, 15% based on **13**) as colorless solid. ^{1}H NMR ($CDCl_3$) δ 9.24 (br s, D_2O exch., 1H), 3.42–2.23 (br m, 28H), 1.45 (m, 27H). HRMS (ESI†) *m*/*z*; found 641.4367 $[M + H]^+$ (calcd 641.4350 for $C_{30}H_{57}N_8O_7$). FTIR: *ν* 3176, 2976, 2820, 2098, 1729, 1668, 1558, 1454, 1368, 1308, 1266, 1163, 1108, 976, 852, 591 cm^{-1}.

N-Chloroacetyl 2-azidoethylamine (15)

2-Azidoethylamine (**14**, prepared according to the literature,[18] 976 mg, 11.34 mmol) was placed in the round bottom flask which was flushed with N_2. Dry THF (25 ml) was added and the

resulting solution was cooled to −78 °C, followed by the addition of Et_3N (4.1 ml, 29.47 mmol). Chloroacetyl chloride (1.17 ml, 14.74 mmol) was added dropwise (*via* syringe, over *ca.* 3 min period), the mixture was stirred in the atmosphere of N_2 while the cooling bath was allowed to warm up gradually and was completely removed after 2 h. After being stirred for further 2 h (N_2 atmosphere) was the mixture diluted with brine (80 ml) and was extracted with EtOAc (3 × 30 ml). Combined organic extract was dried and was concentrated, the residue was subjected to FCC on 60 g SiO_2, hexanes-acetone (2 : 1), the fractions containing the product were identified by anisaldehyde staining. Evaporation of the eluate afforded *N*-chloroacetyl 2-azidoethylamine (**15**, 1.01 g, 55%) as slightly yellow oil. ^{1}H NMR ($CDCl_3$) δ 6.90 (br s, D_2O exch., 1H), 4.07 (s, 2H), 3.50 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 166.2, 50.5, 42.5, 39.1. HRMS (CI) *m*/*z*; found 163.0380 $[M + H]^+$ (calcd 163.0386 for $C_4H_8ClN_4O$); LRMS (CI) *m*/*z* (rel. abundance): 163 $[M^+]$ (83), 120 (18), 106 (100), 72 (29). FTIR: *ν* 3294, 3077, 2937, 2096, 1655, 1534, 1434, 1262, 1102, 762 cm^{-1}.

Alkylation of DO3A-O*t*Bu (8) with *N*-chloroacetyl 2-azidoethylamine (15)

DO3A-O*t*Bu (**8**, 257 mg, 0.5 mmol) and *N*-chloroacetyl 2-azidoethylamine (**15**, 89 mg, 0.55 mmol) were dissolved in MeCN (4 ml), followed by the addition of K_2CO_3 (180 mg, 1.3 mmol). The mixture was stirred for 18 h at 60 °C. The solvent was evaporated, the residue was partitioned between water (20 ml) and EtOAc (2 × 20 ml). Combined organic extract was dried and was concentrated, the residue was triturated with hexanes to afford the azide modified ligand **7a** (314 mg, 98%). Analytical samples can be obtained by FCC as described above, for the spectral characterization of **7a** see above.

Metallation of the azide modified ligand 7a with $CuCl_2{\cdot}2H_2O$, $Ga(NO_3)_3{\cdot}H_2O$ and $InCl_3{\cdot}4H_2O$

A solution of azide modified ligand **7a** (192 mg, 0.3 mmol) in CH_2Cl_2 (3 ml) was treated with TFA (1.5 ml). The mixture was stirred at 18 h at rt, the solvent was evaporated and the excess TFA was removed by coevaporation with $PhCH_3$ (2 × 20 ml) and CH_2Cl_2 (2 × 20 ml), providing fully deprotected azide modified ligand (141 mg, quantitative) as colorless solid. ^{1}H NMR (D_2O) δ 3.73 (m, 8H), 3.37–3.24 (br m, 20H). HRMS (ESI†) *m*/*z*; found 473.2464 $[M + H]^+$ (calcd 473.2472 for $C_{18}H_{33}N_8O_7$).

Separate solutions of deprotected azide modified ligand (47 mg, 0.1 mmol) in water (1.5 ml) were treated with $CuCl_2{\cdot}2H_2O$ (19 mg, 0.11 mmol), $Ga(NO_3)_3{\cdot}H_2O$ (28 mg, 0.11 mmol) and $InCl_3{\cdot}4H_2O$ (32 mg, 0.11 mmol) and the pH of each reaction was adjusted (1 M NaOH) to reach the optimum complexation kinetics (pH 5.5 for Cu^{2+}, pH 3 for Ga^{3+} and pH 6.5 for In^{3+}).[3b] The mixtures were stirred for 30 min at 95 °C, were cooled to room temperature and were neutralized (pH 7, 1 M NaOH). The aqueous solutions were transferred into dialysis bags and were dialyzed (see General experimental procedures). The solutions were then transferred into centrifuge tubes, were frozen and were lyophilized to provide azide building blocks **7b–7d** as solid residues.

Cu^{2+} modified azide building block **7b** (36 mg, 67%), blue solid. HRMS (ESI†) *m*/*z*; found 534.1628 $[M - H]^+$ (calcd 534.1612 for $C_{18}H_{31}N_8O_7Cu$). FTIR: *ν* 3211, 3052, 2937, 2101, 1671, 1554, 1249, 590 cm^{-1}.

Ga^{3+} modified azide building block **7c** (30 mg, 53%), pale yellow solid. HRMS (ESI†) *m*/*z*; found 539.1471 $[M - 2H]^+$ (calcd 539.1493 for $C_{18}H_{30}N_8O_7Ga$). FTIR: *ν* 3375, 2963, 2102, 1669, 1605, 1404, 1312, 1088, 925, 593 cm^{-1}.

In^{3+} modified azide building block **7d** (31 mg, 53%), colorless solid. HRMS (ESI†) *m*/*z*; found 585.1262 $[M - 2H]^+$ (calcd 585.1276 for $C_{18}H_{30}N_8O_7In$). FTIR: *ν* 3400, 2963, 2108, 1629, 1388, 1328, 1087, 931, 585 cm^{-1}.

"Click" CuAAC reaction between the alkyne building block 6b and the azide building blocks 7b–7d

Separate mixtures containing the alkyne building block (**6b**, 68 mg, 8.3 × 10^{-5} mol) and the azide building blocks (**7b** and **7c**, 54 mg; **7d**, 59 mg; 0.1 mmol each) in *i*-PrOH (0.5 ml) and H_2O (1.5 ml) were treated with $CuSO_4{\cdot}5H_2O$ (0.1 M solution, 170 μl, 1.67 × 10^{-5} mol), sodium ascorbate (0.1 M solution, 670 μl, 6.7 × 10^{-5} mol) and DMEDA (11 μl, 0.1 mmol). The mixtures were stirred for 18 h at 60 °C, were cooled to rt and were transferred into centrifuge tubes. Insoluble portions were removed by centrifugation (10,000 rpm for 2 min), the supernatants were transferred into HPLC vials and were subjected to the HPLC purification (Method A) as described in General experimental procedures. The fractions containing the desired heterometallic complexes **5a–5c** were concentrated, followed by dialysis (see General experimental procedures) and lyophilization.

Gd^{3+}/Cu^{2+} heterometallic complex **5a** (25 mg, 22%), pale blue solid. HPLC: t_R 8.4 min. HRMS (ESI†) *m*/*z*; found 1351.3684 $[M - 2H]^+$ (calcd 1351.3666 for $C_{48}H_{72}N_{14}O_{18}CuGd$). FTIR: *ν* 3394, 2925, 1616, 1395, 1322, 1242, 1085, 589 cm^{-1}.

Gd^{3+}/Ga^{3+} heterometallic complex **5b** (22 mg, 19%), colorless solid. HPLC: t_R 7.9 min. HRMS (ESI†) *m*/*z*; found 1357.3512 $[M - 4H]^+$ (calcd 1357.3489 for $C_{48}H_{70}N_{14}O_{18}GaGd$). FTIR: *ν* 3390, 2926, 1673, 1615, 1397, 1318, 1085, 937, 587 cm^{-1}.

Gd^{3+}/In^{3+} heterometallic complex **5c** (42 mg, 36%), colorless solid. HPLC: t_R 8.7 min. HRMS (ESI†) *m*/*z*; found 1402.3400 $[M - 3H]^+$ (calcd 1402.3331 for $C_{48}H_{71}N_{14}O_{18}GdIn$). FTIR: *ν* 3396, 2923, 1621, 1391, 1326, 1085, 936, 584 cm^{-1}.

Optimization of the reaction conditions for the "click" reaction between the alkyne building block 6b and the azide building blocks 7b–7d

Separate vials containing solutions of the alkyne building block **6b** (10^{-5} mol); the azide precursors **7b**, **7c** or **7d** (10^{-5} mol each); $CuSO_4{\cdot}5H_2O$ (2.5 × 10^{-6} mol); sodium ascorbate (1.25 × 10^{-5} mol) and DMEDA (10^{-5} mol) in 1 ml of H_2O were incubated for 1 h at 80 °C in a heating block. Samples (100 μl) were withdrawn prior to the placement of the reaction vial to the heating block, then after being incubated at 80 °C for 15 min and 1 h. The samples were diluted with H_2O to reach the total volume of 500 μl, followed by an HPLC analysis (Method A, General experimental procedures).

Conclusions

With the aim to establish a general methodology for the synthesis of dual MRI/PET(SPECT) molecular probes, we have

presented the preparation of an alkyne modified Gd^{3+} derived complex along with a synthesis of a small series of azide modified complexes metallated with metal ions relevant to PET and SPECT (Cu^{2+}, Ga^{3+}, In^{3+}). ''Click'' CuAAC chemistry was utilized to connect these building blocks. Crucially, a common starting material which is commercially available (DO3A-O*t*Bu) was used to assemble both the alkyne- and azide functionalized counterparts.

A preliminary optimization of the reaction conditions for the metallation of the azide modified building block with PET(SPECT) relevant metal ions was carried out along with a preliminary optimization for the ''click'' CuAAC chemistry utilized to connect the respective building blocks. This methodology is suitable for the preparation of Gd^{3+}/Cu^{2+} and Gd^{3+}/In^{3+} heterometallic complexes; however, it is not suitable for the Gd^{3+}/Ga^{3+} complex for which more study is required.

The MRI sensitivity associated with the potential MRI/PET(SPECT) molecular probes was found to compare well with that associated with a clinically used MRI contrast agent Dotarem. This finding suggests, that the detection of the potential MRI/PET(SPECT) molecular probes described in this study should be feasible using a clinically used MRI scanner.

We believe that the presented methodology is general enough to be useful to scientists involved in the preparation and validation of advanced probes for molecular imaging. It is easy to envision that this approach could be modified for the preparation of NOTA (1,4,7-triaazacyclononane-1,4,7-triacetic acid)-derived azides, which are capable of fast complexation kinetics with Ga^{3+} as well as other transition metal ions relevant to PET(SPECT).[3b] Moreover, this work complements recent efforts to utilize ''click'' CuAAC chemistry for the preparation of luminescent sensors[19,22] or bimodal MRI/time gated luminescence imaging molecular probes.[27] To assemble various DOTA-derived heterometallic complexes,[28] the ''click'' CuAAC approach appears to be more general and easier to perform when compared to the preparation of similar complexes by means of peptide coupling and sequential metallation,[29] or by a multicomponent Ugi reaction based strategy.[30]

We are also looking forward to use the methodology presented in this manuscript for the preparation of ''real'' bimodal MRI/PET(SPECT) molecular probes involving ''hot'' radioactive isotopes. The results of these studies will be described in a due course.

Acknowledgements

We thank Dr Mohamed Moustafa for the preparation of 2-iodoethylammonium iodide, a precursor to **14**. We are indebted to Drs. Francisco Martinez and Timothy J. Scholl (Department of Medical Biophysics, The University of Western Ontario, London, Ontario, Canada) for the acquisition of NMRD profiles. We thank Mr. Pierangello Gobbo for the assistance with the acquisition of FTIR spectra. Mr. Mark Milne is thanked for fruitful discussions.

Financial support for this work has been provided by Petro Canada (Robarts Young Innovator Award for MS), OICR (The Ontario Institute for Cancer Research) and NSERC (National Science and Engineering Council of Canada).

References

1 S. J. Martin, C. Catana, B. K. Swann, S. B. Siegel, W. Jung, R. E. Nutt, S. R. Cherry, C. D. Claussen and B. J. Pichler, *Radiology*, 2007, **244**, 807.
2 For the recent reviews on molecular probes for MRI see: (*a*) B. Yoo and M. D. Pagel, *Front. Biosci.*, 2008, **13**, 1733; (*b*) C. F. G. C. Geraldes and S. Laurent, *Contrast Media Mol. Imaging*, 2009, **4**, 1; (*c*) E. Terreno, D. Delli Castelli, A. Viale and S. Aime, *Chem. Rev.*, 2010, **110**, 3019.
3 For the recent reviews on molecular probes for PET see: (*a*) K. Tanaka and K. Fukase, *Org. Biomol. Chem.*, 2008, **6**, 815; (*b*) T. J. Wadas, E. H. Wong, G. R. Weisman and C. J. Anderson, *Chem. Rev.*, 2010, **110**, 2858; (*c*) M. D. Bartholomä, A. S. Louie, J. F. Valliant and J. Zubieta, *Chem. Rev.*, 2010, **110**, 2903.
4 For an excellent review on the development of various bimodal probes for molecular imaging see: L. E. Jennings and N. J. Long, *Chem. Commun.*, 2009, 3511.
5 There have also been recent efforts to generate nanoparticles capable of acting as bimodal MRI/PET molecular probes, see for example: (*a*) M. Lewin, N. Carlesso, C. H. Tung, X. W. Tang, D. Cory, D. T. Scadden and R. Weissleder, *Nat. Biotechnol.*, 2000, **18**, 410; (*b*) C. Glaus, R. Rossin, M. J. Welch and G. Bao, *Bioconjugate Chem.*, 2010, **21**, 715.
6 L. Frullano, C. Catana, T. Benner, A. D. Sherry and P. Caravan, *Angew. Chem., Int. Ed.*, 2010, **49**, 2382.
7 C. P. Gros, A. Eggenspiller, A. Nonat, J. M. Barbe and F. Denat, *Med. Chem. Commun.*, 2011, **2**, 119.
8 E. Gianolio, L. Maciocco, D. Imperio, G. B. Giovenzana, F. Simonelli, K. Abbas, G. Bisi and S. Aime, *Chem. Commun.*, 2011, **47**, 1539.
9 J. A. Park, J. Y. Kim, H. K. Kim, W. Lee, S. M. Lim, Y. Chang, T. J. Kim and K. M. Kim, *ACS Med. Chem. Lett.*, 2012, **3**, 299.
10 (*a*) M. Suchý, M. Milne, A. X. Li, N. McVicar, D. W. Dodd, R. Bartha and R. H. E. Hudson, *Eur. J. Org. Chem.*, 2011, 6532; (*b*) M. Milne, K. Chicas, A. X. Li, R. Bartha and R. H. E. Hudson, *Org. Biomol. Chem.*, 2012, **10**, 287.
11 M. Milne and R. H. E. Hudson, *Chem. Commun.*, 2011, **47**, 9194.
12 R. E. Mewis and S. J. Archibald, *Coord. Chem. Rev.*, 2010, **254**, 1686.
13 For a recent review on CuAAC chemistry applied to metal chelating systems see: H. Struthers, T. L. Mindt and R. Schibli, *Dalton Trans.*, 2010, **39**, 675.
14 Alkylation of DO3A-OtBu (**8**) with a different electrophile under similar conditions has been previously utilized in our laboratory, see: M. Suchý, R. Ta, A. X. Li, F. Wojciechowski, S. H. Pasternak, R. Bartha and R. H. E. Hudson, *Org. Biomol. Chem.*, 2010, **8**, 2560.
15 E. J. F. Prodhomme, C. Ensch, F. B. Bouche, T. Kaminski, S. Deroo, P. Seck, G. Kirsch and C. P. Muller, *Bioconjugate Chem.*, 2007, **18**, 2045.
16 A. Pasha, M. Lin, G. Tircsó, C. L. Rostollan, M. Woods, G. E. Kiefer, A. D. Sherry and X. Sun, *J. Biol. Inorg. Chem.*, 2009, **14**, 421.
17 Acylation of amines and amino acids with chloroacetyl chloride is a methodology widely used in our laboratory to

prepare electrophiles suitable for the preparation of MRI contrast agents, see for example ref. 10*a* and: (*a*) F. Wojciechowski, M. Suchý, A. X. Li, H. A. Azab, R. Bartha and R. H. E. Hudson, *Bioconjugate Chem.*, 2007, **18**, 1625; (*b*) M. Suchý, A. X. Li, R. Bartha and R. H. E. Hudson, *Bioorg. Med. Chem.*, 2008, **16**, 6156; (*c*) A. E. H. Elmehriki, M. Milne, M. Suchý, R. Bartha and R. H. E. Hudson, *Can. J. Chem.*, DOI: 10.1139/cjc-2012-0358.

18 A. Benalil, B. Carboni and M. Vaultier, *Tetrahedron*, 1991, **47**, 8177.

19 E. Pershagen, J. Nordholm and K. E. Borbas, *J. Am. Chem. Soc.*, 2012, **134**, 9832.

20 M. K. Schultz, S. H. Parameswarappa and F. C. Pigge, *Org. Lett.*, 2010, **12**, 2398.

21 (*a*) Y. Li and A. H. Flood, *Angew. Chem., Int. Ed.*, 2008, **47**, 2649; (*b*) A. H. St. Amant, L. A. Bean, J. P. Guthrie and R. H. E. Hudson, *Org. Biomol. Chem.*, 2012, **10**, 6521.

22 C. Szíjjartó, E. Pershagen and K. E. Borbas, *Dalton Trans.*, 2012, **41**, 7660.

23 E. M. Sletten and C. R. Bertozzi, *Angew. Chem., Int. Ed.*, 2009, **48**, 6974.

24 S. Aime, M. Botta, M. Fasano and E. Terreno, *Chem. Soc. Rev.*, 1998, **27**, 19.

25 P. Caravan, J. J. Ellison, T. J. McMurry and R. B. Lauffer, *Chem. Rev.*, 1999, **99**, 2293.

26 A. Barge, G. Cravotto, E. Gianolio and F. Fedeli, *Contrast Media Mol. Imaging*, 2006, **1**, 184.

27 P. Verwilst, S. E. Eliseeva, L. V. Elst, C. Burtea, S. Laurent, S. Petoud, R. N. Muller, T. N. Parac-Vogt and W. M. De Borggraeve, *Inorg. Chem.*, 2012, **51**, 6405.

28 For the recent reviews on preparation of various bifunctional chelators for Gd^{3+} and other lanthanide(III) ions see :(*a*) L. Frullano and P. Caravan, *Curr. Org. Synth.*, 2011, **8**, 535; (*b*) L. Lattuada, A. Barge, G. Cravotto, G. B. Giovenzana and L. Tei, *Chem. Soc. Rev.*, 2011, **40**, 3019.

29 M. Suchý, A. X. Li, R. Bartha and R. H. E. Hudson, *Tetrahedron Lett.*, 2010, **51**, 1087.

30 L. Tei, G. Gugliotta, S. Avedano, G. B. Giovenzana and M. Botta, *Org. Biomol. Chem.*, 2009, **7**, 4406.