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Synthesis of a novel amphiphilic GdPCTA-[12] derivative as a potential micellar MRI contrast agent

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ABSTRACT

A novel amphiphilic contrast agent, a GdPCTA-[12] derivative containing a dodecyl chain as lipophilic moiety, has been prepared. A convergent synthetic route from commercially available diethylene triamine and 3-hydroxypyridine is described. The target amphiphilic gadolinium complex was obtained in nine steps in 22% overall yield. Physicochemical properties and relaxivity measurements of this new contrast agent are described.

The majority of paramagnetic contrast agents (CA) used for diagnostic magnetic resonance imaging (MRI) comprise a paramagnetic metal core, typically gadolinium(III), which is complexed to a chelating ligand. In order to be considered as a potential CA, a Gd complex must have a high thermodynamic stability and a kinetic inertness, as free gadolinium ion is highly toxic for humans even at low doses.[1]

The high affinity of Gd (III) for a number of polyaminocarboxylic acids, either cyclic or linear, has been exploited to form very stable complexes (up to $\log K_{ML}$ >20), which are developed for clinical applications.

Contrast enhancement is due to the ability of the paramagnetic Gd^{3+} cation to shorten the longitudinal (T_1) and transverse (T_2) relaxation times of water protons in the surrounding tissues. The effectiveness of gadolinium chelates as MRI contrast agents is usually assessed in vitro by measuring the corresponding relaxivities r_1 and r_2, defined as the longitudinal and transverse relaxation rates, respectively, for a millimolar solution of Gd complex.

The commercial CAs routinely used for clinical diagnosis have longitudinal relaxivities (r_1) ranging from 3.5 to 5 $mM^{-1}\,s^{-1}$.[1] Although these substances are widely used in clinical applications, there is still a need to develop new compounds with improved performances in terms of relaxivity.

For this purpose, an efficient approach is to slow the rotational motion of the Gd-chelate and to increase the number of inner sphere water molecules by designing heptadentate chelators. A common approach to slow rotational motion has been to attach the Gd^{III} complex to a slowly tumbling macromolecule such as a dendrimer,[2] linear polymer,[3] or protein[4] or by supramolecular aggregation of amphiphilic complexes.[5,6]

Based on this rational drug design, we synthesized a new heptacoordinate gadolinium complex Gd–PCTA-[12] (3,6,9,15-tetraazabicyclo[9.3.1]pentadeca-1(15),11,13-triene-3,6,9-triacetate) able to coordinate two inner sphere water molecules and containing a dodecyl chain as lipophilic moiety in order to self-assemble into micelles.

We have previously reported the synthesis of 12-membered azapyridinomacrocycle PCTA 12 **1**[7] and a rigidified [PCTA-12] derivative **2**.[8] In this Letter, we describe a convergent synthetic route to a novel amphiphilic [PCTA-12] derivative **3** via a reaction sequence based on macrocyclization involving tri-N-alkylated triamine block (Scheme 1).

The lipophilic moiety is introduced into the 3-position of the pyridine ring via a lipophilic amide function. The 3-position seems to be sufficiently far away to limit any interference with the coordination sphere of Gadolinium.[9]

The synthesis started with commercially available diethylenetriamine **4**, which was selectively dinosylated to form disulfonamide **5** in 74% yield by spontaneous crystallization from the

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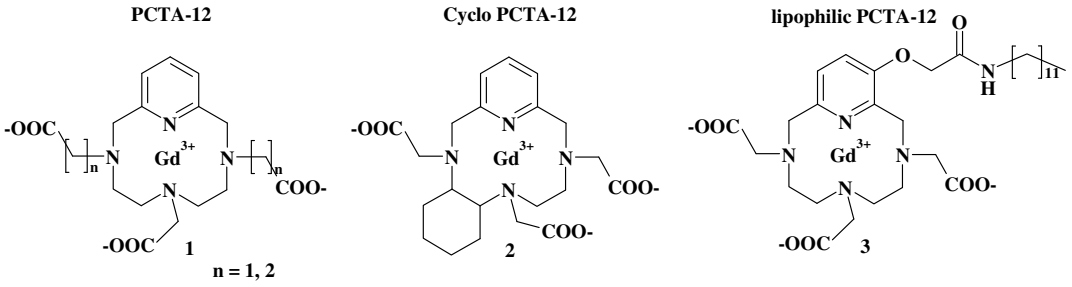
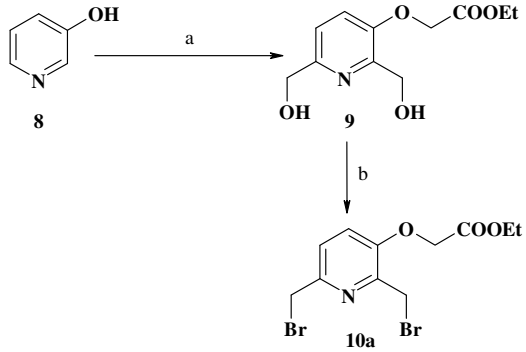


Scheme 1.

reaction medium (Scheme 2) The nosyl group (2-nitrobenzenesulfonamide) plays several important roles in this reaction. In fact, it allows the monoalkylation of either primary or secondary amine functions of compound **4** to give the compound **7a** after the selective protection of the primary amine functions. During the alkylation process, owing to the increased acidity, the deprotonation of the remaining N–H bond of the sulfonamido groups is easily accomplished even with relatively weak bases. On the other hand, in the Richman–Atkins cyclization reaction, the bulky nature of this group is, most likely, involved in a preorganization of the intermediates, which favours the transition state leading to the intramolecular cyclization, decreasing the importance of the alternative intermolecular oligomerization processes. Finally, the nosyl group can be removed under mild conditions with soft nucleophiles.[10]

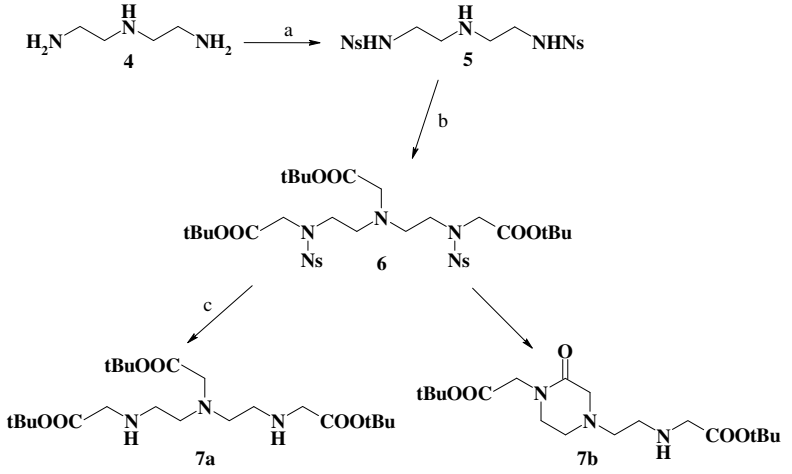
The reaction of 1,7-dinosyl-1,4,7-triazaheptane **5** with *tert*-butyl bromoacetate in refluxing acetonitrile and in the presence of potassium carbonate led quantitatively to the functionalized diprotected compound **6**, which was pure enough to be used in the next step without prior purification. The nosyl groups were removed by treating the crude compound **6** with thiophenol in the presence of sodium carbonate in dimethylformamide at 50 °C for three hours. After purification, the expected functionalized triamine **7a** was isolated in 80% yield. Note that the lactamized product **7b** was preferentially formed under the hardest conditions, i.e. longer reaction times at higher temperatures or more reactive conditions (mercaptoethanol/DBU/1 h/rt/CH_3CN).

The reaction sequence adopted for the other building block is given in Scheme 3. 3-Hydroxypyridine **8** as treated with aqueous formaldehyde and sodium hydroxide to form the expected 2,6-dihydroxymethyl-3-hydroxypyridine, which was purified by crystallization (MeOH) of the corresponding hydrochloride.[9] Regioselective O-alkylation at the phenol hydroxyl group was then performed in a one-pot reaction with ethylbromoacetate in a mixture of acetonitrile/water (Scheme 3). After purification, the resulting compound **9** was obtained in 40% overall yield. The functionalized pyridine **9** was then treated with triphenylphosphine and carbon tetrabromide in anhydrous acetonitrile at room temperature to give the dibromide derivative **10a** in 76% yield.

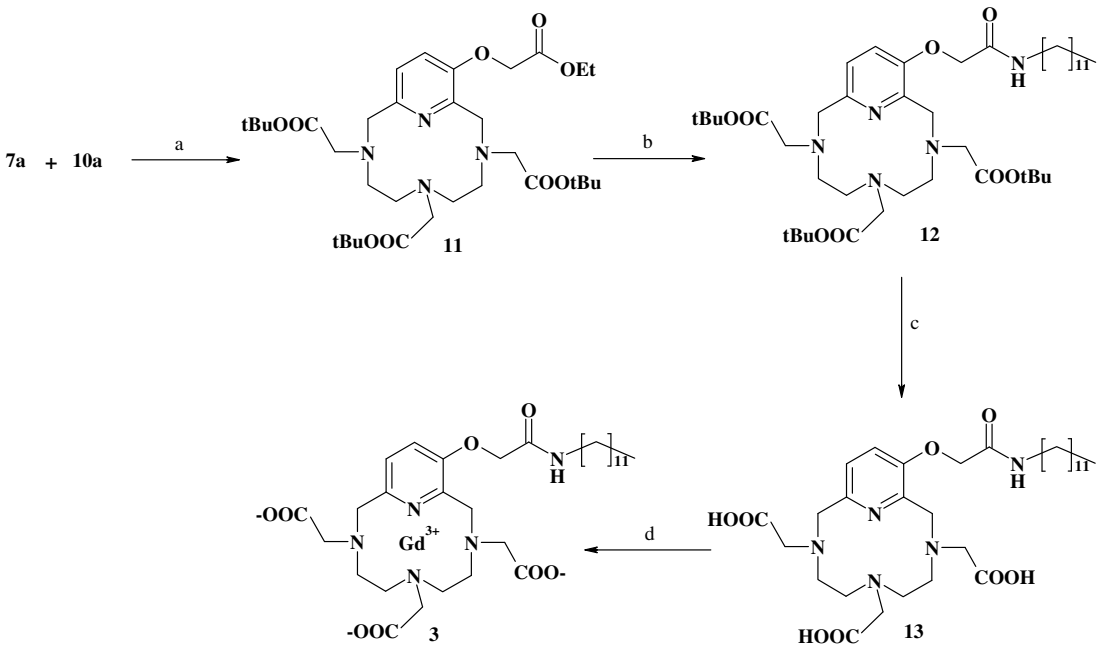


Scheme 3. Reagents and conditions: (a) (i) aqueous formaldehyde 37%, NaOH, H_2O, 90 °C, 6 h; (ii) ethyl bromoacetate, CH_3CN–H_2O, 80 °C, 2 h, 40%; (b) PPh_3, CBr_4, CH_3CN, rt, 4 h, 76%.

The macrocyclization reaction between bis(bromomethyl)pyridine **10a** and the functionalized triamine **7** was carried out in the presence of a base in dimethylformamide at 80 °C for 3 h (Scheme 4). Various parameters were studied to optimize the yield of the reaction (Table 1).



Scheme 2. Reagents and conditions: Ns = 2-NO_2–C_6H_4 (a) (i) NaOH, H_2O, rt; (ii) 2-nitrobenzenesulfonyl chloride, THF–Et_2O (1:6), rt, overnight, 74%; (b) *tert*-butyl bromoacetate, K_2CO_3, CH_3CN, reflux, 2 h, 100%; (c) thiophenol, Na_2CO_3, DMF, 50 °C, 3 h, 80%.



Scheme 4. Reagents and conditions: (a) Na_2CO_3, DMF, 80 °C, 3 h, 87%; (b) $AlMe_3$, dodecylamine, toluene, 50 °C, overnight, 87%; (c) HCl 2 M in Et_2O, CH_2Cl_2, rt, 24 h, 100%; (d) Gd_2O_3, H_2O, 80 °C, 3 h, 50%.

Table 1

Entry	Substrate concentration in DMF[a] (M)	Base[b]	Yield of 8 (%)
1	0.05	K_2CO_3	25–33
2	0.10	K_2CO_3	48–54
3	0.10	Cs_2CO_3	31
4	0.10	Na_2CO_3	65 (87[c])
5	0.10	Li_2CO_3	48

[a] All reactions were performed in DMF at 80 °C for 3 h using 0.77 mmol of substrates introduced at rt.
[b] Four equivalents of base
[c] Isolated in large scale (up to 13 mmol).

First, we studied the influence of the substrate concentration. The macrocyclization reaction was performed with 0.1 and 0.05 M solutions of substrates in the presence of potassium carbonate (Table 1, entries 1 and 2). The best yield was obtained for a 0.1 M solution.

Second, given that the dibromide derivative **10a** is sensitive to temperature, it was introduced by two different methods, at room temperature just before heating or dropwise at 80 °C. No difference in yield was observed between these two methods.

The role of the nature of the base-counterion was then examined during nucleophilic displacement of the halide in the ring closure step. The results (Table 1 entries 2–5) show a template effect from Na^+. After optimization, the macrocyclization reaction was performed with a 0.1 M solution of substrates in DMF at 80 °C and in the presence of sodium carbonate and gave compound **11** in large scale (up to 13 mmol) in 87% yield.

It is to be noticed that when the dibromo derivative **10a** was replaced by the corresponding dichloro compound 10 b in the same conditions as entry 2, compound **11** was obtained in less than 15% yield versus 48–54%.

The last step of synthesis involves the formation of the amide linkage between dodecylamine and the macrocycle derivative **11.** Firstly, by using conventional conditions in the presence of *N*-hydroxysuccinimide/EDCI, a tedious purification of the crude product by chromatography on silica gel must be performed. Secondly, dodecylamine was chemoselectively coupled with macrocycle **11** in the presence of DIBAL-H.[11] Impurities were formed in addition to the expected product, and, as previously, a purification was therefore required. Lastly, we applied a procedure previously described by Weinreb[12] in which trimethylaluminium is used as a condensing agent. The preactivation of one equivalent of dodecylamine with one equivalent of $AlMe_3$ was followed by addition of macrocycle **11**. The reaction was performed in toluene at 50 °C overnight and was followed by a basic treatment. The effective complexation of both the amine function and the ester function by alkylaluminium[13] led chemoselectively to the pure amine derivative **12** in 87% yield. Moreover, these treatments were simplified, as no byproduct is formed.

Finally, ligand **13** was obtained quantitatively by treatment of tri-*tert*-butyl ester **12** in dichloromethane with a 2 M ether solution of hydrochloric acid at room temperature.

The corresponding Gd complex **3**[14] was obtained, after precipitation from the reaction, by heating the ligand with gadolinium oxide (Gd_2O_3) in water at 80 °C while maintaining pH between 5.2 and 5.5 in 50% yield. The electrospray MS data confirmed the presence of the Gd complex. The presence of free gadolinium(III) ions was checked by the usual Arsenazo test, and was estimated to be 0.4 mol %/mol.

The micellar concentration of Gd–PCTA-[12] derivative **3** was determined at 0.19 mM by plotting the T_1 relaxation rate (20 MHz, 37 °C) as a function of Gd–PCTA-[12] derivative **3** concentration.

The r_1-relaxivity value obtained in aqueous solutions at 37 °C and 20 MHz for the new micellar Gd–PCTA-[12] derivative **3** is higher than that for the previously reported micellar Gd(DOTA-C14) complex[6a] (33 vs 18.8 mM^{-1} s^{-1}) under the same conditions and for other DOTA, DTPA and PCTA amphiphilic derivatives cited in Table 2. Most likely, the number of H_2O molecules on the Gd^{3+} is responsible for the increased relaxivity compared to the Gd(DOTA-C14) complex. This new contrast agent has higher r_1-relaxivity than the Gd(PCTA-O-C12) complex[10a] (33 vs 28.5 mM^{-1} s^{-1}, i.e., 16% enhancement). A possible explanation would be related to

Table 2
Comparison of r_1-relaxivities measured at 20 MHz for various amphiphilic Gd complexes

Gd complexes	r_1-relaxivities (mM^{-1} s^{-1}), 37 °C
Gd(PCTA–CH_2CONH–C12) **3**	33
Gd(PCTA–O–C12)[10a]	28.5
Gd(DOTA–C14)[6a]	18.8
Gd(DTPA–CONH–C12) andGd(DTPA–CONH–C14) andGd(DTPA–CONH–C16) andGd(DTPA–CONH–C18)[15]	<15 from NMRD profiles
Gd(DTPA–bisCONH–C14) andGd(DTPA–bisCONH–C16)[16]	<14 from NMRD profiles
Gd(DTPA–bisCONH–C18)[16]	<10 from NMRD profile

the secondary amide function of the lipophilic side arm that can form hydrogen bonds between monomers, resulting in more rigidified micelles. This would be responsible for enhancement of the local rotational correlation time and for an increased relaxivity of the new Gd complex.

In conclusion, the amphiphilic GdPCTA-[12] derivative **3** was synthesized in nine steps from commercially available diethylene triamine **4** and 3-hydroxypyridine **8** in 22% overall yield. In comparison, Hovland et al.[10a] have described the synthesis of a derivative, bearing a dodecylamine chain directly connected to the aromatic hydroxyl on the pyridine, in six steps from two commercially unavailable compounds in 3% overall yield.

The physicochemical properties of compound **3** are being further investigated, and will be published shortly.

Acknowledgement

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14. *Analytical data for all compounds:* Compound **5**: ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.52 (t, J = 6.5 Hz, 4H), 2.92 (t, J = 6.5 Hz, 4H), 7.86–7.91 (m, 4H), 7.97–8.00 (m, 2 H), 8.00–8.07 (m, 2H); ^{13}C (100 MHz, DMSO-d_6): δ 42.6, 47.8, 124.4, 129.5, 132.6, 132.7, 134.0, 147.7. Compound **6**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.34 (s, 18H), 1.44 (s, 9H), 2.86 and 3.45 (2 × t, J = 6.8 Hz, 8H), 3.24 (s, 2H), 4.15 (s, 4H), 7.51–7.61 (m, 2H), 7.62–7.72 (m, 4H), 8.03–8.11 (m, 2H); ^{13}C (100 MHz, $CDCl_3$): δ 27.9, 28.2, 46.8, 49.5, 53.2, 56.2, 81.5, 82.3, 124.0, 131.0, 131.9, 133.3, 133.5, 148.0, 167.8, 170.3; HRMS (ES^+) calcd for $C_{34}H_{50}N_5O_{14}S_2 + H^+$, 816.2796; found, 816.2787. Compound **7**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.39 (s, 9H), 1.41 (s, 18H), 2.61–2.65 (m, 4H), 2.75–2.79 (m, 4H), 3.27 and 3.29 (s, 6H); ^{13}C (100 MHz, $CDCl_3$): δ 28.1, 47.2, 51.2, 171.0, 171.1, 81.0, 81.3, 53.6, 55.8. Compound **9**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.23 (t, J = 7.2 Hz, 3H), 4.19 (q, J = 7.2 Hz, 2H), 4.60, 4.61 and 4.73 (3s, 6H), 7.00 and 7.15 (2d, J = 8.5 Hz, 2H); ^{13}C (100 MHz, $CDCl_3$): δ 14.1, 60.2, 61.7, 64.2, 65.5, 168.4, 148.2, 150.0, 151.3, 119.2, 119.9. Compound **10a**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.30 (t, J = 7.2 Hz, 3H), 4.27 (q, J = 7.2 Hz, 2H), 4.52 and 4.67 (2s, 4H), 4.74 (s, 2H), 7.06 and 7.09 (2 d, J = 8.6 Hz, 2H); ^{13}C (100 MHz, $CDCl_3$): δ 14.2, 28.8, 33.3, 61.8, 65.8, 120.2, 124.5, 146.7, 149.3, 151.7, 167.9. Compound **11**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.27 (t, J = 7.0 Hz, 3 H), 1.42 (s, 9H), 1.46 (s, 9H), 1.47 (s, 9H), 1.99–2.22 (m, 4H), 2.48–2.67 (m, 4H), 3.11 (2d, J = 17.6 Hz, AB, 2H), 3.32 and 3.36 (2d, J = 3.0 Hz, AB, 2H), 3.42 and 3.47 (2 d, J = 7.0 Hz, AB, 2H), 3.68 and 3.93 (2d, J = 14.6 Hz, AB, 2H), 3.74 and 4.13 (2d, J = 15.8 Hz, AB, 2H), 4.21 (q, J = 7.0 Hz, 2H), 4.67 and 4.72 (2d, J = 16.6 Hz, AB, 2H), 7.16 and 7.20 (2d, J = 8.5 Hz, 2H); ^{13}C (100 MHz, $CDCl_3$): δ 13.7, 27.5, 52.7, 53.2, 53.3, 53.5, 55.9, 56.0, 58.9, 59.3, 61.1, 61.0, 65.1, 82.2, 82.37, 82.42, 120.1, 121.8, 147.1, 149.1, 150.6, 167.7, 172.5, 172.6, 173.1; LRMS (IC) calcd for $C_{33}H_{54}N_4O_9 + Na^+$, 673; found, 673.47; LRMS (ES^+) calcd for $C_{33}H_{54}N_4O_9 + H^+$, 651 found, 651.40. Compound **12**: LRMS (IC) calcd for $C_{43}H_{75}N_5O_8 + H^+$, 790; found, 790.44; LRMS (ES^+) calcd for $C_{43}H_{75}N_5O_8 + H^+$, 790; found, 790.51). Compound **13**: LRMS (ES^+) calcd for $C_{31}H_{51}N_5O_8 + H^+$, 622; found, 622.37. Compound **3**: LRMS (ES^+) calcd for $C_{31}H_{48}GdN_5O_8 + H^+$, 776; found, 776.00.
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