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PAPER

A concise synthesis procedure to furnish multi-gram amounts of hexadentate, bivalent DO2A-based chelators

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The synthesis of three bifunctional chelators, namely 1,7-bis-(4-aminobenzyl)-1,4,7,10-tetraaza-cyclododecane-4,10-diacetic acid (**1**), 1,7-bis-(4-isothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane-4,10-diacetic acid (**2**) and 1,7-bis-(4-maleimidobenzyl)-1,4,7,10-tetraazacyclododecane-4,10-diacetic acid (**3**), is presented. Compounds **1–3** are versatile building blocks for the synthesis of bivalent imaging agents for molecular imaging. This straightforward, high yielding synthesis route affords **1–3** on a multi-gram scale.

Introduction

Conjugates of bifunctional macrocyclic metal complexes and bioactive compounds, *i.e.* peptides, antibodies or small molecules are increasingly being used in preclinical and clinical studies. Complexes of paramagnetic metals find application in nuclear magnetic resonance imaging (MRI), a variety of lanthanide complexes are used for optical imaging (OI), radioactive metals emitting low energy γ-radiation are used in single photon emission computed tomography (SPECT), positron emitting radionuclides are used for positron emission tomography (PET) and complexes of α- and β-particle emitting radionuclides are used for tumour therapy (ERT).[1] With respect to PET, clinical ^{68}Ga PET is a rapidly growing alternative to cyclotron-produced positron-emitters like ^{11}C or ^{18}F.[2] Favourable decay properties ($t_{1/2}$: 68 min; β^+(max): 1.8 MeV; δ^+-abundance: 89%) and cost-effective availability of ^{68}Ga *via* a ^{68}Ge/^{68}Ga-radionuclide generator meet the demands of economically strained healthcare systems.[3]

Despite substantial differences in between these different imaging modalities, all of them share a mutual concept for the design and synthesis of the metal complex–biomolecule conjugates. A thermodynamically stable and kinetically inert metal complex is covalently attached to a bioactive molecule, which serves as a homing device to accumulate the metal complex in the desired tissue. Multidentate chelators based on 1,4,7,10-tetraazacyclododecane (cyclen) are the most frequently used chelating agents. Notably, several derivatives of cyclen have been approved for medical applications or are currently being investigated in clinical phase II and phase III studies. In some cases, *i.e.* the somatostatin agonist tyr^3-octreotide a single chelator-peptide conjugate, the cyclen based tyr^3-DOTATOC and its analogues, can be used to synthesise PET or SPECT imaging probes and tailored therapeutic agents.[4]

Multivalent conjugates obtained by a combination of a central chelating moiety with more than one pendant biomolecule have been shown to greatly enhance specificity to biological targets.[5–7] Using a ^{64}Cu-labelled, bivalent RGD-derivative, it was clearly demonstrated that this concept also leads to an increased affinity to the target.[7] However, the development and pre-clinical evaluation of novel multivalent conjugates has been complicated by limited commercial availability and challenging chemical preparation of appropriate bifunctional chelators. Despite the pronounced interest in this class of compounds, few derivatives have been described in literature. Even fewer are available from commercial suppliers. As a result, application has been strictly limited to analogues of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) using amide bonds for conjugation. In an extension of this concept, a convenient, reliable high-yield synthetic route to various orthogonally functionalised derivatives of 1,4,7,10-tetraazacyclododecane-4,10-diacetic acid (DO2A), furnishing multi-gram amounts suitable for conjugation to a variety of functional groups is presented. 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) is an octadentate metal chelator.[8] A variety of medically relevant metals form stable complexes with DOTA and its hexadentate and heptadentate analogues.[9] This opens up the opportunity to substitute two acetate groups of DOTA with functionalized pendant arms to attach targeting vectors to obtain disubstituted DO2A-derivatives.[10,11]

Results and discussion

The synthetic route starting from bis-*tert.*-butyl protected DO2A (**4**), is outlined in Scheme 1. DO2A was synthesized from cyclen in three steps following a known route from the literature.[12]

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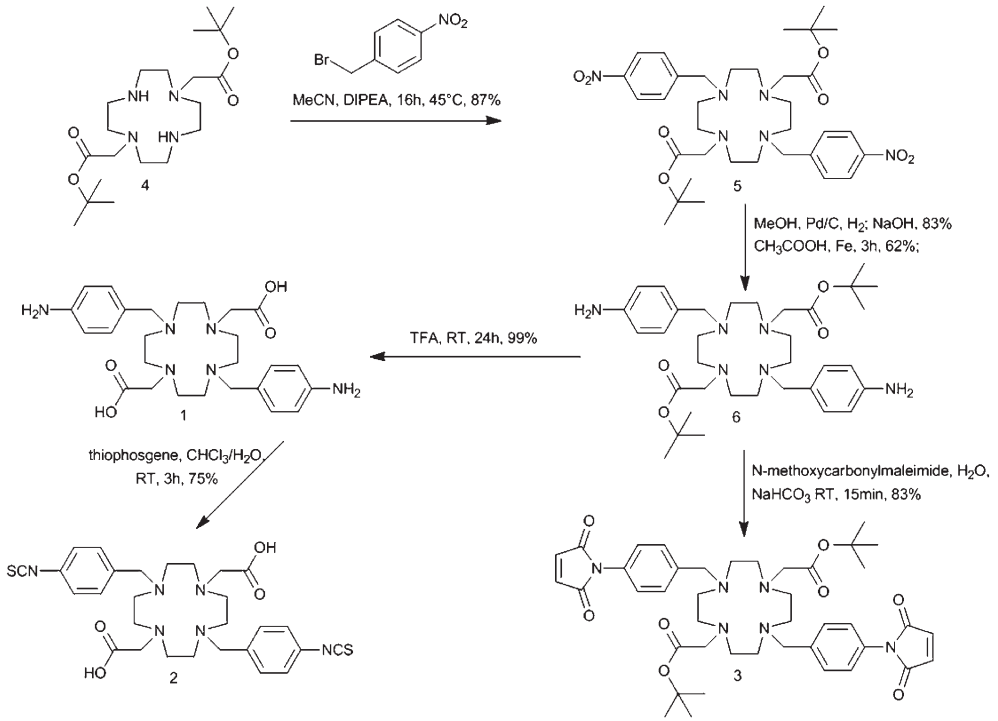

Scheme 1 Synthesis of divalent bifunctional chelators **1**, **2** and **3.**

Alkylation of **4** with 4-nitrobenzyl bromide was achieved in MeCN at 45 °C using diisopropyl ethyl amine as the base. Upon its formation, the product readily precipitates from the reaction mixture in high purity.The pure product **5** is obtained in high purity *via* filtration; elaborate purification can be omitted. Hence, careful optimisation of the concentration of the reaction mixture was conducted to increase the yield of **5**. Key intermediate **6** was obtained by reduction of the nitro-groups. Since the benzyl functions are easily cleaved during catalytic hydrogenations and the *tert*-butyl functions split off rapidly under acidic conditions, several reduction procedures were screened with the aim to achieve optimal conversion of the nitro groups.

Reduction of the nitro groups using $NaBH_4$ in the presence of Cu^I salts did not yield the desired product. The use of Zn^0 in glacial acetic acid and $SnCl_2$ in refluxing $MeOH/H_2O$ led to poor isolated yields due to partial ester cleavage. Catalytic hydrogenation with Pd^0 on carbon in methanol, ethanol or AcOEt at RT resulted in complete removal of the benzyl functions. Increasing the H_2 pressure from 1 to 10 bar did not give better results. The de-benzylation could be avoided by using Fe in glacial acetic acid as the reducing medium. Under these conditions the desired product **6** was obtained, but only moderate yields were achieved due to partial cleavage of the protective groups. It was finally possible to overcome these issues by performing the catalytic hydrogenation under basic reaction conditions. A substantial increase in the yield was achieved since the benzyl-functions were no longer cleaved.

Intermediate **6** provides amino functions that can readily be used for conjugation to electrophiles. Moreover, the amino functions of this key intermediate can be converted into a variety of electrophilic groups like isothiocyanato, maleimido, or bromoacetyl-functions which facilitate conjugation through reactions with nucleophiles. Isothiocyanato and maleimido groups are orthogonally reactive towards certain nucleophiles. Isothiocyanato groups can be converted into thioureas upon reaction with amines. In contrast, maleimido groups can be used for site specific conjugation due to their selectivity for sulfhydryl functions.

Bifunctional chelator **1** was obtained in quantitative yield after deprotection of the carboxylate functions in **6** with trifloroacetic acid. Conversion of the aromatic amino groups into isothiocyanate groups was achieved by reaction of **1** with thiophosgene in a biphasic reaction mixture of chloroform and water. Product **2** was obtained *via* phase separation, lyophilisation of the aqueous phase and purification by preparative HPLC. Compound **3** was synthesized by reacting compound **6** with an excess of maleimidocarboxylic acid methyl ester in a 2 : 1 mixture of sodium bicarbonate solution and dioxane. The desired product was obtained by extraction and purification *via* reversed phase chromatography in an overall yield of 83%. Products **1–3** were obtained in 69% (**1**), 53% (**2**) and 57% yield (**3**), respectively, starting from DO2A (58%, 38% and 41%, respectively, starting from cyclen). All synthetic procedures were performed on a multi-gram scale in our laboratories.

In order to demonstrate the utility of these new chelating agents, compound **2** was labeled using a purified solution of $^{68}Ga^{III}$.[14] The labelling reaction was carried out in Millipore water at pH 3.2 using 10 μg labelling precursor at various temperatures. Quantification of radiochemical yields was performed by radioTLC and radioHPLC. It was found that the ^{68}Ga-labelling procedure of this precursor requires high temperatures to achieve satisfying yields. At 50 °C only moderate yields of about 30% were obtained after 10 min. An increase of

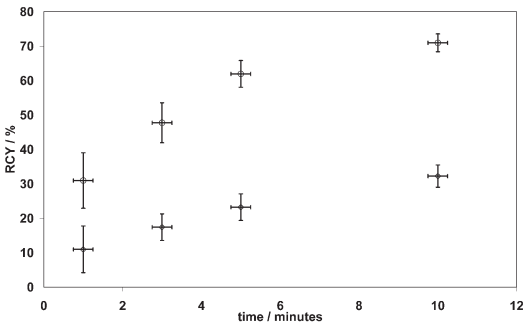

Fig. 1 Time-dependency of ^{68}Ga-labelling in 5 mL Millipore water with 10 mg (17 nmol) labelling precursor at two different temperatures: 50 °C (◇) and 70 °C (×). Errors are given as ± 1 standard deviation.

the reaction temperature to 95 °C furnished the desired product in 71% radiochemical yield within 10 min of heating.

Further improvement of the radiochemical yield was achieved under microwave enhanced conditions. Quantitative incorporation of the ^{68}Ga radioactivity into the desired product was achieved after 2 min at 140 °C at a pressure of 6 bar in a focused microwave reactor.[15] The temperature-dependent time-course of labelling yields is shown in Fig. 1.

The kinetic inertness and thermodynamic stability of ^{68}Ga-chelates conjugated to bioactive molecules for molecular imaging are of utmost importance for imaging.[2a] Since free $^{68}Ga^{3+}$ inside the body ultimately ends up in one of the two Fe^{3+} binding sites of the plasma protein *apo*-transferrin, challenge studies involving an excess of *apo*-transferrin and the ^{68}Ga-complex have emerged as a routine method for determination of the complex stability. Alternatively, challenge of the complexed radionuclide using an excess of DTPA or an excess of trivalent metal ions of similar complexation characteristics are well accepted methods. In order to assess the stability of the novel ^{68}Ga complex towards transchelation, the labeled product was co-incubated with *apo*-transferrin in phosphate buffered saline at pH 7.4 and 37 °C.[15–17] The composition of the mixture was monitored by radioTLC for two hours. [^{68}Ga]-**1** remained intact over the course of this study, indicating sufficient stability for the intended purpose. The results are shown in Fig. 3.

To examine the ^{68}Ga-complex stability, transmetallation and transchelation experiments were conducted with [^{68}Ga]-**1**. Transchelation of ^{68}Ga to DTPA (Fig. 2) or to *apo*-transferrin (Fig. 2) was not observed over a period of sixty minutes. Moreover, a high complex stability in terms of displacement of the $^{68}Ga^{III}$ core *via* transmetallation reactions was observed in the presence of Fe^{III} ions.

No substantial release of ^{68}Ga from the chelates [^{68}Ga]-**1** was observed in physiological *apo*-transferrin solution over a period of two hours (Fig. 3). Thus, the tested compounds possessed sufficient complex stability to warrant synthesis and investigation of chelator-biomolecule conjugates in *in vitro* or *in vivo* experiments.

Experimental

All chemicals were obtained from commercial suppliers Fisher Scientific, STREM, Sigma-Aldrich and VWR and used without further purification. TFA was donated by Solvay. Anhydrous solvents were used for the reactions. ^{1}H-NMR spectra were recorded on Bruker AC 200 and AvanceII NMR spectrometers at 300 and 400 MHz, respectively. ^{13}C-NMR spectra were recorded at 75 and 100 MHz, respectively. Chemical shifts are referred to in the solvent residual signal. Field desorption (FD) mass spectra were recorded on a Finnigan MAT90 FD spectrometer. HRMS spectra were measured on a Micromass QTOF Ultima 3 spectrometer. For purification of the compounds a Dionex P680 HPLC with an UVD170U UV-detector (254 and 360 nm) and a Phenomenex® Synergy Max RP8 15 × 250 mm HPLC-column (H_2O/MeOH 75 : 25, 0.01% TFA) were used. TLC was performed on self-cut Merck Silica 60 F_{254} plates. Column chromatography was carried out on Acros silica gel 60, 0.063–0.200 mesh. ^{68}Ga was obtained from a ^{68}Ge/^{68}Ga-radionuclide generator (Cyclotron AG, Obninsk, Russia). Radio-TLC was conducted on Silica Gel 60 F_{254} TLC-Plates and analysed with a Canberra Instant Imager.

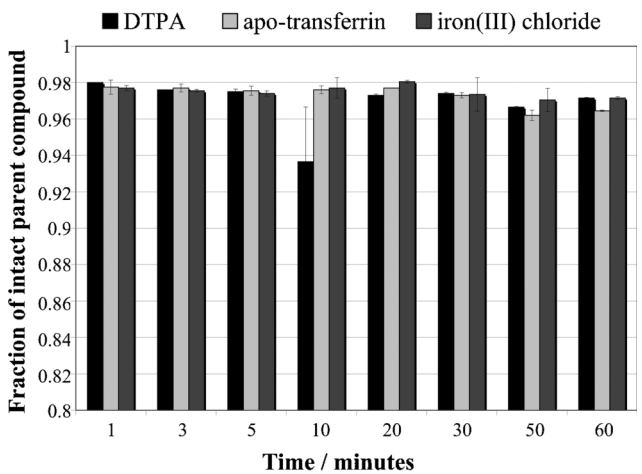

Fig. 2 Stability of [^{68}Ga]-**1** over 60 min.

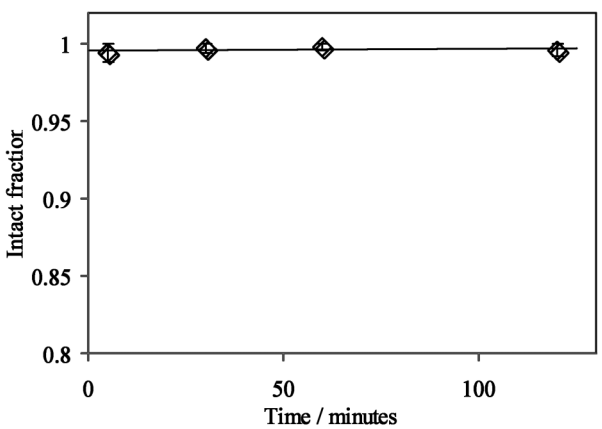

Fig. 3 Validation of the stability of [^{68}Ga]-**1** in *apo*-transferrin solution.

1,7-bis-(4-Nitrobenzyl)-1,4,7,10-tetraazacyclododecane-4,10-diacetic acid *tert*-butylester (5)

Compound **4** (6 g, 15 mmol) and diisopropylethylamine (6.42 g, 48 mmol) were dissolved in acetonitrile (50 ml) and heated to 45 °C. *p*-Nitrobenzyl bromide (7.2 g, 33.3 mmol) dissolved in acetonitrile (15 ml) was added dropwise. The reaction mixture was stirred for 16 h at 45 °C after which TLC indicated complete conversion of compound **4**. The precipitate was filtered off and washed three times with hexane (30 ml). Product **5** (8.76 g, 13.1 mmol, 87%) was obtained after drying *in vacuo* as a colourless amorphous solid. Mp = 126.6 °C;^{1}H NMR (400 MHz, $CDCl_3$): δ (in ppm) 8.18 (d, J = 8.5 Hz, 4H, Ar*H*), 7.68 (d, J = 8.5 Hz, 4H, Ar*H*), 3.65 (s, 4H, ArCH_2-N), 3.09 (s, 4H, N-CH_2CO_2–), 2.83 (m, 8H, N–CH_2), 2.65 (m, 8H, N–CH_2), 1.39 (s, 18H, CO_2–C(CH_3)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ (in ppm) 170.8 (CO_2), 148.4 (C_{Ar}–N), 146.9 (C_{Ar}–CH_2), 129.4 (C_{Ar}–H), 123.4 (C_{Ar}–H), 80.8 (CO_2–C(CH$_3$)$_3$), 59.3 (Ar–CH_2–N), 56.4 (N–CH_2CO_2–), 53.2 (N–CH_2), 52.6 (N–CH_2), 28.2 (C(CH_3)$_3$); MS (FD): *m*/*z* (%): 670.4 (83.9), 671.4 (100), 672.5 (40.2). FT-IR, δ in cm^{-1} = 2978, 2928, 2852, 1731, 1603, 1521, 1456, 1369, 1346, 1228, 1160, 852. Exact mass calculated for $C_{34}H_{50}N_6O_8$: 670.3690, found 670.3688. Anal. Calcd. for $C_{34}H_{50}N_6O_8$ C, 60.88; H, 7.51; N, 12.53; O, 19.08; found C, 60.91; H, 7.54; N, 12.50.

1,7-bis-(4-Aminobenzyl)-1,4,7,10-tetraazacyclododecane-4,10-diacetic acid *tert*-butylester (6)

Method A. Compound **5** (3 g, 4.5 mmol) was dissolved in methanol (70 ml), 1 M NaOH (10 ml) and 5% Pd on charcoal (300 mg) were added. A gentle stream of hydrogen was bubbled through the reaction mixture for three hours with continuous stirring. Upon completion of the reaction, the reaction mixture was filtered through a bed of Celite 545 and the filtrate was concentrated *in vacuo*. The residue was taken up in 0.1 M hydrochloric acid (30 ml) and washed two times with chloroform (30 ml). The aqueous phase was basified with 1 M NaOH (10 ml) and the product was extracted with chloroform (3 × 30 ml). The organic phase was dried over Na_2SO_4 after which the solvent was evaporated *in vacuo*. Product **6** was obtained as a yellow, amorphous solid (2.27 g, 3.7 mmol, 83%).

Method B. Compound **5** (4 g, 6 mmol) iron powder (2.8 g, 50 mmol), glacial acetic acid (25 ml) and water (10 ml) were combined and stirred for 3 h. The solvent was removed *in vacuo*, the residue was taken up in 2 M NaOH (50 ml) and the product was extracted with chloroform (5 × 50 ml). The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo* to furnish **6** as a yellow, amorphous solid (2.26 g, 3.7 mmol, 62%). Mp = 67–70 °C.^{1}H NMR (400 MHz, $CDCl_3$): δ (in ppm) 7.16 (d, J = 8.0 Hz, 4H,Ar*H*), 6.64 (d, J = 8.0 Hz, 4H, Ar*H*), 3.61 (s, 4H, ArCH_2–N), 3.15 (s, 4H, N–CH_2CO_2–), 2.84 (m, 8H, N–CH_2), 2.61 (m, 8H, N–CH_2), 1.40 (s, 18H, CO_2–C(CH_3)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ (in ppm) 171.3 (CO_2), 147.2 (C_{Ar}–N), 145.0 (C_{Ar}–CH_2), 130.2 (C_{Ar}–H), 126.1 (C_{Ar}–H), 80.5 (CO_2–C(CH$_3$)$_3$), 59.6 (Ar–CH_2–N), 56.3(N–CH_2CO_2–), 52.4 (N–CH_2), 52.1(N–CH_2), 28.2 (C(CH_3)$_3$); FT-IR, δ in cm^{-1} = 3417, 2924, 2854, 2587, 1724, 1614, 1514, 1458, 1386, 1219, 1158, 1085, 826. MS (FD): *m*/*z* (%): 611.4 (100), 612.4 (32.7); HRMS: exact mass calculated for $C_{34}H_{54}N_6NaO_4$ 633.4104, found: 633.4086.

1,7-bis-(4-Aminobenzyl)-1,4,7,10-tetraazacyclododecane-4,10-diacetic acid (TFA-salt) (1)

Compound **6** (3 g, 4.9 mmol) was dissolved in trifluoroacetic acid (40 ml) and stirred for 24 h. The reaction mixture was concentrated *in vacuo* to obtain 3.68 g (4.9 mmol, 99%) of the desired product **1**. ^{1}H NMR (300 MHz, D_2O): δ (in ppm) 7.73 (d, J = 8.0 Hz, 4H, Ar*H*), 7.53 (d, J = 8.0 Hz, 4H, Ar*H*), 4.57 (s, 4H, ArCH_2–N), 3.44 (m, 8H, H^+N–CH_2), 3.21–2.87 (m, 12H, N–CH_2CO_2–, H^+N–CH_2); ^{13}C NMR (75 MHz, D_2O): δ (in ppm) 174.1 (CO_2), 132.9 (C_{Ar}–N), 131.7 (C_{Ar}–CH_2), 124.1 (C_{Ar}–H), 117.8 (C_{Ar}–H), 56.9 (Ar–CH_2–N), 53.2 (N–CH_2CO_2–), 50.0 (N–CH_2), 48.1 (N–CH_2); FT-IR, δ in cm^{-1} = 3420, 2924, 2854, 2589, 1720, 1616, 1514, 1460, 1387, 1211, 1158, 1084, 825. MS (ESI): *m*/*z* (%): 499.31 (100), 500.31 (18.46), 521.33 (43.63), 522.32 (9.63); HRMS: exact mass calculated for $C_{26}H_{38}N_6NaO_4$ 521.2852, found: 521.2848.

1,7-bis-(4-Isothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane-4,10-diacetic acid (TFA-salt) (2)

Compound **1** (3 g, 4 mmol) was dissolved in water (20 ml) and the pH value was adjusted to 7–8 with 1 M NaOH. Thiophosgene (1.82 g, 16 mmol) was dissolved in chloroform (20 ml). Both phases were combined and stirred vigorously for three hours at room temperature. The phases were separated and the aqueous phase was washed with chloroform (3 × 15 ml). The aqueous phase was removed by lyophilisation and the residue was purified using HPLC. Compound **2** (1.74 g, 3 mmol) was obtained as a yellow solid. Mp = 79 °C (decomposition),^{1}H NMR (300 MHz, MeOH-d4): δ(in ppm) 7.83 (d, J = 8.5 Hz, 4H, Ar*H*), 7.44 (d, J = 8.5 Hz, 4H, Ar*H*), 4.65 (s, 4H, ArCH_2–N), 3.57 (m, 4H, N–CH_2CO_2–), 3.05–2.03 (m, 16H, H^+N–CH_2); ^{13}C NMR (75 MHz, D_2O): δ (in ppm) 175.1 (CO_2), 145.3 (N=C=S), 134.3 (C_{Ar}–CH_2), 127.7 (C_{Ar}–N), 120.1 (C_{Ar}–H), 116.2 (C_{Ar}–H), 61.1 (Ar–CH_2–N), 59.0 (N–CH_2CO_2–), 54.0 (N–CH_2), 51.6 (N–CH_2); FT-IR, δ in cm^{-1} = 3424, 3071, 2926, 2859, 2110, 1721, 1670, 1609, 1511, 1460, 1387, 1199, 1160, 1085, 929, 847, 798. MS (ESI): *m*/*z* (%): 583.24 (100), 584.27 (31.27), 605.24 (66.39), 606.26 (13.33). Anal. Calcd. for $C_{28}H_{36}N_6O_5S_2$ C, 55.98; H, 6.04; N, 13.99; O, 13.32, S, 10.67; found C, 55.91; H, 6.33; N, 13.90.

1,7-bis-(4-Maleimidobenzyl)-1,4,7,10-tetraazacyclododecane-4,10-diacetic acid (3)

Compound **6** (500 mg, 0.66 mmol) was dissolved in 1 M $NaHCO_3$-solution (20 ml) and *N*-methoxycarbonylmaleimide (3 g, 19.4 mmol) dissolved in dioxane (10 ml) was added. The reaction mixture was stirred for 15 min at room temperature. Subsequently, the pH-value was raised to pH 12 with 2 M NaOH and the product was extracted with 3 portions of chloroform (25 ml). The organic phases were combined and dried over Na_2SO_4. The solvent was removed *in vacuo* to obtain the desired product (**3**) (362 mg, 0.55 mmol, 83%) as a pale yellow solid. Mp = 96 °C (decomposition). ^{1}H NMR (300 MHz, $CDCl_3$): δin ppm) 7.53 (d, J = 8.5 Hz, 4H, Ar*H*), 7.27 (d, J = 8.5 Hz, 4H, Ar*H*), 6.82 (s, 4H, (–*H*C=C*H*–)), 3.58 (s, 4H, ArCH_2–N), 3.14 (s, 4H,

N–C$H_2$$CO_2$–), 2.84 (m, 8H, H^+N–CH_2), 2.64 (m, 8H, H^+N–CH_2), 1.39 (s, 18 H, CO_2–C(CH_3)$_3$); ^{13}C NMR (75 MHz, D_2O): δ (in ppm) 173.3 (CO_2), 159.8 (N–*C*(O)–CH), 138.4 (–H*C*=*C*H–), 132.2 (C_{Ar}–CH_2), 131.7 (C_{Ar}–N), 127.6 (C_{Ar}–H), 121.1 (C_{Ar}–H), 81.8 (CO_2–*C*(CH_3)$_3$), 59.6 (Ar–*C*H_2–N), 57.3 (N–*C*$H_2$$CO_2$–), 53.2 (N–*C*$H_2$), 51.2 (N–*C*$H_2$), 28.2 (C(*C*$H_3$)$_3$); FT–IR, δ in cm^{-1} = 3366, 2977, 2835, 1714, 1596, 1560, 1516, 1457, 1388, 1370, 1309, 1259, 1231, 1157, 1093, 845, 756. MS (FD): *m*/*z* (%): 771.41 (100), 772.40 (38.66). HRMS: Sample decomposed.

General procedure for the ^{68}Ga-radiolabelling of 1,7-diacetic acid-4,10-di-(4-isothiocyanate)-benzyl-1,4,7,10-tetraazacaclododecane

10 μg (17 nmol) of the labelling precursor **2** (10 μL of 1 mg/1 mL stock solution) was added to water (5 ml). The mixture was preheated to the desired labelling temperature. Then 50 μL of the generator-derived and cation-exchanger purified ^{68}Ga was added to the reaction mixture. Samples (1 μL) were withdrawn from the reaction mixture after 1, 3, 5 and 10 min and spotted on a TLC-plate The plate was developed in a mixture of 5% NaCl solution and ethanol (3 : 1) after 10 min.

Stability of the labelled product [^{68}Ga]Ga-1,7-diacetic acid-4,10-di-(4-isothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane

A solution of the ^{68}Ga labelled product [^{68}Ga]-**1** was added to a solution of *apo*-transferrin (3 mg ml^{-1}) in PBS-buffer pH 7.4. The mixture was incubated at 37 °C and the samples were withdrawn at defined time-points (1, 30, 60, 120 min). The composition of the reaction mixture was monitored by radioTLCs. The kinetic inertness and thermodynamic stability of product [^{68}Ga]-**1** were confirmed by challenge studies in DTPA solution (1 μM and 1 mM), $FeCl_3$ solution (1 μM) and *apo*-transferrin solution (1 mg ml^{-1} in DPBS (1 ×)). Aliquots of 1–3 MBq of the purified products were added to the appropriate solutions (200 μl) and incubated under gentle agitation at 37 °C. The composition of the mixtures was monitored by analysing 1 μl aliquots with radio-TLC at selected time-points for up to 2 h.

Conclusions

Herein a straightforward synthetic procedure is described which furnished divalent bifunctional chelators on a multi-gram scale in high yield. The procedure can be carried out using inexpensive, commercially available chemicals, rendering this route very cost-effective. All final compounds are suitable for the synthesis of bivalent chelator–biomolecule conjugates using a ''click-chemistry''-like attachment of targeting vectors to the introduced functional groups. A radiolabeled complex of compound **2** has shown sufficient stability *in vitro* to warrant its use in a PET-study. However, a minor constraint of these DOTA derived chelators are the rather harsh labelling conditions to obtain high yields of the labeled product. These might not be compatible with sensitive compounds such as proteins. In these cases, conjugation of the labeled complex to the protein can be considered as an alternative route, yet avoiding exposure of sensitive molecules to the labelling reaction.

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