Synthesis, *in vitro* evaluation and ^{68}Ga-radiolabeling of CDP1 towards PET/CT imaging of bacterial infection

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KEYWORDS: LL37, NODAGA, PET, Infection, Bacteria, Solid-phase peptide synthesis, CDP1

Running title ^{68}Ga-CDP1 for PET/CT imaging of bacterial infections

Abstract

Bacterial infections are a major concern in the human health sector due to poor diagnosis and development of multi drug resistant strains. PET/CT provides a means for the noninvasive detection and localization of the infectious foci; however, the radiotracers available are either cumbersome to prepare or their exact contribution towards the imaging is not yet established. Human antimicrobial peptides are of interest for development as PET radiotracers as they are an integral component of the immune system, non-immunogenic towards the recipient and show selectivity towards pathogens such as bacteria. Herein we report on the potential of LL37, a human cathelecidin antimicrobial peptide, as a radiotracer for bacterial imaging. Bi-functional chelator 1,4,7-triazacyclononane,1-glutaric acid-4,7-acetic acid (NODAGA) was utilized to functionalize the antimicrobial peptide, which in turn was capable of chelating gallium. The

synthesized [nat]Ga-CDP1 showed bacterial selectivity and low affinity towards hepatic cells, which are favorable characteristics for further preclinical application.

Introduction

Bacterial infections are one of the fastest growing disease risk factors in health care despite significant global developments in antimicrobial chemotherapy. Infection still remains a major cause of morbidity and mortality due to poor diagnosis and increasing drug resistance.[1] Due to the numerous mechanisms of pathogenesis, early stage diagnosis is crucial for the effective prevention and treatment of bacterial infections.[2] The general, bacterial identification procedure involves culturing and examining the microorganism from the suspected site of contagion, which are time consuming, laborious and require skilled personnel.[3-5] Alternatively, Magnetic Resonance Imaging (MRI)[6], Computerized Tomography (CT)[7] and ultrasound can be used for the detection and localization of these pathogens by identifying the inflammatory changes in the local anatomy, water content in tissue or capillary permeability as a result of the infectious lesions.[6, 7] However, it is difficult for these methods to differentiate between sterile inflammation and bacterial infection in the absence of anatomical landmarks.[2, 8, 9] Due to the disadvantages associated with anatomy-based scanning systems, Positron Emission Tomography Computerized Tomography (PET/CT) imaging plays a key role in the diagnosis of diseases.[10] One of the methods to differentiate infection from sterile inflammation is to use radiolabeled agents with affinity for bacterial cell. To date, various biomimetics, antimicrobials, leukocytes, antibodies, bacteriophages, antibiotics, sorbitol, maltose, maltohexaose, and siderophores have been made available for bacterial radiopharmaceutical imaging, but each approach has its own limitations.[3-5, 10-16] Despite of exponential growth in radiopharmaceutics, very few of them are commercially available and approved by Food and Drug Administration (FDA) for bacterial imaging; these includes radiolabeled leukocytes (^{111}In-oxine-leukocyte, ^{99m}Tc-HMPAO-leukocute and ^{99m}Tc-Stannous Colloid), radiolabeled anti-granulocyte antibodies (^{99m}Tc-Sulesomab and ^{99m}Tc-Besilesomab), 2-deoxy-2-(^{18}F)fluoro-D-glucose (^{18}F-FDG) and [67/68]Ga-citrate. From these imaging techniques, ^{18}F-FDG-PET/CT has been considered for infection diagnosis for a long period of time, but its exact contribution has not yet been determined.[17] Radiolabeled leukocytes are considered to be the gold standard of infection imaging; however it is time consuming, labor intensive and requires blood handling.[8] Moreover, commercially available Gallium-67-([67]Ga-) citrate shows low specificity along with high energy γ radiation and longer half-life (78 hr) exposing patients to increased radioabsorption.[18] Due aforesaid disadvantages, antimicrobial peptides can be consider as potential candidate for PET tracer as it tend to accumulate at the infection sites rather than sterile inflammation due to their preferential binding to bacteria over mammalian cells.[4, 19] Since many of the antimicrobial peptides target bacterial cell wall proteins and lipids which are absent in mammalian cells, this feature makes them

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excellent candidates for infection imaging.[20] Recently, ^{68}Ga-DOTA-TBIA101, a small radiolabeled depsidomycin-derived bioconjugate has been used to detect infection sites using PET/CT scanning and is an excellent example of how radiolabeled peptides can be employed to distinguish infection from inflammation.[12] Moreover, ^{68}Ga-NOTA-UBI fragments were also reported to show bacterial selectivity and specificity *in vitro*.[21] Furthermore, human neutrophil peptide, neutrophil elastase inhibitor peptide human β-defensin and human lactoferrin-derived peptide have also been successfully evaluated for imaging bacterial infection.[22-25]

LL37, a linear human cationic antimicrobial peptide (hCAP18) comprising of 37 amino acid residues from the C-terminus[26] is widely associated with the innate immune response. LL37 is found in squamous epithelium and neutrophil granulocytes and is constitutively released into the extracellular space.[26, 27] This peptide neutralizes bioactive molecules present in the bacterial cell wall and is also responsible for bacterial growth inhibition.[28, 29] In addition to its antimicrobial activities as a first line defense mechanism, this peptide is also linked with chemotaxis, histamine release, angiogenesis, cell migration, and cytokine production.[30-32] These biotic roles facilitate the LL37 modulation of the immune response, neovascularization of injured tissues and wound healing.[31, 32] Because of its close association with the innate human immune response, we envisaged the suitability of LL37 as a potential radiotracer for the diagnosis of infection. Furthermore and although the synthesis of this kind of large peptides can be considered a challenge, our group recently reported an optimized solid phase synthesis of LL37.[33]

Presently, LL37 radiolabeled compounds have not been reported for differentiating infection from inflammation. The bifunctional chelator, 1,4,7-triazacyclononane,1-glutaric acid-4,7-acetic acid (NODAGA), has gained popularity for radiolabeling of peptides because of its in vivo stability.[34] In this proof of concept study, we developed NODAGA-functionalized LL37 and conjugated it with [nat]Ga. It must be noted that NODAGA-functionalized LL37 will further be referred to as CDP1. We also present [nat]Ga-CDP1 uptake in different bacteria and a hepatocellular carcinoma (HepG2) cell line. Additionally, the radiosynthesis of CDP1 with the PET radioisotope gallium-68, including its identification by radio-HPLC/UV analysis is reported.

Methods and Materials

Materials

NODAGA(tBu)$_3$ was purchased from CheMatech (Dijon, France). All Fmoc protected amino acids, coupling reagents and the resin Rink Amide-MBHA were purchased from GLS Biochem Systems, Inc., (Shanghai, China). GaCl$_3$ was purchased from sigma-aldrich[®] (Germany).

Peptide synthesis

LL37 was synthesized as described in our previous publication using SPPS and Fmoc chemistry on 0.1 mmol scale.[33] The synthesis was confirmed by cleaving an aliquoted amount of resin followed by matrix-assisted laser-desorption ionization (MALDI) (Autoflex III smartbeam; Bruker Daltonics, Bremen, Germany) analysis. MALDI (positive mode) showed the product with m/z 4493.

Coupling of NODAGA(tBu)$_3$ to the peptide and cleavage

NODAGA(tBu)$_3$ was coupled to LL37 on resin at 0.1 mmol scale using *N,N′*-diisopropylcarbodiimide (DIC)/OxymaPure to synthesize CDP1. The mixture of NODAGA(tBu)$_3$, DIC and OxymaPure was dissolved in tetrahydrofuran (THF) (0.1 ml) and allowed to react for 16 hr at room temperature. The ratio of NODAGA (tBu)$_3$/DIC/OxymaPure was 1:1:1 whereas NODAGA (tBu)$_3$ to free amine ratio was 1.2:1. Excess reagents were removed by washing the resin with 5 ml of THF (2 x) and DCM (2 x), consecutively. The tBu protected NODAGA attached to side chain protected LL37 peptide was finally cleaved from the resin using a cocktail of 1.0 ml TFA:H$_2$O:thioanisole (95:2.5:2.5) over a 2 hr period. During cleavage of the peptide from the resin, all protective groups were also removed. The resin was removed by filtration and washed with TFA (1.0 ml), which was then evaporated upon bubbling of N$_2$ gas through the mixture. The peptide was precipitated in 5.0 ml of ice-cold diethyl ether and centrifuged. The precipitate was then dissolved in 1.0 ml of water and further purified using prep-HPLC. The final product was characterized by MALDI-MS, revealing an m/z 4849 for CDP1 in positive mode.

CDP1 purification

CDP1 was purified on an ACE C18 preparative column (150 x 21.2 mm) by preparative HPLC (Shimadzu, Kyoto, Japan). A two-buffer system consisting of 0.1 % formic acid (FA)/H$_2$O (v/v) and 0.1 % FA/acetonitrile (v/v) were employed. A gradient of 15–80 % of 0.1 % FA/acetonitrile (v/v) over 30 min with a flow rate of 10 ml/min was used and the fractions were characterized by LC-MS (Shimadzu, Kyoto, Japan). Fractions which showed the desired mass were pooled and lyophilized and store for use in further experiments. The retention time of the compound on prep-HPLC was 21.6 min.

Non-radioactive ^{nat}Ga-labeling of CDP1

Non-radioactive natural Gallium(III)chloride (^{nat}Ga; i.e. Gallium has two natural stable isotopes, namely, ^{69}Ga (60 %) and ^{71}Ga (40 %)) was utilized to label the CDP1 as previously described.[35] Therefore, 1.0 mg of CDP1 was dissolved in 828 μl of water; 167 μl 1.9 M sodium acetate and 5.0 μl of 0.7 M ^{nat}GaCl$_3$. The solution was allowed to react for 15 min at room temperature. Quantitative labeling of ^{nat}Ga-CDP1 was confirmed by Q-TOF LC-MS (Bruker Daltonics, Bremen, Germany).

LC-MS method for quantification of [nat]Ga-CDP1

Quantification of [nat]Ga-CDP1 was carried out on a Maxis LC-MS (Bruker Daltonics, Bremen, Germany) coupled with an Agilent 1100 HPLC equipped with a YMC Triart C18 column (3.0 mm x 150 mm). Mobile phase A was water with 0.1% formic acid (FA) and mobile phase B was acetonitrile with 0.1% FA, the HPLC parameters were as follows: the flow rate was 0.3 mL/min with a linear gradient from 5% to 95% phase B over a period of 15 min, hold 2 min at 95% phase B and finally re-equilibration at 5% phase B for 2 min. The mass spectrometer was used in positive ion mode, with a nebulizer pressure of 1.5 bar, dry gas flow rate of 8.0 L/min, drying temperature of 180 °C and capillary voltage of 5500V. The retention time of [nat]Ga-CDP1 was 10.1 min.

Uptake of [nat]Ga-CDP1 by *S.aureus*, *E.coli* and *M. smegmatis*

To determine the uptake of [nat]Ga-CDP1 by *S.aureus, E.coli* and *M.smegmatis*, 10^5 bacterial cells from each bacterial strain were incubated with [nat]Ga-CDP1 at a final concentration of 20 µg/ml. Uptake rates were determined at 4 and 37 ^{o}C temperatures. Samples were collected at 0, 1, 2 and 3 hr time points post inoculation. Samples were then centrifuged at 15000 rpm for 10 min at 4 °C (Hermle, GmBH, Germany, Rotor 221, 23) and the supernatants were collected. The supernatants were treated with equal amounts of ice cold acetonitrile and vortexed for 30 sec followed by a 2 hr cooling step at -20 °C. The samples were centrifuged at 15000 rpm for 10 min at 4 °C and passed through C_{18} SPE cartridge (Waters, Milford, MA, USA) preconditioned with 100 % acetonitrile. All the samples were diluted in the same manner and the recovered amounts of the Ga-CDP1 in the media was determined by the optimized LC-MS method described in the previous section.

Uptake of [nat]Ga-CDP1 by hepatocellular carcinoma (HepG2) cells

HepG2 cells were obtained from Highveld Biologicals (Johannesburg, South Africa). Cells were cultured in Eagle's minimum essential media (Lonza Biowhittaker, Basel, Switzerland) supplemented with 10% foetal calf serum, 1% L-Glutamine and 1% pen/strep-fungizone (Sigma-Aldrich, St Louis, USA); in a humidified incubator at 37 ºC with 5% CO_2. Cells were then seeded in 6-well culture plates (200,000 cells/well) and allowed to adhere overnight prior to treatment. The peptide was administered once the cells had reached approximately 80% confluency. Cells were treated with 20 µg/ml of the compound in triplicate at 4 °C and 37 ºC temperatures. The cell culture supernatant was aspirated at 0, 1, 2 and 3 hr time points and the samples collected for both incubating temperatures. Samples were then centrifuged at 15,000 rpm (Hermle, GmBH, Germany; Rotor 221, 23) for 10 min at 4 °C and the supernatants were collected. The collected supernatants were treated with an equal amount of ice cold acetonitrile and vortexed followed by a 2 hr cooling step at -20 °C. Finally, the samples were centrifuged at 15,000 rpm for 10 min at 4 °C and

passed through C_{18} SPE cartridge preconditioned with 100 % acetonitrile. All samples were diluted in the same manner and the recovered amount of the ^{nat}Ga-CDP1 in the media was determined by LC-MS.

^{68}Ge/^{68}Ga-Generator elution and ^{68}Ga-radiolabeling

Gallium-68 (^{68}Ga: 89 %; EC β$^+$ max. 1.9 MeV, half-life: 68 min) was yielded from an 1850 MBq-loaded, tin-dioxide-based ^{68}Ge/^{68}Ga generator (iThemba LABS, Somerset West, South Africa). The eluate fractionation and the radiolabeling was conducted by adapting a previously described method.[21] Briefly, the ^{68}Ge/^{68}Ga-generator provided radioactivity concentrated in 2 ml 0.6 M HCL which was buffered with 2.5 M sodium acetate trihydrate to yield a selected pH range of 3.5-4.0 such that gallium is in its reactive state, the $[Ga(OH_2)_6)^{3+}]$ ion, which allows rapid complexation to the chelator molecule. CDP1 was dissolved in Millipore water to achieve a peptide stock concentration of 1 μg/μl necessary to develop the radiolabeling protocol. A 0.5 ml aliquot of buffered ^{68}Ga^{3+} (122 - 150 MBq) was mixed with 10-20 nmol CDP1, NODAGA (positive control) and LL37 (negative control) and was incubated at room temperature for at least 5 min followed by HPLC/UV analyses. ^{68}Ga-labeled c(RGDyK)-isothiocyanabenzyl-1,4,7-triazaclononane-1,4,7-triatetic acid (NOTA-RGD), an imaging agent for integrin receptor expression in cancer, was radiolabeled as previously described and employed as a radiolabeling performance reference (to reflect any variability in the generator eluate quality).[36]

Identification of the ^{68}Ga-CDP1 using UV/radio-HPLC analysis

For determination of the % RCP a reverse-phase HPLC column (Zorbax SB C18, 4.6 × 250 mm × 5 μm; Agilent Technologies, CA, USA) using a 5-95% A-B gradient (over 15 min) at a flow rate of 1 mL/min, was employed. Solvent A consisted of 0.1 % aqueous trifluoroacetic acid (TFA); solvent B utilized 0.1 % TFA dissolved in acetonitrile. The HPLC apparatus (Agilent 1200 series HPLC instrument, Agilent Technologies Inc., Wilmington DE, USA) contained a radioactive detector (Gina Star, Raytest, Straubenhardt, Germany) following radioactivity (counts per second) combined with a diode array detector following UV absorbance at 214, 220 and 240 nm. For determination of the radiolabeling efficiency (%LE) HPLC analysis was supported by control measures using radio-ITLC employing a silica-gel based solid phase and 0.1 M sodium citrate as mobile phase (free ^{68}Ga [Rf=0.8] and ^{68}Ga-CDP1 [Rf=0.2]). Both methods were utilized to detect ^{68}Ga-CDP1 and differentiate it from uncomplexed ^{68}Ga, potential radiolabeled by-products (^{68}Ga-NODAGA precursor), and any deteriorated peptide or fractionated LL37-NODAGA where possible. The retention times for ^{68}Ga, NODAGA, CDP1 and NOTA were established and used for identification.

Biostatistics

If not indicated otherwise, results are expressed as mean ± standard deviation of mean (SD). Paired and unpaired Student-t tests were performed to indicate significance to compare different parameters and P values ≤ 0.05 were considered significant.

## Results and Discussions

Synthesis of CDP1 and natGa-conjugation

The peptide LL37 was successfully synthesized with the coupling reagents DIC/OxymaPure in DMF using automatic microwave synthesis except for the coupling of 20th Ile residue in solid phase in which manual coupling in THF was necessary. The resultant product gave the desired m/z of 4493 using MALDI-TOF MS. The peptide functionalization with NODAGA was achieved in solid-phase using DIC/OxymaPure in THF at room temperature for 16 hrs to give the desired m/z of 4849 (Scheme 1). natGa was complexed to the CDP1 using sub-millimolar concentrations of $^{nat}Ga^{3+}$ as previously reported.[35] The complexation was confirmed by HRMS for natGa-CDP1.

Bacterial and Hepatocellular uptake of the natGa-CDP1

The results of the bacterial and hepatocellular uptake of the natGa-CDP1 are presented in Figures 1A and 1B. The uptake was studied at two temperatures 4 °C and 37 °C as previously reported for association/binding assays involving UBI incubation.[37, 38] In our previous publication we also used a fluorescent detection method comparing it with the radio-assay which provided similar in vitro results whilst developing natGa-NOTA-UBI as a tracer.[39] However, in the present study we used the supernatant to quantify the compound reduction on LC-MS, a very sensitive detection method which negates the requirement of a radio-assay. At lower temperature (4 °C), the bacterial cell growth will be slower. Slightly lower uptake of the conjugate natGa-CDP1 at 4 °C was observed compared to 37 °C. The conjugated peptide was also examined for its antimicrobial activity, which showed no bacteriostatic effects to *S. aureus, E. coli and M. smegmatis* at the concentrations applied in this study.

Uptake of natGa-CDP1 by *S.aureus*

The natGa-CDP1 uptake by the gram positive candidate, *S.aureus,* was determined immediately and compared after 1, 2 and 3 incubation periods at 4 °C and 37 °C. At 4 °C the concentration of the compound was found to be 78% in the supernatant after 3 hr. This result showed that 22% natGa-CDP1 was taken up by *S. aureus*. Likewise, at 37 °C the concentration of the conjugated peptide taken up was 20% after 3 hr.
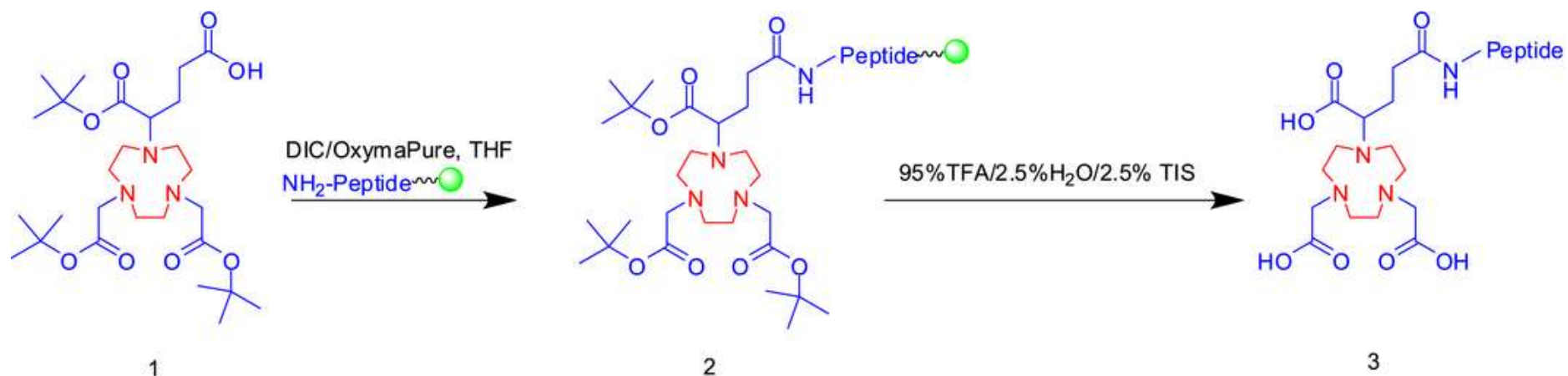
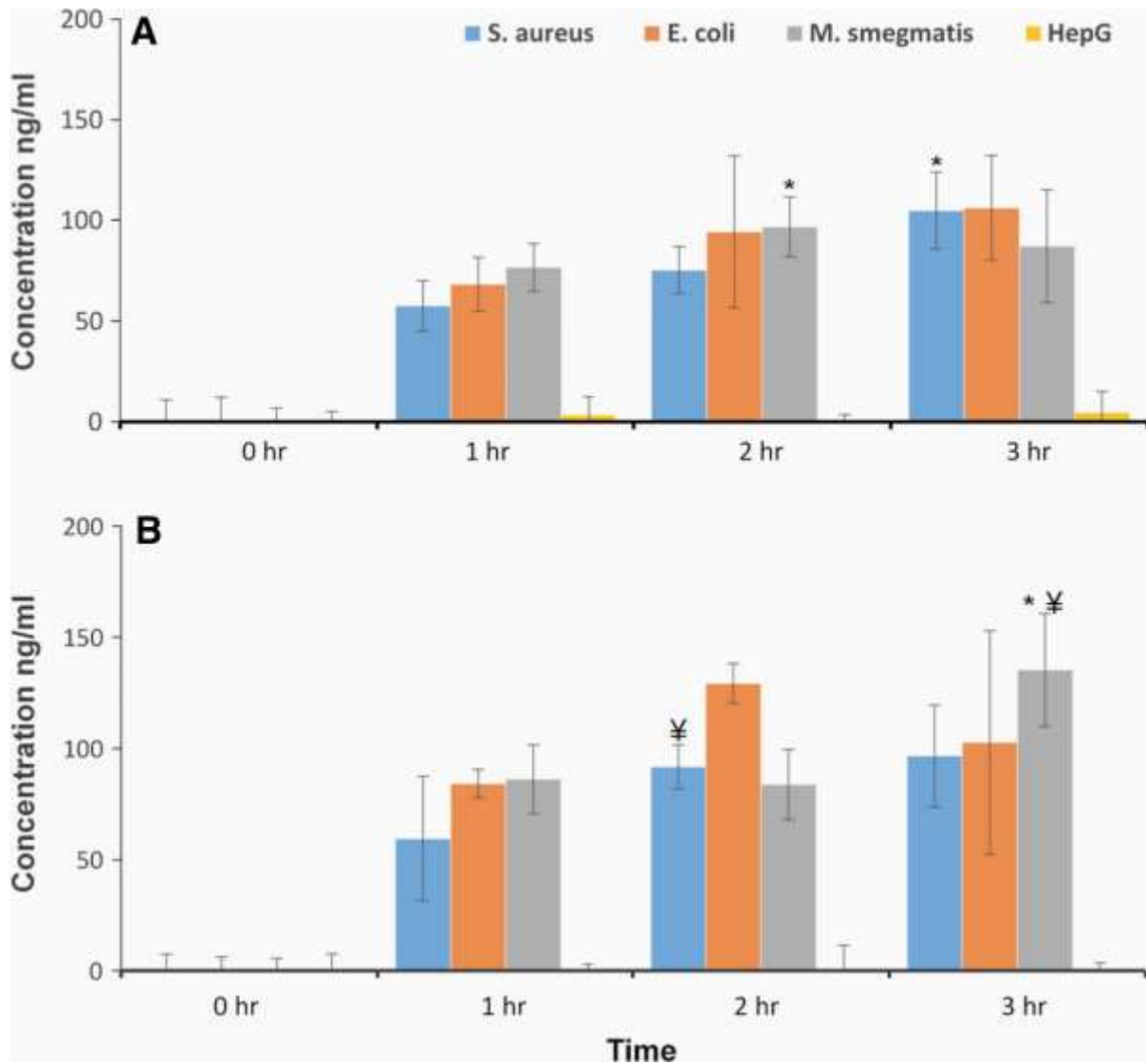**Scheme 1.** Synthesis of CDP1

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Figure 1. Comparison of natGa-CDP1 uptake by cellular *(HepG2)* and bacterial cells at (A) 4 °C and (B) 37 °C. Mean results ± SD (*n*=3) is presented. *Paired one-tailed Student-*t* test on the comparison of consecutive incubation times returned *p*<0.05. ¥) Paired one-tailed Student-*t* test comparing cells incubated at 4°C to 37°C returned *p*<0.05.

All of the data were found to be within the allowed error limit of analytical method development guideline.[40] This bacterial uptake result suggests selectivity of the peptide towards gram positive *S. aureus* over HepG2 cells.

Uptake of [nat]Ga-CDP1 by *E. coli*

The uptake of [nat]Ga-CDP1 by *E. coli* was also determined at the time points described in the previous section. The results revealed that 22% and 21% of the compound was accumulating in *E. coli* cells at 4 °C and 37 °C after 3 hrs of incubation, respectively. This positive uptake of [nat]Ga-CDP1 by *E. coli* cells opens the opportunity to explore the efficacy of this peptide to be used as a PET radiotracer for infection diagnosis against gram-negative bacteria.

Uptake of [nat]Ga-CDP1 by *M. smegmatis*

Acid fast *M. smegmatis* represents mycobacteria for this uptake study because it is non-pathogenic and it has fast replicating time when compared to other members of this family. This bacteria shares a similar cell wall structure and more than 2000 homologous genes with *M. tuberculosis*.[41] Normalization of the data at the 0 hr with the 3 hr incubation time points for the compound showed 18% and 28% uptake by this bacterial cell at 4 °C and 37 °C, respectively. These results indicate potential affinity of [nat]Ga-CDP1 towards *Mycobacterium sp.* and should be further explored.

Uptake of [nat]Ga-CDP1 by HepG2 cells

At both 4 °C and 37 °C, the amount of [nat]Ga-CDP1 determined in the supernatant of the HepG2 cells was >99% after 3 hr of incubation when normalized with the 0 hr time point (**Fig. 1A**). This result reveals that there was <1% uptake of the compound by the HepG2 cells regardless of the incubation temperature. In the same time, bacterial cells consumed 50-120 times more Ga-CDP1 as compared to HepG2 cells. This experiment reveals that the compound has negligible affinity towards the mammalian cells.

The *in vitro* results may allow translating the outcome for potential *in vivo* applications. The high degree of differentiation between bacterial and mammalian cell uptake may be reflected by the high targeting ability of [nat]Ga-CDP1, which may lead to potential detection of bacteria. The results show no significant difference in the uptake behavior between the bacterial strains evaluated, suggesting no bacterial selectivity of [nat]Ga-CDP1 towards bacterial identification. The quantified amounts of [nat]Ga-CDP1 were similar to these found for UBI29-41 and NOTA-UBI (currently in clinical trials) and higher than hLF (which was subsequently discontinued for tracer development).[37-39] In Addition, > ^{68}Ga-CDP1 nM would be injected to animals or humans which supports to conclude that approx. 100 ng/ mL of total compound associated with 10^5 cfu bacteria would be more than a desirable threshold concentration to proceed with animal

infection models (normally 10^8 cfu would be used in animal models of infection).[42] Naturally occurring infection sites in vivo may exceed these cfu counts.[43, 44] Moreover, the design of the study majorly served to aim, whether the tested compound will be bacteria-selective (against mammalian cells). This aim was met based on the results provided. It may also provide a preliminary idea – based on the degree of binding - of the compounds to bacteria of different (gram) status, especially if the bacterial envelope is suggested as a binding target for the compound (such as ^{68}Ga-CDP1). The details of this in vitro study design were adapted from a recently published paper.[42]

^{68}Ge/^{68}Ga-Generator elution and ^{68}Ga-radiolabeling

In ^{68}Ge/^{68}Ga-Generator elution, the fractionation method yielded 78 ± 5 % and 91± 4 % (360- 560 MBq) of the total elutable ^{68}Ga-activity in 1 and 2 ml, respectively. NODAGA and CDP1 were labeled successfully within 5 min at room temperature with a percentage radiochemical purity (% RCP) of > 88 % adapting conditions from a former study radiolabeling NOTA-UBI.[21] ^{68}Ga-CDP1 was stable in its formulation throughout the 60 min period tested. To the best of our knowledge, this is the first (documented approach presenting) report showing the successful ^{68}Ga-radiolabeling of this bioconjugate. The results suggest an optimization of the radiolabeling parameters (pH, CDP1 molarity and purification of the generator eluate) which was not considered within the scope of this study. As a reference peptide NOTA-RGD was radiolabeled successfully achieving >82 % radiolabeling efficiency (%LE).

Identification ^{68}Ga-CDP1

The chromatographic method was capable of confirming successful radiolabeling (peak integration) of ^{68}Ga-CDP1 (Figure 2). The retention times of 11.5-12 min allowed significant differentiation from the unbound buffered ^{68}Ga (2.3-3.5 min). Separately radiolabeled ^{68}Ga-NODAGA just used as a control was detected at retention times of 4- 4.2 min, but no notable ^{68}Ga-activity peak was detected during HPLC analyses of any ^{68}Ga-CDP1 samples at that retention time.

The reference radiolabeled peptide (^{68}Ga-NOTA-RGD) confirmed ^{68}Ga-labeling performance of the CDP1 system (elution of ^{68}Ga-NOTA-RGD was faster on the RP-HPLC column - 7.45 min), which was expected due to the increased polar character of NOTA-RGD compared to CDP1. The % LE for NOTA-RGD was well within the expected range, which indicates that the generator eluate quality was not a compromising parameter to yield the highest possible radiolabeling efficiency and yield. All retention times corresponded well with their respective UV peak maxima (wavelength ranged 212-260 nm). Following incubation with
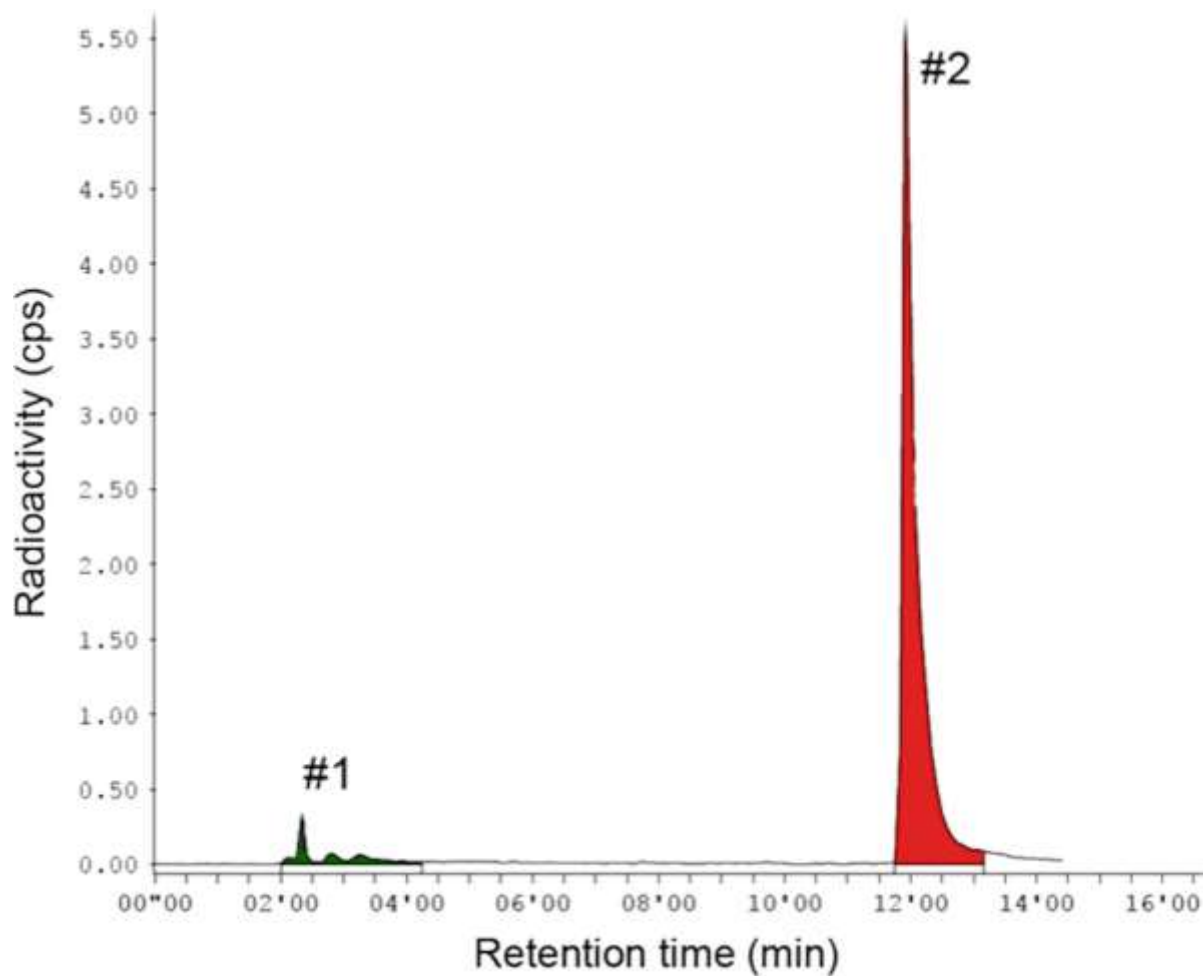
Figure 2. Representative radio-HPLC chromatogram showing successful differentiation of free [68]Ga (#1: 2.3-3.5 min retention) and ^{68}Ga-CDP1 (#2: 11.5-12 min retention). Quantification following radio-peak integration resulted in a 91% RCP for ^{68}Ga-CDP1.

[68]Ga, unfunctionalized LL-37 was detected at 10.50 min (UV 214 and 220 nm) showing no radioactivity peak at this retention time using radio-HPLC.

It should be noted that the use of NODAGA allows for radiolabeling at room temperature, which can be beneficial for radiolabeling heat-vulnerable peptides.[45] Based on this identification of radiolabeled ^{68}Ga-CDP1, further refinements can be carried out to optimize the radiolabeling technique aiming for both, quantitative complexation and thermodynamic stability of ^{68}Ga to CDP1. Radiolabeling of longer peptides and polypeptides may be significantly different in comparison to shorter peptide derivatives (those protocols are reported widely; i.e. for DOTA-TATE). The preliminary radiolabeling protocol was adapted from DOTA-TATE radiolabeling and amounted in sufficient yields to carry out the HPLC characterization of the ^{68}Ga-CDP1, however; to incubate bacterial cells in the presence of ^{68}Ga-CDP1 substantial optimisations of parameters such as specific activity, radiolabeling yield, solvent (ethanol) concentration have to be performed. A cellular assay using gamma-analytical detection methods would also be limited by the half-life of the radioisotope.

Conclusion

In this proof of concept study, we reported the functionalization of LL37 with NODAGA and its usability as a PET radiotracer for potential infection imaging using gallium. [nat]Ga-CDP1 showed significant affinity towards bacterial cells; it also has a low association with HepG2 cells, affording specific bacterial uptake. In conclusion, this complex can potentially be used as a candidate for preclinical studies in infection imaging.

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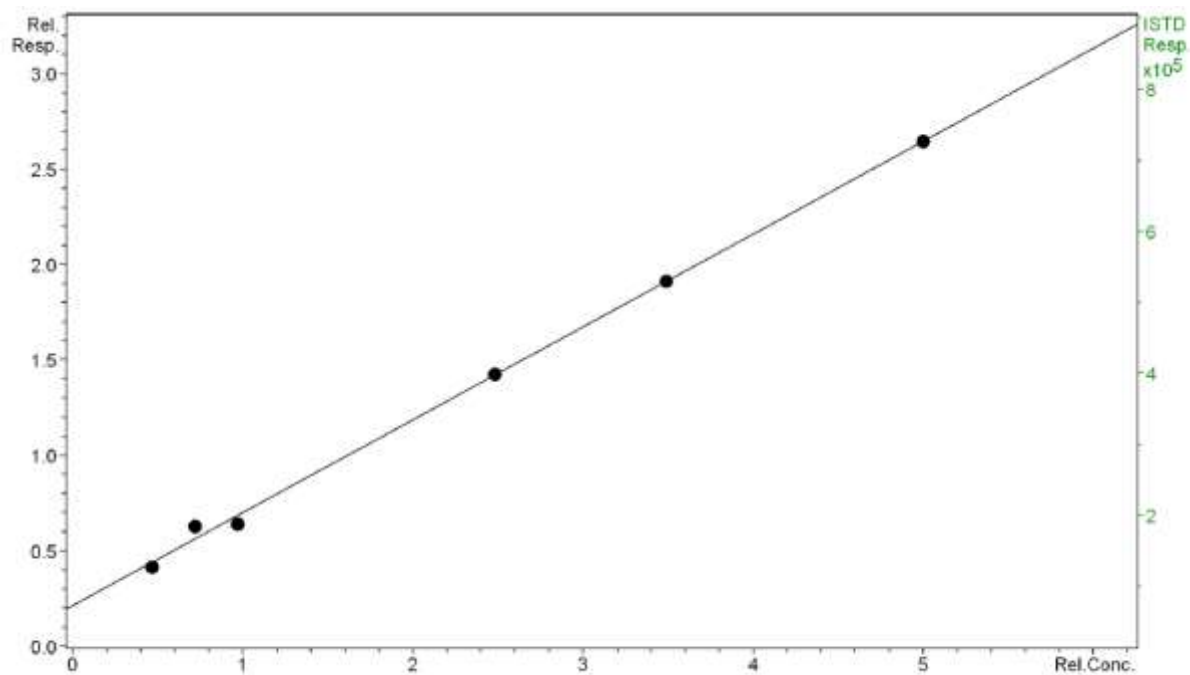
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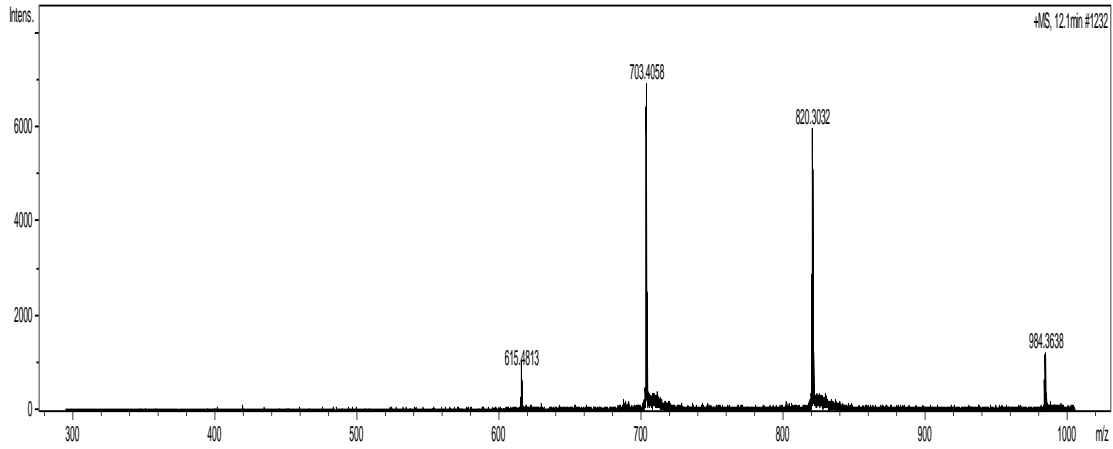
Supplementary figure 1. Product ion spectra of natGa-CDP1 showing 703,41 m/z, which was used for the quantitation of the analyte in bacterial and mammalian cells.
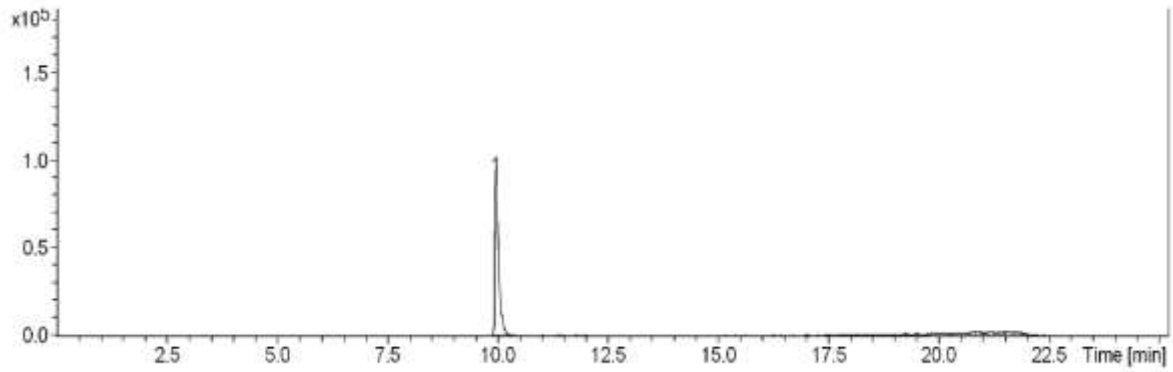
Supplementary figure 2. Typical chromatogram for natGa-CDP1, showing its retention at 10.1 min using a YMC Triart C18 column (150mm x 3.0mm; 3μm i.d.).

Supplementary figure 3. Calibration curve, created using QuantAnalysis (Bruker Daltonics, Bremen, Germany), used for the quantitation of ^{nat}Ga-CDP1.