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Original

The radiometal makes a difference. Synthesis and preliminary characterisation of DOTA-minigastrin analogue complexes with Ga, Lu and Y

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Abstract

BACKGROUND: The minigastrin analogue — CP04: DOTA-$(DGlu)_6$-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2 has been developed for CCK2R targeting. This analogue can be radiolabelled with ^{111}In or ^{68}Ga for imaging, or with ^{90}Y and ^{177}Lu for therapy. However, affinity of the chelator-peptide conjugates to the cell membrane receptors may vary depending on the metal incorporated into the complex. So far, there are no such studies for the ligands of gastrin/cholecystokinin receptor CCK2R. It is supposed that the reason for the differentiation of receptor affinity to the respective receptors is in the changes of structure of chelating system and their influence on the bioactive conformations of the metal conjugated peptides. Herein, we report on the radiolabelling of CP04 with ^{90}Y, ^{177}Lu and ^{68}Ga and synthesis of cold CP04 complexes with respective stable metals for further structural and physico-chemical and biological studies.

MATERIALS AND METHODS: From 200 to 600 MBq of ^{90}Y, ^{177}Lu or ^{68}Ga were used for radiolabelling of 20 μg of CP04 dissolved in ascorbic acid solution (50 mg/mL, pH 4.5). Non-radioactive complexes with Lu and Ga were synthesized in milligram amounts starting from 0.5 mg up to 5 mg of CP04 dissolved in ascorbic acid solution (50 mg/mL, pH 4.5) when using 2-molar excess of the metal ions. Complex formation needed 5 min in microwave oven or 12 min in thermo-block at 95°C. RP-HPLC isocratic method (Kinetex 150/4.6 mm; 25% AcN/0.1% TFA, 1 mL/min) with UV/Vis and radiometric detection was developed for investigation of the radiolabelled and "cold" complexes. For LC-MS investigations, HPLC method was modified replacing TFA by formic acid.

RESULTS AND DISCUSSION: Yields of CP04 radiolabelling were greater than 90% for all three radionuclides. The HPLC method enabled identification of these radio-complexes based on comparison to their non-radioactive equivalents. In all cases, chromatograms revealed peaks that could be attributed to the metal-CP04 complexes and to impurities (including methionine oxidation). LC-MS analysis of Ga and Lu complexes revealed conformity of the observed molecular ions to the predicted formulas (m/z 2116 and 2220 Da for Ga and Lu, respectively). Different chromatographic behaviour observed for Ga-CP04 complex comparing to Lu- and Y- labelled peptide (relative retention to CP04: 1.08, 0.86 and 0.85, respectively) suggest different coordination of the metal ions. Therefore, further studies are planned using the non-radioactive complexes in order to assess their structural conformations.

KEY words: minigastrin, CCK2 receptors, complexes, ^{68}Ga, ^{177}Lu, ^{90}Y, radiochemical synthesis, HPLC, LC-MS

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Background

The cholecystokinin receptor type 2 (CCK-2R) is overexpressed in MTC with very high density and incidence over 90%, as revealed by autoradiographic studies [1]. According to these findings suitable tracers for targeting this particular receptor were developed. From the late 90s last century, a variety of CCK-2/gastrin related peptides (members of the gastrin- and cholecystokinin families, or possessing characteristics of both), were radiolabelled and studied in vitro (in terms of proper peptide sequence synthesis, stability

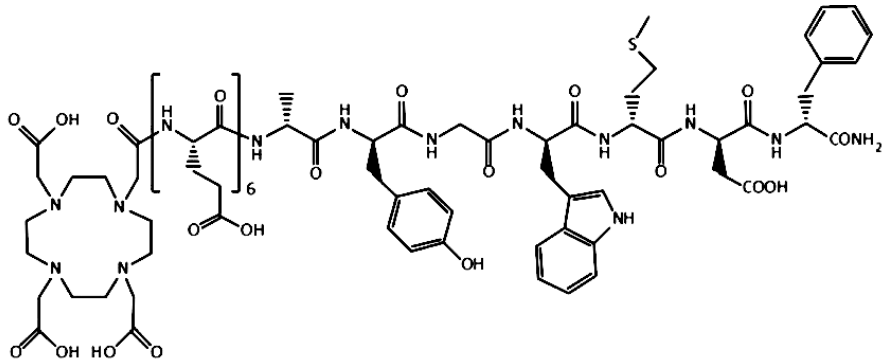

Figure 1. Structure of CP04 peptide

of the compound in serum, receptor affinity and binding) and in preclinical animal models (in terms of biodistribution, excretion pathways and uptake ratios in tumour to various organs) [2–8]. Some of these radiopeptides were tested in small clinical pilot-studies in humans. Receptor targeting was seen to some extent in physiologically CCK-2/gastrin receptor expressing tissues and most importantly, in the tumour tissue with the high tumour to background ratios. In the attempt to enable therapy with CCK-2/gastrin receptor-binding radiolabelled analogues further research was stimulated and coordinated within the European COST BM0607 project. Novel analogues were synthetized and comparatively evaluated both in vitro as well as in vivo regarding stability, receptor binding and tumour targeting [3–8]. As a result of these comparisons one derivative, namely DOTA-DGlu-DGlu-DGlu-DGlu-DGlu-DGlu-Ala-Tyr-Glu-Trp-Met-Asp-Phe-NH_2 (CP04, Fig. 1) showed the most promising characteristics in terms of high stability and receptor affinity, high and persistent tumour uptake and low kidney retention. Therefore, ^{111}In-CP04 was selected for further clinical evaluation [9].

The gastrin and CCK-2 receptors are potential targets for radionuclide therapies, because ligands such as CP04 can be radiolabelled with ^{111}In or ^{68}Ga for imaging, or with ^{90}Y and ^{177}Lu for therapy. Recently, the influence of ^{111}In-radiolabelled DOTA-minigastrin analogues stereochemistry on their in vitro and in vivo behaviour was evaluated [10]. Despite the very small difference in these analogues due to the stereochemistry of the spacers the biological distribution differed significantly in terms of accumulation in the tumour and kidney retention. In order to fully understand the mechanisms responsible for these differences, the authors emphasize the need for further studies on the influence of radiotracer's secondary structure on its pharmacokinetics.

Affinity of the chelator-peptide conjugates to the cell membrane receptors may also vary depending on the metal incorporated into the complex. This phenomenon has been observed both in vitro and in vivo for the radiocomplexes of DOTA conjugated somatostatin and bombesin analogues [11–13]. It is supposed that the reason for the differentiation of receptor affinity to the respective receptors is in the changes of structure of chelating system and their influence on the bioactive conformations of the metal conjugated peptides [14]. However, stability of the metal complexes may be an additional factor that influences the affinity to the receptors [15]. So far, there are no such studies for the ligands of gastrin/cholecystokinin receptor CCK2R.

In line with the above need, the aim of our project was to investigate the influence of selected metals on the structure and properties of DOTA-$(DGlu)_6$-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2 (CP04), a gastrin/CCK analogue. Herein, we report on the radiolabelling of CP04 with ^{90}Y, ^{177}Lu and ^{68}Ga, the synthesis of cold CP04 complexes with respective stable metals, and on their preliminary characterisation by HPLC and LC-MS methods.

Materials and methods

Optimization of CP04 radiolabelling with ^{90}Y by microwave synthesis

The microwave oven was used for investigation of the influence of temperature and reaction time on the ^{90}Y-labelling yield of CP04 and on its oxidation. For the radiolabelling 600 MBq of ^{90}Y (ItraPol, NCBJ OR POLATOM) was added to 20 μg of CP04 (Pichem, Austria) dissolved in 0.2 mL of ascorbic acid buffer (50 mg/mL, pH 4.5) and incubated in microwave oven (Initiator, Biotage) at 95°C and 98°C from 5 to 25 min.

Radiolabelling of CP04 with ^{90}Y, ^{177}Lu and ^{111}In

For radiolabelling 200-600 MBq of ^{90}Y (ItraPol, NCBJ OR POLATOM), ^{177}Lu (LutaPol, NCBJ OR POLATOM) or ^{111}In (Mallinckrodt) was added to 20 μg of CP04 (Pichem, Austria) dissolved in 0.2 mL of ascorbic acid buffer (50 mg/mL, pH 4.5) and incubated in the microwave oven (Initiator, Biotage) for 5 min or in thermo-block at 95°C for 12 min.

Radiolabelling of CP04 with ^{68}Ga

200 μL of ^{68}Ga eluate (200 MBq) from ^{68}Ge/^{68}Ga generator (iThemba) was added to 20 μL of CP04 (1 mg/mL) and 70 μL of 2.5 M CH_3COONa, followed by 12 min incubation at 95°C. It was then diluted with 710 μL of ascorbic acid (50 mg/mL, pH 4.5).

Synthesis of the cold complexes of CP04 with Ga, Lu and Y for LC-MS study

The non-radioactive complexes of CP04 with Lu, Y, In and Ga were synthesized in milligram amounts starting from 0.5 mg up to 5 mg of peptide dissolved in ascorbic acid buffer (50 mg/mL, pH 4.5) using 2-fold molar excess of the metal ion. Incubation was carried out for 5 min in microwave oven or 15 min in thermo-block at 95°C followed by purification on SPE C18 columns.

Table 1. Radiolabelling yields and radiochemical purity of ^{90}Y-CP04 complexes synthesized in microwave oven

Parameters: temp., time	Yield [%]	Radiochemical Purity of ^{90}Y-CP04 [%]	Oxidized CP04 [%]
95°C, 5 min	99.4	94.4	1.2
95°C, 15 min	99.7	92.0	3.8
95°C, 25 min	99.6	89.6	5.8
98°C, 5 min	99.7	93.3	2.5

Determination of partition coefficient (log P)

For the determination of octanol/water partition coefficients, the aliquots (50 μL) of radiolabelled CP04 complexes were diluted with PBS (pH 7.4) to 500 μL and added to 500 μL of octanol in a testing tube, each in six replicates. The mixture was vigorously vortexed over a period of 15 min at room temperature (RT). After centrifugation, the radioactivity of 50 μL aliquots of both layers was measured in an automatic gamma counter (1470 Wizard, Wallac) and the log P value was calculated.

Analytical method

RP-HPLC isocratic method (Kinetex 150/4.6 mm; 25% AcN/0.1% TFA, 1 mL/min; oven temp. 40°C) with UV/Vis and radiometric detection was devised for investigation of the radiolabelled and "cold" CP04 complexes.

LC-MS study

LC-MS investigations were performed using the UFLC Shimadzu (Kioto, KYT, Japan) system coupled with ABI 4000 QTRAP linear ion trap quadrupole LC/MS/MS mass spectrometer (AB Sciex, Foster City, CA).

Analysis parameters: Kinetex 5u C18 100A, 4.6 × 150 mm column; 25%AcN/0.1% TFA (in some experiments TFA was replaced with formic acid); flow rate 1 mL/min; t = 30°C; ionization electrospray (ESI)/positive; ion spray voltage, 5.5 kV; desolvatation temperature, 700°C; curtain gas, 10 psi. Optimal spectra were obtained from Enhanced MS (EMS) scans at low (40V) declustering potential.

Results and discussion

A macrocyclic chelator DOTA (1,4,7,10-tetraazocyclododecane-1,4,7,10-tetraacetic acid) is one of the most often used chelators for coupling to peptides for PRRT (peptide receptor radionuclide therapy) [16, 17], however the reaction kinetics differ for each radiometal. Hence, depending on whether the ^{90}Y, ^{111}In or ^{177}Lu are planned to be used, the optimization of radiolabelling conditions is required. In addition, the elevated temperature and incubation time may influence the rate of undesired radiochemical species formation. Contrary to traditional heating devices used for radiolabelling, the microwave heating assures that desired temperatures are reached rapidly in a fully controlled radiolabelling process. Thus, microwave heating shortens incubation time, which might be critical in case of oxidation-sensitive peptides.

Methionine present in the amino acid sequence of CP04 peptide might be oxidised during labelling at elevated temperature, resulting in reduced receptor affinity. Therefore control of oxidation was critical for the quality of potential CP04 based radiopharmaceutical. The microwave oven used in our experiments (Initiator, Biotage) allowed precise control of heating temperature with an infrared sensor. Already after 5 min incubation in the microwave oven at 95°C the radiolabelling yield greater than 99% (calculated in reference to unbound ^{90}Y radioactivity) was obtained. The isocratic HPLC method with Kinetex 5u C18 column provided good resolution and allowed the quantitation of observed peaks. The peaks revealed in the radiochromatograms could be attributed to radiolabelled CP04 and to the impurities such as a free radiometal and oxidized form of CP04 due to the methionine oxidation. Radiochemical purity of ^{90}Y-CP04 depended on the time of incubation and was decreasing with increase of incubation time (Table 1). At 5, 15 and 25 min incubation the radiochemical purity was 94.4%, 92% and 89.6%, respectively, while the contribution of radioactive oxidized forms of CP04 (Rt ca. 3.5–3.8 min) increased (from 1.2% to 5.8% after 5 and 25 min, respectively). Therefore, for further studies we kept the incubation time as short as possible to obtain ^{90}Y-DOTA-gastrin of high RCP and to avoid of the methionine residue oxidation.

Cold complexes of CP04 with Y, Lu and Ga were synthesized in sufficient quantities to allow their stability and LC-MS studies. Radiometal complexes of CP04 with ^{90}Y, ^{177}Lu and ^{68}Ga were obtained with yields greater than 90% and their identity was confirmed by HPLC in comparison to their non-radioactive equivalents. In all cases, HPLC chromatograms revealed peaks that could be attributed to the metal-CP04 complexes and to the impurities (including complexes with the oxidized peptide, Rt ca. 2.3–2.9 min). Different chromatographic behaviour observed for Ga- complex comparing to Lu- and Y-CP04 (relative retention to CP04: 1.08, 0.86 and 0.85, respectively) suggests different coordination of the metal ions (Fig. 2). The partition coefficient Log P values of -2.92 ± 0.005 were determined for ^{90}Y-CP04 as well as for ^{177}Lu-CP04 while it was -2.68 ± 0.03 for ^{68}Ga-CP04, what suggests a slightly higher lipophilicity of the gallium complex.

LC-MS analysis of Ga and Lu complexes revealed conformity of the observed molecular ions to the predicted formulas (m/z 2116

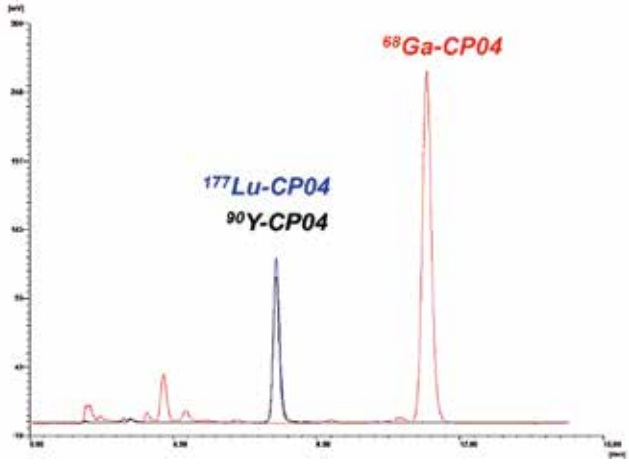

Figure 2. Radiochromatograms of ^{177}Lu-CP04, ^{90}Y-CP04 and ^{68}Ga--CP04 (Kinetex 150/4.6 mm; 25%AcN/0.1% TFA, 1 mL/min)

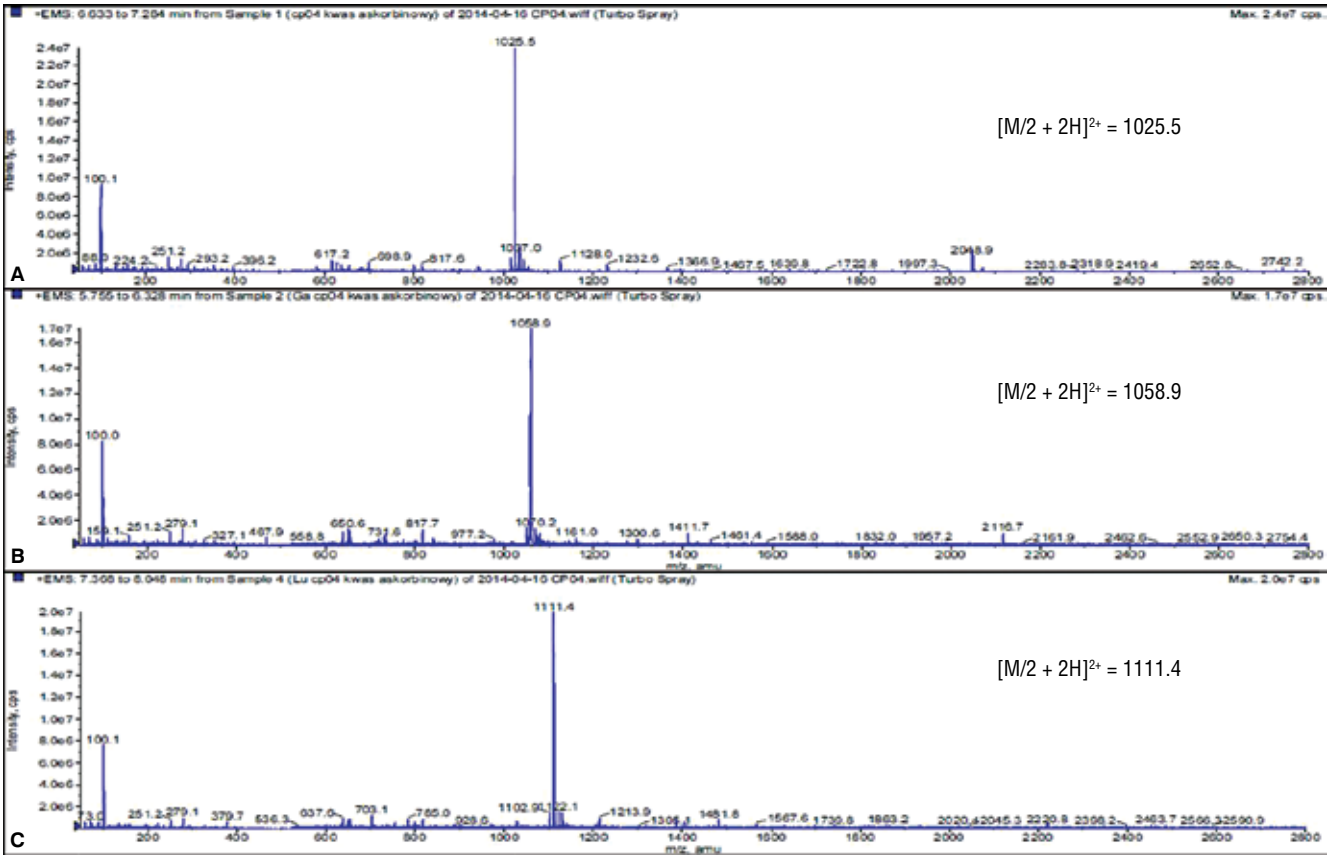

Figure 3. The mass spectra of: **A.** CP04 peptide; **B.** Ga-CP04 complex; **C.** Lu-CP04 complex

Table 2. Characteristic of metal complexes with CP04

Compound	Relative retention (r) (mob. phase with TFA)	Molecular mass [g/mol]	Number of carboxylic groups of DOTA coordinating metal ion
CP04	1	2049	–
Ga-CP04	1.08	2116	2
Lu-CP04	0.86	2220	3
Y-CP04	0.85	2135	3
In-CP04	0.86	2161	3

and 2220 Da for Ga and Lu, respectively; Fig. 3, Table 2). In the Ga complex only 2 carboxylic groups of DOTA are involved in the coordination, whereas 3 carboxylic groups are needed for Lu, Y and In. This results in different geometry of complexes with CP04. Our observations are in accordance with those by Viola-Villegas et al. [18], who proposed different structures for Ga- and Lu/Y-DOTA complexes.

Conclusions

Our study showed that the DOTA conjugated minigastrin analogue, CP04, can be easily and efficiently radiolabelled both with positron emitting radionuclide ^{68}Ga, as well as with beta minus emitting radionuclides such as yttrium-90 or lutetium-177. However, different coordination of radiometals may lead to different physico-chemical and biological properties of these radiocomplexes. LC-MS study revealed that in case of yttrium and lutetium complexes, 3 carboxylic groups of DOTA are involved in the radiometal coordination, whereas in case of gallium only 2. This structural difference causes differential hydrophilicity of the Ga- and Y/Lu- complexes what has been observed in chromatographic and partition coefficient studies.

Further in vitro and in vivo studies are in progress to indicate whether the type of coordination of radiometal by DOTA bifunctional chelator significantly influences the biological properties of radiolabelled CP04 and its interaction with CCK-2 receptors in vivo.

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References

1. Reubi JC, Waser B. Concomitant expression of several peptide receptors in neuroendocrine tumours: molecular basis for in vivo multireceptor tumour targeting. Eur J Nucl Med Mol Imaging 2003; 30: 781–793.
2. Behe M, Behr TM. Cholecystokinin-B (CCK-B)gastrin receptor targeting peptides for staging and therapy of medullary thyroid cancer and other CCK-B receptor expressing malignancies. Biopolymers (Pept Sci) 2002; 66: 299–418.
3. Mather SJ, McKenzie AJ, Sosabowski JK, et al. Selection of radiolabeled gastrin analogs for peptide receptor-targeted radionuclide therapy. J Nucl Med 2007; 48: 615–622.
4. Breeman WAP, Froberg AC, de Blois E et al. Optimised labelling, preclinical and initial clinical aspects of CCK-2 receptor-targeting with 3 radiolabeled peptides. Nucl Med Biol 2008; 35: 839–849.
5. Von Guggenberg E, Rangger C, Sosabowski J et al. Preclinical evaluation of radiolabeled DOTA-derivatized cyclic minigastrin analogs for targeting cholecystokinin receptor expressing malignancies. Mol Imaging Biol 2012; 14: 366–375.
6. Ocak M, Helbok A, Rangger C et al. Comparison of biological stability and metabolism of CCK2 receptor targeting peptides, a collaborative project under COST BM0607. Eur J Nucl Med Mol Imaging 2011; 38: 1426–1435.
7. Laverman P, Joosten L, Eek A et al. Comparative biodistribution of 12 ^{111}In-labelled gastrin/CCK2 receptor-targeting peptides. Eur J Nucl Med Mol Imaging 2011; 38: 1410–1416.
8. Aloj L., Aurilio M, Rinaldi V et al. Comparison of the binding and internalization properties of 12 DOTA-coupled and ^{111}In-labelled CCK2/gastrin receptor binding peptides: a collaborative project under COST Action MB0607. Eur J Nucl Med Mol Imaging 2011; 38: 1417–1425.
9. ERA-NET TRANSCAN/01/2013 in the framework of international project "Phase I clinical trial using a novel CCK-2/gastrin receptor-localizing radiolabelled peptide probe for personalized diagnosis and therapy of patients with progressive or metastatic medullary thyroid carcinoma". EudraCT number: 2015-000805-38.
10. Kolenc-Peitl P, Tamma ML, Kroselj M et al. Stereochemistry of Amino Acid Spacers Determines the Pharmacokinetics of ^{111}In-DOTA-Minigastrin Analogues for Targeting the CCK2/Gastrin Receptor. Bioconjugate Chem 2015; 26: 1113–1119.
11. Reubi JC, Schar JC, Waser B et al. Affinity profiles for human somatostatin receptor subtypes SST1-SST5 of somatostatin radiotracers selected for scintigraphic and radiotherapeutic use. Eur J Nucl Med 2000; 27: 273–282.
12. Koumarianou E, Loktionova NS, Fellner M et al. ^{44}Sc-DOTA-BN[2-14]NH_2 in comparison to ^{68}Ga-DOTA-BN[2-14]NH_2 in pre-clinical investigation. Is ^{44}Sc a potential radionuclide for PET? Appl Radiat Isot. 2012; 70: 2669–2676.
13. Koumarianou E, Pawlak D, Korsak A, Mikolajczak R. Comparison of receptor affinity of ^{nat}Sc-DOTA-TATE versus ^{nat}Ga-DOTA-TATE. Nucl Med Rev Cent East Eur 2011; 14: 85–89.
14. Fani M, Del Pozzo L, Abiraj K et al. PET of somatostatin receptor-positive tumors using ^{64}Cu- and ^{68}Ga-somatostatin antagonists: the chelate makes the difference. J Nucl Med 2011; 7: 1110–1118.
15. Price E.W, Orvig C. Matching chelators to radiometals for radiopharmaceuticals. Chem Soc Rev 2014; 43: 260–290.
16. Stasiuk GJ, Long NJ. The ubiquitous DOTA and its derivatives: the impact of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid on biomedical imaging. Chem Commun (Camb) 2013; 49: 2732–2746.
17. Parus JL, Pawlak D, Mikolajczak R, Duatti A. Chemistry and bifunctional chelating agents for binding ^{177}Lu. Curr Radiopharm 2015 Mar 12 [Epub ahead of print].
18. Viola-Villegas N, Doyle RP. The coordination chemistry of 1,4,7,10-tetraazacyclododecane-N',N'',N,N'''-tetraacetic acid (H_4DOTA): Structural overview and analyses on structure–stability relationships. Coord Chem Rev 2009; 253: 1906–1925.