TAME-Hex A − A Novel Bifunctional Chelating Agent for Radioimmunoimaging

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Keywords: Chelating agents / Radioimmunoimaging / Positron emission tomography / Gallium chelate / TAME derivative

Two novel (bifunctional) chelating agents, TAME-Hex A and B, which are polyaminopolycarboxylic acids based on the tripodal TAME [tris(aminomethyl)ethane] structure, have been designed and synthesized. The chelators show very good stability with gallium(III) ions and thus are highly effective candidates for use in radioimmunoimaging (RII), especially in positron emission tomography (PET).

Introduction

Bifunctional chelating agents continue to be an interesting topic in medicinal and chemical research because of their usefulness in the diagnosis and therapy of cancer diseases.[1] Radioimmunoimaging (RII) and radioimmunotherapy (RIT) rely on the ability of a molecule with a chelating functionality, which will sequester a radioactive metal ion, to combine with a monoclonal antibody or any other receptor-specific substrate, like an oligopeptide[2] or a Lewis^x structure analog.[3] This approach allows radiopharmaceuticals to be delivered specifically to malignant tissue while minimizing the risk of unspecific irradiation of sane tissue.

Chelating agents useful for RII and RIT should be able to form metal-chelates with high thermodynamic stability as well as high kinetic inertness in vivo to reduce intoxication arising from the loss of the radioactive (heavy-)metal ion. Derivatives of the chelating structures of DTPA (diethylenetriaminetetraacetic acid)[4] and DOTA (1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*′′′,*N*′′′-tetraacetic acid), like BAD[5] (Figure 1), are most frequently used for this purpose at present, but new chelators with improved or altered physical properties for different biological applications are desirable.

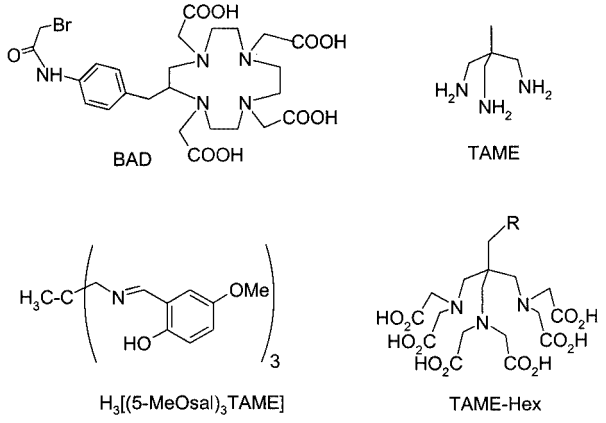

Figure 1. The structures of BAD, TAME, H_3[(5-MeOsal)$_3$TAME] and TAME-Hex

1,1,1-Tris(aminomethyl)ethane (TAME) (Figure 1) is a tridentate ligand which is used as a starting material in the synthesis of more complex ligands.[6,7] Salicylaldimines of TAME are used as chelating agents in the complete encapsulation of Ga^{3+} ions.[8] Together with the positron emitting isotope ^{68}Ga, which is available from a parent/daughter generator system (^{68}Ge/^{68}Ga) independently of an in-house cyclotron, these compounds are useful in positron emission tomography (PET), a very powerful technique used in medical diagnosis. An example of such a chelating agent, H_3[(5-MeOsal)$_3$TAME], which can be used to assess myocardial blood flow, is shown in Figure 1.[9]

Astonishingly, no bifunctional chelating agents based on the tripodal TAME structure, which could be useful for radioimmuno-imaging or -therapy, have been reported in the literature. In this paper, we report the synthesis of two novel, tripodal chelators, one bifunctional and the other monofunctional, which are derivatives of tris(aminomethyl)-

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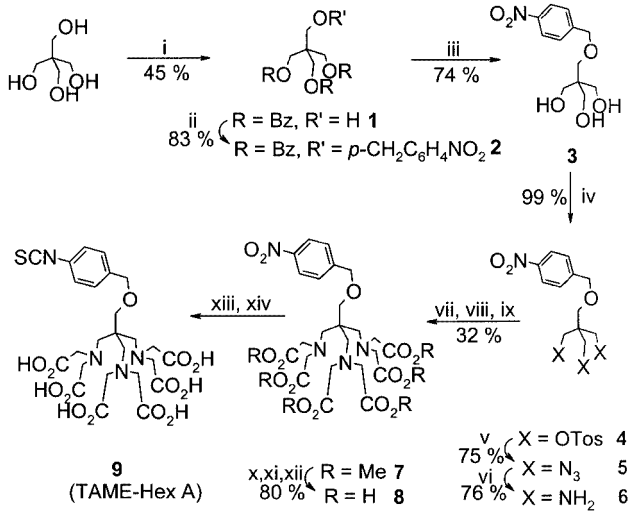
ethane-*N,N,N′,N′,N″,N″*-hexaacetic acid (TAME-Hex) (Figure 1). The Ga^{3+} complexes of these chelators should be very stable and are expected to be very useful in positron emission tomography.

Results and Discussion

TAME-Hex A

To obtain a bifunctional chelating agent, it was decided to introduce the *p*-nitrobenzyloxy moiety into the 2-position of TAME-Hex, which could then be easily transformed into the corresponding isothiocyanate for the purpose of conjugation to a monoclonal antibody or peptide. Starting from commercially available pentaerythritol, it was necessary to differentiate between one of the four chemically equivalent hydroxy groups of this compound. This was accomplished by acylation of pentaerythritol with three equivalents of benzoyl chloride in pyridine (Scheme 1). The threefold benzoylated product **1** could easily be isolated from the other over- and underacylated products by silica gel chromatography. As *O*-alkylation with *p*-nitrobenzyl bromide is usually not possible with sodium hydride as base, silver(I) oxide[10] was used for the transformation of **1** into the alkylated compound **2**. Deacylation of **2** with NaOMe delivered the desired *O*-(4-nitrobenzyl)pentaerythritol (**3**) in 28% yield over three steps.



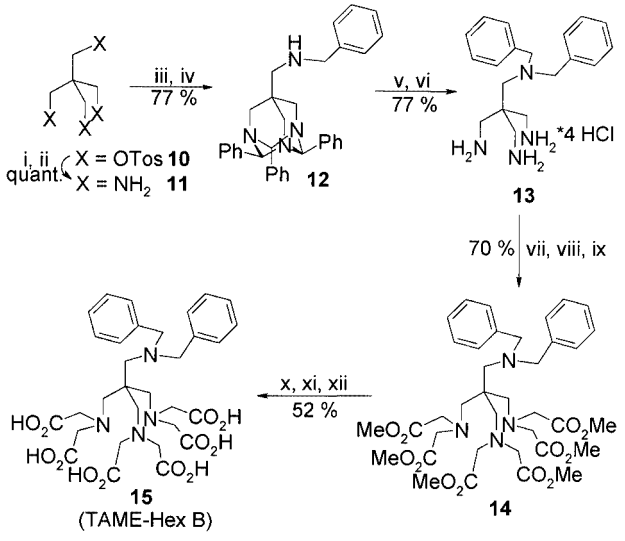
Scheme 1. Synthesis of TAME-Hex A (**9**). Reagents and conditions: i) BzCl (3 equiv.), Py; ii) *p*-nitrobenzyl bromide, Ag_2O, CH_2Cl_2; iii) NaOMe, MeOH/CH_2Cl_2; iv) TosCl, Py, DMAP; v) NaN_3, DMSO, 90 °C; vi) Ph_3P, THF/H_2O; vii) $BrCH_2CO_2tBu$, Na_2CO_3, DMF, 65 °C; viii) TFA; ix) CH_2N_2, MeOH; x) 1 N NaOH, MeOH/H_2O; xi) HCl; xii) RP-18 column; xiii) H_2/Pd-C, H_2O, Na_2CO_3; xiv) Cl_2CS, $CHCl_3$/H_2O

The next crucial step was the transformation of the three hydroxy groups of compound **3** into amines. This goal was best achieved by the threefold tosylation of **3** (→ **4**), nucleophilic substitution with sodium azide in DMSO (→ **5**) and the subsequent reduction of the triazide **5** to the triamine **6** by use of the Staudinger reaction.[11]

The carboxymethyl functionalities were introduced next. Although direct alkylation of **6** with bromoacetic acid should, in principle, be possible, the isolation of the highly polar product from the reaction mixture as well as the monitoring of the progress of the reaction would be difficult. Thus, the use of a bromoacetic ester is preferable. *tert*-Butyl bromoacetate was the reagent of choice because it will not undergo intramolecular lactam formation like many other esters. The alkylation of triamine **6** with *tert*-butyl bromoacetate and sodium carbonate in DMF delivered the corresponding sixfold *tert*-butyl ester. However, to achieve greater purity, deprotection of the ester groups by TFA and subsequent reaction with diazomethane to yield the hexamethyl ester **7** was necessary. Pure **7** was then saponified with 1 N NaOH in methanol/water, acidified with HCl to pH 1.5 and submitted to RP-18 column chromatography to obtain the chelator **8** in the free-acid form. Finally, the transformation of **8** to the isothiocyanate form **9** (TAME-Hex A) was performed by catalytic reduction and subsequent reaction with thiophosgene.

Tame-Hex B

Furthermore, a monofunctional derivative of TAME-Hex, TAME-Hex B, was synthesized (Scheme 2), which is also of interest for use in imaging methods like PET.



Scheme 2. Synthesis of TAME-Hex B (**15**). Reagents and conditions: i) NaN_3, DEG, 120 °C; ii) H_2/Pd-C, MeOH, HCl; iii) benzaldehyde, MeOH; iv) $NaBH_4$, $CHCl_3$/MeOH; v) BnBr, DMF, DIPEA; vi) 1.5 N HCl; vii) $BrCH_2CO_2tBu$, Na_2CO_3, DMF, 65 °C; viii) TFA; ix) CH_2N_2, MeOH; x) 1 N NaOH, MeOH/H_2O; xi) HCl; xii) RP-18 column

The advantage of this synthetic procedure is that one of the four amino groups in tetrakis(aminomethyl)methane (**11**),[12] which is obtained from the tetratosylate of pentaerythritol (**10**),[13] can be differentiated by the reaction with four equivalents of benzaldehyde and subsequent reduction

of the resulting aldimine to the triazaadamantane **12.**[14] Compound **12** was then benzylated and the benzylidene protecting groups removed by 1.5 N hydrochloric acid to afford the TAME derivative **13** in 77% yield. Alkylation with *tert*-butyl bromoacetate as mentioned above, deprotection with TFA and esterification with diazomethane delivered the hexamethyl ester **14** in 70% yield. Alkaline saponification and work up as described above for compound **9** finally led to the desired chelating agent **15** (TAME-Hex B).

Note that this synthesis can be is easily varied to give a bifunctional derivative of TAME-Hex B. To achieve this, it is necessary to use an alkylating or acylating reagent that contains a potential anchoring group for the derivatization of compound **12**.

Radiolabeling and Stability Testing of New Chelators

The stabilities of the $^{111}In^{3+}$- and $^{67}Ga^{3+}$-chelates of **8** and **15** were tested by performing *trans*-chelation experiments using DTPA (diethylenetriaminepentaacetic acid) as the competing ligand. Compound **9** could not be employed in these investigations because the isothiocyanate group transforms under the experimental conditions.

Both chelators could be radiolabeled with either ^{111}In or ^{67}Ga with less than 0.1% unchelated radiometal remaining at the end of the radiolabeling process. ^{111}In-**8** and ^{111}In-**15** had similar stabilities with about 10% of the metal-chelates remaining intact after 24 hours.

The stabilities of the ^{67}Ga chelates were significantly better: ^{67}Ga-**8** was very stable with 94% remaining intact after 10 days (Figure 2). ^{67}Ga-**15** was even more stable with around 99% still intact after 10 days (Figure 3).

TAME-Hex B **15** has nine potential donors to fully saturate the coordination spheres of either Ga^{III} or In^{III}; the higher stability of Ga^{III} chelates is probably due to its smaller ionic radius and higher charge density. This new TAME-Hex derivative is now being evaluated for application within nuclear medicine to stably chelate ^{67}Ga and ^{68}Ga.

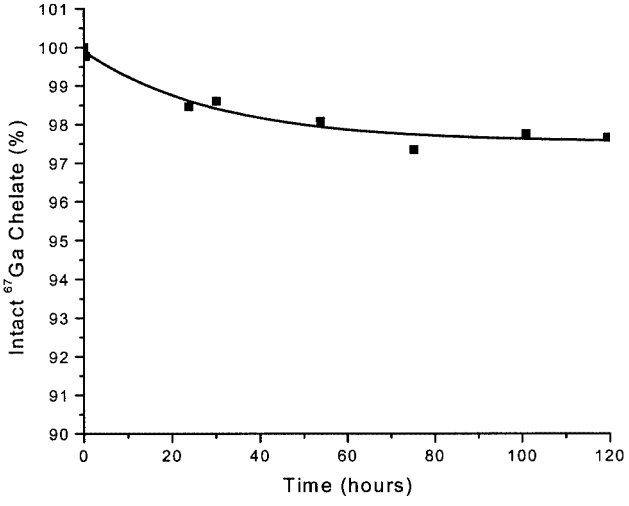


Figure 2. Stability of ^{67}Ga-**8** to *trans*-chelation by a 1000-fold excess of DTPA at an ambient temperature

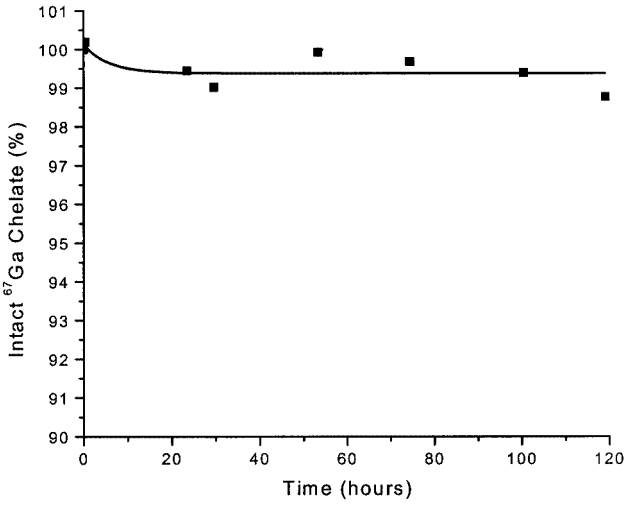


Figure 3. Stability of ^{67}Ga-**15** to *trans*-chelation by a 1000-fold excess of DTPA at an ambient temperature

Conclusions

In conclusion, we have designed and synthesized two novel chelating agents belonging to the class of polyaminopolycarboxylic acids based on the tripodal TAME structure. The first stability tests showed that the new gallium chelates are stable against *trans*-chelation by a 1000-fold excess of DTPA and thus are potentially highly effective candidates for use in radioimaging. Further investigations into the exact nature of the thermodynamics, kinetics and biological stabilities of the chelates are currently under investigation.

Experimental Section

General Remarks: Twice-distilled water was used for the reactions leading to and with chelating agents. Merck "Plastikfolien Kieselgel 60 F_{254}" plates were used for thin layer chromatography. Detection was achieved by UV absorption at λ = 254 nm or by dipping the plates in a solution of ammonium molybdate (20 g) and cerium(IV) sulfate in 10% sulfuric acid (Mostain, 400 mL) or a 1% solution of ninhydrin in ethanol and subsequent heating to 150 °C. Preparative flash column chromatography was performed with a pressure of 1.3−1.4 bar using Silica 60 (40−63 μm, 230−400 mesh ASTM) from Baker Co. or Knauer Europrep C_{18} (600pm, 35−70 μ, irregular). Melting points were determined in a Gallenkamp apparatus and are uncorrected. NMR spectra were measured with a Bruker AC 250 (250 MHz) or Bruker DRX 600 (600 MHz) spectrometer. MALDI mass spectra were measured with a KRATOS Analytical Kompact MALDI 2 spectrometer. 2,5-Dihydroxybenzoic acid (DHB) was used as the matrix in the positive mode unless otherwise stated, and azathiothymine (ATT) in the negative mode.

Radiolabeling: The chelators were radiolabeled with both ^{111}In and ^{67}Ga by heating them at elevated temperatures at a neutral pH and by using a transfer ligand of ammonium acetate. ^{111}In (2 μL, 1 mCi, 0.05 M HCl) or ^{67}Ga (2 μL, 1mCi, 0.05 M HCl), chelator **8** or **15** (5 μL, 2 mg·mL^{-1}) and 3 M NH_4OAc (50 μL, pH 7.0) were heated at 100 °C for 10 minutes. The radiochemical yield was assayed by mixing an aliquot of the reaction mixture with 5 mM DTPA (pH 7.0) immediately prior to C_{18} RP HPLC analysis with an eluent gradient of 50 mM NH_4OAc (pH 7.0) to 50 mM NH_4OAc (pH 7.0)/80% acetonitrile over 10 minutes at 0.7 mL·min^{-1}.

Stability Testing: The ^{111}In or ^{67}Ga chelates were diluted to 5 μM with 5 mM DTPA (pH 7.0) and stored at ambient temperature. Periodically 10 μL samples were removed and analyzed by C_{18} RP HPLC as outlined above.

Tri-*O*-benzoylpentaerythritol (1): Benzoyl chloride (3.6 mL, 31 mmol) was slowly added to a well-stirred suspension of pentaerythritol (1.3 g, 9.5 mmol) in dry pyridine (15 mL) at room temperature (gentle cooling was necessary after a while). After 3 h of stirring, water (120 mL) was added and the mixture was extracted with ethyl acetate (120 mL). The organic layer was washed with 5% HCl and water (100 mL each), dried with $MgSO_4$ and the solvents evaporated. The residue was purified by flash chromatography on a silica column (toluene/acetone, 10:1) to afford 1.9 g (4.3 mmol, 45%) of **1** as a viscous, colorless oil. ^1H NMR (250 MHz, $CDCl_3$): δ = 2.76 (t, ^3J = 6.8 Hz, 1 H, CH_2OH), 3.68 (d, ^3J = 6.8 Hz, 2 H, CH_2OH), 4.60 [s, 6 H, $C(CH_2O)_3$], 7.38−8.04 (m, 15 H, 3 Ph) ppm. $C_{26}H_{24}O_7 \cdot H_2O$ (466.5): calcd. C 66.94, H 5.62; found C 66.47, H 5.30.

Tri-*O*-benzoyl-*O*-(4-nitrobenzyl)pentaerythritol (2): A mixture of **1** (4.25 g, 9.47 mmol), 3-Å molecular sieves (4.0 g), silver oxide (3.30 g, 14.2 mmol) and 4-nitrobenzyl bromide (67 mg, 0.31 mmol) in dry CH_2Cl_2 (35 mL) was stirred for 40 h, after which it was filtered and the filtrate evaporated. The residue was applied on a silica column and chromatography was performed using CH_2Cl_2 as eluent, to give **2** (4.60 g, 7.88 mmol, 83%) as a nearly colorless, glassy mass. ^1H NMR (250 MHz, $CDCl_3$): δ = 3.77 (s, 2 H, CH_2OCH_2Ar), 4.60 (s, 2 H, OCH_2Ar), 4.64 [s, 6 H, $C(CH_2O)_3$], 7.37−8.02 (m, 19 H, Ar, 3 Ph) ppm. $C_{33}H_{29}NO_9$ (583.6): calcd. C 67.92, H 5.01, N 2.40; found C 67.18, H 5.04, N 2.53.

***O*-(4-Nitrobenzyl)pentaerythritol (3):** The tribenzoate **2** (4.55 g, 7.80 mmol) was dissolved in a mixture of CH_2Cl_2 (20 mL) and MeOH (40 mL) and NaOMe (300 mg, 5.5 mmol) was added to the well-stirred solution. After stirring for 24 h at room temperature, the mixture was neutralized with acidic resin (Amberlite IR-120), filtered and concentrated under reduced pressure. The residue was crystallized from toluene (40 mL) to afford **3** (1.56 g, 5.77 mmol, 74%) as a colorless solid, m.p. 107 °C. ^1H NMR (250 MHz, $CDCl_3$): δ = 2.23 (t, ^3J = 6.7 Hz, 3 H, 3 CH_2OH), 3.56 (s, 2 H, CH_2OCH_2Ar), 3.75 [d, ^3J = 6.7 Hz, 6 H, $C(CH_2OH)_3$], 4.60 (s, 2 H, OCH_2Ar), 7.46 (d, ^3J = 8.8 Hz, 2 H, Ar), 8.80 (d, ^3J = 8.8 Hz, 2 H, Ar) ppm. $C_{12}H_{17}NO_6$ (271.3): calcd. C 53.13, H 6.32, N 5.16; found C 53.23, H 5.92, N 4.74.

***O*-(4-Nitrobenzyl)-tris[*O*-(4-methylphenylsulfonyl)]pentaerythritol (4):** Compound **3** (3.03 g, 11.2 mmol) was dissolved in dry pyridine (90 mL) and tosyl chloride (8.60 g, 45.1 mmol) was added, followed by DMAP (140 mg, 1.15 mmol). The reaction mixture was stirred for 8 h at room temperature and for a further 8 h at 40 °C, after which it was poured into ice-water (250 mL) and extracted with CH_2Cl_2 (250 mL). The organic layer was washed with water (250 mL), dried with $MgSO_4$ and concentrated. The solid residue was purified by treatment with warm MeOH (100 mL) to afford **4** (8.14 g, 11.1 mmol, 99%) as a colorless solid, m.p. 134 °C. ^1H NMR (250 MHz, $CDCl_3$): δ = 2.44 (s, 9 H, 3 CH_3 {Tos}), 3.40 (s, 2 H, CH_2OCH_2Ar), 4.42 (s, 2 H, OCH_2Ar), 3.94 [s, 6 H, $C(CH_2O)_3$], 7.30−8.18 (m, 16 H, Ar, 3 Tos) ppm. $C_{33}H_{35}NO_{12}S_3$ (733.8): calcd. C 54.01, H 4.81, N 1.91; found C 54.01, H 4.94, N 1.61.

3-Azido-2,2-bis(azidomethyl)-1-(4-nitrobenzyloxy)propane (5): Sodium azide (5.3 g, 81 mmol) was added to a solution of the tritosylate **4** (4.96 g, 6.76 mmol) in dry DMSO (100 mL) and the mixture was stirred for 20 h at 90 °C. After cooling to room temperature, the mixture was poured into cold water (300 mL) and extracted with diethyl ether twice (200 and 100 mL). The organic layer was washed twice with brine and dried with Na_2SO_4. Concentration under reduced pressure afforded triazide **5** (2.03 g, 5.10 mmol, 75%) as a yellowish oil which could be used without further purification in the next step. ^1H NMR (250 MHz, $CDCl_3$): δ = 3.39 [s, 6 H, $C(CH_2O)_3$], 3.50 (s, 2 H, CH_2OCH_2Ar), 4.62 (s, 2 H, OCH_2Ar), 7.45−8.24 (m, 4 H, Ar) ppm.

2-(Aminomethyl)-2-[(4-nitrobenzyl)oxymethyl]propane-1,3-diamine (6): Triazide **5** (347 mg, 0.87 mmol) was dissolved in THF (5 mL) and triphenylphosphane (913 mg, 3.5 mmol) in THF (5 mL) was added to the stirred, ice-cooled solution under an Ar atmosphere. Water (2 mL) was added and the mixture was stirred at room temperature for 12 h and for a further 6 h at 50 °C. The solution was cooled and then neutralized by addition of conc. HCl (0.2 mL) and then concentrated under reduced pressure. The residue was partitioned between CH_2Cl_2 (40 mL) and 0.5 M HCl (40 mL), and the aqueous phase was extracted twice with CH_2Cl_2 (30 mL each). The aqueous phase was made alkaline (pH = 9−10) with NaOH and the product re-extracted with CH_2Cl_2 (4 × 30 mL). The organic layer was dried (Na_2SO_4) and concentrated to afford triamine **6** (211 mg, 0.66 mmol, 76%) as a colorless oil, and was used directly in the next step.

Tetramethyl 2-[Bis(methoxycarbonylmethyl)aminomethyl]-2-[(4-nitrobenzyl)oxymethyl]propylene-1,3-dinitrilotetraacetate (7): Triamine **6** (211 mg, 0.66 mmol) was dissolved in dry DMF (5 mL) and, after saturation of the solution with Ar, alkylated with *tert*-butyl bromoacetate (0.97 mL, 6.5 mmol) and Na_2CO_3 (687 mg, 6.5 mmol) at 65 °C for 18 h. The reaction mixture was stirred for 12 h at room temperature and then diluted with diethyl ether (50 mL) and water (50 mL). The organic layer was washed with brine (50 mL), dried with Na_2SO_4, concentrated and submitted to flash chromatography (petroleum ether/ethyl acetate, 10:1) to afford the hexaalkylated product as a colorless oil (253 mg, 0.27 mmol, 41%); R_f = 0.68 (petroleum ether/ethyl acetate, 4:1).

To achieve greater purity, this product was converted into the corresponding hexa(methyl ester) by cleavage of the *tert*-butyl ester groups with TFA (3 mL, overnight) and by subsequent methylation of the vacuum-concentrated residue with diazomethane. Therefore, an excess amount of CH_2N_2 in ether was added to the solution of the reactant in 90% MeOH. After stirring for several minutes, the excess of CH_2N_2 was destroyed by addition of acetic acid. The mixture was concentrated in vacuo and the residue purified by flash chromatography (toluene/acetone, 10:1, +0.5% Et_3N) to yield **7** (150 mg, 0.21 mmol, 81%) as a colorless oil. Total yield from **7** (3 steps): 32%. ^1H NMR (250 MHz, $CDCl_3$): δ = 2.78 [s, 6 H, $C(CH_2N)_3$], 3.52 (s, 2 H, 2-H) 3.59 (s, 12 H, 6 NCH_2CO_2), 3.67 (s, 18 H, 6 OMe), 4.49 (s, 2 H, OCH_2Ar), 7.47 (d, ^3J = 8.7 Hz, 2 H, Ar), 8.21 (d, ^3J = 8.7 Hz, 2 H, Ar) ppm. MALDI-MS (positive mode): *m/z* = 701.6 $[MH]^+$ (calcd. 701.7), 722.6 $[MNa]^+$ (calcd. 723.7). $C_{30}H_{44}N_4O_{15} \cdot 2H_2O$ (736.0): calcd. C 50.13, H 6.73, N 7.80; found C 50.17, H 6.39, N 7.68.

2-[Bis(carboxymethyl)aminomethyl]-2-[(4-nitrobenzyl)oxymethyl]-propylene-1,3-dinitrilotetraacetic Acid (8): Hexakis(methyl ester) **7** (70 mg, 0.10 mmol) was dissolved in MeOH (2 mL), and a solution of NaOH (160 mg, 4.0 mmol) in twice-distilled water (1 mL) was added. After stirring for 24 h, the mixture was acidified with HCl to approximately pH 1.5, and concentrated under reduced pressure. The solid residue was dissolved in a minimum amount of MeOH/H_2O (1:3), loaded onto a RP-18 column and eluted with MeOH/H_2O (1:3 → 1:1, +1% HOAc). Lyophilization of the collected and partially concentrated fractions afforded a fluffy, pale-yellow solid (51 mg, 0.08 mmol, 80%). ^{1}H NMR (250 MHz, D_2O): δ = 3.36 [s, 6 H, C(CH_2N)$_3$], 3.74 (s, 12 H, 6 NCH_2CO$_2$), 3.95 (s, 2 H, 2-H), 4.73 (s, 2 H, OCH_2Ar), 7.62 (d, ^3J = 8.2 Hz, 2 H, Ar), 8.26 (d, ^3J = 8.2 Hz, 2 H, Ar) ppm. MALDI-MS (positive mode): *m*/*z* = 639.5 [MNa]$^+$ (calcd. 639.5). $C_{24}H_{32}N_4O_{15}$·H_2O (634.6): calcd. C 45.43, H 5.40, N 8.83; found C 45.43, H 5.71, N 8.81.

2-[Bis(carboxymethyl)aminomethyl]-2-[(4-isothiocyanatobenzyl)oxymethyl]propylene-1,3-dinitrilotetraacetic Acid (9): Compound **8** (6.2 mg, 9.8 μmol) and sodium carbonate (4.3 mg, 40 μmol) were dissolved in water (1.5 mL) and the mixture added to a suspension of Pd on charcoal (10%, 6 mg) in water (1.5 mL), which had been previously stirred under hydrogen. After 4.5 h of hydrogenation under a slight pressure (balloon), the mixture was filtered through a pad of Celite (which had previously been washed with water). Thiophosgene (5 μL, 25 μmol) in $CHCl_3$ (3 mL) was added to the filtrate with vigorous stirring. After stirring for 1 h, the $CHCl_3$ layer was separated and the aqueous layer was partially evaporated in vacuo (30 °C) to remove any volatiles and lyophilized to afford **9** (9.8 mg, quant.) as an amorphous, nearly colorless solid. ^{1}H NMR (250 MHz, D_2O): aromatic protons: δ = 7.31−7.51 (m, 4 H) ppm. MALDI-MS (positive mode, ATT-matrix) of an acidified (HCl) sample: *m*/*z* = 630.2 [MH]$^+$ (calcd. 629.5), 652.3 [MNa]$^+$ (calcd. 651.5).

2-(Aminomethyl)-2-[(dibenzylamino)methyl]propane-1,3-diamine Tetrahydrochloride (13): DIPEA (1.22 mL, 7.0 mmol) and benzyl bromide (0.71 mL, 6.0 mmol) were added to a stirred solution of compound **12** (1.64 g, 3.37 mmol) at room temperature. After stirring overnight, the reaction mixture turned to a white suspension, which was diluted with diethyl ether and toluene (80 mL each) and washed with water (80 mL) and brine (80 mL). The organic layer was dried with $CaCl_2$ and concentrated under reduced pressure. The residue was suspended in a small amount of diethyl ether, filtered and washed with methanol to yield 7-[(dibenzylamino)methyl]-2,4,6-triphenyl-1,3,5-triazaadamantane (1.66 g, 2.88 mmol, 85%) as a white powder. This was dissolved in freshly distilled THF (40 mL) and 1.5 N HCl (50 mL) was added under vigorous stirring, which was continued for 30 min. The THF was removed by rotary evaporation, and the resulting aqueous solution was extracted with CH_2Cl_2 (2 × 50 mL). Evaporation of the aqueous layer under reduced pressure delivered **13** (1.27 g, 2.57 mmol, 77%) as an amorphous, colorless solid. ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 3.15 [br. s, 6 H, C(CH_2N)$_3$], 3.21 (br. s, 2 H, 2-H), 3.67 [br. s, 4 H, N(CH_2Ph)$_2$], 7.29−7.43 (m, 10 H, 2 Ph), 8.55 (br. s, 9 H, 3 NH_3^+) ppm. $C_{19}H_{22}N_4$·4HCl·2H_2O (494.3): calcd. C 46.10, H 7.34, N 11.33; found C 46.00, H 7.20, N 11.43.

Tetramethyl 2-[Bis(methoxycarbonylmethyl)aminomethyl]-2-[(dibenzylamino)methyl]propylene-1,3-dinitrilotetraacetate (14): Compound **13** (1.25 g, 2.53 mmol) was dissolved in dry DMF (15 mL) and, after saturation of the solution with Ar, alkylated with *tert*-butyl bromoacetate (3.2 mL, 21 mmol) and Na_2CO_3 (2.8 g, 26 mmol) at 65 °C for 8 h. The reaction mixture was stirred for a further 12 h at room temperature and then diluted with dichloromethane (80 mL) and water (60 mL). The organic layer was washed with brine (50 mL), dried with Na_2SO_4, concentrated and submitted to flash chromatography (petroleum ether/ethyl acetate, 10:1) to afford the hexaalkylated product as a colorless oil (1.12 g, 1.12 mmol, 44%); R_f = 0.67 (petroleum ether/ethyl acetate, 4:1).

To achieve greater purity, this product was converted into the corresponding hexa(methyl ester) by cleavage of the *tert*-butyl ester groups with TFA (10 mL, overnight) and by subsequent methylation of the vacuum-concentrated residue with diazomethane. Therefore, an excess amount of CH_2N_2 in diethyl ether was added to the solution of the reactant in 90% MeOH. After stirring for several minutes, the excess of CH_2N_2 was destroyed by addition of acetic acid. The mixture was concentrated in vacuo and the residue purified by flash chromatography (toluene/acetone, 10:1, +1% Et_3N) to yield **14** (570 mg, 0.77 mmol, 70%) as a colorless oil. ^{1}H NMR (250 MHz, $CDCl_3$): δ = 2.72 (s, 2 H, 2-H), 2.90 [s, 6 H, C(CH_2N)$_3$], 3.59 [s, 16 H, 6 NCH_2CO$_2$, N(CH_2Ph)$_2$], 3.65 (s, 18 H, 6 OMe), 7.27−7.34 (m, 10 H, 2 Ph) ppm. $C_{37}H_{52}N_4O_{12}$·H_2O (762.9): calcd. C 58.26, H 7.14, N 7.34; found C 58.32, H 7.03, N 7.25.

2-[Bis(carboxymethyl)aminomethyl]-2-[(dibenzylamino)methyl]-propylene-1,3-dinitrilotetraacetic Acid (15): Hexa(methyl ester) **14** (350 mg, 0.46 mmol) was dissolved in MeOH (5 mL), and a solution of NaOH (480 mg, 12.0 mmol) in twice-distilled water (6 mL) was added. After stirring for 3 d in a Teflon vessel, the mixture was acidified with HCl to approximately pH 1 and concentrated under reduced pressure. The solid residue was dissolved in a minimum amount of MeOH/H_2O (1:4) containing 1% of Et_3N (without Et_3N the residue would not dissolve), loaded onto a RP-18 column and eluted with MeOH/H_2O (1:4 → 10:1, +1% HOAc). Lyophilization of the collected fractions afforded a colorless solid (171 mg, 0.24 mmol, 52%), which contained 1 equiv. of HOAc (and 0.1 equiv. of Et_3N). Crystallization of a part of this product from a little water delivered **15** free of HOAc. ^{1}H NMR (250 MHz, D_2O; **15**·HOAc): δ = 2.07 (s, 3 H, OAc), 3.17 [br. s, 6 H, C(CH_2N)$_3$], 3.41 (br. s, 2 H, 2-H), 3.54 (br. s, 12 H, 6 NCH_2CO$_2$), 4.40 [br. s, 4 H, N(CH_2Ph)$_2$], 7.53 (br. s, 10 H, 2 Ph) ppm. ^{1}H NMR (250 MHz, D_2O; pH = 12): δ = 1.92 (s, 3 H, OAc), 2.88 (br. s, 2 H, 2-H), 2.98 [br. s, 6 H, C(CH_2N)$_3$], 3.37 (br. s, 12 H, 6 NCH_2CO$_2$), 3.70 [br. s, 4 H, N(CH_2Ph)$_2$], 7.27−7.48 (m, 10 H, 2 Ph) ppm. MALDI-MS (positive mode): *m*/*z* = 661.4 [MH]$^+$ (calcd. 660.7), 683.5 [MNa]$^+$ (calcd. 683.7). $C_{31}H_{40}N_4O_{12}$ (660.7): calcd. C 56.36, H 6.10, N 8.48; found C 56.01, H 6.53, N 8.31.

Acknowledgments

This work was supported by the Ludwig Institute for Cancer Research, New York, and by the Fonds der Chemischen Industrie.

[1] G. Sgouros, in *Encyclopedia of Cancer* (Ed.: J. R. Bertino), 2nd ed., vol. 4, Academic Press, Elsevier Science, San Diego, **2002**, pp. 29−40.
[2] A. Heppeler, S. Froidevaux, H. R. Mäcke, E. Jerman, M. Behe, P. Powell, M. Henning, *Chem. Eur. J.* **1999**, *5*, 1974−1981.
[3] Y. Fu, S. Laurent, R. N. Muller, *Eur. J. Org. Chem.* **2002**, 3966−3973.
[4] M. W. Brechbiel, O. Gansow, *J. Chem. Soc., Perkin Trans. 1* **1992**, 1173−1178.
[5] L. Kukis, G. L. DeNardo, S. J. DeNardo, G. R. Mirick, L. A. Miers, D. P. Greiner, Cl. F. Meares, *Cancer Research* **1995**, *55*, 878−884.

[6] R. J. Geue, G. Searle, *Aust. J. Chem.* **1983**, *36*, 927−935.
[7] R. Vignier, G. Serratrice, A. Dupraz, C. Dy, *Eur. J. Inorg. Chem.* **2001**, 1789−1795.
[8] A. Green, M. J. Welch, J. C. Huffman, *J. Am. Chem. Soc.* **1984**, *106*, 3689−3691.
[9] A. Green, M. J. Welch, C. J. Mathias, K. A. Fox, R. M. Knabb, J. Huffman, *J. Nucl. Med.* **1985**, *26*, 170−180.
[10] K. Fukase, H. Tanaka, S. Torii, S. Kusumoto, *Tetrahedron Lett.* **1990**, *31*, 389−392.
[11] M. Vaulter, N. Knouzi, R. Carrié, *Tetrahedron Lett.* **1983**, *24*, 763−764.
[12] R. Riemschneider, O. Göhrig, P. Groß, A. Rook, K. Breutel, C. Faria, *Monatsh. Chem.* **1965**, 147−158.
[13] E. B. Fleischer, A. E. Gebala, A. Levey, P. A. Tasker, *J. Org. Chem.* **1971**, *36*, 3042−3044.
[14] T. J. Dunn, W. L. Neumann, M. M. Rogic, S. R. Woulfe, *J. Org. Chem.* **1990**, *55*, 6368−6373.

Received April 2, 2004

