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AAZTA⁵/AAZTA⁵-TOC: synthesis and radiochemical evaluation with ⁶⁸Ga, ⁴⁴Sc and ¹⁷⁷LuJean-Philippe Sinnes, Johannes Nagel and Frank Rösch* 

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Abstract

Purpose: AAZTA (1,4-bis (carboxymethyl)-6-[bis (carboxymethyl)]amino-6-methylperhydro-1,4-diazepine) based chelators were initially developed in the context of magnetic resonance imaging. First radiochemical studies showed the capability of AAZTA to form stable complexes with radiolanthanides and moderately stable complexes with ⁶⁸Ga. For a systematic comparison of the labelling capabilities with current diagnostic and therapeutic trivalent radiometals, AAZTA⁵ (1,4-bis (carboxymethyl)-6-[bis (carboxymethyl)]amino-6-[pentanoic-acid]perhydro-1,4-diazepine) was synthesized representing a bifunctional version with a pentanoic acid at the carbon-6 atom. To evaluate the effect of adding a targeting vector (TV) to the bifunctional chelator on the complex formation, AAZTA⁵-TOC was synthesized, radiolabeled and tested in comparison to the uncoupled AAZTA⁵.

Methods: AAZTA⁵ was synthesized in a 5-step synthesis. It was coupled to the cyclic peptide TOC (Phe¹-Tyr³ octreotide) via amide bond formation. AAZTA and AAZTA⁵-TOC complex formations with ⁶⁸Ga, ⁴⁴Sc and ¹⁷⁷Lu were investigated at different pH, temperature and precursor amounts. Stability studies against human serum, PBS buffer, EDTA and DTPA were performed.

Results: AAZTA⁵ and AAZTA⁵-TOC achieved quantitative labelling (> 95%) at room temperature in less than 5 min with all three nuclides at pH ranges from 4 to 5.5 with low precursor amounts of 1 to 10 nmol. [⁴⁴Sc]Sc-AAZTA⁵ complexes as well as [⁴⁴Sc]Sc-AAZTA⁵-TOC were completely stable. The ¹⁷⁷Lu complexes of AAZTA⁵ and AAZTA⁵-TOC showed high stability comparable to the ⁴⁴Sc complexes. In contrast, the [⁶⁸Ga]Ga-AAZTA⁵ complex stability was rather low, but interestingly, [⁶⁸Ga]Ga-AAZTA⁵-TOC was completely stable.

Conclusion: AAZTA⁵ appears to be a promising bifunctional chelator for ⁶⁸Ga, ⁴⁴Sc and ¹⁷⁷Lu with outstanding labelling capabilities at room temperature. Complex stabilities are high in the case of ⁴⁴Sc and ¹⁷⁷Lu. While [⁶⁸Ga]Ga-AAZTA complexes alone lacking stability, [⁶⁸Ga]Ga-AAZTA⁵-TOC demonstrated high stability. The latter indicates an interesting feature of [⁶⁸Ga]Ga-AAZTA⁵-labelled radiopharmaceuticals.

Keywords: Gallium-68, Scandium-44, Lutetium-177, AAZTA⁵-TOC, AAZTA⁵, TOC, Somatostatin, PET, Theranostic

Introduction

The ongoing development in molecular imaging is focusing more and more on theranostic approaches as they link imaging directly with therapy as well as monitoring of the treatment (Baum and Kulkarni 2012; Baum and Rösch 2013; Rösch et al. 2017). In this context, patient-individual dosimetry is important for application of long-lived therapeutic nuclides. This emphasizes the advantage of a longer-lived PET-nuclide like ^{44}Sc over ^{68}Ga (Khawar et al. 2018; Roesch 2012a; Kerdjoudj et al. 2016).

Most of the commonly used nuclides for PET imaging and therapy are trivalent metals Me(III), thus have a positive charge of 3 as ions in solution. The most frequently used bifunctional chelators (BFC) are macrocyclic derivatives based on DOTA and NOTA. Bifunctionalization of DOTA normally uses one of the acid groups to connect to the targeting vector (TV). DOTA-conjugated TV's are typically ^{68}Ga -labeled at ca. 95 °C for 10–15 min to achieve high labelling yields (Tsiou et al. 2017; Price and Orvig 2014; Roesch 2012b). NOTA- or NODAGA-conjugated analogues allow ^{68}Ga -labelling at lower temperature, if larger amounts of the labelling precursor are utilized, yet for many compounds at lower concentration heating to 95 °C is common to achieve fast and quantitative labelling (Eisenwiener et al. 2002). For theranostic strategies with ^{177}Lu only DOTA can be used, since NOTA derivatives are not suitable for complexing ^{177}Lu . Again, ^{177}Lu -labelling of DOTA-peptides is achieved at temperatures close to 100 °C and optimal labelling pH is around 4.

In addition, some relevant molecular targeting vectors (e.g. based on proteins) are temperature and pH sensitive. Accordingly, optimal chelators for radiolabeling sensitive biomolecules with trivalent radiometals (^{68}Ga , ^{44}Sc , ^{177}Lu , ^{90}Y , ^{213}Bi , ^{225}Ac) at room temperature and pH 5–6 would be extremely important. There are some proper candidates for labelling at room temperature. HBED (Schuhmacher et al. 1992), THP (Berry et al. 2011), H₂dedpa (Boros et al. 2010, 2012), DATA (Waldron et al. 2013; Seemann et al. 2017; Farkas et al. 2017; Seemann et al. 2015) show the desired labelling capabilities mostly for ^{68}Ga , while THP and DATA have optimum labelling parameters reaching fast quantitative yields at room temperature on wide pH ranges up to pH 6 (Tsiou et al. 2017). AAZTA⁵/AAZTA^{CN} were reported to provide good labelling with not only ^{68}Ga , but also ^{44}Sc and ^{177}Lu (Pfister et al. 2015; Nagy et al. 2017; Manzoni et al. 2012).

In this work, we want to evaluate the AAZTA lead structure in several aspects. First, a bifunctional derivative (AAZTA⁵), needed to later covalently attach the chelator to a TV, was synthesized. Here, our strategy is to replicate the approach we successfully introduced for the DATA chelator (Seemann et al. 2017). Second, we systematically optimize radiolabeling protocols for Me(III)-AAZTA⁵ complexes with ^{68}Ga , ^{44}Sc and ^{177}Lu . Third, we correlate labelling yield with Me(III)-AAZTA⁵ complex stability in vitro. Fourth, we synthesized a proof-of-principle radiopharmaceutical, namely AAZTA⁵-TOC, to again determine radiolabeling efficiency and in vitro stability.

Materials and methods

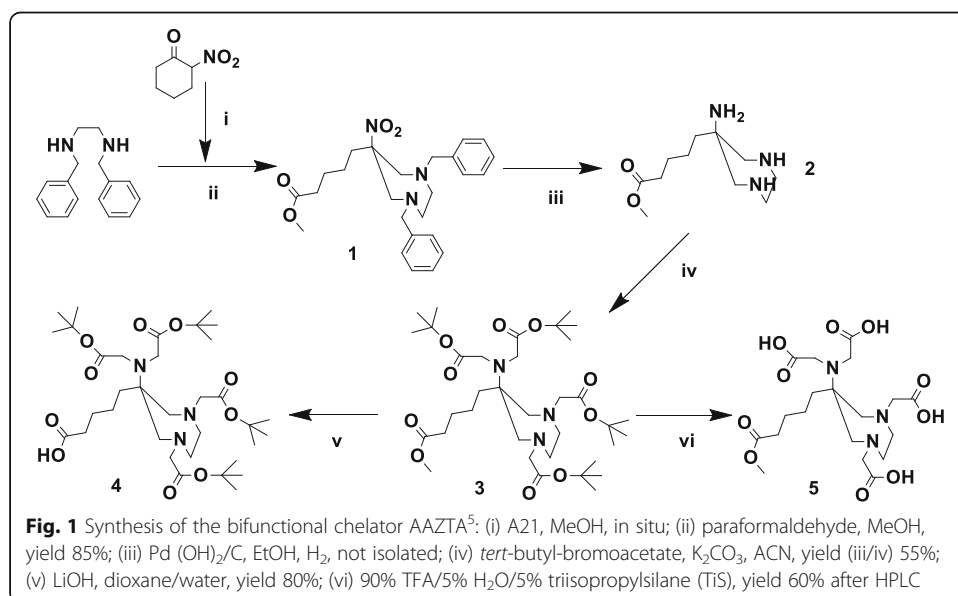
Synthesis of AAZTA⁵

All standard chemicals were acquired from Sigma-Aldrich, Merck and VWR. AAZTA⁵ (2,2'-(6-(bis (carboxymethyl)amino)-6-(4-carboxybutyl)-1,4-diazepane-1,4-diyl) diacetic

acid) was synthesized in a 5-step synthesis. The reactions to form the diazepine ring 1 via ring opening of nitrocyclohexanon and the full reduction to 2 has been published before as it is also part of the DATA synthesis (Fig. 1) (Seemann et al. 2017). To form the fully alkylated AAZTA, the ongoing reaction was catalyzed by KI and moderate temperatures of 40 °C as well as a huge excess of *tert*-butyl bromoacetate. By doing so reaction, yields could be pushed to 50–60%. Analytics of 3 lead to: $^1\text{H-NMR}$ (CDCl_3 , 400 MHz, δ [ppm]): 3.65 (s, 4 H); 3.61 (s, 4 H); 3.22 (s, 3 H); 2.99 (d, $J = 14.1$ Hz, 2 H); 2.85–2.65 (m, 4 H); 2.63 (d, $J = 14.1$ Hz, 2 H); 2.31 (t, $J = 7.4$ Hz, 2 H); 1.62–1.52 (m, 4 H); 1.44 (s, 18 H); 1.43 (s, 18 H); 1.25 (m, 2 H); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz, δ [ppm]): 174.37 (s); 172.89 (s); 170.94 (s); 80.86 (s); 80.38 (s); 65.29 (s); 63.17 (s); 62.61 (s); 59.39 (s); 52.09 (s); 51.56 (s); 37.34 (s); 34.26 (s); 28.31 (s); 28.25 (s); 25.89 (s); 21.83 (s). To deprotect the bifunctional acid group, the methyl ester is cleaved with LiOH in dioxane/water to afford 4, while for the evaluation of the free chelator 5 the protective *tert*-butyl groups were cleaved with TFA leaving the methyl ester intact.

Synthesis of AAZTA⁵-TOC

A standard amide coupling was performed to couple the AAZTA⁵ to TOC. The TOC (Phe¹-Tyr³-octreotide) was purchased from ABX Germany as H-D-Phe-Cys-Tyr (^tbutyl)-D-Trp (boc)-Lys (boc)-Thr (^tbutyl)-Cys-Thr (ol) protected for amide coupling on the N-terminal amine of D-phenylalanine. As coupling reagent HBTU with an excess of HOBT was added to a solution of 4 in DMF. DIPEA was used as organic base. The formation of the active ester could be observed on LC-MS (column used: ZORBAX with 5% to 95% ACN (+ 0.1% MeCOOH)/water (+ 0.1% MeCOOH) in 5 min) as the mass of 4 (ESI-MS ($M + H^+$): 672.45, 673.45, 674.46) with a retention time of 3.4 min changed to the active ester (ESI-MS ($M + H^+$)) with a retention time of 3.8 min. Monitoring the formation of the active ester showed full formation of the active ester after 15–20 min and the TOC was added. After coupling, all protective groups were cleaved with TFA. HPLC purification was performed on a LUNA column



(Phenomenex® Luna® 10 µm C18(2) 100 Å) with isocratic conditions of 21% ACN (+ 0.1% TFA)/79% water (+ 0.1% TFA). The final product AAZTA⁵-TOC was collected at a retention time of 15–17 min in an yield of 45% over the coupling reaction, deprotection and purification. ESI-MS showed the pure product at 1464,6 (M + H)⁺ (C₆₇H₉₄N₁₃O₂₀S₂ predicted 1464,62).

Radiolabeling with ⁶⁸Ga, ⁴⁴Sc and ¹⁷⁷Lu

For radiolabeling with ⁶⁸Ga a ⁶⁸Ge/⁶⁸Ga generator (TiO₂-based matrix, Cyclotron Co., Obninsk, Russia) was used with acetone post-processing separating iron and zinc impurities as well as ⁶⁸Ge breakthrough (Zhernosekov et al. 2007). Radiolabeling with ⁴⁴Sc was performed with a ⁴⁴Sc/⁴⁴Ti generator (Filosofov et al. 2010; Pruszyński et al. 2010) located at the Institute of Nuclear Chemistry in Mainz. ¹⁷⁷Lu was provided by itg Munich following the carrier-free production pathway ¹⁷⁶Yb(n,γ)¹⁷⁷Yb → ¹⁷⁷Lu (Lebedev et al. 2000).

Radiolabeling for all radiometals was performed in 1 ml of 0.2 M ammonium acetate buffer pH 4.5 and 5.5. Due to the post processing for ⁴⁴Sc the nuclide is provided in 0.25 M ammonium acetate buffer pH 4 (Pruszyński et al. 2010) and for first studies the pH was not adjusted above pH 4. With 1 ml labeling volume for ⁶⁸Ga and ⁴⁴Sc precursor concentrations of 5 and 10 pmol/l and for ¹⁷⁷Lu lower concentrations of 0.5 and 1 pmol/l were used. To show the mild labelling capability of the AAZTA⁵ chelator, the reaction temperature was adjusted to 25 °C with a BT 03 heater from HLC BioTech (Germany). Labelling studies were performed with 50 MBq for ⁴⁴Sc and 100 MBq for ⁶⁸Ga and ¹⁷⁷Lu. At different time points of 1, 3, 5 and 10 min aliquots for TLC and HPLC analytics were taken. The pH was controlled at start of labelling and after labelling, was finished.

For reaction control TLC (TLC Silica gel 60 F₂₅₄ Merck®) with citrate buffer (pH 7) and ammonium acetate buffer (pH 7)/MeOH 50/50 was used and compared to radio HPLC (Chromolith flush column, water: ACN with 0.1% TFA, 5 to 95% ACN in 10 min). TLCs were measured in RITA TLC imager (Elysia Raytest). The citrate TLC showed free radio metal with an R_f of 0.9 and all labelled compound stucked to an R_f of 0.1 to 0.3. As TLC would show colloidal radiometals sticking to an R_f of 0.1 to 0.2 as labelled compound, radio-HPLC was used to exclude the presence of colloidal radiometals, as colloidal radiometals cannot be eluted from the used HPLC columns.

Stability studies

Stability studies were performed in HS, PBS and EDTA/DTPA solution (pH adjusted to 7 by PBS buffer) in triplicate, starting from Me(III)-AAZTA⁵/AAZTA⁵-TOC batches of > 95% radiochemical purity. Time points were adjusted to the nuclides half-life: for ⁶⁸Ga 0.5, 1, 2 h; for ⁴⁴Sc 0.5, 1, 4, 8 h, 24 h; for ¹⁷⁷Lu 0.5, 1 and 2 h, 1 and 7 days. Stabilities against HS were also tested for adsorption to HS as fractions of the stability study at given time points were added to acetonitrile. After precipitation and centrifugation, the supernatant solution was removed and residue was measured for activity. HS (human male AB plasma, USA origin) was bought from Sigma Aldrich, PBS was prepared with a BupH™ Phosphate Buffered Saline Pack (PIERCE), EDTA and DTPA

solution were prepared using the prepared PBS buffer by adding EDTTA and DTPA to a 0.01 M concentration.

Results

Synthesis of AAZTA⁵ and AAZTA⁵-TOC

The established synthesis for DATA^{5m} and DATA^{5m}-TOC (Seemann et al. 2017) was successfully transferred to AAZTA⁵ and AAZTA⁵-TOC. The key step, attaching four *tert*-butyl-acetate-arms to the diazepine backbone, was optimized to work in good yields by forcing the reaction with slightly increased temperatures of 40 °C and the addition of KI. Without the exchange from bromine to iodine on the *tert*-butyl bromoacetate nearly no alkylation was observed. Even with KI the reaction is still relatively slow and needs to be stirred over two days. Deprotection of the methyl group on the AAZTA⁵ was done similar to that of DATA^{5m}. Purification by extraction (dichloromethane against 0.1 M NaCO₃ in water) gave the pure product ready for coupling in the dichloromethane phase. Ready for coupling AAZTA⁵ could be isolated with a yield of 35% calculated over all reaction steps from the *N,N'*-dibenzylethylenediamine used in the first reaction step.

Formation of the AAZTA⁵ active ester with HBTU (in DMF with 7 eq of DIPEA) was fast and could be monitored by LC-MS showing the active ester. After 15 min and positive LC-MS control, the active ester was added to the TOC solved in DMF. After one night, the DMF was removed and residues were resolved in 95% TFA/2.5% water/2.5% TiS (triisopropylsilane) to start the deprotection step. Lower concentrations of TFA with dichloromethane as solvent were not acidic enough to fully deprotect the chelator, leaving 1–2 *tert*-butyl groups even after 1 night. After 3–4 h of reaction the TFA were removed and the residue solved in 80% water/20% acetonitrile for HPLC. Yields after the HPLC were in a solid range of 30–40%.

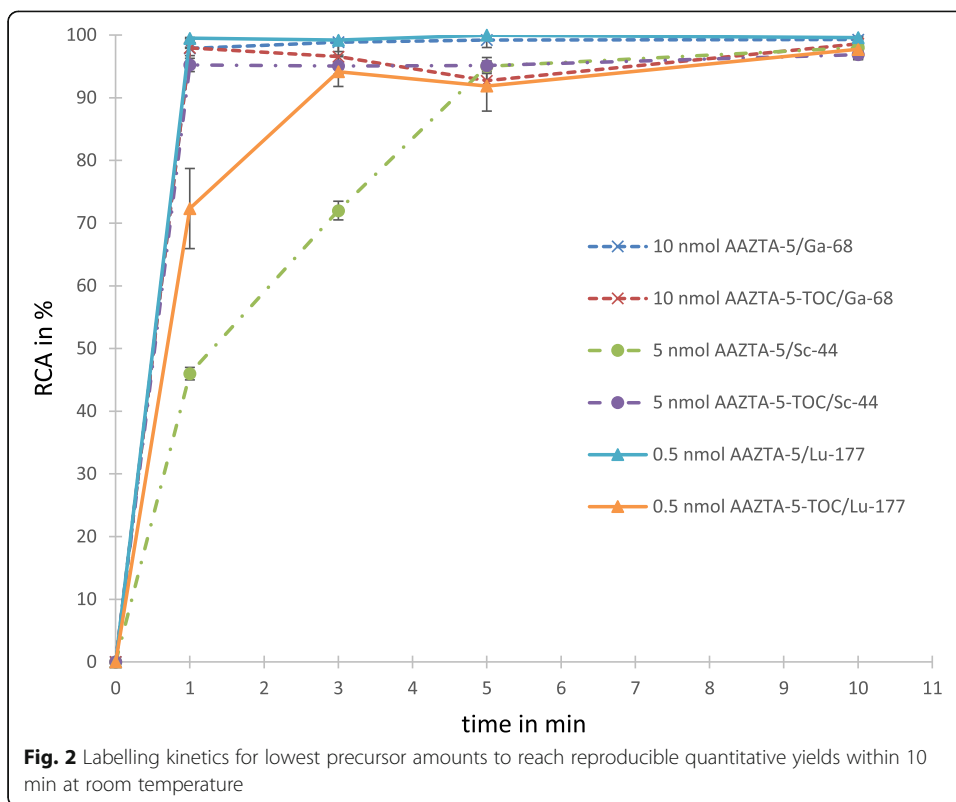
Radiochemical evaluations with ⁶⁸Ga

For both the free chelator AAZTA⁵ as well as the AAZTA⁵-TOC, radiolabelling with 10 nmol precursor showed an almost quantitative yield of >98% in less than 5 min; most of the time reaching >95% even after 1 min at room temperature (Fig. 2). At 5 nmol of precursor amount, a slower kinetic was observed, but still yielding >90% after 15 min most of the time, yet reproducibility became a problem. Variation of the pH from 4.5 to 5.5 showed no difference in the kinetics or the overall yield. Thus, 10 nmol was found to be the optimal precursor amount to prepare [⁶⁸Ga]Ga-AAZTA⁵ and [⁶⁸Ga]Ga-AAZTA⁵-TOC, ready to be investigated in subsequent stability studies.

Stability of [⁶⁸Ga]Ga-AAZTA⁵ against HS and PBS were high for 1 h, but decreased slowly at 2 h to a value of 85% and 79%, respectively. Adding EDTA or DTPA decreased the stability further up to 40–50% after 2 h. In contrast to the free chelator, the [⁶⁸Ga]Ga-AAZTA⁵-TOC was completely stable against HS, PBS, EDTA and DTPA over two hours (Table 1).

Radiochemical evaluation with ⁴⁴Sc

Labelling both AAZTA⁵ and AAZTA⁵-TOC with ⁴⁴Sc showed a quantitative yield of >97% with 10 nmol in less than 5 min, most of the time reaching >95% even after 1 min,



all at room temperature. Lowering the precursor amount to five nmol induced slower kinetics for the free chelator, still yielding > 95% after 10 min for AAZTA⁵. In contrast, radiolabelling with five nmol of AAZTA⁵-TOC was always > 95% in less than 5 min at room temperature. Labelling with five nmol was stable enough to use it for stability studies. Stability studies showed for both the free chelator as well as the AAZTA⁵-TOC stability against HS, PBS, EDTA and DTPA over 8 h. [⁴⁴Sc]Sc-AAZTA⁵-TOC remained stable with > 90% in HS and PBS even over 24 h (Table 2).

Radiochemical evaluation with ¹⁷⁷Lu

General labelling procedures for DOTA conjugated radiopharmaceuticals with n.c.a. ¹⁷⁷Lu showed that DOTA is capable of complexing ¹⁷⁷Lu in molar ratios from 1: 5 to 1: 10 for save reproducible quantitative labelling. 100 MBq of ¹⁷⁷Lu is around 0.1 nmol. ¹⁷⁷Lu-labelling with 0.5 nmol AAZTA⁵ (ratio 1: 5) showed quantitative yields of > 98% already after 1 min at room temperature. Formation of [¹⁷⁷Lu]Lu-AAZTA⁵-TOC had lower kinetics, yet also leading to yields of > 95% after 5 min. Both showed rapid

Table 1 Radiolabelling data for [⁶⁸Ga]Ga-AAZTA⁵, [⁶⁸Ga]Ga-AAZTA⁵-TOC, [⁴⁴Sc]Sc-AAZTA⁵, [⁴⁴Sc]Sc-AAZTA⁵-TOC after 10 min (15 min for 5 nmol AAZTA⁵) at room temperature

	⁶⁸ Ga-Radiolabelling				⁴⁴ Sc-Radiolabelling	
	AAZTA ⁵		AAZTA ⁵ -TOC		AAZTA ⁵	AAZTA ⁵ -TOC
pH	4.5	5.5	4.5	5.5	4.0	4.0
5 nmol	92.3 ± 1.8	89.8 ± 2.4	94.3 ± 1.7	91.7 ± 3.5	98.0 ± 0.6	96.9 ± 0.9
10 nmol	98.5 ± 0.8	99.1 ± 0.5	99.0 ± 0.7	98.6 ± 0.9	99.5 ± 0.3	96.7 ± 1.2

Table 2 Stability data for ^{68}Ga , ^{44}Sc and ^{177}Lu complexes of AAZTA⁵ and AAZTA⁵-TOC against human serum, PBS buffer and EDTA and DTPA in PBS buffer in percent of stable complex at specific time points (% labelled compound compared to free activity found at given time point)

	^{68}Ga			^{44}Sc			^{177}Lu		
	time	AAZTA ⁵	AAZTA ⁵ -TOC	time	AAZTA ⁵	AAZTA ⁵ -TOC	time	AAZTA ⁵	AAZTA ⁵ -TOC
HS	30 min	93.0 ± 0.8	98.1 ± 0.2	1 h	93.7 ± 0.9	95.1 ± 0.6	1 h	99.8 ± 0.1	92.5 ± 1.9
	1 h	92.7 ± 1.3	96.9 ± 0.2	4 h	94.3 ± 1.7	94.3 ± 0.8	2 h	99.7 ± 0.1	92.3 ± 2.6
	2 h	85.3 ± 3.4	95.0 ± 0.3	8 h	93.2 ± 1.1	95.0 ± 1.1	24 h	99.9 ± 0.1	91.5 ± 2.1
				24 h	91.3 ± 1.8	93.8 ± 1.0	7 d	82.5 ± 3.2	86.3 ± 3.2
PBS	30 min	97.5 ± 1.0	99.1 ± 0.2	1 h	95.3 ± 0.9	97.3 ± 0.5	1 h	97.8 ± 0.5	92.0 ± 1.4
	1 h	88.7 ± 0.5	99.2 ± 0.1	4 h	92.7 ± 1.3	98.5 ± 0.9	2 h	98.6 ± 0.4	93.5 ± 1.3
	2 h	78.5 ± 4.5	99.3 ± 0.1	8 h	95.9 ± 1.2	96.7 ± 1.4	24 h	99.1 ± 0.6	91.0 ± 2.2
				24 h	96.0 ± 0.8	94.7 ± 1.6	7 d	96.0 ± 0.8	93.1 ± 1.8
EDTA	30 min	93.0 ± 0.2	98.9 ± 0.4	1 h	93.7 ± 1.3	96.0 ± 0.4	1 h	98.9 ± 0.2	95.7 ± 2.4
	1 h	50.3 ± 7.4	99.4 ± 0.2	4 h	90.0 ± 3.2	97.6 ± 0.7	2 h	97.9 ± 0.3	95.1 ± 2.6
	2 h	49.5 ± 2.5	99.6 ± 0.1	8 h	92.3 ± 2.1	96.9 ± 1.0	24 h	79.2 ± 2.9	86.8 ± 2.6
				24 h	91.9 ± 1.8	95.4 ± 2.1	7 d	48.9 ± 1.5	77.5 ± 2.3
DTPA	30 min	84.3 ± 0.9	97.8 ± 0.2	1 h	94.3 ± 0.9	98.7 ± 0.3	1 h	99.1 ± 0.2	98.9 ± 0.5
	1 h	78.0 ± 1.4	99.4 ± 0.1	4 h	92.5 ± 0.5	98.9 ± 0.7	2 h	99.1 ± 0.1	96.2 ± 3.2
	2 h	45.7 ± 1.3	99.6 ± 0.3	8 h	93.8 ± 1.2	96.9 ± 1.5	24 h	80.4 ± 5.4	82.8 ± 4.7
				24 h	93.4 ± 1.1	92.6 ± 3.2	7 d	59.0 ± 14.3	72.9 ± 5.6

labelling with ^{177}Lu in less than 1 min at one nmol precursor. Labelling behaviour was the same for pH 4.5 and 5.5. Labelling of 500 MBq ^{177}Lu with 5 nmol precursor (for stability studies) were also performed yielding > 98% after 1 min reaction time at room temperature (Table 3).

Stability studies showed for both the free chelator as well as the AAZTA⁵-TOC good stabilities of > 90% against HS and PBS over 24 h. Stabilities against EDTA and DTPA were slightly lower with > 85% after 24 h. Longer studies of 7 d proved good stability against PBS with > 90% and a small degradation in HS with > 85% (mostly adsorption on serum proteins, visible by precipitation). 7 d stability values for EDTA and DTPA gave increasing instability down to 75 and 70% respectively for AAZTA⁵-TOC and to 60 and 50% respectively for the free chelator.

Discussion

Our experience concerning the synthesis of DATA^{5m}-TOC (Seemann et al. 2017) could be transferred, with adjustments on the alkylation step, to prepare the free chelator AAZTA⁵ and its TOC derivative AAZTA⁵-TOC in good yields. Radiolabelling with ^{68}Ga showed good and rapid complexation at room temperature with quantitative

Table 3 Radiolabelling data for [^{177}Lu]Lu-AAZTA⁵ and [^{177}Lu]Lu-AAZTA⁵-TOC

pH	^{177}Lu -Radiolabelling			
	4.5		5.5	
ligand: Lu ratio	AAZTA ⁵	AAZTA ⁵ -TOC	AAZTA ⁵	AAZTA ⁵ -TOC
5: 1	97.9 ± 1.6	98.9 ± 0.5	99.9 ± 0.2	97.7 ± 1.5
10: 1	98.6 ± 0.8	98.7 ± 1.1	99.3 ± 0.7	99.6 ± 0.1

yields in less than 5 min for both the free chelator as well as the AAZTA⁵-TOC. The complexation also worked on pH 5.5 without showing issues forming colloidal ⁶⁸Ga, giving the same high yields after 5 min as for pH 4.5. This proves that elevation of the pH does not affect the labelling in a negative way. This rapid labelling at room temperature represents a significant advantage of AAZTA⁵ over DOTA. Second, fast labelling kinetics of DOTA also demand higher temperatures and precursor amounts and this demonstrates the most relevant feature of AAZTA⁵ compared to DOTA-derivatives.

Stability of the free [⁶⁸Ga]Ga-AAZTA⁵ complex was not 100% over the 2 h, releasing 10–15% of the ⁶⁸Ga against HS and PBS consistent with literature (Waldron et al. 2013; Baranyai et al. 2013). However, and extremely interesting, [⁶⁸Ga]Ga-AAZTA⁵-TOC was completely stable over two hours against HS and PBS, showing a positive effect of the TOC on the stability of the ⁶⁸Ga-complex. Due to the short spacer between the chelator and the TV, this could be a steric influence of the peptide moiety, but also the contribution of the amide formed in the coupling process may influence ⁶⁸Ga coordination. Adding EDTA or DTPA to the PBS increased the instability for AAZTA⁵, whereas AAZTA⁵-TOC remained fully stable. It appears to be necessary to study the impact of moieties other than TOC to further understand this phenomenon.

Rapid and quantitative ⁴⁴Sc-AAZTA⁵ complex formation (both AAZTA⁵ and AAZTA⁵-TOC) at room temperature with low precursor amounts of five nmol demonstrate that the AAZTA⁵ structure is almost ideal for labelling ⁴⁴Sc. With 100 MBq, ⁶⁸Ga in contrast to 50 MBq ⁴⁴Sc the effective amount of radiometal used per labelling was even higher for ⁴⁴Sc due to its longer half-life. In fact, 50 MBq ⁴⁴Sc contain near double the nmol of radio metal compared to 100 MBq ⁶⁸Ga. Using half of the precursor amount on double the nmol radio metal leads to a chelator to radio metal ratio in the labelling solution that is 4 times lower in the ⁴⁴Sc labelling than for ⁶⁸Ga one. In addition, less precursor is also lowering the chelator concentration in the labelling solution. Consequently, AAZTA⁵ and AAZTA⁵-TOC have outstanding labelling capabilities for ⁴⁴Sc. [⁴⁴Sc]Sc-AAZTA⁵ and [⁴⁴Sc]Sc-AAZTA⁵-TOC were completely stable against HS and PBS over two times the half-lives of ⁴⁴Sc and even stable after 24 h. Only the addition of EDTA and DTPA showed a small release of the ⁴⁴Sc after 24 h while the complexes are stable over 8 h. Combining the rapid quantitative radiolabelling with the good stability, AAZTA⁵ and AAZTA⁵-TOC offer optimal labelling capabilities for ⁴⁴Sc.

Radiolabelling with ¹⁷⁷Lu was performed in equimolar ratios showing that even a ratio of 1: 5 between radiometal and chelator gave quantitative yields. Using ratios of 1: 10, rapid labelling is observed after one minute already at room temperature. Adjusting the pH to 5.5 had no influence on labelling speed or yields showcasing the same stable labelling as seen before with ⁶⁸Ga and ⁴⁴Sc. Stability over 24 h showed both the AAZTA⁵ as well as the AAZTA⁵-TOC to be stable against HS and PBS with a slight decomplexation against EDTA and DTPA. After seven days, both complexes stay intact against PBS whereas the degradation by addition of EDTA and DTPA increases. For HS, some adsorption to serum proteins was observed by precipitation, while most of the complex stays intact. Overall, the complex stability is good for PBS and needs further evaluation in vivo to see the influence of the data from the HS.

Conclusion

The chimerical triaza-type AAZTA⁵ chelator showed rapid quantitative radiolabelling at room temperature with low precursor amounts up to pH 5.5 for ⁶⁸Ga, ⁴⁴Sc and ¹⁷⁷Lu. Attaching TOC forming the AAZTA⁵-TOC had no influence on the labelling proving the good labelling capabilities can be transferred. The ⁶⁸Ga complex of AAZTA⁵ was not stable as predicted, but AAZTA⁵-TOC showed complete stability revealing a strong positive effect of the TV on the stability. This effect needs further research. The [⁴⁴Sc]Sc-AAZTA⁵ complex as well as [⁴⁴Sc]Sc-AAZTA⁵-TOC were completely stable followed by the ¹⁷⁷Lu complexes being stable over 24 h with small degradation after one week. AAZTA⁵ appears to be an excellent chelator for mild rapid labelling of both ⁴⁴Sc and ¹⁷⁷Lu forming a theranostic pair.

Abbreviations

ACN: Acetonitrile; BFC: Bifunctional chelators; DMF: Dimethylformamide; e.g.: Exempli gratia; EtOH: Ethanol; HPLC: High performance liquid chromatography; HS: Human serum; MeOH: Methanol; PBS: Phosphate buffered saline; PET: Positron Emission Tomography; TFA: Trifluoroacetic acid; TIS: Triisopropylsilane; TLC: Thin-layer chromatography; TV: Targeting vector

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Authors' contributions

JPS were in charge for chelator and AAZTA-TOC synthesis, purification and radiochemical evaluation. JN supported and helped with further development on purification steps and radiochemical questions. FR supervised the whole part of the present study. All authors read and approved the manuscript.

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Availability of data and materials

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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