Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 16 (2008) 2606–2616
Bioorganic & Medicinal Chemistry

Improved syntheses and applicability of different DOTA building blocks for multiply derivatized scaffolds

C. Wängler,[a] B. Wängler,[a] M. Eisenhut,[a] U. Haberkorn[b] and W. Mier[b,*]

[a]*German Cancer Research Center, Radiopharmaceutical Chemistry, INF 280, 69120 Heidelberg, Germany*
[b]*Universtiy Clinics of Heidelberg, Radiologische Klinik INF 400, Heidelberg, Germany*

Received 7 September 2007; revised 12 November 2007; accepted 16 November 2007
Available online 22 November 2007

Abstract—DOTA (1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid), which forms extremely stable complexes with a large number of metal ions, is one of the most important and most commonly used chelators for in vivo applications such as cancer diagnosis and therapy. However, many of the published synthesis protocols for DOTA derivatives are complicated and give the products in low yields. Here we report improved synthesis routes for tris-*t*Bu-DOTA, tris-benzyl-DOTA, and thiol-DOTA, and also describe the synthesis of the novel compound tris-4-nitro-benzyl-DOTA. In addition, we determined the applicability of the DOTA derivatives tris-*t*Bu-DOTA, thiol-DOTA, tris-benzyl-DOTA, tris-4-nitrobenzyl-DOTA, tris-allyl-DOTA, DOTA-PFP-ester, and DOTA-PNP-ester for multimerization reactions using amino functionalized PAMAM dendrimers of different sizes. Thiol-DOTA was found to be the best compound for efficient reactions with dendritic scaffolds generating highly homogeneous DOTA-multimers. This DOTA derivative could be quantitatively conjugated to a 128-mer dendrimer.

1. Introduction

DOTA (1,4,7,10-tetraaza-cyclododecane-1,4,7,10-tetra-acetic acid) is one of the most important chelators in radiochemistry as it forms complexes with extremely high stability constants with a large number of main group and transition metal ions. Due to this, DOTA is the most commonly used chelator for in vivo applications such as cancer therapy and clinical diagnosis. For this purpose, it is introduced into biomolecules such as peptides and antibodies and subsequently labeled with radionuclides.[1–6] Furthermore, some of the currently used MRI contrast agents such as Gadovist®, ProHance®, and Dotarem® are based on DOTA-gadolinium complexes. Gadomer-17®, a recently developed MRI contrast agent, uses a multiply DOTA-derivatized dendrimer which shows much longer retention times and improved visualization properties due to higher signals compared to low molecular weight gadolinium chelates.[7]

This kind of multimerization of DOTA is also advantageous for the synthesis of protein conjugates as it enables an enhanced specific activity of biomolecules.[8–10] With these compounds, a much higher specific activity can be obtained by modifying only some sites of the carrier molecule.

As DOTA is the chelator of choice, protocols are required that allow the facile synthesis of derivatives that are applicable for multimeric derivatizations of biomolecules. However, many of the published synthesis procedures are complex or give the products in low yields.

In the current work, some synthesis protocols are improved to enable faster and reliable DOTA-synthon syntheses. Further on, several DOTA derivatives were studied with regard to their suitability for multimerization reactions. PAMAM dendrimers were used as model compounds for these reactions and were reacted with the different DOTA derivatives to define the most suitable synthon for quantitative derivatizations of multimeric scaffold molecules.

2. Results and discussion

2.1. Improved synthesis of tris-*t*Bu-DOTA, tris-benzyl-DOTA and thiol-DOTA and synthesis of the novel compound tris-4-nitro-benzyl-DOTA

Conjugation reactions with sensitive biomolecules, such as antibodies or cytokines, have to be conducted under

Keywords: DOTA; Chelators; Synthesis; Multivalency; Dendrimers.
* Corresponding author. Tel.: +49 6221 567720; fax: +49 6221 5633629; e-mail: walter.mier@med.uni-heidelberg.de

0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2007.11.044

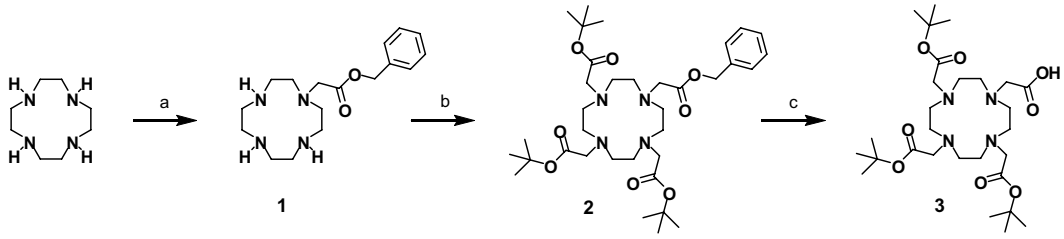
mild conditions. Therefore, the high number of syntheses that could be used for conjugation reactions is limited to biocompatible reactions.[11] For the multiple conjugation to dendritic structures the spectrum of applicable reactions is broader and a large spectrum of activated chelators can be used. DOTA derivatives have gained high importance in radiochemistry and therefore protocols are required that allow the facile and uncomplicated synthesis of these compounds.

Besides the in situ activation,[12] two different kinds of DOTA-derivatives are known: active esters which do not need to be deprotected after coupling[13] and protected derivatives that are reacted using additional coupling reagents and deprotected after the coupling.[14,15] Many of the published synthesis protocols for DOTA-derivatives are complicated with difficult purification steps or give the products in low yields. Especially for tris-*t*Bu-DOTA (**3**), which is the derivative of choice for the introduction of DOTA in solid phase synthesis, several synthesis protocols have been described in the literature.[16,17] However, these protocols do not emphasize the critical points of the synthesis: the low temperature required during the reaction and purification of the first intermediate product (1,4,7,10-tetraaza-cyclododec-1-yl)-acetic acid benzyl ester (**1**) and a rapid purification in all steps of the synthesis (Scheme 1).

Higher reaction temperatures and long incubation with the silica chromatography material lead to intramolecular ring formations or fragmentations of the products requiring complex purification steps and reduced synthesis yields.

The synthesis protocol described for thiol-DOTA (**5**)[18] starts from tris-*t*Bu-DOTA which is either expensive or has to be synthesized according to the multi-step protocol described above. Furthermore, the slow deprotection of the *t*Bu-groups and fragmentation products produced during the long incubation time in TFA result in low yields (Fig. 1).

The synthesis can be simplified by starting from DOTA that is directly reacted with *S*-trityl-protected mercap-



Scheme 1. Synthesis of tris-*t*Bu-DOTA. Reagents and conditions: (a) benzyl-bromoacetate, $CHCl_3$, rt, 3 h, 86%; (b) *tert*-butyl bromoacetate, K_2CO_3, acetonitrile, rt, 2–5 h, 67%; (c) Pd/C, H_2, THF/MeOH 1:1, rt, 2–8 h, 71%.

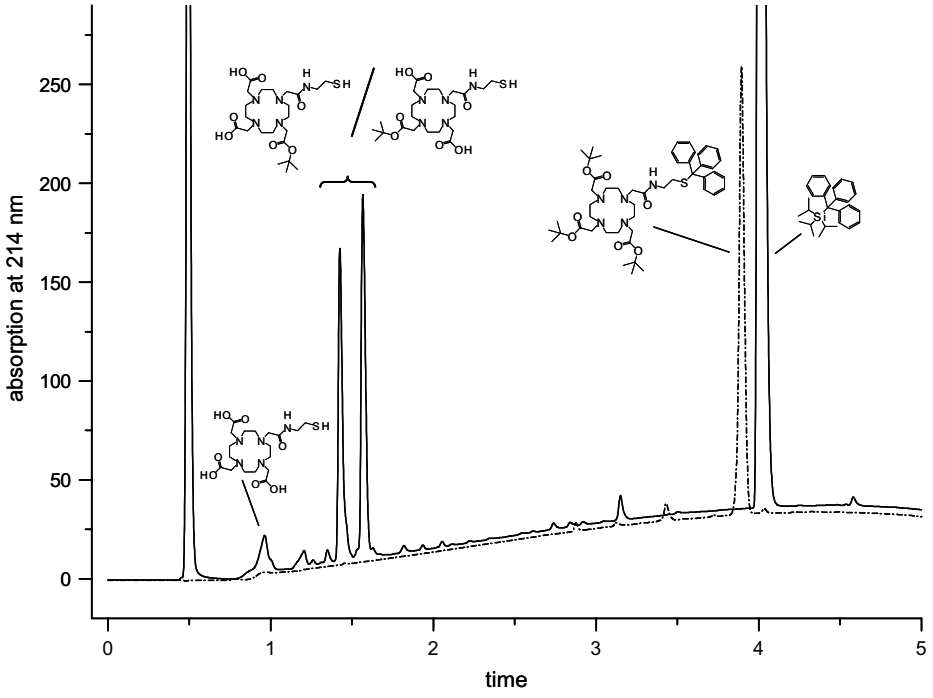
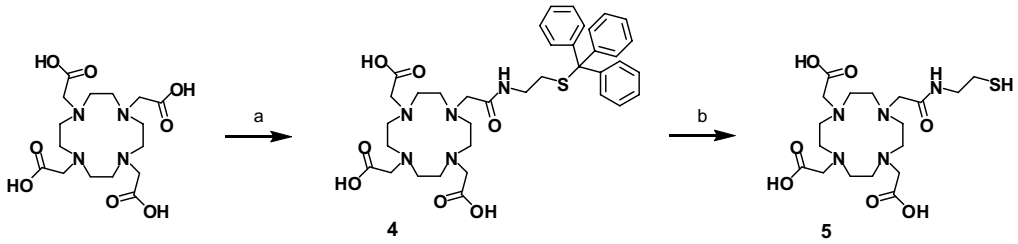


Figure 1. Chromatograms of the deprotection reaction of *S*-trityl-mercapto-*tris*-*t*Bu-DOTA with concentrated TFA after 16 h (compact line) and the educt (dashed line). Elution gradient: 0–100% MeCN + 0.1% TFA in 5 min.



Scheme 2. Synthesis of thiol-DOTA starting from DOTA. Reagents and conditions: (a) *S*-trityl-mercapto-ethanolamine trifluoroacetate, DCC, water/MeCN 1:1, pyridine, rt, 10 h, 23%; (b) TIS, TFA, rt, 5 min, 56%.

toethanol[19] and subsequently deprotected with concentrated TFA (Scheme 2) leading to much less side products in the final deprotection step (Fig. 2).

Tris-benzyl-DOTA (**8**) is a valuable DOTA synthon especially if a mild deprotection step of the coupled DOTA is required. The synthesis of this compound[20] can be facilitated by using an allyl-protected acetic acid derivative instead of a *tert*-butyl-protected one in the first step of the synthesis. The allyl protecting group can easily be removed with tetrakis-(triphenylphosphine)-palladium as the catalyst (Scheme 3).

This is advantageous because the benzyl protecting groups are not inert against the harsh deprotection conditions required for the *tert*-butyl-groups with 12 N HCl/dioxane 1:1 in the last step of the synthesis leading to fragmentations of the product and low reaction yields (Fig. 3).

However, using the synthesis approach described above, much less side products can be observed in the final deprotection step (Fig. 4).

Moreover, a new DOTA-derivative, tris-4-nitro-benzyl-DOTA (**10**), was synthesized. As the 4-nitro-benzyl-group can readily be cleaved with TBAF (tetrabutylammonium fluoride) in organic solvents,[21] this compound should be deprotectable under very mild conditions and should therefore be suitable for the derivatization of sensitive molecules with DOTA. The compound could be obtained in a three-step synthesis in moderate yields. However, it was found to be highly sensitive, and therefore it could not successfully be used for coupling experiments.

2.2. Applicability of different DOTA derivatives for multimerization reactions

For some applications it is necessary to introduce a high number of chelators into one molecule. This is, for

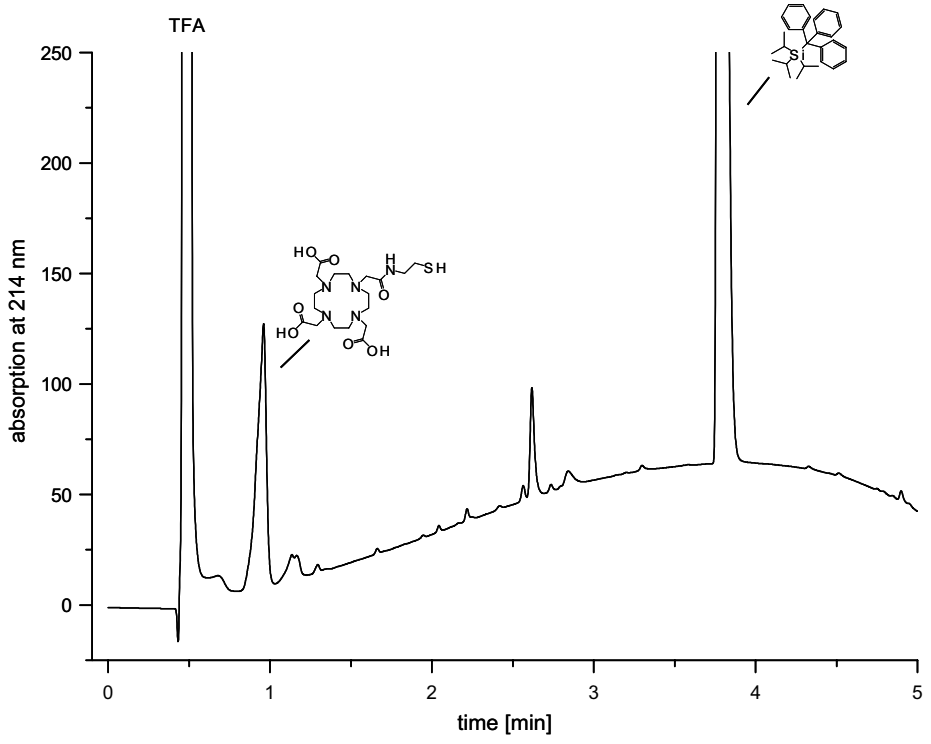
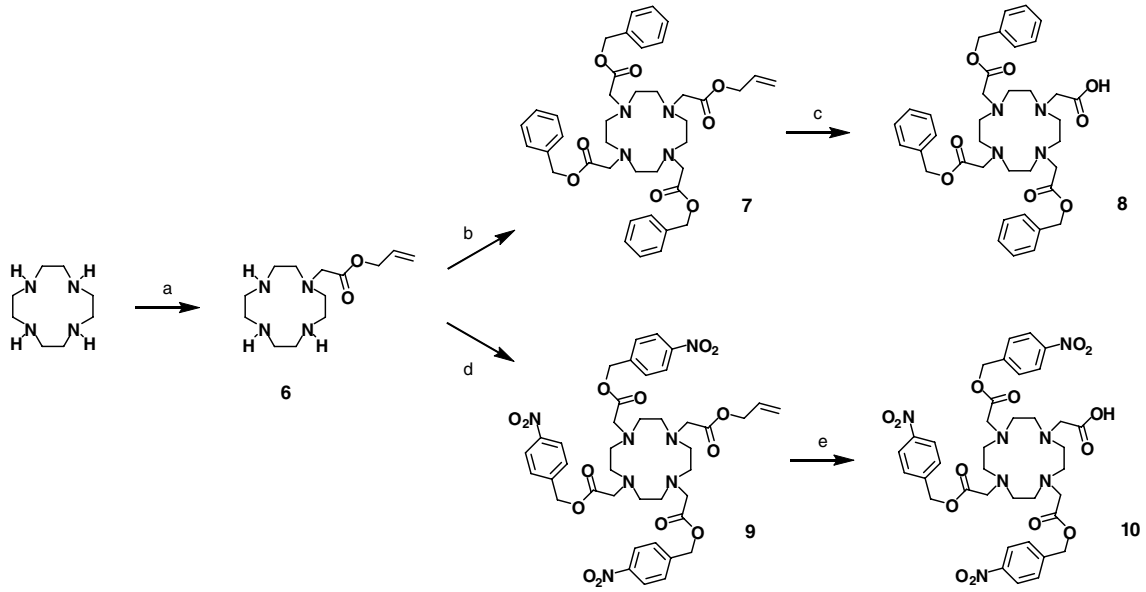


Figure 2. Chromatogram of the deprotection reaction of *S*-trityl-mercaptoethylamino-DOTA with concentrated TFA after 5 min. Elution gradient: 0–100% MeCN + 0.1% TFA in 5 min (note that the product does not contain a strong chromophore absorbing at 214 nm).



Scheme 3. Synthesis of tris-benzyl-DOTA and tris-4-nitro-benzyl-DOTA. Reagents and conditions: (a) allyl-chloroacetate, $CHCl_3$, 1 h 4 °C, 6 h, rt, 66%; (b) benzyl-bromoacetate, K_2CO_3, MeCN, rt, 10 h, 44%; (c) tetrakis-(triphenylphosphine)-palladium, morpholine, CH_2Cl_2, rt, 3 h, 71%; (d) 4-nitrobenzyl-bromoacetate, K_2CO_3, MeCN, rt, 2 h, 78%; (e) tetrakis-(triphenylphosphine)-palladium, morpholine, CH_2Cl_2, rt, 1 h, 27%.

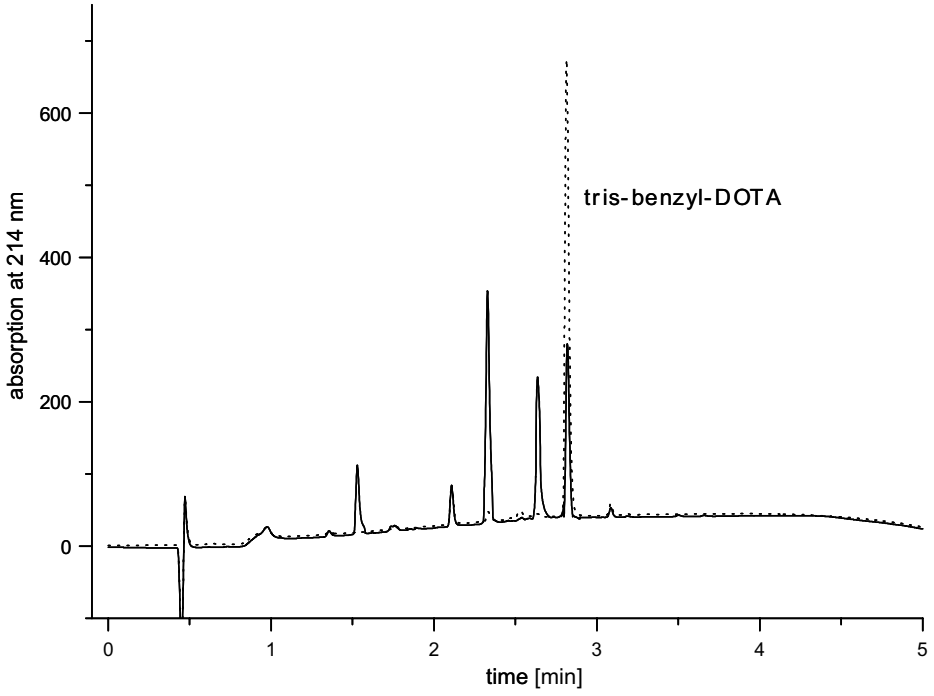


Figure 3. Decomposition of tris-benzyl-DOTA with 12 N HCl/dioxane 1:1 after 10 min (dashed line) and 12 h (compact line). Elution gradient: 0–100% MeCN + 0.1% TFA in 5 min.

example, favorable for the synthesis of MRI contrast agents or radiotracers which require high amounts of metal ions, for example, gadolinium, per molecule to accomplish high contrasts or for achieving high specific activities of radiolabeled compounds.

Therefore, several DOTA-derivatives were investigated with regard to their coupling yields in multimerization reactions. For the multimerization reactions PAMAM dendrimers containing a polyethyleneglycol linker with a terminal protected thiol group and one to 128 amine functions were used.[8] These dendrimers were reacted with tris-*t*Bu-DOTA (**3**), thiol-DOTA (**5**), tris-benzyl-DOTA (**8**), tris-4-nitrobenzyl-DOTA (**10**), tris-allyl-DOTA (**11**),[22] DOTA-PFP-ester (**12**)[13], and DOTA-PNP-ester (**13**)[13] (Scheme 4).

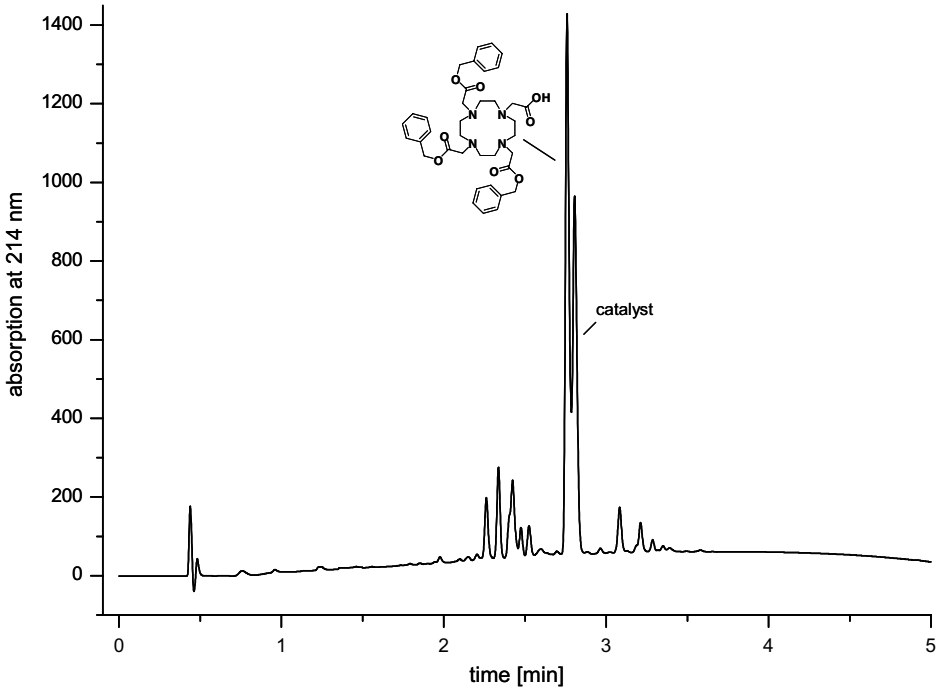


Figure 4. Chromatogram of the final deprotection step of **7** to tris-benzyl-DOTA with tetrakis-(triphenylphosphine)-palladium as catalyst after 3 h. Elution gradient: 0–100% MeCN + 0.1% TFA in 5 min.

These experiments showed that tris-*t*Bu-DOTA and tris-allyl-DOTA could be coupled to the dendrimers containing one to eight amino functions giving homogeneous products in good yields (Table 1 and Fig. 5). However, the subsequent deprotection steps of the DOTA-multimers were problematic. In the case of the tris-*t*Bu-DOTA-multimers, the deprotection with concentrated TFA gave rise to fragmentations of the polyethyleneglycol linker of the dendrimer molecules within 10 min. Furthermore, the deprotection reaction was very slow and led to heterogeneous products due to incomplete removal of the protecting groups. In the case of the tris-allyl-DOTA-multimers, the deprotection reaction was quantitative within short reaction times but gave reaction mixtures that were difficult to purify as the catalyst tetrakis-(triphenylphosphine)-palladium gave very high UV-signals which prevented the HPLC purification of the products.

The active esters of DOTA should be advantageous building blocks for multimerization reactions, as they can be coupled directly without the use of additional coupling reagents and furthermore, subsequent deprotection steps are dispensable. DOTA-PFP-ester and DOTA-PNP-ester were reacted with dendrimers containing one to eight amino functions. It could be observed that the active esters reacted inefficiently with the amino groups of the dendrimers producing inhomogeneous products even when applied in high excess of up to five equivalents of active ester per amino function (Table 1). However, it could be seen that the DOTA-PFP-ester showed a much higher reactivity than the DOTA-PNP-ester (Table 1).

Table 1. Coupling yields determined by HPLC of the different DOTA derivatives with dendrimers containing a different number of amino functions

DOTA-derivative	Coupling yields in % with *n* amino functions per dendrimer							
	n = 1	*n* = 2	*n* = 4	*n* = 8	*n* =16	*n* = 32	*n* = 64	*n* = 128
Tris-*t*Bu-DOTA (**3**)	100 (83)[a]	100 (89)[a]	100 (73)[a]	95 (70)[a]	n.d.	n.d.	n.d.	n.d.
Thiol-DOTA (**5**)	100 (91)[a]	100 (79)[a]	100 (83)[a]	100 (89)[a]	100 (73)[a]	100 (85)[a]	100 (70)[a]	100 (65)[a]
Tris-benzyl-DOTA (**8**)	60	4	0	n.d.	n.d.	n.d.	n.d.	n.d.
Tris-allyl-DOTA (**11**)	100 (79)[a]	100 (76)[a]	94 (84)[a]	93	n.d.	n.d.	n.d.	n.d.
DOTA-PFP-ester (**12**)	56	43	28	10	n.d.	n.d.	n.d.	n.d.
DOTA-PNP-ester (**13**)	60	40	6	0	n.d.	n.d.	n.d.	n.d.

n.d., not determined.
[a] Yields of isolated products.

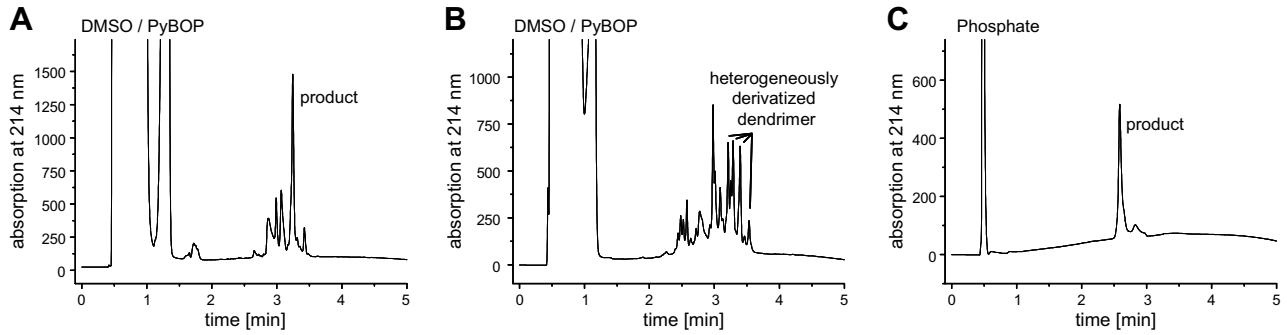


Figure 5. Chromatograms of the derivatization reactions of an 8 amino functions containing dendrimer with tris-*t*Bu-DOTA (A), tris-benzyl-DOTA (B), and thiol-DOTA (C). Elution gradient: 0–100% MeCN + 0.1% TFA in 5 min.

Tris-benzyl-DOTA showed coupling properties similar to the active esters of DOTA in the multimerization reactions and also gave inhomogeneous products due to insufficient coupling yields (Table 1 and Fig. 5).

In contrast to this, thiol-DOTA could be introduced quantitatively after previous derivatization of the dendrimer amino groups with maleimido caproic acid[8] (Table 1). This approach produces DOTA-multimers that do not require an additional deprotection step and is therefore also highly suited for the derivatization of biomolecules. Furthermore, the coupling reaction was complete within a few minutes and the products could be obtained in high homogeneity (Fig. 5).

In summary, tris-*t*Bu-DOTA and tris-allyl-DOTA can be coupled quantitatively even to high numbers of amino functions but show unfavorable deprotection properties. Due to the high UV-signals produced by the deprotection catalyst, tris-allyl-DOTA is especially suited for solid phase chemistry. Tris-benzyl-DOTA, DOTA-PFP-ester, and DOTA-PNP-ester show insufficient reactivities when coupled to a high number of amino functions and are therefore not suited for multimerization reactions, although the active esters give good results when conjugated to biomolecules. Thiol-DOTA quantitatively reacts with maleimide-derivatized dendrimers and the products can be obtained in good yields and high homogeneity. Due to this, thiol-DOTA is a suitable compound for generating DOTA-multimers applicable in molecular imaging providing high contrasts.

3. Conclusion

In summary, the synthesis routes for tris-*t*Bu-DOTA, tris-benzyl-DOTA, and thiol-DOTA could be improved and the synthesis of the novel compound tris-4-nitrobenzyl-DOTA could be established.

Further on, we determined the applicability of the DOTA derivatives tris-*t*Bu-DOTA, thiol-DOTA, tris-benzyl-DOTA, tris-4-nitrobenzyl-DOTA, tris-allyl-DOTA, DOTA-PFP-ester, and DOTA-PNP-ester for multimerization reactions of PAMAM dendrimers of different sizes. It was found that tris-*t*Bu-DOTA and tris-allyl-DOTA could be multimerized and gave homogeneous coupling products. However, the deprotection of the products was problematic showing that the application of tris-*t*Bu-DOTA and tris-allyl-DOTA is restricted to non-sensitive molecules and solid phase syntheses, respectively. Tris-benzyl-DOTA, DOTA-PNP-ester, and DOTA-PFP-ester showed low reactivities toward the dendritic core molecules producing heterogeneous products and thus were not suitable for multimerization reactions.

However, it was found that thiol-DOTA has superior coupling properties regarding its applicability for a multiple introduction into dendritic molecules: it could be introduced quantitatively generating highly homogeneous DOTA-multimers which do not require deprotection after coupling. Thus, thiol-DOTA is suitable for the derivatization of multimeric scaffolds and the obtained DOTA-multimers could, when introduced into carrier molecules, generate high contrasts in molecular imaging.

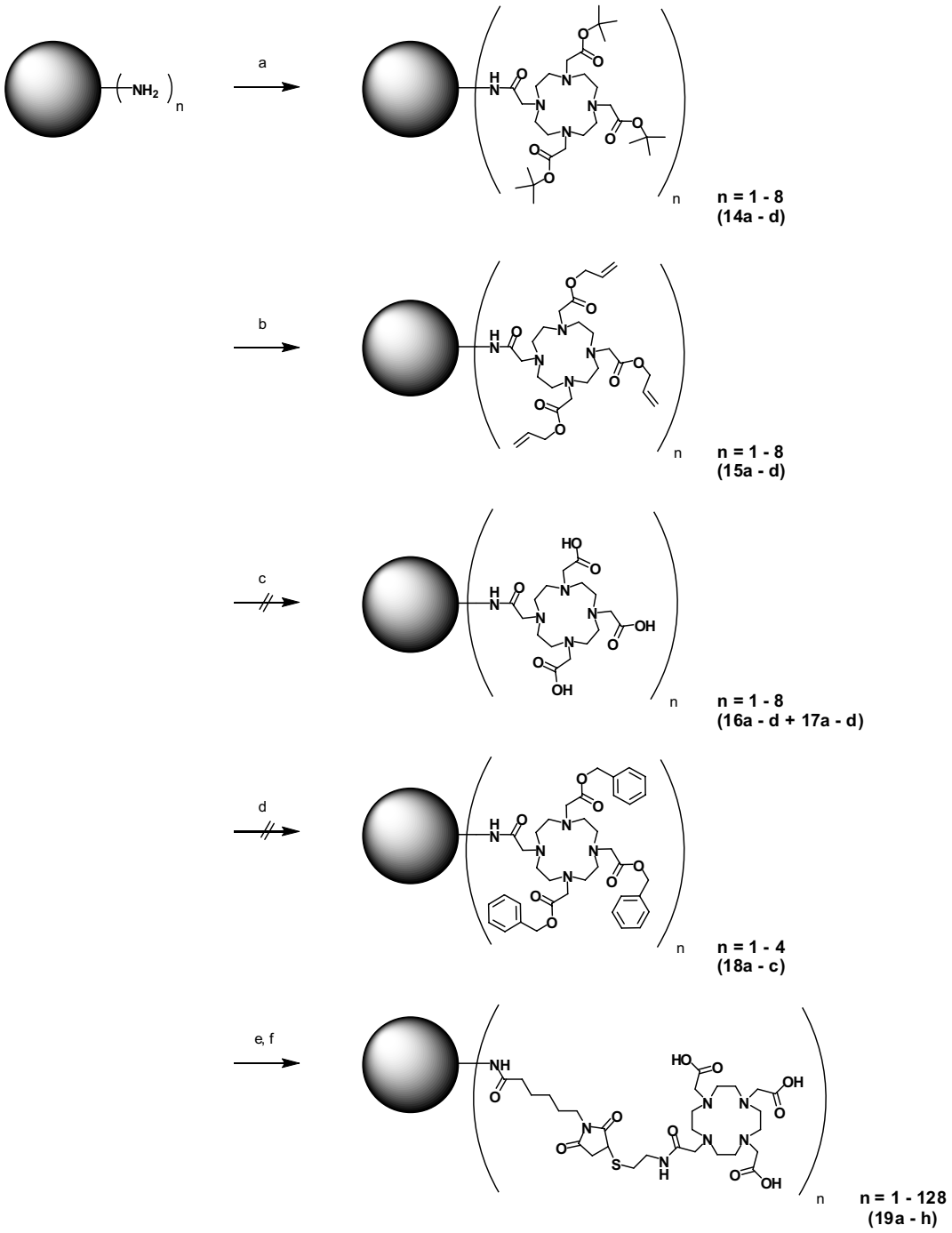
4. Experimental

4.1. General

All commercially available chemicals were of analytical grade and used without further purification prior to use.

An Agilent 1100® system with a Chromolith® Performance (RP-18e, 100–4.6 mm, Merck, Germany) column was used for analytical purposes. Semi-preparative HPLC purifications were performed using a Gyncotech P-580 system (Germering, Germany) equipped with a variable SPD 6-A UV detector and a C-R5A integrator (both Shimadzu, Duisburg, Germany). The column applied was a Chromolith® (RP-18e, 100–10 mm, Merck, Germany).

The MALDI-TOF spectra were obtained on a Kratos Analytical Compact Maldi III system. ESI spectra were obtained using a Triple–Quadrupole-mass spectrometer TSQ 7000 (Thermo Fisher Scientific, Bremen). NMR spectra were taken using a Varian Mercury Plus 300 MHz and a Varian NMR System 500 MHz, respectively.



Scheme 4. Reaction of the PAMAM dendrimers containing 1–128 amine functions with the different DOTA derivatives. Reagents and conditions: (a) tris-*t*Bu-DOTA, PyBOP, DIPEA, DMF, rt, 3 h, 70–83%; (b) tris-allyl-DOTA, PyBOP, DIPEA, DMF, rt, 2.5 h, 76–84%; (c) tris-benzyl-DOTA, PyBOP, DIPEA, DMF, rt, 5 h, products not isolated; (d) DOTA-PFP-ester or DOTA-PNP-ester, DIPEA, DMF, rt, 1 h or 10 h, products not isolated; (e) maleimido caproic acid, PyBOP, DIPEA, DMF, rt, 40 min, 29–84%; (f) thiol-DOTA, PBS (0.05 M P, 0.15 M NaCl, pH 7.2), rt, 10 min, 65–91%.

Tris-allyl-DOTA, DOTA-PFP-ester, and DOTA-PNP-ester were synthesized according to the literature methods.[13,22]

4.2. (1,4,7,10-Tetraaza-cyclododec-1-yl)-acetic acid benzyl ester (1)

To a solution of cyclene (3 g, 17.4 mmol) in 20 mL $CHCl_3$, a solution of benzyl-bromoacetate (2 g, 8.7 mmol) in 5 mL $CHCl_3$ was added dropwise over a period of one hour at room temperature. After two hours, the solvent was evaporated at room temperature and the product was purified via column chromatography on silica with $CHCl_3$/EtOH/$NH_4OH_{(conc.)}$ 8:9:4 as the eluent ($R_f = 0.6$).

Special care has to be taken that the temperature of the soluted product never rises above 25 °C as this leads to

an unwanted internal ring formation. The product was obtained as a colorless oil (2.38 g, 7.44 mmol, 86%). ^{1}H NMR (DMSO-d_6) (δ, ppm; *J*, Hz): 7.41 (s, 5H); 5.21 (s, 2H); 3.51–3.43 (m, 2H); 2.64–2.38 (m, 16H). ^{13}C NMR (DMSO-d_6) (δ, ppm): 175.8; 145.1; 130.1; 129.3; 127.5; 75.6; 57.8; 54.9; 53.1; 53.0; 49.2. MALDI-MS (*m*/*z*) for $[M+H]^+$(calculated): 320.6 (321.4).

4.3. (4,7,10-Tris-*tert*-butoxycarbonylmethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-acetic acid benzyl ester (2)

To a suspension of (1,4,7,10-tetraaza-cyclododec-1-yl)-acetic acid benzyl ester (2.38 g, 7.44 mmol) and powdered K_2CO_3 (4.27 g, 30.5 mmol) in 40 mL acetonitrile, a solution of *tert*-butyl bromoacetate (5.8 g, 29.76 mmol) in 10 mL acetonitrile was added dropwise over a period of one hour at room temperature. The reaction was monitored by TLC with $CHCl_3$/EtOH 9:1 as mobile phase. When the reaction was complete, the solids were removed and the volatile components were evaporated. As the product showed a considerable decomposition when incubated for longer than two hours with silica, it was rapidly purified via column chromatography on silica with first $CHCl_3$ and subsequently $CHCl_3$/EtOH 9:1 as the eluent (R_f = 0.5). The product was obtained as a colorless foam (3.29 g, 4.97 mmol, 67%). ^{1}H NMR (DMSO-d_6) (δ, ppm; *J*, Hz): 7.35 (s, 5H); 5.13 (s, 2H); 3.46–3.39 (m, 2H); 3.31 (s, 2H); 3.29–2.71 (bs, 7H); 2.43–1.94 (bs, 6H); 1.44 (s, 9H); 1.40 (bs, 18H). ^{13}C NMR (DMSO-d_6) (δ, ppm): 174.3; 172.9; 144.7; 129.8; 128.7; 127.3; 85.7; 75.3; 58.3; 57.1; 53.8; 52.6; 52.3; 50.5; 31.2. MALDI-MS (*m*/*z*) for $[M+H]^+$(calculated): 662.8 (663.4).

4.4. (4,7,10-Tris-*tert*-butoxycarbonylmethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-acetic acid (tris-*t*Bu-DOTA) (3)

To a solution of (4,7,10-tris-*tert*-butoxycarbonylmethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-acetic acid benzyl ester (830 mg, 1.25 mmol) in 40 mL THF/MeOH 1:1, palladium on carbon (10% Pd, 114 mg) was added and the reaction mixture was stirred in a hydrogen atmosphere at room temperature. The reaction was monitored by HPLC. After completion of the reaction, the catalyst was removed by filtration, the solvents were evaporated, and as the product showed a considerable decomposition when incubated for longer than three hours with silica it was rapidly purified via column chromatography on silica with a gradient of water/MeCN + 0.1% TFA starting with MeCN (R_f = 0.5 with water/MeCN 1:1 + 0.1% TFA). The product was obtained as a colorless solid (507 mg, 0.89 mmol, 71%). ^{1}H NMR (DMSO-d_6) (δ, ppm; *J*, Hz): 3.46 (m, 2H); 3.36 (s, 6H); 3.33 (bs, 4H); 2.87 (bs, 4H); 2.72 (bs, 4H); 2.64 (bs, 4H); 1.41 (s, 18H); 1.40 (s, 9H). ^{13}C NMR (DMSO-d_6) (δ, ppm): 179.63; 179.30; 89.53; 65.63; 64.61; 61.88; 60.78; 58.59; 56.09; 36.88; 36.83. MALDI-MS (*m*/*z*) for $[M+H]^+$(calculated): 572.6 (573.4). ESI-MS (*m*/*z*) for $[M+H]^+$ (calculated): 573.4 (573.4).

4.5. *S*-Trityl-mercaptoethylamino-DOTA (*S*-trityl-thiol-DOTA) (4)

To a solution of *S*-trityl-mercaptoethanolamine trifluoroacetate (2.54 g; 5.9 mmol) and DOTA (3 g; 5.9 mmol) in 40 mL water/MeCN 1:1, a solution of DCC (1.21 g; 5.86 mmol) in 5 mL pyridine was added dropwise at room temperature. After reaction overnight, the solvents were evaporated and the product was purified via column chromatography on silica with water/MeCN 1:1 + 0.1% TFA as the eluent (R_f = 0.8). The product was obtained as a light yellow solid (952 mg, 1.4 mmol, 23%) after lyophilization. ^{1}H NMR (DMSO-d_6) (δ, ppm; *J*, Hz): 7.35–7.21 (m, 15H); 3.96–3.04 (m, 18H); 3.02–2.97 (m, 8H); 2.24 (t, 2H, J^3 = 6.7). ^{13}C NMR (DMSO-d_6) (δ, ppm): 158.87; 158.45; 145.00; 129.71; 128.74; 127.45; 66.73; 46.54; 46.48; 26.62. MALDI-MS (*m*/*z*) for $[M+H]^+$(calculated): 706.1 (706.3).

4.6. Mercaptoethylamino-DOTA (thiol-DOTA) (5)

S-Trityl-thiol-DOTA (952 mg, 1.4 mmol) was dissolved in a mixture of 200 μL TIS and 5 mL TFA and reacted for 5 min at room temperature. The volatile components were evaporated and the product was purified using semi-preparative HPLC with 0–20% MeCN + 0.1% TFA in 3 min as the gradient (R_t = 1.1 min). The product was isolated as a yellow oil (363 mg, 0.78 μmol, 56%) after lyophilization. ^{1}H NMR (DMSO-d_6) (δ, ppm; *J*, Hz): 3.98 (bs, 2H); 3.84 (bs, 2H); 3.59 (bs, 6H); 3.37–3.09 (m, 16H); 2.54 (q, 2H, J^3 = 7.5); 2.41 (t, 1H, J^3 = 7.6). ^{13}C NMR (DMSO-d_6) (δ, ppm): 158.93; 158.51; 54.61; 53.47; 51.22; 51.07; 49.29; 48.99; 42.96; 23.97. MALDI-MS (*m*/*z*) for $[M+H]^+$(calculated): 463.9 (464.1). ESI-MS (*m*/*z*) for $[M+H]^+$ (calculated): 464.2 (464.1).

4.7. (1,4,7,10-Tetraaza-cyclododec-1-yl)-acetic acid allyl ester (6)

To a solution of cyclene (560 mg, 3.26 mmol) in 40 mL $CHCl_3$, a solution of allyl-chloroacetate (190 μL, 1.63 mmol) in 10 mL $CHCl_3$ was added dropwise over one hour at 4 °C. After six hours at room temperature, the solvent was evaporated and the product was purified via column chromatography on silica with $CHCl_3$/EtOH/$NH_4OH_{(conc.)}$ 8:9:4 as the eluent (R_f = 0.5). The product was obtained as a colorless oil (290 mg, 1.07 mmol, 66%). ^{1}H NMR (DMSO-d_6) (δ, ppm; *J*, Hz): 5.97–5.84 (m, 1H); 5.33–5.17 (m, 2H); 4.55–4.53 (m, 2H); 3.38 (s, 2H); 2.64–2.38 (m, 16H). ^{13}C NMR (DMSO-d_6) (δ, ppm): 171.46; 133.30; 118.47; 64.95; 55.93; 51.83; 47.42; 46.45; 45.51. MALDI-MS (*m*/*z*) for $[M+H]^+$(calculated): 270.0 (271.2). ESI-MS (*m*/*z*) for $[M+H]^+$ (calculated): 271.2 (271.2).

4.8. (4-Allyloxycarbonylmethyl-7,10-bis-benzyloxycarbonylmethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-acetic acid benzyl ester (7)

To a solution of (1,4,7,10-tetraaza-cyclododec-1-yl)-acetic acid allyl ester (290 mg, 1.07 mmol) in 40 mL

MeCN, powdered K_2CO_3 (600 mg, 4.28 mmol) and a solution of benzyl-bromoacetate (671 μL, 4.28 mmol) in 10 mL MeCN were added at room temperature. After reaction overnight, the suspension was filtered, the solvent evaporated and the product purified via column chromatography on silica with $CHCl_3$/EtOH 9:1 as the eluent ($R_f = 0.2$). The product was obtained as a colorless foam (340 mg, 0.48 mmol, 44%). ^{1}H NMR (DMSO-d_6) (δ, ppm; *J*, Hz): 7.41–7.29 (m, 15H); 6.00–5.85 (m, 1H); 5.38–5.22 (m, 2H); 5.17–5.07 (m, 6H); 4.62–4.57 (m, 2H); 3.97–3.94 (m, 5H); 3.49–2.54 (m, 16H). ^{13}C NMR (DMSO-d_6) (δ, ppm): 174.04; 173.81; 136.45; 132.97; 129.09; 128.85; 128.66; 118.76; 66.67; 66.57; 65.78; 65.44; 55.51; 55.40; 53.21; 49.12. MALDI-MS (*m*/*z*) for $[M+H]^+$(calculated): 714.6 (715.4). ESI-MS (*m*/*z*) for $[M+H]^+$ (calculated): 715.3 (715.4).

4.9. (4,7,10-Tris-benzyloxycarbonylmethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-acetic acid (tris-benzyl-DOTA) (8)

To a solution of (4-allyloxycarbonylmethyl-7,10-bis-benzyloxycarbonylmethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-acetic acid benzyl ester (340 mg, 0.48 mmol) in 40 mL CH_2Cl_2, morpholine (830 μL; 827 mg, 9.5 mmol) and tetrakis-(triphenylphosphine)-palladium (165 mg, 0.14 mmol) were added at room temperature. After three hours, the solvent was evaporated and the product was purified via column chromatography on silica with water/MeCN 45:55 + 0.1% TFA as the eluent ($R_f = 0.5$). The product was obtained as a colorless foam (230 mg, 0.34 mmol, 71%). ^{1}H NMR (DMSO-d_6) (δ, ppm; *J*, Hz): 7.37–7.30 (m, 15H); 5.14–5.09 (m, 6H); 4.11–4.02 (m, 2H); 3.80–3.74 (m, 6H); 3.42–3.07 (m, 16H). ^{13}C NMR (DMSO-d_6) (δ, ppm): 170.80; 169.72; 136.22; 129.12; 128.84; 128.75; 66.70; 63.92; 54.29; 53.65; 51.48; 49.17. MALDI-MS (*m*/*z*) for $[M+H]^+$(calculated): 673.9 (675.3). ESI-MS (*m*/*z*) for $[M+H]^+$ (calculated): 675.3 (675.3).

4.10. (4-Allyloxycarbonylmethyl-7,10-bis-4-nitrobenzyloxycarbonylmethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-acetic acid 4-nitrobenzyl ester (9)

To a solution of (1,4,7,10-tetraaza-cyclododec-1-yl)-acetic acid allyl ester (291 mg, 1.08 mmol) in 40 mL MeCN, powdered K_2CO_3 (604 mg, 4.31 mmol) and a solution of 4-nitrobenzyl-bromoacetate (1.18 g, 4.31 mmol) in 10 mL MeCN were added at room temperature. After two hours, the suspension was filtered, the solvent evaporated, and the product purified via column chromatography on silica with $CHCl_3$/EtOH 9:1 as the eluent ($R_f = 0.1$). The product was obtained as a colorless foam (720 mg, 0.87 mmol, 78%). ^{1}H NMR (DMSO-d_6) (δ, ppm; *J*, Hz): 8.23 (d, 6H, $J^3 = 8.8$); 7.69 (d, 6H, $J^3 = 8.8$); 5.98–5.85 (m, 1H); 5.39–5.21 (m, 8H); 4.61–4.58 (m, 2H); 4.05 (s, 6H); 3.96 (s, 2H); 3.24 (s, 16H). ^{13}C NMR (DMSO-d_6) (δ, ppm): 169.06; 168.41; 147.85; 144.07; 132.85; 129.39; 124.23; 118.99; 65.51; 65.34; 53.74; 53.21; 49.15. MALDI-MS (*m*/*z*) for $[M+H]^+$(calculated): 850.2 (850.3). ESI-MS (*m*/*z*) for $[M+H]^+$ (calculated): 850.4 (850.3).

4.11. (4,7,10-Tris-4-nitrobenzyloxycarbonylmethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-acetic acid (tris-4-nitrobenzyl-DOTA) (10)

To a solution of (4-allyloxycarbonylmethyl-7,10-bis-4-nitro-benzyloxycarbonylmethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-acetic acid 4-nitrobenzyl ester (720 mg, 0.87 mmol) in 40 mL CH_2Cl_2, morpholine (1.48 mL; 1.47 g, 17 mmol) and tetrakis-(triphenylphosphine)-palladium (294 mg, 0.25 mmol) were added at room temperature. After one hour, the solvent was evaporated and the product was purified via column chromatography on silica with water/MeCN 1:1 + 0.1% TFA as the eluent ($R_f = 0.5$). The product was obtained as a colorless foam (190 mg, 0.23 mmol, 27%). ^{1}H NMR (DMSO-d_6) (δ, ppm; *J*, Hz): 8.23 (d, 6H, $J^3 = 8.8$); 7.69 (d, 6H, $J^3 = 8.8$); 5.37–5.24 (m, 6H); 4.12–4.04 (m, 2H); 4.03 (s, 6H); 3.21 (s, 16H). ^{13}C NMR (DMSO-d_6) (δ, ppm): 168.96; 168.21; 147.63; 144.01; 129.28; 124.18; 66.84; 65.49; 65.30; 53.69; 53.19; 49.11. MALDI-MS (*m*/*z*) for $[M+H]^+$(calculated): 809.7 (810.3).

4.12. Reaction of PAMAM dendrimers with tris-*t*Bu-DOTA (14a–d)

A solution of PyBOP (2.9 equiv per amine function of the respective dendrimer) and DIPEA (3 equiv per amine function) in 300 μL DMF was added to tris-*t*Bu-DOTA (**3**) (3 equiv per amine function) and reacted for two minutes. Subsequently, this mixture was added to a solution of the respective full generation dendrimer containing 1–8 amine functions (50 μmol) in 200 μL DMF. After 3 h, the products were purified using semi-preparative HPLC using a gradient of 10–100% MeCN + 0.1% TFA over 12 min. The products were isolated as pale yellow solids in yields of 70–83% after lyophilization.

G_0—1 DOTA: MALDI-MS (*m*/*z*) for $[M+H]^+$ (calculated): 1137.5 (1138.6). ESI-MS (*m*/*z*) for $[M+H]^+$ (calculated): 1138.8 (1138.6).
G_1—2 DOTA: MALDI-MS (*m*/*z*) for $[M+H]^+$ (calculated): 1921.5 (1921.2). ESI-MS (*m*/*z*) for $[M+H]^+$ (calculated): 1921.4 (1921.2).
G_2—4 DOTA: MALDI-MS (*m*/*z*) for $[M+H]^+$ (calculated): 3489.2 (3487.2). ESI-MS (*m*/*z*) for $[M+H]^+$ (calculated): 3486.8 (3487.2).
G_3—8 DOTA: ESI-MS (*m*/*z*) for $[M+H]^+$ (calculated): 6618.4 (6619.4).

4.13. Reaction of PAMAM dendrimers with tris-allyl-DOTA (15a–d)

A solution of PyBOP (2.4 equiv per amine function of the respective dendrimer) and DIPEA (5 equiv per amine function) in 300 μL DMF was added to tris-allyl-DOTA (2.5 equiv per amine function) and reacted

for two minutes. Subsequently, this mixture was added to a solution of the respective full generation dendrimer containing 1–8 amine functions (50 μmol) in 200 μL DMF. After 2.5 h, the products were purified using semi-preparative HPLC using a gradient of 15–100% MeCN + 0.1% TFA over 12 min. The products containing 1–4 DOTA moieties were isolated as yellow solids in yields of 76–84% after lyophilization.

G_0—1 DOTA: MALDI-MS (*m*/*z*) for $[M+H]^+$ (calculated): 1089.9 (1090.6).
G_1—2 DOTA: MALDI-MS (*m*/*z*) for $[M+H]^+$ (calculated): 1825.2 (1825.0).
G_2—4 DOTA: MALDI-MS (*m*/*z*) for $[M+H]^+$ (calculated): 3298.0 (3295.9). ESI-MS (*m*/*z*) for $[M+H]^+$ (calculated): 3295.2 (3295.9).
G_3—8 DOTA: ESI-MS (*m*/*z*) for $[M+H]^+$ (calculated): 6232.7 (6233.6).

4.14. Reaction of PAMAM dendrimers with DOTA-PNP-ester (16a–d)

A solution of DOTA-PNP-ester (2.5 equiv per amine function of the respective dendrimer) and DIPEA (6 equiv per amine function) in 300 μL DMF was added to a solution of the respective full generation dendrimer containing 1–8 amine functions (50 μmol) in 200 μL DMF and reacted overnight. The products were not isolated.

4.15. Reaction of PAMAM dendrimers with DOTA-PFP-ester (17a–d)

A solution of DOTA-PFP-ester (2.5 equiv per amine function of the respective dendrimer) and DIPEA (6 equiv per amine function) in 300 μL DMF was added to a solution of the respective full generation dendrimer containing 1–8 amine functions (50 μmol) in 200 μL DMF and reacted for one hour. The products were not isolated.

4.16. Reaction of PAMAM dendrimers with tris-benzyl-DOTA (18a–c)

A solution of PyBOP (2.9 equiv per amine function of the respective dendrimer) and DIPEA (5 equiv per amine function) in 300 μL DMF was added to tris-benzyl-DOTA (**8**) (3 equiv per amine function) and reacted for two minutes. Subsequently, this mixture was added to a solution of the respective full generation dendrimer containing 1–4 amine functions (50 μmol) in 200 μL DMF. The products were not isolated.

4.17. Reaction of PAMAM dendrimers with thiol-DOTA (19a–h)

To a solution of the respective maleimide-derivatized dendrimer containing 1–128 maleimide functions (10 μmol) in 200 μL MeCN was added a solution of thiol-DOTA (4 equiv per maleimide) in 250 μL PBS (0.05 M, 0.15 M NaCl, pH 7.2) and the mixture was reacted for 10 min. After acidification with 1 M HCL (200 μL) the products were purified using semi-preparative HPLC using a gradient of 20–100% MeCN + 0.1% TFA over 8 min. The products were isolated as white to ocher solids in yields of 65–91% after lyophilization.

Acknowledgment

The authors thank T. Timmermann from the Faculty of Pharmacy at the University of Heidelberg for recording NMR spectra.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.11.044.

References and notes

1. de Jong, M.; Breeman, W. A. P.; Valkema, R.; Bernard, B. F.; Krenning, E. P. *J. Nucl. Med.* **2005**, *46*, 13S.
2. Smith, C. J.; Volkert, W. A.; Hoffman, T. J. *Nucl. Med. Biol.* **2005**, *32*, 733.
3. Reubi, J. C.; Waser, B. *Eur. J. Nucl. Med. Mol. Imaging* **2003**, *30*, 781.
4. Meyer, A.; Auernheimer, J.; Modlinger, A.; Kessler, H. *Curr. Pharm. Des.* **2006**, *12*, 2723.
5. Witzig, T. E.; Molina, A.; Gordon, L. I.; Emmanouilides, C.; Schilder, R. J.; Flinn, I. W.; Darif, M.; Macklis, R.; Vo, K.; Wiseman, G. A. *Cancer* **2007**, *109*, 1804.
6. Ringhoffer, M.; Blumstein, N.; Neumaier, B.; Glatting, G.; von Harsdorf, S.; Buchmann, I.; Wiesneth, M.; Kotzerke, J.; Zenz, T.; Buck, A. K.; Schauwecker, P.; Stilgenbauer, S.; Döhner, H.; Reske, S. N.; Bunjes, D. *Br. J. Haematol.* **2005**, *130*, 604.
7. Kobayashi, H.; Kawamoto, S.; Choyke, P. L.; Sato, N.; Knopp, M. V.; Star, R. A.; Waldmann, T. A.; Tagaya, Y.; Brechbiel, M. W. *Mag. Reson. Med.* **2003**, *50*, 758.
8. Wängler, C.; Moldenhauer, G.; Eisenhut, M.; Haberkorn, U.; Mier, W. Unpublished results.
9. Kobayashi, H.; Sato, N.; Saga, T.; Nakamoto, Y.; Ishimori, T.; Toyama, S.; Togashi, K.; Konishi, J.; Brechbiel, M. W. *Eur. J. Nucl. Med.* **2000**, *27*, 1334.
10. Mamede, M.; Saga, T.; Kobayashi, H.; Ishimori, T.; Higashi, T.; Sato, N.; Brechbiel, M. W.; Konishi, J. *Clin. Cancer Res.* **2003**, *9*, 3756.
11. Hermanson, G. T. *Bioconjugate Techniques*; Academic Press, 1996.
12. Lewis, M. R.; Kao, J. Y.; Anderson, A.-L. J.; Shively, J. E.; Raubitschek, A. *Bioconj. Chem.* **2001**, *12*, 320.
13. Mier, W.; Hoffend, J.; Krämer, S.; Schuhmacher, J.; Hull, W. E.; Eisenhut, M.; Haberkorn, U. *Bioconj. Chem.* **2005**, *16*, 237.
14. Antunes, P.; Ginj, M.; Walter, M. A.; Chen, J.; Reubi, J.-C.; Maecke, H. R. *Bioconj. Chem.* **2007**, *18*, 84.
15. Ginj, M.; Schmitt, J. S.; Chen, J.; Waser, B.; Reubi, J.-C.; de Jong, M.; Schulz, S.; Maecke, H. R. *Chem. Biol.* **2007**, *13*, 1081.
16. Heppeler, A.; Froidevaux, S.; Mäcke, H. R.; Jermann, E.; Béhé, M.; Powell, P.; Hennig, M. *Chem. Eur. J.* **1999**, *5*, 1974.
17. Mukai, T.; Namba, S.; Arano, Y.; Ono, M.; Fujioka, Y.; Uehara, T.; Ogawa, K.; Konishi, J.; Saji, H. *J. Pharm. Pharmacol.* **2002**, *54*, 1073.

18. Mattila, K.; Siltainsuu, J.; Balaspiri, L.; Ora, M.; Lönnberg, H. *Org. Biomol. Chem.* **2005**, *3*, 3039.
19. Maltese, M. *J. Org. Chem.* **2001**, *66*, 7615.
20. Anelli, P. L.; Lattuada, L.; Gabellini, M.; Recanati, P. *Bioconj. Chem.* **2001**, *12*, 1081.
21. Kocieński, P. *Protecting Groups*; Thieme: Stuttgart, 1994, p. 138.
22. Wängler, B.; Beck, C.; Wagner-Utermann, U.; Schirrmacher, E.; Bauer, C.; Rösch, F.; Schirrmacher, R.; Eisenhut, M. *Tet. Lett.* **2006**, *47*, 5985.