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Polysaccharides as carriers for magnetic resonance imaging contrast agents: synthesis and stability of a new amino acid linker derivative

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Abstract

The relative hydrolytic stability of contrast agents for magnetic resonance imaging (MRI), consisting of paramagnetic metal chelates bound to polysaccharides through an ester bond, has been investigated. Four preparations of biodegradable, cross-linked starch particles were studied as model compounds: diethylenetriaminepentaacetic acid (DTPA)–starch particles (**1**), two batches of gadolinium–DTPA (GdDTPA)–starch particles (**2a**,**2b**) with different Gd content, and *N*-(2-phenylethyl)succinamoyl starch ester particles (**4**). In a study of hydrolytic rates in water suspension, the derivatives with GdDTPA bound directly to the particle via the carboxylic acid groups in DTPA (**2a**,**2b**) showed 74 and 86% remaining matrix-bound GdDTPA, respectively, after 21 days. The unchelated derivative (**1**) showed 96% remaining matrix-bound DTPA, while for the succinamoyl-linked derivative (**4**), no significant hydrolysis took place during the same time span. To investigate the corresponding stability of ester bonds in water-soluble, blood-pool agents for MRI, the degradation rate of the macromolecular derivatives dextran–DTPAGd (**5**) and dextran–β-alanine–DTPAGd (**6c**) were compared in artificial blood plasma. The remaining fraction of undegraded ester bond in **6c** was approximately 95% after 100 min, while **5** was approximately fully degraded over the same time span. These results indicate that the conjugate with the β-alanine spacer may have a more suitable degradation rate for blood-pool MRI contrast purposes than the derivatives with GdDTPA directly ester bound. It was also shown by relaxation measurements that gadolinium–ethylenediaminetetraacetic acid (GdEDTA) was demetalated in a test solution of phosphate (3 mM) at 37 °C. No demetalation was observed for GdDTPA derivatives of water-soluble polysaccharides, represented by the dextran–GdDTPA conjugate **5**

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and aminoethyldextran–GdDTPA **7**, lacking an ester bond between GdDTPA and the dextran matrix. © 1996 Elsevier Science Ltd.

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1. Introduction

Particular carriers and macromolecular agents alter the pharmacokinetic profile of low-molecular-weight, water-soluble agents. Paramagnetic particles have been evaluated as potential carriers of paramagnetic moieties in magnetic resonance imaging (MRI) contrast agents for the reticuloendothelial system, i.e. in liver and spleen [1]. Water-soluble macromolecules like human serum albumin [2–4], immunoglobulins [5], monoclonal antibodies [6], and polysaccharides [7–11] have been suggested as potential MRI contrast agents for the blood pool. These approaches have received much attention during recent years, as the development of MRI contrast agents for medical imaging tends to move towards more organ-specific agents. The most important parameters for MRI contrast agents include high efficacy, high safety index, and pharmacokinetic properties which make them suitable for the intended diagnostic use.

The starch particles used in this study can be prepared within well-defined limits of particle characteristics, degree of cross-linking, and biodegradability [12]. They behave like gels when swelled in water. Few reports describe the use of similar particles as carriers of contrast agents for diagnostic imaging, but dextrans are extensively used clinically as blood plasma substitutes [13]. Several of the products described have GdDTPA directly linked to the carbohydrate via an ester bond [14], while others have reported amide [15] or urethane [7] bonds. No comparative stability studies of these different DTPA derivatives have been reported earlier, but stability studies of dextran–drug derivatives have been reported [16].

We have previously described water-soluble polysaccharides [17] and cross-linked starch microspheres [18] as carriers of paramagnetic contrast agents for MRI. As a continuation, the aims of this study were to synthesise some ester derivatives of polysaccharide carriers, evaluate the relative in vitro stability, and identify linkers with a hydrolytic stability sufficient to provide clinically useful MRI contrast enhancement in the reticuloendothelial system and the blood-pool compartment.

2. Experimental

General.—Dextran was purchased from Sigma, gadolinium chloride ($GdCl_3$) from Ventron or Aldrich, and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) from Novabiochem. DTPA, *N*-(9-fluorenylmethoxycarbonyl)-β-alanine (FMOC-β-alanine), and all other chemicals were purchased from Fluka or E. Merck. Artificial blood plasma (Seronorm®), containing standard values of all major human blood plasma components, was purchased from Nycomed Pharma AS. Spherical starch particles were prepared by cross-linking hydrolysed potato starch with epichlorohydrin by a modifica-

tion of the bead polymerisation method [19]. The particles were estimated to have a mean number of "glucose unit" equivalents of 5.5 mmol/g of dry solid. The number mean diameter of the particulate starting material was 1.4 μm, measured by Coulter Counter.

Ultrasound treatment means sonication in an ultrasonic bath at 20 kHz and 50 W.

Analysis of metal ion content was performed using inductively coupled plasma atomic emission spectrophotometry (ICP). Analysis of water content was performed using the Karl Fischer titration method.

Relaxation measurements were performed at 0.24 tesla (T) and 37 °C on a Radx NMR spectrometer (Radx proton spin analyser), using an inversion recovery sequence. The spin–lattice relaxivity, denoted r_1, is given as the slope of the linear least-squares regression analysis of the relaxation rate R_1 $(1/T_1)$ plotted versus the concentration. All relaxivity data are expressed per Gd atom.

High-performance liquid chromatography (HPLC) analyses were performed using a Hewlett–Packard or a Perkin–Elmer system, an RP-18 column, and an LC-15B absorbance detector. As discussed in an earlier report [17], monitoring of molecular weight (MW) for the dextran derivatives using gel permeation chromatography (GPC) showed that the EDC coupling method used in the present work does not give significant increase in MW. Free or uncomplexed DTPA and free Gd ions were quantified by titration with DTPA or $GdCl_3$, respectively, in the presence of Xylenol Orange indicator.

The NMR spectra were recorded using a Varian XL-300 instrument.

DMF, Me_2SO, and $CHCl_3$ were dried by storage over 4 Å molecular sieves. Water in the text is equivalent to distilled water. DTPA bisanhydride (DTPA-A) [20], GdEDTA [21,22], and GdDTPA [22] were prepared as described in the literature.

The amounts of $GdCl_3$ given in the procedures are net weights after correction for water content.

DTPA–starch particles (**1**).—The derivative was prepared using a slightly modified, previously described method [18]: starch particles (2.00 g, 11.1 mmol "glucose units") were washed with Me_2SO (3×60 mL) by successive centrifugation and decantation, and suspended in Me_2SO (100 mL). After treatment of the suspension with ultrasound (10 min), DTPA-A (179 mg, 0.5 mmol) was added and the suspension was shaken for 24 h. The suspension was washed with water (6×60 mL), left standing for 3 days, and then washed again with water (6×60 mL). The DTPA-starch particles were suspended in water to 100 mL. Lyophilisation yielded a white powder (2.15 g). Anal. Found: N, 1.14%.

GdDTPA–starch particles (**2a** and **2b**).—The derivatives **2a** and **2b** with different content of Gd were prepared from DTPA–starch particles as previously described [18], except that the products were isolated by lyophilisation after the dialysis. Analytical data for **2a** and **2b** are given in Table 1.

N-(2-Phenylethyl)succinamic acid (**3**).—The compound was prepared using a slightly modified literature procedure [23]. Succinic anhydride (4.96 g, 49.5 mmol) was dissolved in $CHCl_3$ (100 mL) at 0 °C. 2-Phenylethylamine (5.00 g, 41.3 mmol) and *N*-methylmorpholine (5.00 g, 49 mmol) were added. The mixture was warmed to room temperature, stirred at ambient temperature for 24 h, and then extracted with water

Table 1
Analytical data and r_1 of GdDTPA starch microspheres (**2a**,**2b**)

Product	Gd content (%)	Water content (%)	r_1 (s^{-1} mM^{-1}) in water
2a	2.6	7.1	14.3
2b	5.1	6.5	8.4

(2 × 100 mL). After adjustment of pH to 1.0 with 12 M HCl, the precipitate was filtered off and washed on the filter with 1 M HCl. The precipitate was suspended in water (100 mL), and the pH was adjusted to 7.0 with 10 M NaOH, and then to 1.0 with 12 M HCl, to reprecipitate the product. The precipitate was filtered off, washed on the filter with 1 M HCl, and dried in vacuo at 50 °C for 24 h, to give a white powder (5.0 g, 54%). Reprecipitation of a part of the product (2.94 g) yielded white flakes (1.83 g, 34%); mp. 119–123 °C, ref. [23] 125–126 °C; ^{1}H NMR (300 MHz, D_2O, NaOD): δ 7.4–7.3 (m, 5 H), 3.4 (t, 2 H), 2.8 (t, 2 H), 2.4 (s, 4 H); ^{13}C NMR (75.5 MHz, D_2O, NaOD): δ 183.8, 178.3, 142.1, 131.7, 131.4, 129.2, 43.3, 37.3, 36.0, 35.2. Anal. Calcd for $C_{12}H_{15}NO_3$: C, 65.14; H, 6.83; N, 6.33. Found: C, 65.16; H, 6.81; N, 6.19.

N-(2-Phenylethyl)succinamoyl starch ester particles (**4**).—A suspension of starch particles (3.00 g, 16.7 mmol "glucose units") in DMF (50 mL) was treated with ultrasound for 15 min, and washed with DMF (3 × 30 mL) by successive centrifugation and decantation. The particles were resuspended in DMF (200 mL), and *N*-(2-phenylethyl)succinamic acid (**3**) (179 mg, 0.81 mmol), EDC (171 mg, 0.89 mmol), and 4-pyrrolidinopyridine (12 mg, 0.08 mmol) were added. After 20 h with shaking at ambient temperature, the particles were washed with DMF (6 × 50 mL), then with water (6 × 50 mL), and resuspended in water to 80 mL. An aliquot (2 mL) was taken out for stability studies. A part of the suspension (20 mL) was lyophilised to yield **4** (490 mg). Anal. Found: N, 0.53%. The yield in the coupling reaction was estimated to be 60%.

GdDTPA–dextran conjugate (**5**).—DTPA (7.71 g, 19.6 mmol) was dissolved in Me_2SO (300 mL) by slight warming, and 4-pyrrolidinopyridine (291 mg, 1.96 mmol) and EDC (4.13 g, 21.6 mmol) were added at ambient temperature. This solution was added dropwise to a stirred solution of dextran (21.0 g, mol. wt. 70,000, 116.7 mmol "glucose units") in Me_2SO (600 mL). After stirring for 18 h at ambient temperature, a mixture of $CHCl_3$ and diethyl ether (7:8, 1000 mL) was slowly added. After 2 h of cooling in an ice–water bath, the white precipitate was collected by decanting, and washed with the $CHCl_3$–diethyl ether mixture (200 mL). After decantation, the product was redissolved in citrate buffer (600 mL) at pH 4.4 and the water phase was washed with $CHCl_3$ (150 mL). $GdCl_3$ (5.17 g, 19.6 mmol) was dissolved in water (250 mL), and this solution was added dropwise during 20 min to the solution of the dextran derivative while the pH was kept at 5–5.5 by adding 2 M NaOH. When the pH had stabilised at around 5, the solution was dialysed using a Hollow Fiber cartridge H1P10-20 until the T_1 in the filtrate was above 2000 ms. The solution was concentrated to 800 mL using the Hollow Fiber system, and lyophilised for 3 days. The white porous solid was dried in vacuo for 48 h at ambient temperature, and then at 50 °C for 24 h, to yield 21.8 g of a white porous solid. Anal. Found: Gd, 4.4% (corr. for water); water,

Table 2
Added reactants and solvents in the preparation of **6a–c**. Calculated starting molarity of β-alanine functionality in all preparations was 2.8 mmol

Added reactant/solvent	**6a**	**6b**	**6c**
Activated DTPA solution used (mmol DTPA)	1.4	2.8	4.2
7:8 $CHCl_3$–diethyl ether (mL)	125	150	175
Citrate buffer pH 5.5 (mL)	50	80	125
$GdCl_3$ in water (mmol Gd/mL)	1.4/30	2.8/60	4.2/90

9.9%; free Gd, 0.02%; uncomplexed DTPA, $< 0.1\%$; r_1 in water: 10.4 s^{-1} mM^{-1}; r_1 in artificial blood plasma: 8.6 s^{-1} mM^{-1}.

GdDTPA–β-alanine–dextran conjugate (**6a–c**).—A slight modification of the reported procedure [24] was used: To a solution of dextran (4.73 g, mol. wt. 70,000, 26.3 mmol "glucose units") in Me_2SO (100 mL) was added a solution of FMOC-β-alanine (2.75 g, 8.82 mmol), EDC (1.85 g, 9.7 mmol), and 4-pyrrolidinopyridine (133 mg, 0.9 mmol) in Me_2SO (50 mL). After stirring at ambient temperature for 20 h, piperidine (25 mL, 253 mmol) was added. The solution was stirred for 55 min, and then extracted with hexane (3 × 150 mL, and then 2 × 100 mL). The volume of the Me_2SO solution was adjusted to 300 mL. An aliquot of 13 mL was taken out and the volume of this solution was adjusted with water (65 mL). The aqueous solution was dialysed against water for 36 h and lyophilised to yield a white solid (150 mg). Anal. Found: N, 0.39%. The remaining Me_2SO solution containing dextran–β-alanine conjugate was split into three (3 × 95.7 mL), and to each portion were added the amounts of reagents below, as given in Table 2. The solution of activated DTPA was prepared from DTPA (3.93 g, 10 mmol), 4-pyrrolidinopyridine (148 mg, 1 mmol), and EDC (2.1 g, 11 mmol) in Me_2SO (175 mL). After addition of this activated DTPA solution to the solution of the dextran derivative and stirring for 18 h at ambient temperature, a mixture of ether–$CHCl_3$ (7:8) was added in portions. After 2 h, the yellow precipitates were collected by decantation and centrifugation. The precipitates were dissolved in citrate buffer, and a solution of $GdCl_3$ in water was added. The pH was adjusted to ca. 5 with 2 M NaOH. After 15 min, the solutions were dialysed until T_1 in the dialysis water was above 2900 ms. The solutions were lyophilised and analysed; analytical data for all products are given in Table 3.

GdDTPA–aminoethyldextran (**7**).—The synthesis of the starting material aminoethyldextran (mol. wt. 80,000, d.s. 0.35) was described earlier [17]. The material was estimated to have a mean number of "glucose unit" equivalents of 5.1 mmol/g of dry solid. To a solution of aminoethyldextran (2.00 g, 3.59 mmol amine equivalents) in Me_2SO (100 mL) was added DTPA-A (1.35 g, 3.76 mmol, 5% excess to estimated equivalents of amino groups). The solution was stirred at ambient temperature for 24 h, then cooled in an ice–water bath. From this point, the procedure for the preparation of **5** was followed. Lyophilisation yielded a white powder (2.15 g). Anal. Found: N, 1.14%; Gd, 7.2%; water, 2.4%; r_1 in water: 8.9 s^{-1} mM^{-1}.

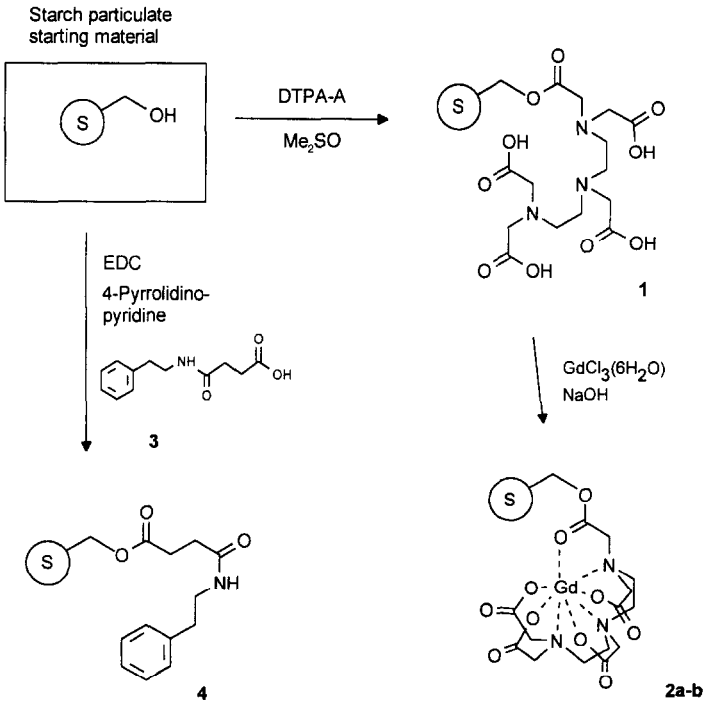
The synthetic routes are given in Schemes 1 and 2.

Table 3
Analytical data for dextran derivatives (**6a–c**)

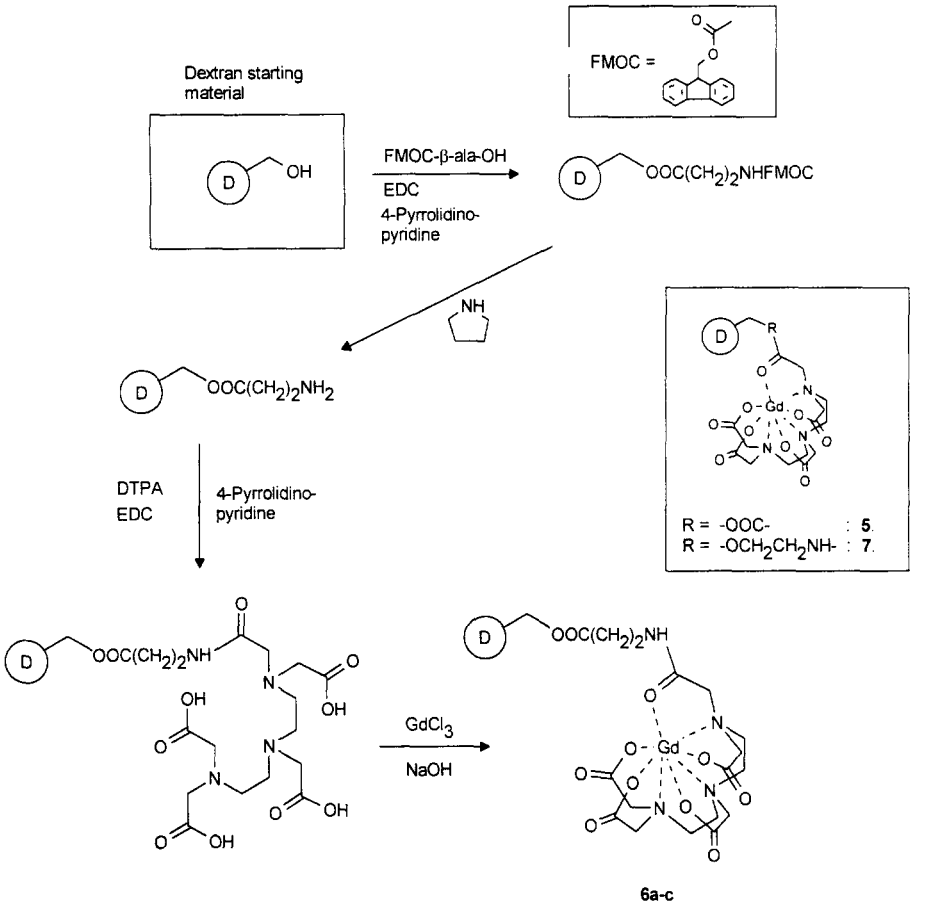
	6a	6b	6c
Yield (g)	1.01	1.13	1.04
Water (%)	4.1	1.7	2.5
Gd (%)	1.7	2.2	3.4
N (%)	1.06	1.38	1.85
N (%) [a]	0.75	0.97	1.5
r_1 (s^{-1} mM^{-1}) water, 10 MHz	9.4	8.7	8.7
r_1 (s^{-1} mM^{-1}) artificial plasma, 10 MHz	6.9	6.9	6.9

[a] Expected N percentage, calculated from the content of Gd, if all β-alanine moieties on dextran were completely 1:1 (mol:mol) substituted with GdDTPA.

Methods for evaluation of stability.—The rate of ester hydrolysis of the particulate derivatives **1**, **2a**,**2b**, and **4** in water was calculated from the ratio between the amount of DTPA, GdDTPA, or **3** in the dialysis water of particle suspensions, and the remaining amount of substituent attached to the carbohydrate carrier versus time. All analyses of kinetics and calculation of hydrolytic degradation rates and half-lives were performed using the computer program Siphar version 4.0. Curve fitting was performed using the computer program Sigmaplot version 1.02.



Scheme 1. Synthesis and structures of derivatives **1**, **2a**,**2b**, **3**, and **4**.



Scheme 2. Synthesis and structures of derivatives **5**, **6a–c**, and **7**.

Ester hydrolysis of GdDTPA from **2a,2b** was monitored by measuring relaxation times T_1 in an aqueous dispersion of the products, estimating the concentration of the contrast agent by using GdDTPA as an external standard. Relaxation rates R_1 were plotted against time. The studies were performed using distilled water, keeping the pH between 5.5 and 6 during the study by exchanging the dialysis water at each recording of T_1. The cleavage products (DTPA and **3**, respectively) from **1** and **4** were monitored using HPLC.

Hydrolysis of the water-soluble dextran derivatives **5** and **6c** in artificial blood plasma was estimated by measuring the change in R_1 at 37 °C as a function of time. The measurements were performed in NMR tubes, and the Gd concentrations in all test solutions were 0.2 mM. The corresponding cleavage product from **5**, GdDTPA, was measured as an external standard under the same conditions. The difference in r_1 for **5** and **6c** (8.6 and 6.9 mM^{-1} s^{-1}, respectively, in artificial blood plasma) was corrected for; this was performed by weighing for the difference in R_1, relative to the estimated

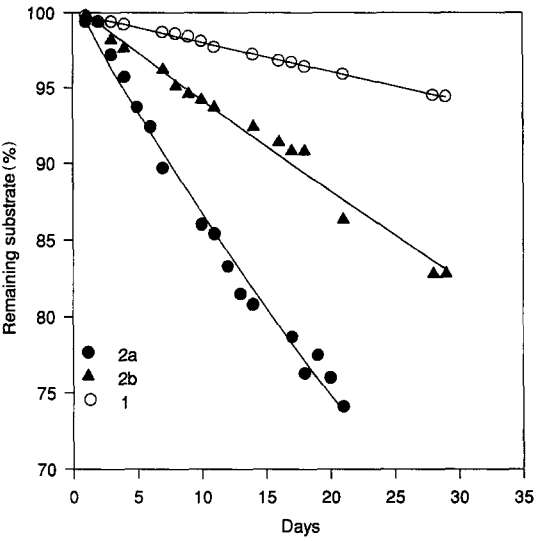


Fig. 1. Remaining substrate (%) of **1**, **2a**, and **2b** in water at ambient temperature at pH 5.5–6. The data are consistent with pseudo-first-order processes for all products.

fraction of hydrolysed product. The difference in r_1 of the anticipated degradation product β-alanine–DTPAGd and GdDTPA was assumed not to be significant.

A test system for evaluation of resistance to metal decomplexation of the dextran derivatives towards inorganic phosphate consisted of a solution of 3 mM sodium hydrogen phosphate at pH 7 in 1:2.3 (v/v) glycerol–water at 37 °C. $Gd_2(PO_4)_3$, GdEDTA, and GdDTPA were used as standards. The T_1 relaxation times were measured over a time scale of 3 days, and the respective plateaus of R_1 calculated.

3. Results and discussion

The results of the hydrolysis measurements of **1**, **2a**, and **2b** in distilled water are given in Fig. 1. The results of analysis of the hydrolysis data for **1**, **2a**, and **2b** are given in Table 4.

No evidence of biexponential behaviour of the hydrolysis profile, as a sign of any

Table 4
Results from the degradation study on particulate derivatives **1** and **2a**,**2b**

Product	Approximate half-life (days)	$10^3 \times k_1$ (days^{-1}) [a]	Remaining undegraded fraction (approx.) after 21 days	Corr.
1	343	2	96	0.9900
2a	48	14	74	0.9922
2b	100	7	86	0.9915

[a] Apparent first-order-rate constant.

compartmentalisation of the metal chelate moiety in the heterogenous particulate system, was found. The derivative **2a** had approximately half the Gd content of **2b** (2.6 and 5.1% Gd, respectively), which was correlated inversely to the degradation rate. This may be related to the fact that the ester bond is surrounded by a substantially lower local concentration of neighbouring carbohydrate hydroxy groups in **2b** compared to **2a**. Influence on the mechanism of ester hydrolysis from carbohydrate hydroxy groups in dextran–drug derivatives has been reported earlier [16]. However, further studies are needed to investigate such a relationship more thoroughly. The Gd-free derivative (**1**) had a degree of substitution corresponding to a Gd content of 4.2% in a chelated conjugate. The estimated degradation half-life for **1** was significantly longer than for the Gd-containing derivatives (**2a**,**2b**). The coordination number for Gd is reported to be 8–9 [25], and the ester carbonyl is probably coordinated to the Gd, which still has a position free for water access (Scheme 1). There are several studies reporting an enhanced rate of hydrolysis for metal-chelated amino acid esters, as a consequence of the electron-withdrawing effect of the coordinated metal atom [26,27]. In the conjugate **4**, *N*-(2-phenylethyl)succinamic acid (**3**) is attached to the carbohydrate via an ester bond, where electronic effects from an amino acid moiety and catalytic effects from a chelated Gd ion were eliminated. Significant amounts of **3** were not detected in the suspension medium of **4** during the time scale of the study. Lipophilicity of the aromatic group or low water-solubility of **3** could affect the hydrolysis rate because of lower accessibility for water, but the monoamide **3** is easily soluble in water above pH 3 and the phenyl group is spaced 6 atoms away from the ester bond.

In summary, these results have shown that the rate of hydrolysis of the ester bond in these derivatives (*a*) is enhanced for the amino acid ester derivative with DTPAGd directly bound to the carbohydrate matrix, and especially for the metal-chelated amino acid moiety, as compared to the unconjugated aliphatic ester bond, and (*b*) seems to be higher with lower degree of substitution.

As a consequence of these results, the question arises whether the stability of the ester bond in the derivatives **2a** and **2b** is sufficient for use in a linker between a contrast agent and a macromolecular carrier. The rate of hydrolysis may not be critical for particulate delivery of paramagnetic metal chelates to liver and spleen, since the residence time of particulate products in the blood pool is usually very short. However, the hydrolysis is critical for the shelf-life of the particulate products in water suspension.

The half-life of the ester bond hydrolysis was expected to be longer for the particulate carbohydrate derivatives than for water-soluble derivatives, because of the heterogenous nature of the particulate suspensions and better accessibility of water to the ester bond in the water-soluble derivative. As a consequence of this, and the results above, a linker between the metal chelate part and the carbohydrate part containing an ester bond would be expected to reduce the destabilising effect from the metal ion and the amino acid moiety, but maintain degradability. The hydrolytic half-life of a dextran derivative in 80% human blood plasma at 37 °C, where metronidazole was linked to dextran 70,000 via a succinic acid linker, was found to be 57.8 h [28]. To evaluate the stability of the analogous metal chelate derivative, the dextran derivatives **5** and **6a–c** were prepared. The background for preparation of three samples of the linker derivative with increasing Gd content (**6a–c**) was to investigate the control in the synthetic method. The rationale

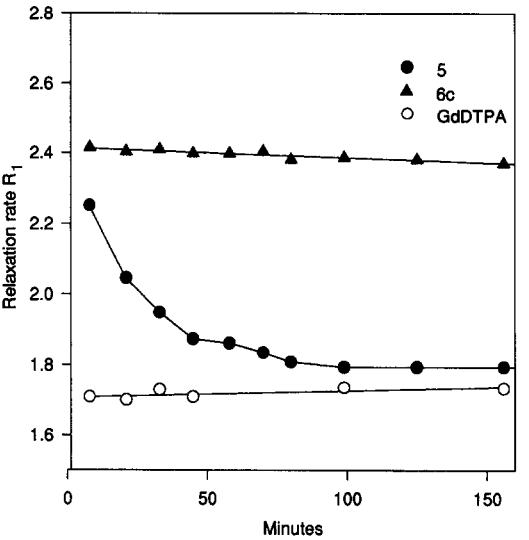


Fig. 2. Evaluation of stability of **5** and **6c** in artificial blood plasma at 37 °C. The degradation data are consistent with pseudo-first-order processes for both products.

for choosing amino acid linkers for this purpose was their endogenic nature, their synthetic versatility, and our experience with other biodegradable starch particles where glycine and β-alanine are used as linkers; in this study [29] the latter amino acid linker resulted in significantly more stable derivatives than the former. Conformational differences as a result of a different linker chain-length are expected to make minor contributions to the variations in half-life for these derivatives. The major effect is anticipated to be the electronic influence from the amino group in **6a–c**, which is also reflected in the difference in carboxylic pK_a values for glycine and β-alanine (2.34 and 3.60, respectively [30]), and the fact that the methyl β-alaninate is significantly more hydrolytically stable than methyl glycinate [27].

The results from the degradation rate measurements for **5** and **6c** in artificial blood plasma are given in Fig. 2.

Because of the reduced tumbling rate τ_R of the metal chelate when attached to the macro-molecular carbohydrate in **5** and **6c**, R_1 is higher than for the GdDTPA standard with equal Gd content. Consequently, complete degradation of **5** or **6c** would result in R_1 values approaching the level of the standard if no transesterification reactions or amine acylation reactions of proteins occur, instead of clean hydrolytic cleavage to GdDTPA.

The results of analysis of the cleavage data for **5** and **6c** are given in Table 5. The degradation of **5** is nearly completed within 100 min, with a half-life of approximately 0.36 h. During the same time span, ca. 95% of **6c** was intact, and the half-life was approximately 87 h. As a comparison, the degradation of the aminoethyldextran derivative (**7**), where GdDTPA was bound to the carbohydrate matrix via an amide bond, was measured in another study using the same method, and it showed no significant change corresponding to any degradation process of the substrate.

Table 5
Results from the hydrolytic degradation study of dextran derivatives (**5**) and (**6c**)

Compound	Approximate half-life (h)	$10^3 \times k_1$ (h^{-1}) [a]	Remaining undegraded fraction (approx %) after 100 min	Corr.
5	0.36	1925	~ 0	0.9983
6c	87	8	95	0.9570

[a] Apparent first-order-rate constant.

The number of free carboxylic acid groups in DTPA derivatives is critical for the metal stability constant for the gadolinium chelate. Sherry et al. reported that the conversion of one carboxyl group in DTPA to an amide or ester bond did not affect the Gd^{3+} binding constant drastically [31]. Further, in the same study, the thermodynamic stability constants for GdEDTA and GdDTPA were found to be 17.55 and 22.25, respectively, in accordance with earlier published results [32]. GdDTPA is stable in vivo, but GdEDTA is reported to be unstable in rat serum, and inorganic phosphate is anticipated to be one of the most efficient competitors for Gd in vivo [33]. These arguments indicate that GdDTPA with one of the carboxylic acid groups bound to a macromolecule via a linker containing an ester or amide bond may be stable in vivo.

A decomplexation of **5** and **6c** in artificial blood plasma, simultaneously with the ester hydrolysis, would interfere with the interpretation of the results of the degradation studies, so it was important to eliminate this possibility in the present work. Consequently, as a test for the relative stability of the metal chelate part in the dextran derivatives relative to GdEDTA and GdDTPA, the relaxation times of **5** and **7** were measured in 3 mM phosphate buffer at pH 7.0 and 37 °C. As controls, the relaxation times of $GdCl_3$, GdEDTA, and GdDTPA were measured under the same conditions. The results are summarised in Table 6.

If no significant tendency of change in R_1 occurred for the dextran derivatives, the metal chelate was expected to be stable in this system, since r_1 of the corresponding Gd phosphate salt is very low (an R_1 plateau of 0.7 $s^{-1} \times 10^{-3}$ at a Gd concentration of 0.1 mM). The data for **5** and **7** in Table 6 indicate a 6–20% reduction in R_1 in 3 mM

Table 6
R_1 during 3 days in 3 mM phosphate buffer (pH 7) at 37 °C and 10 MHz

Sample	Gd concn (mM)	$10^3 \times R_1$ plateau (s^{-1})	
		Without phosphate	With phosphate
GdEDTA	0.1	1.9	0.7 [a]
GdDTPA	0.1	1.3	1.2
Gd^{3+}	0.1	2.1	0.7
Gd^{3+}	0.15	3.2	0.8
5	0.15	2.4	2.0
7	0.15	1.6	1.5

[a] A decrease towards 0.7 $s^{-1} \times 10^{-3}$ (the level for 0.1 mM Gd^{3+}) over 3 days was observed.

phosphate. This may be attributed to removal of more loosely dextran-bound Gd, or DTPA-bisester moieties in the dextran matrix. From the results given in Table 6, it is concluded that the stability limit for decomplexation of the derivatives **5** and **7** towards a physiologically relevant concentration of inorganic phosphate is between GdEDTA and GdDTPA. Furthermore, the phosphate concentration in the artificial blood plasma used in the degradation study of the dextran derivatives (Fig. 2) was 1.15 mM. The Gd concentration in **5** and **6** was 0.2 mM in this study. Complete demetalation of **5** and **6c** during the time span in Fig. 2 would result in an R_1 level of ca. 0.9 $s^{-1} \times 10^{-3}$. The data given in Fig. 2 show no tendency for reduction in R_1 towards this level. It is concluded that degradation of the ester bond in **5** and **6c** is the only important process for the short-term stability of those products in these in vitro systems.

In conclusion, it has been shown that when the contrast agent GdDTPA is linked covalently via a carboxylic acid group in DTPA with an ester bond to the carbohydrate matrix, the ester bond stability may be insufficient for use in a water-soluble macromolecular carbohydrate agent for blood-pool contrast purposes. To support this conclusion, in vivo clearance studies with different derivatives in this class of compounds have been performed, and will be reported elsewhere [34]. Suitable linker units for spacing the contrast agent away from the ester bond, a method for their preparative use, and a derivative (**6c**) with a sufficiently long half-life in artificial blood plasma have been identified. For the particulate products (**2a–b**), the stability of the ester bond is not critical for their use as contrast agents for MRI since their residence time in the blood pool is much shorter than for the soluble derivatives, and a high degradation rate after cellular uptake in the liver/spleen may be an advantage. A distinct difference in stability towards inorganic phosphate between GdEDTA and the macromolecular GdDTPA derivatives **5** and **7** has been observed, but biological studies with radiolabelled derivatives are needed to show whether the metal chelate part in these derivatives is less resistant to decomplexation in vivo than GdDTPA.

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