

CHEMISTRY

A European Journal

A Journal of



Accepted Article

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Authors: Thomas Price, Steven Yap, Raphaël Gillet, Huguette Savoie, Loïc Charbonnière, Ross Boyle, Aline Nonat, and Graeme J. Stasiuk

This manuscript has been accepted after peer review and appears as an Accepted Article online prior to editing, proofing, and formal publication of the final Version of Record (VoR). This work is currently citable by using the Digital Object Identifier (DOI) given below. The VoR will be published online in Early View as soon as possible and may be different to this Accepted Article as a result of editing. Readers should obtain the VoR from the journal website shown below when it is published to ensure accuracy of information. The authors are responsible for the content of this Accepted Article.

To be cited as: *Chem. Eur. J.* 10.1002/chem.201905776

Link to VoR: <http://dx.doi.org/10.1002/chem.201905776>

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Evaluation of a bispidine-based chelator for gallium-68 and of the porphyrin conjugate as PET/PDT theranostic agent

Dr Thomas W. Price,^[a,b] Dr Steven Y. Yap,^[c] Dr Raphaël Gillet,^[d] Huguette Savoie,^[c] Dr Loïc J. Charbonnière,^[d] Prof. Ross W. Boyle,^{*,[c]} Dr Aline M. Nonat,^{*,[d]} Dr Graeme J. Stasiuk^{*,[a,b]}

Abstract: In this study a bispidine ligand has been applied to the complexation of gallium(III) and radiolabelled with gallium-68 for the first time. Despite its 5-coordinate nature, the resulting complex is stable in serum for over two hours, demonstrating a ligand system well matched to the imaging window of gallium-68 positron emission tomography (PET). To show the versatility of the bispidine ligand and its potential use in PET, the bifunctional chelator was conjugated to a porphyrin, producing a PET/PDT-theranostic, which showed the same level of stability to serum as the non-conjugated gallium-68 complex. The PET/PDT complex killed >90% of HT-29 cells upon light irradiation at 50 μM . This study shows bispidines have the versatility to be used as a ligand system for gallium-68 in PET.

Introduction

Positron emission tomography (PET) is a highly sensitive imaging technique with high tissue penetration.^[1-3] This technique can allow for the *in vivo* imaging of diseased tissues by targeting biochemical processes; thus allowing for detection of disease before physical changes occur. Recently, gallium-68 (⁶⁸Ga) has found significant interest as a PET radionuclide due to its generator based production allowing for ease of access.^[3, 5] Incorporation of ⁶⁸Ga into a radiotracer is typically achieved through the use of a chelator that complexes the PET isotope.^[3, 6, 7] When designing these chelators, special attention must be brought to the three following points: (i) they should form a unique radiolabeled complex, ideally in mild conditions; (ii) with high

kinetic/thermodynamic stability; and (iii) strong resistance to hydrolysis and to transchelation reactions, occurring in particular with transferrin, and to transmetallation or competition with metals such as Cu(II) or Zn(II). Despite numerous attempts and studies only a few ligands fulfill all the criteria.^[6, 8] These ligands are depicted in Figure 1. DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) and its conjugates (with octreotide and derivatives) are clinically in use for imaging neuroendocrine tumours^[9] although they require high temperatures and acidic conditions for radiolabeling.^{[10][11]} The triazacyclononane derivative (NOTA), its analogue with phosphinic pendant arms (TRAP) and the acyclic chelator H₂dedpa also need acidic conditions but no heating.^[6, 12, 14] All these chelators are however subject to competition with Cu(II) and Zn(II),^[15, 16] although TRAP displays an apparent improved selectivity for Ga(III).^[13] 6-amino-1,4-diazepine triacetate (DATA) chelators^[17] as well as siderophores (such as deferoxamine),^[18] N,N'-Bis(2-hydroxybenzyl)ethylenediamine-N,N'-diacetic acid (HBED)^[18] and tris(hydroxypyridinone) (THP)^[18] have the advantage to be radiolabeled in a wide pH range, THP being the most promising as quantitative radiochemical yields can be obtained in mild conditions.^[19, 20] Several THP-bioconjugates have been studied *in vivo*, demonstrating either very promising tumor/body ratio^[21, 22] or disappointing results.^[23, 24] These observations demonstrate that both radiocomplex and biological vector have a synergic effect on the biodistribution of the radiopharmaceutical.

For all these considerations, it is of high interest to investigate the potential of other families of ligands such as bispidines. Bispidines (chelators based on a 3,7-diazabicyclo[3.3.1]nonane core) are widely used chelators with a highly preorganised coordinating site (Figure 1).^[25, 26] They have been used to complex a range of metals, including radiometals such as ⁶⁴Cu, complexes of which were found to be remarkably inert.^[27, 28] Their application to Ga(III) complexation, and particularly ⁶⁸Ga complexation, remains relatively unexplored.^[29]

The field of theranostics aims at developing agents with combined therapeutic activity and diagnostic properties in a single agent.^[30] Such theranostic agents allow for monitoring the uptake and real-time distribution of the therapeutic agent, the progression of the disease, as well as the therapeutic response. This enables an individualized treatment strategy which has been shown to be very efficient in selecting the optimal treatment, limiting adverse reactions, and implementing optimal dosing regimes.^[31, 33] In the ← systems to the tumor are considered as a very promising alternative to conventional treatment, the effectiveness of which is limited by their absence of specificity. For these drugs, triggered by externally applied stimuli (e.g. radiation or light), monitoring their uptake and distribution is vital to optimize their application as

- [a] Dr T.W. Price, Dr G.J. Stasiuk
School of Life Sciences, Faculty of Health Sciences,
University of Hull,
Cottingham Road, Hull, HU6 7RX, UK
E-mail: G.Stasiuk@hull.ac.uk
- [b] Dr T.W. Price, Dr G.J. Stasiuk
Positron Emission Tomography Research Center,
University of Hull,
Cottingham Road, Hull, HU6 7RX, UK
- [c] Dr S.Y. Yap, H. Savoie, Prof. R.W. Boyle
Chemistry, School of Mathematical and Physical Sciences,
University of Hull,
Cottingham Road, Hull, HU6 7RX, UK
E-mail: R.Boyle@hull.ac.uk
- [d] Dr R. Gillet, Dr L.J. Charbonnière, Dr A.M. Nonat
Equipe de Synthèse pour l'Analyse (SynPA),
Université de Strasbourg,
CNRS, IPHC UMR 7178, F-67000 Strasbourg, France
E-mail: aline.nonat@unistra.fr

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it will allow for the appropriate timing of their trigger, hence maximizing their effectiveness.

Photodynamic therapy (PDT) is an example of such a therapeutic technique in which a drug is administered, and then an external trigger (in this case irradiation with a high intensity light source, such as a laser) causes the therapeutic effect. PDT agents are typically photosensitizers that are activated by absorption of visible light which first populate their excited singlet state and then, after energy transfer, their long-lived excited triplet state. This triplet state can undergo photochemical reactions in the presence of oxygen to form reactive oxygen species (ROS), including singlet oxygen.^[34] The localized production of these highly reactive species in the diseased tissues further causes the destruction of the neoplasm. Thus, PDT can allow for highly targeted toxicity with minimal off-target toxicity by only irradiating the target tissue.^[34] Tetrapyrrole structures such as porphyrins, chlorins, bacteriochlorins and phthalocyanines derivatives have been widely investigated in PDT.^[35] Porphyrins have been selected in this study due to their low toxicity, high phototoxicity, ease of synthesis, and innate tumour targeting properties.^[34,36,40]

In view of the multimodal biomedical applications of metalloporphyrin, and in particular for PET/PDT purposes, ⁶⁸Ga has also been used previously with porphyrins.^[41,47] Work in this area started with the incorporation of ⁶⁸Ga into the porphyrin core directly; however this involved vigorous heating using a microwave for efficient radiolabeling.^[41,44] Furthermore, this prevents the incorporation of other metals into the porphyrin cavity, reducing the options available for optimizing the system.^[48] More recent work has therefore involved conjugating a chelator to the porphyrin and then radiolabeling the chelator.^[45,46] First experiments were performed using DOTA and NOTA. However, although they are commonly used for ⁶⁸Ga complexation, they were only poorly labelled when conjugated to the porphyrin.^[45] The acyclic chelator H₃Dpa was able to be readily radiolabeled with ⁶⁸Ga when conjugated to a porphyrin;^[46] however, the resulting complex was insufficiently stable.^[46,49] As for bioconjugates, there is a need of finding a good chelator/porphyrin match in order to optimize both radiolabeling and PET imaging properties and PDT efficiency.

In this study we investigated the ability of a bispidine chelator to complex Ga(III) and to be radiolabeled with ⁶⁸Ga. Further, we conjugated this chelator to a water soluble porphyrin to produce a PET/PDT theranostic agent.

Results and Discussion

Complex Synthesis: The bispidine ligand, L₁, was prepared as previously described.^[28] This pentadentate ligand is expected to coordinate to Ga(III) via the two ternary amines of the diazabicyclononane, the two nitrogen of the pyridyl groups and the acetate arm, in a similar fashion to that observed with Zn(II) for an analogue of L₁ bearing a glycine substituent instead of the (L)-lysine.^[27] Complexation of Ga(III) by L₁ was performed at pH 4.5 at reflux (Scheme 1). Upon complexation of Ga(III), the pyridyl protons are significantly deshielded (Figure 2), with a downfield

shift of 0.3-0.8, indicating the donation of electrons from the ring due to complexation of the cationic metal. The methyl group attached to the amine of the bispidine ring is also significantly deshielded (CH₃ δ_H = 0.9 ppm); this suggests that the amine of the ring is involved in complexation. The proton alpha to the carboxylate of the lysine unit (H₁₀) is also greatly shielded; with an upfield shift of 1.2 ppm (Figure S1).

The backbone of the cyclic structure is also locked in place, as by the coordination of the carboxylate giving an asymmetric nature evidenced by many of the resonances corresponding to protons in these environments; the resonances of H₂ and H₄ are distinct; these are also significantly deshielded compared to the analogous resonances in the free ligand with an upfield shift of 1.1 ppm. The proton at the apex of the ligand ring structure (H₉) is also deshielded by 0.7 ppm. Protons in the 6 and 8 positions show geminal coupling of 13.5 Hz and are also deshielded.

Radiochemistry: Radiolabeling of L₁ with ⁶⁸Ga was followed by radio-TLC. Achieving a high radiochemical yield required heating due to the rigid nature of the chelator. Furthermore, an acidic pH was required for effective radiolabeling; pH 4 was found to be optimal (Figure 3a), likely due to the formation of kinetically inert Ga(III) hydroxides at higher pHs.^[4,50] A relatively high ligand concentration was also required; a radiochemical yield of 89% was achieved at a ligand concentration of 100 μM whereas at 200 μM a radiochemical yield of 94% was achieved (Figure 3b). These results are comparable to those previously reported for macrocyclic chelators such as DOTA, which shows a 95% RCY under similar conditions (upon heating at 85 °C at pH 4.0 for 30 min).^[3,51,52] In terms of complexation kinetics and radiolabeling efficiency, ligand L₁ is not as efficient as NOTA (95% RCY are obtained with no heating at pH 3.5 for 10 minutes with a ligand concentration of 100 μM) and the phosphinic analogue TRAP,^[13] which can be radiolabeled at much lower concentrations (c < 3 μM, 5 min, pH 3.2) at 95°C or even at room temperature when using a large excess of ligand. A similar trend is observed, when comparing to acyclic ligands such as THP (5 min, pH 6.5 at 25°C), H₂dedpa (5-10 min, pH 4.5 at 25°C) and H₃dpa (5-10 min, pH 4.5 at 25°C) (concentration). These differences are not surprising when looking at the chemical structure of ligand L₁, which is a pentadentate ligand and therefore not optimized for Ga(III) complexation in terms of kinetic, selectivity and thermodynamic stability.

However, based on previous observations with ⁶⁴Cu-analogues,^[53] promising results in terms of kinetic inertness were expected when using a bispidine scaffold. This was indeed the case when assessed for radiochemical stability against foetal bovine serum (FBS) no decomplexation was observed over 2 hours incubation at 37°C (Figure S5). This is the ideal imaging window time for gallium-68 half-life, thus showing that the bispidine chelator is suitable for translation to *in vivo* PET applications with ⁶⁸Ga. In addition, it is foreseen that radiochemical yields and labelling conditions may be further improved by utilizing other bispidine derivatives, in particular with hexadentate coordination mode.

Conjugation to porphyrin: To show the bispidine ligand can be utilized for applications in PET, not only as a chelator for gallium-68 but as a functional tool, we conjugated the ligand to a water-soluble porphyrin to produce a PET/PDT theranostic agent. L₁

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was coupled to a water soluble porphyrin through the terminal lysine residue. The NHS-ester of the water soluble porphyrin, **L**₂, was prepared as previously described^[46,54,55] and the amide bond formation was undertaken in DMF. Following semi-preparative HPLC purification, the desired bispidine-porphyrin conjugate, **L**₃, was obtained in a 48% yield (Scheme 2). Conjugate formation was confirmed by mass spectrometry ($m/z = 405.3$ [M]³⁺, Figure S11).

It is evident from the ¹H NMR (Figure S9) that the product contains both porphyrin and bispidine moieties; the aromatic porphyrin ¹H resonances, $\delta_{\text{H}} = 9.46, 9.04$ and 8.29 for the three pyridyl units and the beta hydrogens of the porphyrin ring. The bispidine is evident through the additional aromatic resonances, corresponding to 8 protons, at 7.48 and 8.70 due to the pyridyl arms.

Conjugate complexation reaction: Complexation of Ga(III) was undertaken under the same conditions as for **L**₁. Evidence for complexation was obtained from the ¹H NMR of the complex due to the increased shielding of the bispidine pyridyl protons (downfield shift of 0.3 ppm). The retention of the protons within $\delta_{\text{H}} = -3.08$ confirms that complexation did not take place within the porphyrin ring (Figure S12). While complexation of Ga(III) by porphyrins has been previously reported,^[42] this required more forcing conditions such as microwave heating and as such complexation within the porphyrin ring was not expected.

Radiolabelling of conjugate: Radiolabelling of the conjugate, **L**₃, was achieved under the optimized conditions determined for the ligand **L**₁. Complete complexation of the ⁶⁸Ga was achieved by $200 \mu\text{M}$ **L**₃ at pH 4.5 within 15 minutes when heated to 95°C . This radiolabelled conjugate was assessed for its stability in FBS. All of the activity was retained within the complex over 2 hours (Figure S15). As a control, the porphyrin **L**₂ was radiolabeled under the same conditions; radiochemical yields $<30\%$ were achieved, showing that the conjugate **L**₃ has a selectivity for ⁶⁸Ga in the bispidine chelator, with a stability to FBS within the PET imaging window.

Phototoxicity: To assess the viability of this system as a potential theranostic agent, the photo- and cytotoxicities of both the conjugate, **L**₃, and the Ga(III) complex, [**Ga(L**₃)], were assessed in human adenocarcinoma (HT-29) cells (Figure S16). Cells were incubated with either **L**₃ or [**Ga(L**₃)] at varying concentrations and irradiation was carried out using a constant dose of visible light (20 J cm^{-2} ; $400\text{-}700 \text{ nm}$). The results were compared to a non-irradiated control. Although in a clinical setting red light is more commonly used for PDT, clinical lasers used for PDT are significantly more powerful than the quartz tungsten halogen light source used in this study. To compensate for the lower power, white light was used covering the whole porphyrin absorbance band including the strong Soret band at 422 nm .

Under these conditions, $>90\%$ cell death was seen at a concentration of $50 \mu\text{M}$ for [**Ga(L**₃)] when irradiated (Figure 4). Minimal dark toxicity was observed at all concentrations tested. This shows phototoxicity at a similar concentration to Photofrin®, a clinically relevant porphyrin PDT agent, in HT-29 cells.^[56]

Conclusions

We describe the application of a bispidine ligand, **L**₁, to the complexation of Ga(III). Furthermore, we demonstrate that this ligand can be successfully radiolabeled with ⁶⁸Ga producing a serum stable complex for the first time. Radiolabeling required high temperature (95°C) and concentrations ($200 \mu\text{M}$) to achieve near-quantitative yields (94%). Although a higher ligand concentration than traditional chelators for gallium-68, further optimization of the denticity of the ligand and of the functional groups attached to the bispidine core may improve upon this in the future.^[25]

The bifunctional bispidine, **L**₁, was conjugated to a water-soluble porphyrin; the resulting conjugate, **L**₃, was also applied to gallium(III) complexation and radiolabeling was achieved under the same conditions as for **L**₁. These conditions are milder than those previously reported for insertion of ⁶⁸Ga into the porphyrin core as microwave heating was not required. Furthermore, ¹H NMR analysis of the Ga(III) complex confirms the presence of the protons within the porphyrin ring; this confirms that the radiolabeling is taking place at the chelator site and not at the porphyrin site. This will allow for future developments of this system to potentially incorporate alternate metals into the porphyrin ring.

L₃ and [**Ga(L**₃)] were shown to have low toxicity in the absence of light. Upon irradiation these systems were significantly more toxic with over 90% of HT-29 cells being killed by $50 \mu\text{M}$ of [**Ga(L**₃)], and 79% by **L**₃, upon irradiation.

This work demonstrates the viability of the bispidine framework for Ga(III) complexation and radiolabeling with ⁶⁸Ga for applications in PET imaging. The combination of bispidine and porphyrin produces a PDT agent that can be effectively radiolabeled with ⁶⁸Ga to produce a serum stable theranostic probe for PET/PDT.

Experimental Section

NMR spectra were recorded on a JEOL ECP 400 MHz/JEOL Lambda 400 MHz spectrometer using the residual protic solvent signal as an internal reference. Chemical shifts (δ) are given in ppm (ppm) and coupling constants (J) are given in Hertz (Hz). Mass spectrometry data were obtained from the EPSRC National Mass Spectrometry Facility at Swansea University. UV-vis spectroscopy was carried out on a Varian Cary 50 Bio UV-vis spectrophotometer. pH measurements were carried out using a Jenway model 3520 pH/mV/temperature meter with a three point calibration. All commercially available starting material used in synthesis were obtained from Sigma Aldrich, Fluorochem, and Alfa Aesar and were used without further purification. Deionised water was obtained from a Millipore Milli-Q reagent water system. All solvents were obtained from Fisher Scientific and VWR. HPLC analysis were performed on Agilent HPLC system. The column was a $150 \times 4.6 \text{ mm}$ (Phenomenex, UK) at a flow rate of 1 mL min^{-1} , with a mobile phase consisting of 0.1% TFA in water (solvent A) and 0.1% TFA in acetonitrile (solvent B). Gradient [time/min](solvent A:solvent B): [0-2](95:5). [2-3](95:5 . 78:22). [3-23](78:22 . 77:23). [23-24](77:23 . 70:30). [24-25](70:30 . 5:95). [25-26](5:95). [26-27](5:95 . 95:5). [27-30](95:5).

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Radiochemistry: The IGG100 generator was eluted with 0.6 M aq. HCl (3 mL). This eluate (300. 200 MBq) was diluted with H₂O (15 mL) and passed through a Strata-X-ÔÁHÁ T ÁÔÁÁ ÁT ÁÁ-á-mode polymeric support. The activity was liberated from the column using 98:2 acetone:0.1 M aq. HCl (1 mL). Aliquots (~30 MBq) of this solution were dried under a stream of inert gas at 90 °C and allowed to cool before use. 100 µL of ligand solution was added to the dried ⁶⁸Ga and analysis by TLC. TLC analysis was performed on Kieselgel 60 F₂₅₄ radiolabelling solution was added to 1.5 mL of foetal bovine serum and incubated at 37 °C. Aliquots were taken every 30 minutes for TLC analysis.

Cytotoxicity assays: A stock solution was made by dissolving L₃ or [Ga(L₃)] in medium (2 mL). The stock was sterilized by filtration through a Millex filter unit (Millex-GP). The concentration of the stock was calculated by UV-vis spectroscopy using the extinction coefficient of the conjugate. The stock was diluted further into appropriate cells (HT-29 colon adenocarcinoma, adjusted to a concentration of 1x10⁶ cells /ml in medium with L-glutamine, was seeded into 96 well plates (Falcon). The cells were allowed to incubate in the dark for 1 hour at 37 °C and 5% CO₂, after which they were centrifuged with 3x excess of medium to remove unbound L₃ or [Ga(L₃)]. The pellet of cells was resuspended in 100 µL of medium. The concentration of the radiolabelled cells was put in two 96 wells plates. One plate was irradiated with white light to a dose of 20 J cm⁻² while the other serves as a dark control. 100 µL of foetal bovine serum (FBS) was added to each well and the plates are returned to the incubator overnight. After 18 to 24 hours, the cell viability was determined using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. Cells were seeded into 96 well plates (100 µL/well) and incubated between 1 and 4 hours at 37 °C to allow MTT uptake. The cells were then washed with 100 µL of acid-alcohol mixture (0.04M HCl in absolute 2-propanol). The absorbance at 570 nm was measured on a Biotek ELX800 Universal Microplate Reader. The results were expressed with respect to control values.

Synthesis of [Ga(L₁): L₁ (5 mg, 42 µmol) and L₂ (5 mg, 80 µmol) were taken up in dry DMF (5 mL) and triethylamine (50 µL, 360 µmol) was added. The reaction was allowed to proceed at room temperature overnight protected from light. Solvent was removed under reduced pressure and the reaction was purified using semi-preparative HPLC. The white solid was washed with acetonitrile before being dissolved in 1:1 Acetonitrile:water, filtered, and dried to give a white solid (18 mg, 90%). ¹H NMR (400 MHz, D₂O, 298 K), δ: 8.75 (br s, 2 H, H_a), 8.37 (t, 1 H, H_c, ³J_{HH} = 7.8 Hz), 8.31 (t, 1 H, H_c, ³J_{HH} = 7.6 Hz), 8.02 (d, 1 H, H_b/H_d, ³J_{HH} = 7.8 Hz), 7.81 (7.92 (m, 3 H, H_b, H_d), 5.52 (s, 1 H, H₂/H₄), 5.48 (s, 1 H, H₂/H₄), 4.48 (s, 1 H, H₉), 3.41 (d, 1 H, H₁₀, ³J_{HH} = 8.9 Hz), 3.36 (d, 1 H, H₆/H₈, ²J_{HH} = 13.7 Hz), 3.14 (d, 1 H, H₆/H₈, ²J_{HH} = 13.3 Hz), 2.88 (br s, 2 H, H₁₄), 2.67 (d, 1 H, H₆/H₈, ²J_{HH} = 13.5 Hz), 2.59 (d, 1 H, H₆/H₈, ²J_{HH} = 13.5 Hz), 2.45 (s, 3 H, -CH₃), 1.70 (br s, 1 H, -CH₂-), 1.59 (br s, 4 H, -CH₂-), 1.29 (br s, 1 H, -CH₂-). ¹³C NMR (100 MHz, D₂O, 298 K), δ: 175.14, 171.35, 171.14, 151.31, 151.04, 147.09, 144.50, 128.21, 128.03, 70.58, 68.69, 64.29, 63.79, 57.81, 51.55, 51.26, 50.79, 46.16, 38.99, 26.68, 24.52, 22.83. MS (ESI) *m/z* 297.58 [M]²⁺.

Synthesis of L₃: L₂ (50 mg, 42 µmol) and L₁ (50 mg, 80 µmol) were taken up in dry DMF (5 mL) and triethylamine (50 µL, 360 µmol) was added. The reaction was allowed to proceed at room temperature overnight protected from light. Solvent was removed under reduced pressure and the reaction was purified using semi-preparative HPLC.

The solvent was concentrated under reduced pressure, washed with diethyl ether, and dried under vacuum. The residue was precipitated by addition of diethyl ether over a methanol solution to yield a purple solid (27 mg, 20 µmol, 48%). ¹H NMR (400 MHz, DMSO-*d*₆) δ: 9.46 (s, 6H, porphyrin-m-Py), 9.16-9.10 (m, 6H, bispidine-Py), 8.81-8.52 (m, 2H, bispidine-Py), 8.29 (d, *J* = 16.4 Hz, 4H, porphyrin-o,m-Ph), 7.00-7.08 (m, 6H, bispidine-Py), 5.02 (br s, 2H), 4.69 (s, 9H, N-CH₃), 3.95 (2, 1H), 3.60-3.43 (m, 4H), 3.20-2.94 (m, 4H), 2.28-1.51 (m, 8H), -3.06 (s, 2H, NH). ¹³C NMR (100 MHz, DMSO-*D*₆) δ: 204.09, 198.95, 198.73, 172.05, 166.35, 158.37, 158.06, 156.99, 149.46, 148.08, 144.76 (C), 137.45, 135.04, 132.66 (C), 127.03, 126.58, 126.22, 123.33, 123.19, 122.42, 119.43, 118.86, 116.43, 115.26, 75.01, 74.27, 67.65, 62.26, 61.38, 58.60, 50.57, 48.29, 44.07, 43.32, 42.06, 29.27, 19.70. MS (ESI) *m/z* 405.3 [M]³⁺, HRMS (ESI) *m/z* 405.1727 (calculated for C₇₁H₆₇N₁₂O₈, 405.1729). UV-Vis (H₂O), *nm*: 422, 519, 558, 582, 639. ε(422 nm) = 258300 M⁻¹ cm⁻¹.

Synthesis of [Ga(L₃): L₃ (1 mg, 0.76 µmol) was dissolved in water (0.4 mL). To this solution was added acetate buffer (pH 4.5, 1 M, 50 µL) and a solution of GaCl₃ (70 µL, 57 mM, 4.0 µmol). The reaction was allowed to proceed overnight at 100 °C. Concentration of the reaction solution, followed by purification by semi-preparative HPLC, yielded the product as a purple solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 9.49 (s, 6H, porphyrin-m-Py), 9.23-8.67 (m, 16H, bispidine-*U*-porphyrin-o-Py), 8.62 (s, 1H, bispidine-Py), 8.42-8.11 (m, 6H, bispidine-Py, porphyrin-o,m-Ph), 7.94-7.64 (m, 3H, bispidine-Py), 5.48-4.99 (m, 2H), 4.69 (s, 9H, N-CH₃), 4.04 (s, 1H), 3.10-2.52 (m, 4H), 2.36-0.70 (m, 12H), -3.08 (s, 2H, NH). MS (ESI), *m/z* 433.8 [M-3Cl+H₂O]³⁺, HRMS (ESI), *m/z* 433.8077 (calculated for C₇₁H₆₆GaN₁₂O₈, 433.8107). UV-Vis (H₂O) *nm*: 422, 519, 556, 585, 640. ε(422 nm) = 240000 M⁻¹ cm⁻¹.

Keywords: Gallium-68 Bispidine PET PDT Theranostic

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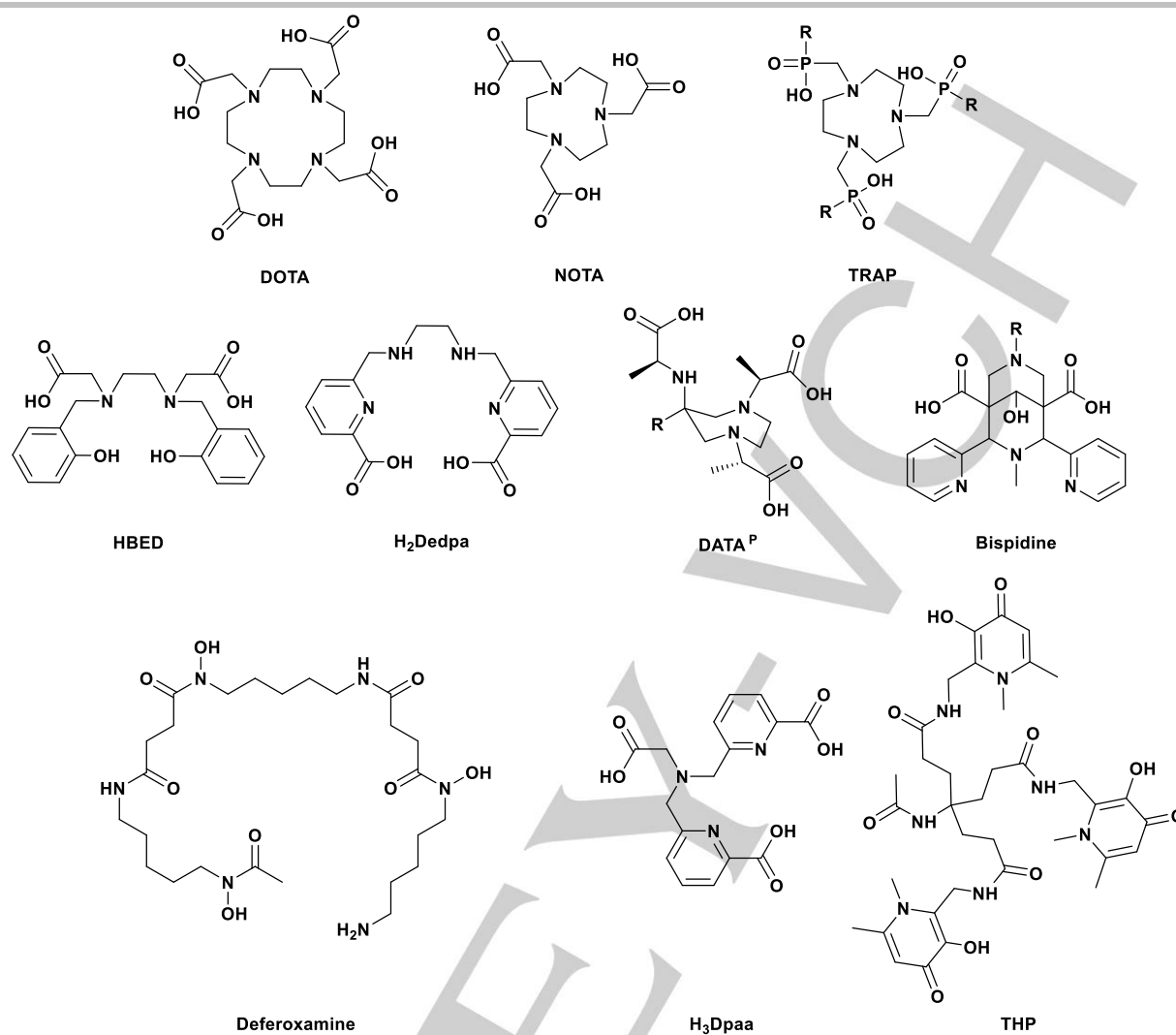
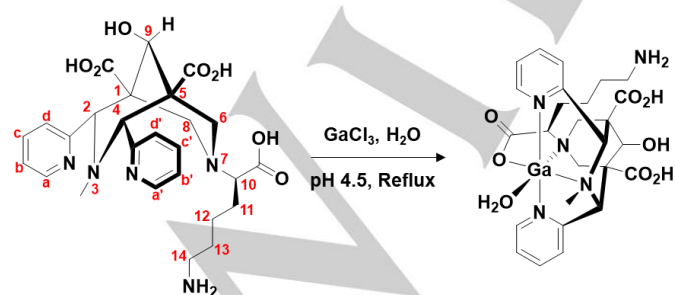


Figure 1. Ligands discussed

Scheme 1. Complexation of Ga(III) by bispidine L₁.

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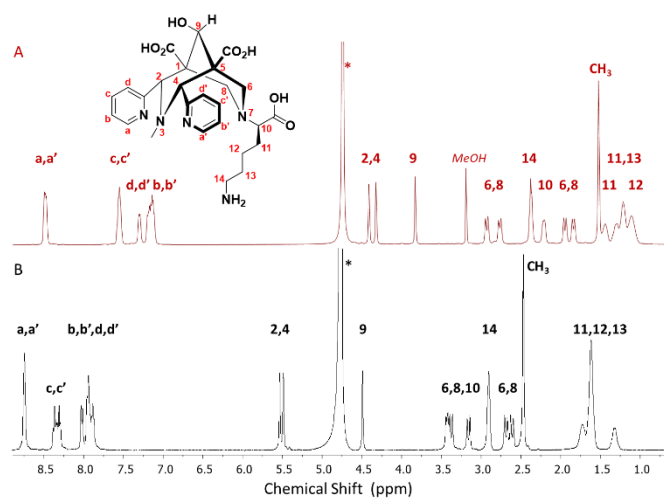


Figure 2. ^1H NMR (400 MHz, 298 K) of A) L_1 (d_4 -MeOH) and B) $[\text{Ga}(\text{L}_1)]$ (D_2O , $\text{pD} = 5.4$). Figure Caption.

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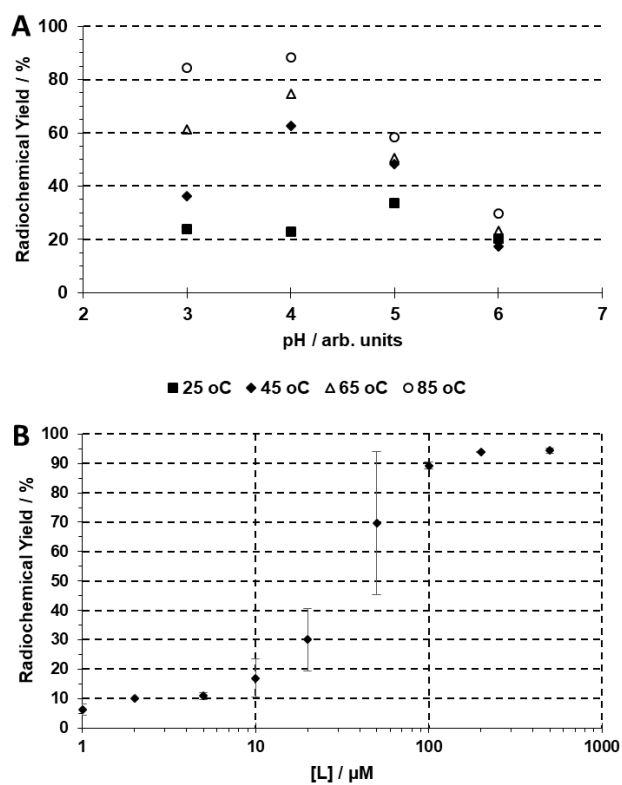
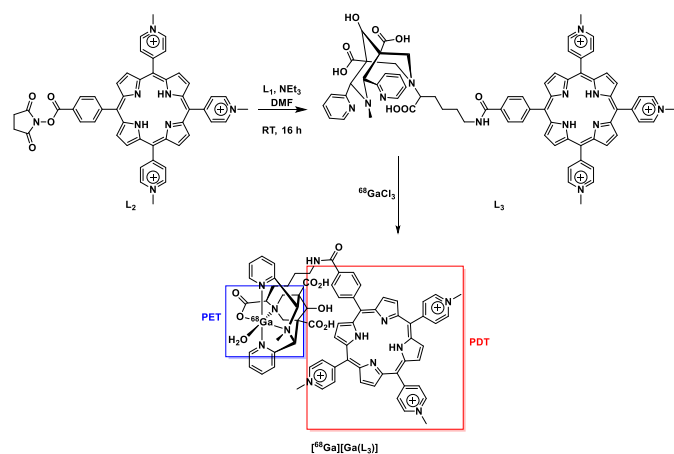


Figure 3. Radiolabelling of L_1 with ^{68}Ga . A) Effect of pH and temperature on radiolabelling. $[L_1] = 100 \mu\text{M}$, $t = 15$ minutes, $l = 0.1 \text{ M NH}_4\text{OAc}$. B) Effect of concentration on radiolabelling. $pH = 4$, $T = 95 \text{ }^\circ\text{C}$, $t = 15$ minutes, $l = 0.1 \text{ M NH}_4\text{OAc}$.

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Scheme 2. Synthesis and radiolabelling of L_3 to produce a PET/PDT theranostic agent.

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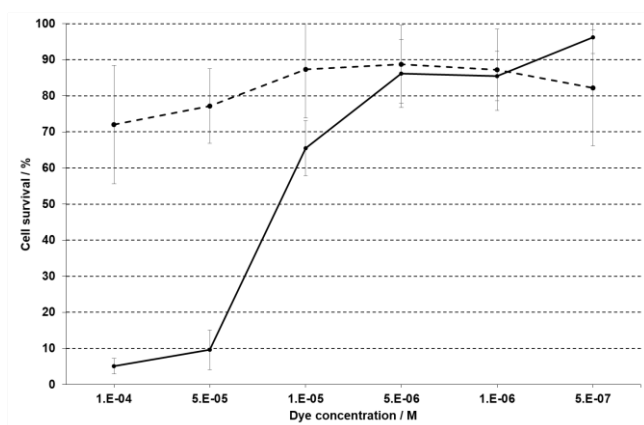


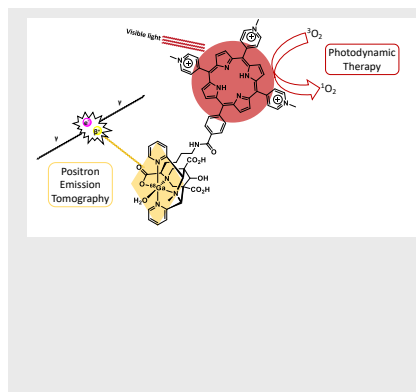
Figure 4. Toxicity of $[\text{Ga}(\text{L}_3)]$ in HT-29 cells as measured by MTT assay. Solid line indicates irradiated toxicity, dashed line indicates non-irradiated toxicity.

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In this study a bispidine ligand has been applied to the complexation of gallium(III) and radiolabelled with gallium-68 for the first time. The resulting complex is stable in serum for over two hours, showing a ligand system perfectly matched to the imaging window of PET. To show the versatility of ligand the bifunctional chelator was conjugated to porphyrin, producing a PET/PDT-theranostic. This killed >90% of HT-29 cells upon light irradiation at 100 μ M.



Dr Thomas W. Price,^[a,b] Dr Steven Y. Yap,^[c] Dr Raphaël Gillet,^[d] Huguette Savoie,^[c] Prof. Loïc J. Charbonnière,^[d] Prof. Ross W. Boyle,^[c] Dr Aline M. Nonat,^[d] Dr Graeme J. Stasiuk^[a,b]

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Title: Evaluation of a bispidine-based chelator for gallium-68 and of the porphyrin conjugate as PET/PDT theranostic agent

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