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Synthesis of a novel 'smart' bifunctional chelating agent 1-(2-[β,D-galactopyranosyloxy]ethyl)-7-(1-carboxy-3-[4-aminophenyl]propyl)-4,10-bis(carboxymethyl)-1,4,7,10-tetraazacyclododecane (Gal-PA-DO3A-NH_2) and its Gd(III) complex

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Abstract—A new synthetic pathway to 1-(2-[β,D-galactopyranosyloxy]ethyl)-7-(1-carboxy-3-[4-aminophenyl]propyl)-4,10-bis(carboxymethyl)-1,4,7,10-tetraazacyclododecane (Gal-PA-DO3A-NH_2) and 1-(2-[β,D-galactopyranosyloxy]ethyl)-4,7,10-tris(carboxymethyl)-1, 4,7,10-tetraazacyclododecane (Gal-DO3A) chelating agents was developed involving full hydroxyl- and carboxyl-group protection in precursors to product. Two sequences of cyclen-N-functionalisation were subsequently investigated, one successfully, towards synthesis of the novel 'smart' bifunctional Gal-PA-DO3A-NH_2 chelate. The longitudinal proton relaxivities of the neutral [Gd-(Gal-PA-DO3A-NH_2)] and [Gd-(Gal-DO3A)] complexes were increased by 28% and 37% in the presence of β-galactosidase, respectively.

1. Introduction

Since the emergence of paramagnetic contrast agents (PCA) as clinical aids in magnetic resonance imaging (MRI) protocols,[1–4] approaches to introducing selectivity of action have fallen into two broad categories; those generating selective tissue distribution of the PCA by conjugation to physiologically relevant macromolecules, and those relying on metabolic PCA-activation to achieve localisation of differential water relaxation characteristics. The former approaches are exemplified in the development of bifunctional chelating agents capable of conjugating macromolecular systems such as human serum albumin[5,6] and the membrane translocation signal peptide 'Tat'.[7,8] Related strategies have also been applied in the fields of radioimmunoimaging and therapy, whereby ligands such as 1-(1-carboxy-3-[4-(nitro/amino/isothiocyanato)phenyl]propyl)-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane (PA-DOTA, an *N*-functionalised DOTA derivative) have been conjugated with monoclonal antibody CC49 and complexed with radioisotopes such as ^{177}Lu.[9–12]

Meanwhile gadolinium(III) complexes of 1-([β,D-galactopyranosyloxy]alkyl)-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane ([β,D-galactopyranosyloxy]alkyl-DO3A conjugates, e.g., EGad, EGadMe) have been developed as substrates to β-galactosidase, an expression-product of the reporter gene *Lac-Z* commonly co-administered intracellularly with the subject-gene of gene-therapy studies.[13–15] Metabolic cleavage of the galactopyranosyl moiety of EGad/EGadMe unveils a free coordinate site on the central Gd^{3+} ion, allowing binding of water with an increase in relaxivity. While significant differentials in water-relaxation-time have been obtained by this method, introduction to the intracellular domain has thus far been achieved by microinjection alone. Since intracellular delivery is a prerequisite for the practical success of this strategy, it is reasoned that

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additional functionalisation of the chelate moiety, facilitating appendage to macromolecular carrier systems, could enhance efficacy of action in vivo, representing a confluence of the two broad approaches to PCA-design described above. In this context, investigation of appropriate synthetic strategies to the bifunctional conjugate 1-[β,D-galactopyranosyloxy]ethyl)-7-(1-carboxy-3-[4-aminophenyl]propyl)-4,10-bis(carboxymethyl)-1,4,7,10-tetraazacyclododecane (Gal-PA-DO3A-NH_2), and the associated Gd(III) complex, was carried out, reporting characterisations of species isolated. The longitudinal proton relaxivities of both Gd(III) complexes of Gal-PA-DO3A-NH_2 and Gal-DO3A were determined in aqueous medium and in the presence of β-galactosidase.

2. Results and discussion

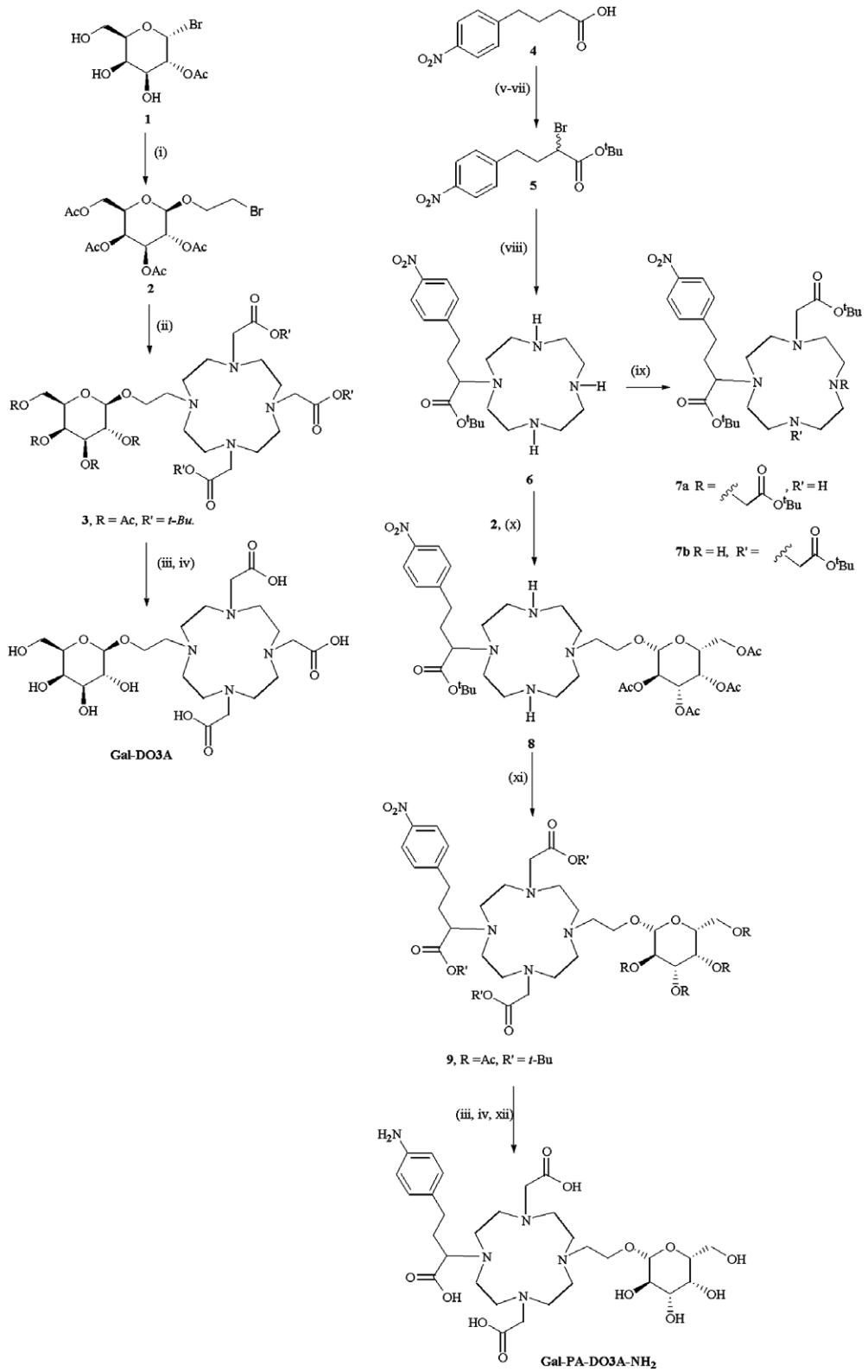
2.1. Ligand synthesis and characterisation

Solubility considerations arising from the presence of pendant-group aromatic functionality in the targeted Gal-PA-DO3A-NH_2 conjugate, and its precursors, encouraged investigation of synthetic pathways retaining fully protected DO3A-carboxyl and pyranosyl-hydroxyl groups in the developing molecular framework up to the point of product generation. Such a methodology was investigated in the first instance towards a simple conjugate β,D-galactopyranosyloxy)ethyl-DO3A (Gal-DO3A, Scheme 1) coupling chelate and sugar moieties as the DO3A-*tert* butyl ester and galactopyranoside tetraacetate, respectively, prior to a final, deprotection step to product. This constitutes a significant departure from the approach adopted during pioneering work to generate the latter conjugate,[13,15] in which hydroxyl sites were deprotected on the cyclen-galactopyranoside adduct, and an aqueous medium was employed for subsequent cyclen-*N*-alkylation with unprotected carboxymethyl groups. The presence of additional functionality on one carboxymethyl-arm in the target Gal-PA-DO3A-NH_2 subsequently necessitated investigation of various sequences of *N*-alkylation, directed primarily by steric factors, and successful modifications to the approach ascertained in the preliminary studies concerning the Gal-DO3A are also outlined in Scheme 1.

Working from 2,3,4,6-tetra-*O*-acetyl-α,D-galactopyranosyl bromide (**1**), bromoethyl-β,D-galactopyranoside tetraacetate (**2**) was isolated anomerically pure in 52% yield from AgOTf/pyranosyl bromide procedure, employing the non-nucleophilic base 2,6-di-*tert*-butyl-4-methylpyridine[16–19] in anhydrous diethyl ether. Organobromide **2** was subsequently used to functionalise the 4th *N*-site of DO3A *tert*-butyl ester[20,21] in the initial investigations, employing K_2CO_3 (2.1 equiv) base in DMF solvent at room temperature under strictly anhydrous conditions to furnish the corresponding fully protected (β,D-galactopyranosyloxy)ethyl-DO3A chelate. The strong propensity for intramolecular hydrogen-bonding associated with the cyclen ring system[22,23] necessitated complete deprotonation of the alicyclic system in order to maintain nucleophilic *N*-function during these alkylation procedures, characterising also the tenacity of solvent retention in free amine products. This latter observation necessitated isolation of protected conjugate **3** from chromatographed solutes as hydrochloride salt (35%) for analytical purpose; achieved through product-precipitation from strictly anhydrous Et_2O/CH_2Cl_2 solution (50:4 v/v) via application of dry HCl gas. Appropriate chromatographic fractions (eluent—CH_2Cl_2/MeOH 89:11 v/v) of free amine were easily identified for this purpose via ^1H NMR analysis on the basis of the integral ratios of resonances associated with *tert*-butyl and acetyl CH_3 groups, respectively. *N*-(tetra-acetyl-β,D-galactopyranosyloxy)ethyl-DO3A-*tert*-butyl esters **3** were subsequently deprotected to give (β,D-galactopyranosyloxy)ethyl-DO3A (Gal-DO3A ~56%) as a hygroscopic solid, through consecutive treatments with trifluoroacetic acid reagent (TFA, cleaving *tert*-Bu groups)[24] and NaOMe/MeOH solution under Zemplen conditions (cleaving Ac groups).[25] The glycosyl linkage remained intact and no loss of anomeric integrity at *C*-1 was detected throughout this coupling/deprotection process, as indicated by $^3J_{HH}$ values associated with anomeric protons; $J = 7.7$ Hz (**3**) and $J = 7.4$ Hz (Gal-DO3A).

As with approaches reported in pursuit of the bifunctional chelating agent PA-DOTA,[12,23,26] mono-alkylation of free cyclen with the N-functionalisation group (PA) was envisaged as the first in the sequence of cyclen-derivatisation steps to the proposed Gal-PA-DO3A-NH_2 conjugate. To this end, the alkyl bromide of the N-functionalisation pendant group was constructed from 4-(4-nitrophenyl)butyric acid (**4**), through α-halogenation of the acyl chloride by treatment with *N*-bromosuccinimide, and HBr (aq 48%) catalyst.[27] Consistent with the formulations of carboxymethyl groups to be appended subsequently, the pendant-group carboxyl function was protected as a *tert*-butyl ester using *tert*-butanol, employing the poor nucleophile *N*,*N*-dimethylaniline as the base of choice, generating *tert*-butyl (*R*,*S*)-2-bromo-4-(4-nitrophenyl)butanoate (**5**) in 42% yield from **4**. Mono-alkylation of cyclen was subsequently achieved in a gently refluxing acetonitrile/K_2CO_3 heterologous system, employing cyclen in 2:1 molar excess of the organobromide, giving **6** in 66% yield.

From this point, both possible sequences of cyclen-*N*-derivatisation to Gal-PA-DO3A-NH_2 were investigated; one involving alkylation of two *N*-sites with carboxymethyl functions and subsequent appendage of the galactopyranosyl moiety, and another more felicitous pathway involving initial linkage of the sugar, followed by appendage of carboxymethyl functions. In the former approach, chromatographically inseparable isomeric mixtures of 1-(PA)-4,7/10-bis(DO3A-*tert*-butyl ester)-1,4,7,10-tetraazacyclododecane (**7a/b**, Scheme 1) were generated from **6** in exhaustive procedures (i.e., 21–23 days, rt) employing strictly stoichiometric quantities of *tert*-butyl bromoacetate in a *N*,*N*-dimethylacetamide/NaOAc (solvent/base) system. Consistent with the observations of Chappell et al.,[12] as structures



Scheme 1. Reagents and conditions: (i) $AgOTf/Et_2O$, −78 °C; then $HO(CH_2)_nBr$; (ii) DO3A-^{t}Bu ester/K_2CO_3 (2.1 equiv)/dry DMF, rt; (iii) TFA, rt; (iv) NaOMe/MeOH, rt; then Amberlite IR-120 (H^+); (v) $SOCl_2/CCl_4$, Δ; (vi) NBS (1.2 equiv)/HBr (aq 48%, cat), Δ; (vii) $PhN(Me)_2/^t$BuOH, 0 °C → rt; (viii) cyclen (2 equiv)/K_2CO_3 (1.4 equiv)/MeCN, Δ; (ix) $BrCH_2CO_2{}^t$Bu (2 equiv)/NaOAc (2 equiv)/dry DMA, rt, 21–23 days; (x) K_2CO_3 (1.1 equiv)/dry DMF, rt; (xi) $BrCH_2CO_2{}^t$Bu (excess)/K_2CO_3 (excess)/dry DMF, rt; (xii) Pd/C (10%, cat)/aq/H_2, 1 atm.

containing one chiral centre, the isomeric mixtures of **7a/b** were readily identifiable in ^{1}H NMR spectra as multiple (exceeding two) downfield doublets associated with aromatic protons. In addition, steric congestion

precluded subsequent alkylation at the fourth *N*-site with bromoethyl-β,D-galactopyranoside tetraacetate **2** (available in minor stoichiometric excess only).

Meanwhile, alkylation of **6** with bromoethyl-β,D-galactopyranoside tetraacetate **2** in minor stoichiometric excess, using the DMF/K_2CO_3 system, achieved greater site-selectivity of alkylation (presumably due to steric factors), giving chelate **8** which was isolated as the hydrochloride salt from chromatographic fractions of free amine (eluent—CH_2Cl_2/MeOH 9:1 v/v, analysed for *tert*-Bu and Ac-CH_3 integral ratios ^{1}H NMR spectra). While the presence of two chiral centres in **8** inevitably complicates the ^{1}H NMR spectrum, broadening multiplets associated with aromatic protons therein, resonance profiles associated with methyl protons of the *tert*-butyl (δ_H = 1.51, 1.55 [1.54sh]) and acetyl groups (δ_H = 1.99, 2.05, ~2.11 br m., 2.17; 4× CH_3 [Ac], also CH_2C$_\alpha$ [PA]) are consistent with the identification of hydrochloride **8** as the 1,7-disubstituted cyclen formulation to good isomeric purity. Treatment with large excess of *tert*-butyl bromoacetate in an anhydrous DMF/K_2CO_3 system over an exhaustive period (15 days, rt) subsequently yielded **9**, which was isolated as the hydrochloride salt. Steric congestion in this structure was evidenced in the broadening of resonance signatures observed in both ^{1}H and ^{13}C NMR spectra resulting from slow conformational interconversion, however spectral characteristics were comparable to those of **8**, displaying the appropriate modification of *tert*-butyl CH_3 resonance integral in the ^{1}H NMR spectrum, and assignments were carried out according to chemical shift criteria. Consecutive carboxyl and hydroxyl group-deprotections were carried out as for **3**, and catalytic (Pd/C) hydrogenation in aqueous medium at atmospheric pressure effected transformation of the nitrophenyl function to the aniline. This was evidenced in the upfield shift of (broad) multiplets associated with aromatic protons in the ^{1}H NMR spectrum of the analytically homogeneous Gal-PA-DO3A-NH_2 ligand, obtained in 20% overall yield for the two deprotection steps and the catalytic hydrogenation.

2.2. Gadolinium(III) complexes and their longitudinal proton relaxivities (R_1)

Complexations of ligands Gal-PA-DO3A-NH_2 and Gal-DO3A were carried out by refluxing in aqueous suspensions of $Gd_2(CO_3)_3$ over 24 h, the reaction progress indicated by gradual disappearance of the suspension. Elemental analyses of the Gd(III) complexes of Gal-PA-DO3A-NH_2 and Gal-DO3A (EGad)[13] were consistent with the anticipated 1:1 Gd(III):ligand stoichiometry forming neutral complexes. Both Gal-PA-DO3A-NH_2 and Gal-DO3A ligands consist of 4-N (cyclen) and 3-O (carboxylate) donor atoms and one ethyl-galactose pendant arm. The $\mathbf{R}_1$ of the neutral [Gd-(Gal-PA-DO3A-NH_2)] and [Gd-(Gal-DO3A)] complexes were 1.47 and 1.52 $mM^{-1}\,s^{-1}$ in water, respectively, and, 1.89 and 2.09 $mM^{-1}\,s^{-1}$ in the presence of β-galactosidase, respectively. The increases in relaxivities were 28% and 37% in the presence of the enzyme. The $\mathbf{R}_1$ of [Gd-(DOTA)$(H_2O)]^-$ and [Gd-(DO3A)$(H_2O)_2$] are 3.59 and 4.82 $mM^{-1}\,s^{-1}$, respectively, in water which are higher than those of [Gd-(Gal-PA-DO3A-NH_2)] and [Gd-(Gal-DO3A)] indicating that the number of water molecule coordinating to the Gd(III) is likely less than one for the latter complexes. The number of water molecules that are in fast exchange (q) with the complex [Tb-(Gal-DO3A)] was reported to be 0.7 by fluorescence study, and the enzymatically cleaved terbium complex was 1.2.[13] These data have supported the q value of [Gd-(Gal-PA-DO3A-NH_2)] is probably similar to that of [Gd-(Gal-DO3A)].

2.3. Conclusion

A novel neutral Gd(III) complex was prepared with the capability of conjugating to a peptide and biomacromolecule, and responding to the presence of a specific enzyme effecting a change in water relaxation time in magnetic resonance imaging. The synthetic methodology and the characterisation of the cyclen based ligands with four pendant arms and their precursors were reported in detail.

3. Experimental

3.1. General

NMR spectra were recorded at ambient temperature on a Bruker AM-250 or a Bruker Avance-500 spectrometer. All ^{13}C spectra are ^{1}H broadband decoupled. Chemical shifts (δ) are expressed in ppm and J values are given in Hz. ^{1}H and ^{13}C NMR spectra were referenced internally to Me_4Si apart from those carried out in D_2O, where TSP-d_4 was used. Infrared spectra were recorded on a Bruker Vector 22 FT-IR spectrophotometer, with samples prepared as 16 mM diameter KBr discs. Mass spectrometry was performed using a Kratos Profile HV3 mass spectrometer in liquid secondary ionisation mass spectrometry mode (employing glycerol as a matrix). Melting points were obtained on an Electrothermal Eng. Ltd digital melting point apparatus, and are uncorrected. A Carlo Erba 1108 elemental analyser was used for C, H and N microanalyses. TLC was performed on pre-coated silica plates (Whatman Al Sil G/UV, 250-μm layer) in the solvent systems stated. Column chromatography was performed over Aldrich silica gel (Merck grade 10180, 70–230 mesh 40 Å).

Reagents were used as purchased. Solvents (DMF, acetonitrile, diethyl ether and methanol) were dried and distilled before use. All other solvents were used as purchased. Filtrations were carried out through scinter-glass, or in the case of Pd/C suspensions and fine suspensions of dessicants, etc. (e.g., Na_2SO_4 and $MgSO_4$), through Celite filter-aid (521) on scinter-glass.

3.2. Syntheses

Cyclen, 1,4,7,10-tetraazacyclododecane (bis H_2SO_4 salt), was prepared according to the method of Atkins et al.[28] and the free amine isolated by continuous extraction from conc. KOH solution employing chloroform.[22]

1,4,7-Tris(carboxymethyl-*tert*-butyl ester)-1,4,7,10-tetraazacyclododecane, HBr salt (DO3A-*tert*-butyl ester, HBr salt) was prepared as a white solid from cyclen according to the method of Schultze and Bulls.[20]

3.2.1. 1-*O*-(2-Bromoethyl)-2,3,4,6-tetra-*O*-acetyl-β,D-galactopyranoside (2).[29–31] A yellow oil (34%); (Found: C 42.1; H 5.18. Calcd. for $C_{16}H_{23}O_{10}Br$: C, 42.2; H, 5.12%): $\delta_H(CDCl_3)$: 4.55 [1H, d, *J* 7.9, *H*-1]; lit.[21] 4.51 [1H, d, *J* 7.9, *H*-1]; $\delta_C(CDCl_3)$.[29–31]

3.2.2. 1-(2-[2,3,4,6-Tetra-*O*-acetyl-β,D-galactopyranosyloxy]ethyl)-4,7,10-tris(carbo-[1,1-dimethyl]ethoxymethyl)-1,4,7,10-tetraazacyclododecane, hydrochloride salt (3). An anhydrous DMF suspension (25 mL) of **2** (1.9 mmol scale, 1.1 equiv), DO3A-*tert*-butyl ester, HBr salt (1.0 equiv) and anhydrous K_2CO_3 (2.1 equiv) was vigorously stirred under anhydrous conditions at rt for 5 days. The mixture was decanted into dichloromethane (400 mL), washed with deionised water (6× 200 mL) and saturated brine (3× 200 mL), and dried over $MgSO_4/Na_2SO_4$. Evaporation yielded a yellow oil which was subjected to column chromatography (eluent—CH_2Cl_2/MeOH 89:11 v/v; TLC—R_f ~0.42), leaving a colourless oil (**3**) of a free amine product. A cooled dichloromethane solution (4 mL) of amine, diluted with anhydrous diethyl ether (50 mL) to the point of turbidity, was bubbled with dry HCl gas for 5 min, precipitating crude hydrochloride salt as a white solid. Immediate isolation of solid and thorough washing with anhydrous ether, followed by dissolution in dichloromethane and evaporation of the filtered solution, generated the product as a cream/yellow solid (1.05 g, 52%): mp 126–131 °C (dec): (Found: C, 47.8; H, 6.75; N, 4.3. Calcd for $C_{42}H_{72}N_4O_{16}{\cdot}HCl{\cdot}KHCO_3{\cdot}7/4MeOH{\cdot}CH_2Cl_2{\cdot}1/4Et_2O$: C, 47.4; H, 7.2; N, 4.7%): ν_{max}/cm^{-1} (KBr): 3433, 3096, 2981, 2936, 1745, 1638, 1460, 1370, 1253, 1228, 1163, 1079; $\delta_H(CDCl_3)$: 1.21 [trace, t, C*H*$_3$ (Et_2O)]; 1.45 [18H, s, 6× C*H*$_3$ (^{t}Bu)]; 1.49 [9H, s, 3× C*H*$_3$ (^{t}Bu)]; 1.98, 2.07, 2.13, 2.18 [12H, 4× s, 4× C*H*$_3$ (Ac)]; 2.40–4.80 [~38H, br m, C*H*$_2$C*H*$_2$ (Et linker), 11× NC*H*$_2$ (DO3A ester), *H*-1,5,6,6′ (gal), 1/2× C*H*$_2$ (Et_2O), 7/4× C*H*$_3$O*H*] in which 4.58 [1H, d, *J* 7.7, *H*-1 (gal)]; 5.02 [1H, dd, J_1 10.4, J_2 2.9, *H*-3 (gal)]; 5.13 [1H, dd, J_1 10.4, J_2 7.7, *H*-2 (gal)]; 5.31 [2H, s, C*H*$_2$Cl$_2$]; 5.39 [1H, d, *J* 2.9, *H*-4 (gal)]; $\delta_C(CDCl_3)$: 20.53, 20.73, 21.11 [*C*H$_3$'s (Ac)]; 28.08, 28.14 [*C*H$_3$'s, ^{t}Bu]; 42.56, 48.10, 50.78, 51.51, 52.31, 53.46, 53.99, 54.33, 54.66, 54.89 [NC*H*$_2$'s]; 61.15 [*C*-6 (gal)]; 63.93 [OC*H*$_2$ (Et linker)]; 66.97, 68.56, 70.63, 71.07 [*C*-2,3,4,5 (gal)]; 82.44, 82.62, 84.65, 84.77 [*C*Me$_3$'s, ^{t}Bu]; 100.77 [*C*-1 (gal)]; 165.04, 165.39, 169.61, 169.87, 170.14, 170.38 [*C*=O's]; MS (LSIMS): 890, 889, 888 (27, 51, 18% resp. $[MH]^+$).

3.2.3. 1-(2-[β,D-Galactopyranosyloxy]ethyl)-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane (Gal-DO3A). An anhydrous dichloromethane solution (5 mL) of **3** (0.85 g, 0.72 mmol) was charged with trifluoroacetic acid (TFA, 100 mL) under N_2, and the reaction mixture was stirred under N_2 at rt for 3 h. Azeotropic evaporation with chloroform (2× 50 mL) yielded a residue which was treated with methanolic NaOMe solution (0.25 M, 100 mL), and the mixture stirred under N_2 at rt for 1.5 h thereafter. The mixture was cooled to 5 °C and neutralised with Amberlite IR-120 (H^+) ion-exchange resin. Immediate filtration, full removal of solvent and subsequent addition of methanol (2 mL), yielded a yellow solution from which crude solid was precipitated by addition of diethyl ether (50 mL). Two repetitions of the latter precipitation procedure and a further precipitation from aqueous solution (2 mL) by dropwise addition to 2-propanol (75 mL, seeded with diethyl ether), followed by consecutive washings of isolated material with 2-propanol and diethyl ether, yielded a hygroscopic white solid which was stored under N_2 (320.1 mg, 56%): mp 132–135 °C (dec): (Found: C, 42.8; H, 6.7; N, 7.25. Calcd for $C_{22}H_{40}N_4O_{12}.5/4\ ^i$PrOH.3/2TFA: C, 43.2; H, 6.5; N, 7.00%): ν_{max}/cm^{-1} (KBr): 3412, 2972, 2867, 1685, 1635, 1460, 1401, 1355, 1321, 1204, 1160, 1131, 1082; $\delta_H(D_2O)$: 1.18, 1.20 [10H, 2× s, C*H*$_3$'s (^{i}Pr)]; 2.83–3.62 [18H, br m's, NC*H*$_2$ (Et linker), 8× C*H*$_2$ (cyclen ring)]; 3.62–3.92 [12H, br m., OC*H*$_2$ (Et linker), 3× C*H*$_2$CO$_2$H, *H*-3,5,6,6′ (gal)]; 3.92–4.12 [13/4 H, m., *H*-2,4 (gal), 5/4× Me$_2$C*H*OH] in which 3.93 [1H, d, *J* 3.0, *H*-4]; 4.42 [1H, d, *J* 7.4, *H*-1 (gal)]; also 4.14–4.35 [trace, br]; $\delta_C(D_2O)$: 26.44 [*C*H$_3$'s, ^{i}Pr]; 52.43br., 58.10 [NC*H*$_2$'s]; 63.73 [*C*-6 (gal), OC*H*$_2$ (Et linker)]; 66.99 [*C*H (^{i}PrOH)]; 71.43, 73.40, 75.50, 77.92 [*C*-2,3,4,5 (gal)]; 105.78 [*C*-1 (gal)]; 117.00 [*C*F$_3$, TFA]; 177.50 [*C*=O's]; MS (LSIMS): 576, 575 (55, 94% resp. $[MNa]^+$); 554, 553 (5, 8% resp. $[MH]^+$).

3.2.4. Gd(III) complexes of Gal-DO3A. An aqueous suspension (20 mL) of Gal-DO3A (145.8 mg, 0.182 mmol) and $Gd_2(CO_3)_3$ (0.5 equiv) was refluxed for 24 h, then filtered. Azeotropic evaporation with 2-propanol yielded crude material. Consecutive triturations in 2-propanol and diethyl ether generated a cream/white powder which was stored under N_2 (114.8 mg, 89%): mp 175–178 °C (dec); (Found: C, 37.5; H, 5.3; N, 7.85. Calcd for $C_{22}H_{37}N_4O_{12}$.Gd: C, 37.4; H, 5.2; N, 7.9%): ν_{max}/cm^{-1} (KBr): 3409, 2873, 1630sh., 1601, 1384, 1326, 1244, 1158, 1085, 939; MS (LSIMS): 709, 708 (2, 2% resp. $[MH]^+$); 577, 576 (9, 8% resp. $[LigandH]^+$).

3.2.5. (1,1-Dimethyl)ethyl (*R*,*S*)-2-bromo-4-(4-nitrophenyl)butanoate (5). The intermediate (*R*,*S*)-2-bromo-4-(4-nitrophenyl)butanoyl chloride was obtained by refluxing 4-(4-nitrophenyl)butyric acid (**4**, 11.86 g, 56.7 mmol) with thionyl chloride in CCl_4 under N_2, followed by α-halogenation of the acyl chloride according to the literature method,[26,27] generating a mobile green oil. A mixture of *N*,*N*-dimethylaniline (20 mL) and 2-methyl-2-propanol (40 mL) was cooled to 5 °C under N_2, and the crude α-brominated acyl chloride was added dropwise. Thereafter, the reaction mixture was warmed to rt (1 h), stirred under N_2 for 18 h and decanted into anhydrous diethyl ether (400 mL). The resulting suspension was washed with 1*N* HCl solution (2× 200 mL), deionised water (2× 200 mL), saturated $NaHCO_3$ solution (2× 200 mL) and saturated brine (200 mL), and the organic phase dried over $MgSO_4$. Evaporation gave a brown oil which was subjected to flash chromatography (eluent—CH_2Cl_2; TLC—R_f 0.83) to yield **5** as a yellow

oil (8.02 g, 41%); (Found: C, 48.95; H, 5.4; N, 4.2. Calcd for $C_{14}H_{18}NO_4Br$: C, 48.8; H, 5.2; N, 4.1%): ν_{max}/cm^{-1} (KBr): 2980, 2936, 1733, 1601, 1521, 1456, 1370, 1347, 1260, 1149, 1110, 853; $\delta_H(CDCl_3)$: 1.49 [9H, s, 3× CH_3 (^{t}Bu)]; 2.16–2.46 [2H, m, CH_2C$_\alpha$]; 2.71–3.04 [2H, m, CH_2Ar]; 4.07 (0.8H, maj.) and 4.14 (0.2H, min.) [1H, 2× dd, J_1 8.0, J_2 6.3 and J_1 8.2, J_2 6.0 resp. C$_\alpha$H]; 7.38 [2H, d, J 8.5, ArH-2,6]; 8.17 [2H, d, J 8.5, ArH-3,5]; $\delta_C(CDCl_3)$: 27.73 [CH$_3$'s, ^{t}Bu]; 33.15 [CH$_2$C$_\alpha$]; 35.66 [ArCH$_2$]; 45.49 (min., DEPT-135), 46.47 (maj.) [C$_\alpha$]; 82.79, 83.00 [CMe$_3$, ^{t}Bu]; 123.88 [ArC-3,5]; 129.38 [ArC-2,6]; 146.68 and 147.91 [ArC-4,1 resp.]; 168.32 [C=O]; also 57.36, 95.66 (DEPT-135); MS (LSIMS): 346, 344 (20, 24% resp. $[MH]^+$).

3.2.6. *N*-(1-Carbo-[1,1-dimethyl]ethoxy-3-[4-nitrophenyl]-propyl)-1,4,7,10-tetraazacyclododecane (6). An anhydrous acetonitrile suspension (30 mL) of cyclen (0.92 g, 5.4 mmol) and fine-ground anhydrous K_2CO_3 (0.51 g, 3.7 mmol) was heated under N_2 to the point of reflux, and an anhydrous acetonitrile solution (10 mL) of α-bromobutanoate **11** (0.91 g, 2.65 mmol) was added. Thereafter, the mixture was gently refluxed under anhydrous conditions (changing quickly from deep blue, to pea-green colour) for 24 h, cooled to rt and filtered. The filtrate was evaporated and the residual brown oil subjected to column chromatography (eluent—CH_2Cl_2/MeOH/0.88 aq NH_3, 15:5:1 v/v/v) and consecutive azeotropic evaporations with 2-propanol and chloroform (50 mL) left residue **6** as a translucent static red oil (0.80 g, 66%); (Found: C, 57.3; H, 8.3; N, 15.3. Calcd for $C_{22}H_{37}N_5O_4.0.2$ KBr: C, 57.5; H, 8.1; N, 15.3%): ν_{max}/cm^{-1} (KBr): 3386, 2933, 2846, 1720, 1601, 1519, 1459, 1345, 1255, 1151, 853; $\delta_H(CDCl_3)$: 1.48 [9H, s, 3× CH_3 (^{t}Bu)]; 1.86–3.09 [3H, v.br., 3× NH]; 1.86–1.98 and 2.01–2.18 [2H, 2× m, CH_2C$_\alpha$]; 2.44–2.61 [2H, m, CH_2Ar]; 2.62–3.09 [16H, m, 8× CH_2 (cyclen ring)]; 3.27 [1H, dd, J_1J_2 7.2, C$_\alpha$H]; 7.39 [2H, d, J 8.8, ArH-2,6]; 8.14 [2H, d, J 8.8, ArH-3,5]; $\delta_C(CDCl_3)$: 28.35 [CH$_3$'s, ^{t}Bu]; 31.08 [CH$_2$C$_\alpha$]; 32.99 [CH$_2$Ar]; 45.44, 45.93, 47.34, 49.11 [CH$_2$'s (cyclen ring)]; 63.15 [C$_\alpha$]; 81.42 [CMe$_3$, ^{t}Bu]; 123.71 [ArC-3,5]; 129.32 [ArC-2,6]; 146.50 and 149.73 [ArC-4,1 resp.]; 171.57 [C=O]; MS (LSIMS): 436 (100%, $[MH]^+$).

3.2.7. Alkylation of 6 with tert-butyl bromoacetate (2 equiv); mixture of 1-(1-[carbo-(1,1-dimethyl)ethoxy]-3-[4-nitrophenyl]propyl)-4,7-bis(carbo-[1,1-dimethyl]ethoxymethyl)-1,4,7,10-tetraazacyclododecane (7a) and 1-(1-[carbo-(1,1-dimethyl)ethoxy]-3-[4-nitrophenyl]propyl)-4,10-bis(carbo-[1,1-dimethyl]ethoxymethyl)-1,4,7,10-tetraazacyclododecane (7b). An anhydrous *N*,*N*-dimethylacetamide solution (10 mL, DMA) of *N*-functionalised cyclen derivative **6** (1.89 g, 4.0 mmol) was charged with anhydrous sodium acetate (0.66 g, 8.0 mmol), cooled under N_2 to 5 °C, and *tert*-butyl bromoacetate (1.56 g, 8.0 mmol) added dropwise (residue washed in with 2 mL DMA). The reaction mixture was stirred under anhydrous conditions for 21 days at rt, evaporated, and a chloroform solution of the residue (200 mL) was washed with deionised water (3× 200 mL) and saturated brine (200 mL), before the organic phase was dried over $MgSO_4$ and evaporated. The residual oil was then refluxed in anhydrous acetonitrile/K_2CO_3 (large excess) suspension for 18 h under anhydrous conditions, filtered, evaporated, and the residue subjected to column chromatography (eluent – CH_2Cl_2/MeOH 9:1 v/v; TLC—R_f ~0.60) to give the free-amine–isomer mixture **7a/b** as a dark red oil (0.42 g): ν_{max}/cm^{-1} (KBr): 3448, 2978, 2933, 1726, 1671, 1603, 1520, 1458, 1369, 1346, 1253, 1221, 1155, 854; $\delta_H(CDCl_3)$: 1.42, 1.43, 1.45, 1.45, 1.48, 1.49, 1.49 [27H, 7× s, 9× CH_3 (^{t}Bu)]; 1.75–2.25 [2H, br m., CH_2C$_\alpha$]; 2.20–4.30 [~23H, br m's, CH_2Ar, C$_\alpha$H, 8× CH_2 (cyclen ring), 2× CH_2CO$_2$ ^{t}Bu]; 7.22, 7.37br.d, 7.44 [2H, 3× d, J 9.1, ~5.4, 8.7 resp., ArH-2,6]; 8.13, 8.14, 8.16, 8.17 [2H, 4× d, J 6.6, 6.6, 6.7, 6.7, ArH-3,5]; 8.00–10.50 [~1H, br, NH]; also 2.15 [1.5H, s] and 4.50 [0.75H, s]; $\delta_C(CDCl_3)$: 28.02, 28.12 [CH$_3$'s, ^{t}Bu]; 32.80 [DEPT-135, CH$_2$CH$_2$ (Pr)]; 42.00 and 42.70 (both DEPT-135), 47.00–52.00w.br, 51.76 (DEPT-135), 55.00–57.00 br., 59.10 [NCH$_2$'s]; 67.10 [C$_\alpha$]; 81.18, 81.64, 81.94, 82.14, 82.38 [CMe$_3$'s, ^{t}Bu]; 123.70, 123.76 [ArC-3,5]; 129.33 [ArC-2,6]; 146.57, 148.93 [ArC-4,1 resp.]; 166.90, 169.37, 170.28, 170.46, 171.12 [C=O's], also 61.15; MS (LSIMS; $C_{34}H_{57}N_5O_8$): 665, 664 (1, 1% resp. $[MH]^+$).

3.2.8. 1-(2-[2,3,4,6-Tetra-*O*-acetyl-β,D-galactopyranosyloxy]ethyl)-7-(1-carbo-[1,1-dimethyl]ethoxy-3-[4-nitrophenyl]propyl)-1,4,7,10-tetraazacyclododecane, hydrochloride salt (8). A DMF solution (5 mL) of *N*-functionalised cyclen derivative **6** (0.70 g, 1.5 mmol) was added dropwise to the anhydrous DMF suspension (5 mL) of protected bromoethyl-β,D-galactopyranoside **2** (0.83 g, 1.8 mmol) and anhydrous K_2CO_3 (0.28 g, 2.0 mmol) under an atmosphere of N_2. Thereafter, the mixture was vigorously stirred at rt under anhydrous conditions for 16 days (progress monitored by TLC; eluent—CH_2Cl_2/MeOH 9:1 v/v), decanted into dichloromethane (300 mL) and washed with deionised water (6× 300 mL) and saturated brine (300 mL). The organic phase was dried over $MgSO_4$, evaporated and subjected to column chromatography (eluent—CH_2Cl_2/MeOH 9:1 v/v; TLC—R_f 0.38–0.46) to give a red oil of free amine. This was treated briefly in dichloromethane/diethyl ether solution (1:5, 18 mL) with dry HCl gas and isolated as for **3**, yielding hydrochloride **8** as a red glass (0.48 g, 35%); (Found: C, 50.8; H, 7.30; N, 7.6. Calcd for $C_{38}H_{59}N_5O_{14}\cdot 2HCl\cdot 1/4CH_2Cl_2\cdot 0{\cdot}2Et_2O$: C, 51.0; H, 6.9; N, 7.6%): ν_{max}/cm^{-1} (KBr): 3433, 2978, 1749, 1669, 1519, 1370, 1346, 1228, 1153, 1058, 855; $\delta_H(CDCl_3)$: 1.21 [1.2H, t, J 7.1, CH_3 (Et_2O)]; 1.51, 1.55 (1.54sh) [9H, 2× s, 3× CH_3 (^{t}Bu)]; 1.99, 2.05, ~2.11 br m., 2.17 [14H, 3× s and br m., 4× CH_3 (Ac) and CH_2C$_\alpha$]; 2.40–4.80 [~27.8H, br m's, C$_\alpha$H, CH_2Ar, CH_2CH_2 (Et linker), 8× CH_2 (cyclen ring), H-1,5,6,6′ (gal), 0.4× CH_2 (δ = 3.47, q, J 7.1, Et_2O)]; 4.85–5.20 [2H, br m, H-2,3 (gal)]; 5.31 [0.5H, s, 0.25× CH_2Cl_2]; 5.41 [1H, br.s, H-4 (gal)]; 7.32–7.60 [2H, br m., ArH-2,6]; 7.95–8.30 [2H, br.d, ArH-3,5]; $\delta_C(CDCl_3)$: 20.51, 20.70 [CH$_3$'s (Ac)]; 28.13, 28.35 [CH$_3$'s, ^{t}Bu]; ~33.00 br, 34.40 [(CH$_2$)$_2$Ar]; ~47.00 w.br (DEPT-135), 53.50 [CH$_2$'s (cyclen ring), NCH$_2$ (Et linker)]; 61.03 [C-6 (gal), OCH$_2$ (Et linker)]; 66.90, ~69.00 br., 70.46, 71.05 [C-2,3,4,5 (gal), C$_\alpha$]; 101.50 br [DEPT-135, C-1 (gal)]; 123.91 [ArC-3,5];

129.55 [Ar*C*-2,6]; 147.00 [Ar*C*-4,1]; 169.88, ~170.13, 170.41 [*C*═O's]; also 95.70 [DEPT-135]; MS (LSIMS): 810, 811 (3, 3% resp. $[MH]^+$).

3.2.9. 1-(2-[2,3,4,6-Tetra-*O*-acetyl-β,D-galactopyranosyloxy]ethyl)-7-(1-carbo-[1,1-dimethyl]ethoxy-3-[4-nitrophenyl]propyl)-4,10-bis(carbo-[1,1-dimethyl]ethoxymethyl)-1,4,7,10-tetraazacyclododecane, hydrochloride salt (9) (Gal-PA-DO3A-NO_2 ester conjugate). An anhydrous DMF suspension (5 mL) of **8** (0.35 g, 0.39 mmol) and anhydrous K_2CO_3 (0.78 g, 5.6 mmol) was stirred at rt under N_2 as *tert*-butyl bromoacetate (0.78 g, 4.0 mmol) was added dropwise. Thereafter, the reaction mixture was stirred under anhydrous conditions for 15 days, decanted into dichloromethane (400 mL) and washed with deionised water (6× 200 mL) and saturated brine (200 mL). The organic phase was dried over $MgSO_4$, evaporated and subjected to gradient chromatography (eluent—0% → 2% → 4% → 5% MeOH in CH_2Cl_2 v/v). Purified free amine was treated for 2 min in dichloromethane/diethyl ether solution (1:27, 84 mL) with dry HCl gas, and isolated as for **3**, yielding hydrochloride **9** as a brown glass (102.7 mg, 22%); (Found: C, 50.9; H, 6.6; N, 5.8. Calcd for $C_{50}H_{79}N_5O_{18}{\cdot}2HCl{\cdot}KHCO_3{\cdot}0.1Et_2O$: C, 50.7; H, 6.8; N, 5.75%): ν_{max}/cm^{-1} (KBr): 3432, 2979, 2937, 1748, 1669, 1520, 1457, 1370, 1346, 1229, 1155, 1075, 845; $\delta_H(CDCl_3)$: 1.21 [0.6H, t, *J* 7.0, 0.2× C*H*$_3$ (Et_2O)]; 1.46, 1.48, 1.49 [27H, 3× s (overlapped), 9× C*H*$_3$ (^{t}Bu)]; 1.99, 2.06, ~2.11 br m., 2.16 [14H, 3× s and br m., 4× C*H*$_3$ (Ac) and C*H*$_2$C$_\alpha$]; 2.40–4.80 [~31.4H, br m's, C$_\alpha$*H*, C*H*$_2$Ar, C*H*$_2$C*H*$_2$ (Et linker), 8× C*H*$_2$ (cyclen ring), *H*-1,5,6,6′ (gal), 2× C*H*$_2$CO$_2$^{t}Bu, 0.2× C*H*$_2$ (δ = 3.48, q, *J* 7.0, Et_2O)]; 4.85–5.20 [2H, br m, *H*-2,3 (gal)]; 5.41 [1H, br m, *H*-4 (gal)]; 7.32–7.65 [2H, br m, Ar*H*-2,6]; 7.95–8.30 [2H, br,m, Ar*H*-3,5]; $\delta_C(CDCl_3)$: 20.53, 20.71 [*C*H$_3$'s (Ac)]; 28.10 [*C*H$_3$'s, ^{t}Bu]; 31.20 br, 32.70, 34.70 [(*C*H$_2$)$_2$Ar]; [*C*H$_2$'s not discernable from noise; δ_C = 40–50 ppm]; 61.09, 65.87 [*C*-6 (gal), O*C*H$_2$ (Et linker)]; 64.20 [DEPT-135, C$_\alpha$]; 66.96, 68.49, 70.70 br [*C*-2,3,4,5 (gal)]; 101.50 [*C*-1 (gal)]; 123.82 [Ar*C*-3,5]; 129.61 [Ar*C*-2,6]; 146.61, ~149.00 br [Ar*C*-4,1 resp.]; 169.94, 170.43 [*C*═O's]; MS (LSIMS): 1039, 1038 (3, 3% resp. $[MH]^+$).

3.2.10. 1-(2-[β,D-Galactopyranosyloxy]ethyl)-7-(1-carboxy-3-[4-aminophenyl]propyl)-4,10-bis(carboxymethyl)-1,4,7,10-tetraazacyclododecane (Gal-PA-DO3A-NH_2). A chloroform solution (4 mL) of [β,D-galactopyranosyloxy]ethyl-PA-DO3A-NO_2 ester (**9**, 100.2 mg, 0.085 mmol) was added dropwise to trifluoroacetic acid (TFA, 20 mL) under N_2, and the reaction mixture was stirred at rt for 3.5 h, before being evaporated alone, and azeotropically with chloroform (2× 25 mL). The residue was treated with methanolic NaOMe solution (0.7 M, 10 mL) and the mixture was stirred under N_2 for 1 h, cooled to 5 °C and neutralised with Amberlite IR-120 (H^+) ion-exchange resin. The resin was immediately separated by filtration, washed with methanol, and the filtrate/washings reduced down to 10 mL before azeotropic evaporation with chloroform (20 mL) yielded a brown oil. Consecutive precipitations from methanol solutions (0.25 mL) by addition of diethyl ether (50 mL) yielded a cream/pink solid. This was dissolved in deionised water (10 mL), filtered and stirred with Pd/C (10% w/w, 50 mg) catalyst under H_2 (replenished at regular intervals) at atmospheric pressure in a gas chamber until uptake of H_2 had ceased (48 h). The reaction mixture was filtered and the filtrate azeotropically evaporated with absolute ethanol. Crystallisation from EtOH/H_2O (10:1 v/v, 11 mL) over 3 days yielded a pink/brown solid (17.8 mg, 21%): mp 178–185 °C (dec); (Found: C, 44.2; H, 7.2; N, 6.7. Calcd for $C_{30}H_{49}N_5O_{12}{\cdot}2TFA{\cdot}3EtOH{\cdot}3H_2O$: C, 44.0; H, 6.9; N, 6.4%): ν_{max}/cm^{-1} (KBr): 3422, 2929, 1633, 1519, 1385, 1078; $\delta_H(H_2O)$: 1.03 [9H, t, *J* 7.5, C*H*$_3$ (EtOH)]; 1.25–1.45 [2H, br m, C*H*$_2$C$_\alpha$]; 1.60–4.80 [~40H, v.br., C$_\alpha$*H*, C*H*$_2$Ar, C*H*$_2$C*H*$_2$ (Et linker), 8× C*H*$_2$ (cyclen ring), 7× gal-*H*'s, 2× C*H*$_2$CO$_2$H, 3× C*H*$_2$ (δ = 3.57, q, *J* 7.5, EtOH)] in which 4.42 [1H, br m., *H*-1 (gal)]; 6.60–7.50 [~4H, br m, Ar*H*'s]; also 7.70–8.05 [trace, br.]; $\delta_C(D_2O)$: 16.73, 16.78 [*C*H$_3$ (Et)]; 27.43, 31.00–32.00 br [*C*H$_2$*C*H$_2$Ar]; 40.50, 42.00–56.00 br, 57.38, 57.42, 58.80–60.30 br. [*C*H$_2$'s]; 61.04 [*C*-6 (gal)], 62.80 br [O*C*H$_2$ (Et linker), C$_\alpha$]; 68.63, 70.65, 72.60, 75.27 [*C*-2,3,4,5 (gal)]; 102.81 [*C*-1 (gal)]; 120.00–123.00 br, 130.11 [*C*F$_3$ (TFA), Ar*C*'s]; (*C*═O's not discernable from noise; δ_C = 160–180 ppm); MS (LSIMS): 673, 672 (2, 2% resp. $[MH]^+$).

3.2.11. Gd(III) complex of Gal-PA-DO3A-NH_2. An aqueous suspension (2 mL) of Gal-PA-DO3A-NH_2 (11.4 mg, 0.011 mmol) and $Gd_2(CO_3)_3$ (2.7 mg, 0.0055 mmol) was refluxed for 24 h, then filtered and the brown filtrate azeotropically evaporated with absolute ethanol. Precipitation from EtOH over several days yielded the Gd(III) complex as a brown solid (7.2 mg, 62%): mp 223–227 °C (dec); (Found: C, 38.0; H, 4.8; N, 5.8. Calcd for $C_{30}H_{46}N_5O_{12}Gd{\cdot}2TFA{\cdot}5/4EtOH{\cdot}11/4H_2O$: C, 37.75; H, 5.1; N, 6.0%): ν_{max}/cm^{-1} (KBr): 3423, 2926, 1619, 1515, 1385, 1079.

3.3. R_1 Relaxivity measurements

Stock solutions (17.5 mM) of [Gd-(Gal-PA-DO3A-NH_2)] and [Gd-(Gal-DO3A)] complexes were prepared in water. Both complexes (2 mM) in phosphate buffer (pH 7.4) were incubated with β-galactosidase (5 μM from Sigma EC 3.2.1.23) at 37 °C for 20 h prior to measurements. Samples of the above solutions were placed in Eppendorf tubes and imaged on a Varian Inova (Palo Alto, CA, USA) 9.4 T horizontal bore MRI scanner. The tubes were placed in a holder within a 63 mm inner diameter RF coil. A spin-echo sequence was used with the following scan parameters: TR = 50, 100, 200, 300, 500, 700, 1200, 3000, 5000, 7000 ms, TE = 10 ms, number of signal averages = 2, 3 slices coronal slices 2 mM thick with a 1 mM gap between the slice, FOV; 100×100 mm^2, matrix of 256×128. The T_1 value of each compound was determined by fitting the data to Eq. (1), the T_1 Saturation Recovery equation.

$$S_i = S_0(1 - e^{x/T_1}) \quad (1)$$

where x is the TR value, and S_i is the measured signal for a given TR value). To ensure reproducibility and consistency, each measurement was carried out three times under identical conditions.

References and notes

1. Brady, T. J.; Gebhardt, M. C.; Pykett, I. L.; Buonanno, F. S.; Newhouse, J. H.; Burt, C. T.; Smith, R. J.; Mankin, H. J.; Kistler, J. P.; Goldman, M. R.; Hinshaw, W. S.; Pohost, G. M. *Radiology* **1982**, *144*, 549–552.
2. Goldman, M. R.; Brady, T. J.; Pykett, I. L.; Burt, C. T.; Buonanno, F. S.; Kistler, J. P.; Newhouse, J. H.; Hinshaw, W. S.; Pohost, G. M. *Circulation* **1982**, *66*, 1012–1016.
3. Carr, D. H.; Brown, J.; Bydder, G. M.; Steiner, R. E.; Weinmann, H. J.; Speck, U.; Hall, A. S.; Young, I. R. *American Journal of Roentgenology* **1984**, *143*, 215–224.
4. Carr, D. H.; Brown, J.; Bydder, G. M.; Weinmann, H. J.; Speck, U.; Thomas, D. J.; Young, I. R. *Lancet* **1984**, *1*, 484–486.
5. Lauffer, R. B.; Parmelee, D. J.; Dunham, S. U.; Ouellet, H. S.; Dolan, R. P.; Witte, S.; McMurry, T. J.; Walovitch, R. C. *Radiology* **1998**, *207*, 529–538.
6. Caravan, P.; Cloutier, N. J.; Greenfield, M. T.; McDermid, S. A.; Dunham, S. U.; Bulte, J. W. M.; Amedio, J. C.; Looby, R. J.; Supkowski, R. M.; Horrocks, W. D.; McMurry, T. J.; Lauffer, R. B. *Journal of the American Chemical Society* **2002**, *124*, 3152–3162.
7. Bhorade, R.; Weissleder, R.; Nakakoshi, T.; Moore, A.; Tung, C. H. *Bioconjugate Chemistry* **2000**, *11*, 301–305.
8. Josephson, L.; Tung, C. H.; Moore, A.; Weissleder, R. *Bioconjugate Chemistry* **1999**, *10*, 186–191.
9. Mulligan, T.; Carrasquillo, J. A.; Chung, Y.; Milenic, D. E.; Schlom, J.; Feuerstein, I.; Paik, C.; Perentesis, P.; Reynolds, J.; Curt, G.; Goeckeler, W.; Fordyce, W.; Cheng, R.; Riseberg, D.; Cowan, K.; Oshaughnessy, J. *Clinical Cancer Research* **1995**, *1*, 1447–1454.
10. Meredith, R. F.; Partridge, E. E.; Alvarez, R. D.; Khazaeli, M. B.; Plott, G.; Russell, C. D.; Wheeler, R. H.; Liu, T. P.; Grizzle, W. E.; Schlom, J.; LoBuglio, A. F. *Journal of Nuclear Medicine* **1996**, *37*, 1491–1496.
11. Alvarez, R. D.; Partridge, E. E.; Khazaeli, M. B.; Plott, G.; Austin, M.; Kilgore, L.; Russell, C. D.; Liu, T. P.; Grizzle, W. E.; Schlom, J.; LoBuglio, A. F.; Meredith, R. F. *Gynecologic Oncology* **1997**, *65*, 94–101.
12. Chappell, L. L.; Rogers, B. E.; Khazaeli, M. B.; Mayo, M. S.; Buchsbaum, D. J.; Brechbiel, M. W. *Bioorganic & Medicinal Chemistry* **1999**, *7*, 2313–2320.
13. Moats, R. A.; Fraser, S. E.; Meade, T. J. *Angewandte Chemie (International ed. in English)* **1997**, *36*, 726–728.
14. Louie, A. Y.; Huber, M. M.; Ahrens, E. T.; Rothbacher, U.; Moats, R.; Jacobs, R. E.; Fraser, S. E.; Meade, T. J. *Nature Biotechnology* **2000**, *18*, 321–325.
15. Meade, T. J.; Fraser, S. E. U. S. Patent 2003/0053954, **2003**.
16. Hirschmann, R.; Nicolaou, K. C.; Pietranico, S.; Leahy, E. M.; Salvino, J.; Arison, B.; Cichy, M. A.; Spoors, P. G.; Shakespeare, W. C.; Sprengeler, P. A.; Hamley, P.; Smith, A. B.; Reisine, T.; Raynor, K.; Maechler, L.; Donaldson, D.; Vale, W.; Freidinger, R. M.; Cascieri, M. R.; Strader, C. D. *Journal of the American Chemical Society* **1993**, *115*, 12550–12568.
17. Kronzer, F. J.; Schuerch, C. *Carbohydrate Research* **1973**, *27*, 379–390.
18. Lucas, T. J.; Schuerch, C. *Carbohydrate Research* **1975**, *39*, 39–45.
19. Burk, R. M.; Gac, T. S.; Roof, M. B. *Tetrahedron Letters* **1994**, *35*, 8111–8112.
20. Schultze, L.; Bulls, A. R. WO Patent 96/28433, **1996**.
21. Berg, A.; Almen, T.; Klaveness, T.; Rongved, T.; Thomassen, H. D. U.S. Patent 5,531,978, **1996**.
22. Buoen, S.; Dale, J.; Krane, J. *Acta Chemica Scandinavica Series B Organic Chemistry and Biochemistry* **1984**, *38*, 773–778.
23. Kruper, W. J.; Rudolf, P. R.; Langhoff, C. A. *Journal of Organic Chemistry* **1993**, *58*, 3869–3876.
24. Kocienski, P. J. *Protecting Groups*, 3rd ed.; Georg Thieme Verlag: Stuttgart, 2005.
25. Zemplen, G.; Pacsu, E. *Berichte Derdeutschen Chemischen Geserschaft* **1929**, *62*, 1613–1614.
26. Cheng, R. C.; Fordyce, W. C.; Goeckeler, W. F.; Kruper, W. J.; Baughman, S.; Garlich, J. R.; Kiefer, G. E.; McMillan, K.; Simon, J.; Wilson, D. A. WO Patent 89/12631, **1989**.
27. Harpp, D. N.; Bao, L. Q.; Black, C. J.; Gleason, J. G.; Smith, R. A. *Journal of Organic Chemistry* **1975**, *40*, 3420–3427.
28. Atkins, T. J.; Richman, J. E.; Oettle, W. F.; Noland, W. E. Eds.; J. Wiley and Sons Chichester, 1988; pp 652–662.
29. Dahmen, J.; Frejd, T.; Gronberg, G.; Lave, T.; Magnusson, G.; Noori, G. *Carbohydrate Research* **1983**, *116*, 303–307.
30. Jung, M. E.; Yang, E. C.; Vu, B. T.; Kiankarimi, M.; Spyrou, E.; Kaunitz, J. *Journal of Medicinal Chemistry* **1999**, *42*, 3899–3909.
31. Davis, B. G.; Maughan, M. A. T.; Green, M. P.; Ullman, A.; Jones, J. B. *Tetrahedron: Asymmetry* **2000**, *11*, 245–262.