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Improved synthesis of DOTA tetraamide lanthanide(III) ligands: tool for increasing the repertoire of potential PARACEST contrast agents for MRI and/or fluorescent sensors

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Abstract

The synthesis of new DOTA tetra-amide (DOTAMR₄) compounds is of great interest given their application in the formation of Ln(III) complexes which are potential PARACEST contrast agents in MRI or fluorescent molecular probes. In this context amino acid and peptide DOTAMR₄ derivatives are particularly attractive since the amino-acid and/or peptide moiety can show responsive properties dependent on a given stimuli which might translate to changes in water exchange rates of the corresponding Ln(III) complex. Current synthesis of DOTAMR₄ derivatives is typically carried out by reacting haloacetamide intermediates with cyclen. However, this method fails to generate the tetra-substituted products when bulky substituents are present in the haloacetamide and in some cases this intermediate cannot be prepared by conventional acylation procedures limiting the number of DOTAMR₄ compounds available for study. As a solution to these limitations, an improved methodology for the synthesis of DOTAMR₄ by coupling DOTA to an appropriate amine containing reagent (i.e. protected amino-acids with the α -amino group free) is presented in this work. Several DOTAMR₄ derivatives which are difficult or impossible to prepare with the traditional methodologies were easily obtained starting with DOTA. A new protocol was derived using this methodology for the solution phase synthesis of DOTA peptide derivatives. With this methodology, many other DOTAMR₄ peptide and non-peptide derivatives have been prepared in our laboratories with several of these new compounds showing interesting properties for molecular imaging.

Keywords

DOTA; amide; peptide; synthesis; molecular sensors; PARACEST agents; MRI

1. Introduction

Synthesis of DOTA-tetraamide (DOTAMR₄) derivatives have found wide application in the formation of lanthanide (Ln³⁺) complexes, given their potential use as PARACEST contrast agents in MRI (1–2) and/or as luminescent probes (3). In the former case, the presence of tetraamide units is essential for slowing down the water exchange rate ($1/\tau_M$) of the water molecule directly coordinated to the metal ion (eg. τ_M for Eu³⁺DOTA is ~200 ns while for Eu³⁺DOTA-(gly)₄, it is ~160 μ s at 25°C) (4), a necessary prerequisite for a complex to show

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appropriate water signal saturation effects. In this respect a large number of Ln^{3+} - DOTAMR₄ complexes have been reported in the literature and, among these, amino acid and peptide-based derivatives are increasingly attracting attention due to their interesting biochemical and responsive properties (eg. temperature, pH CEST agents) (5–6). The latter can be attributed to changes of the metal-coordinated water exchange rates and its proton chemical shift, modulated by the environment provided by the amino acid or peptide sequence present given a certain stimuli (7–8). Despite their potential interesting properties DOTA-tetraamide amino acid and peptide derivatives are scarce in the literature partly due to the difficulty of their synthesis.

While the synthesis of simple symmetrically substituted DOTA-tetraamide compounds is straightforward, the synthesis of DOTAMR₄ with bulky substituents (eg. peptide derivatives) has proven to be difficult. Usually, the synthesis of DOTAMR₄ ligands has been carried out by reacting 1,4,7,10-tetraazacyclododecane (cyclen) or other substituted cyclen derivatives with a bromo or chloro acetamide intermediate (Figure 1) in the presence of a base, yielding the final tetra-substituted products in moderate to poor yields. This methodology suffers further problems whenever the amide intermediate contains a tertiary amine that could undergo intermolecular quaternization and/or when bulky haloacetamide derivatives are used, giving the final tetra-substituted product in even lower yields. The latter has proven to be particularly important when making di- or tri-peptide DOTAM derivatives where cyclen tetra-substitution is highly dependent on the peptide sequence. To circumvent this problem, the use of iodo acetamide di-peptide derivatives has been proposed (9–10). Here, the synthesis of a chloroacetyl di-peptide sequence is carried out first in solution by acylating an amino acid residue (with functional groups of the side chains protected) with chloroacetyl chloride or O-chloroacetyl-N-hydroxysuccinimide. The acylated intermediate is then coupled to the N- α amino group of the next appropriately protected amino acid, followed by substitution of the chloride with an iodide in acetone. The iodoacetamide derivative is then reacted with cyclen giving the tetra-substituted product in excellent single step yields (>90%) and finally the respective protecting groups are removed. Though successful, the global yields of this protocol are still poor (20–40%) and the whole procedure is lengthy and tedious. Surprisingly, in some instances only the tri-substituted derivative of cyclen was obtained (DOTAM-GlyArgNO₂-OMe) even when the iodoacetyl di-peptide was used. These results led to the following hypothesis: the nucleophilic substitution rate of the secondary amino groups of cyclen decreases upon substitution which might explain the lower yields for DOTAMR₄ compounds when chloro or bromo acetyl derivatives are used compared to the iodoacetyl derivatives. Alternatively, N-substitution of cyclen as a first step increases the steric hindrance around the macrocycle and thereby restricts further substitutions.

2. Results and Discussion

In this work, we demonstrate a simple solution to the problems described in the syntheses of DOTAMR₄ derivatives by coupling 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) with an appropriate derivative containing a free amino group (Scheme 1a). Using this strategy, the following problems are circumvented: 1) quaternization of compounds containing tertiary amines is eliminated, 2) steric hindrance is minimal since the reaction centers will be the non-constrained acetate substituents of DOTA instead of the amino groups of cyclen, and 3) the reaction rates are faster for condensation type reactions than for nucleophilic substitutions. Even with the potential advantages of this method, the use of DOTA to obtain DOTAMR₄ compounds has been scarcely explored. The few examples found in literature report very poor to moderate yields for DOTAM tetra-substituted compounds (11–12) and to the best of our knowledge no report for the synthesis of

DOTAMR₄ amino acid or peptide derivatives when using DOTA as starting building unit can be found.

The limited use of DOTA to obtain DOTAMR₄ compounds may be attributed to its poor solubility in common organic solvents and/or the choice of the coupling agent. While working with different coupling agents, we found that the use of carbodiimide reagents to prepare activated esters of DOTA-*tris-tert*-butyl ester fails to give the desired product while benzotriazole type reagents generate the product in excellent yields (13). This can be attributed to the existence of the macrocycle in a zwitterionic form, making it difficult for proton transfer to carbodiimide reagents. This step is not required with benzotriazole reagents where the carboxylate is the starting reactive unit (14). Consequently, it is important to consider that DOTA will be in a zwitterionic form and therefore common peptide coupling agents such as (HBTU, TBTU, HATU, BOP, PyBOP) should be used. Of these, O-(1H-benzotriazol-1-yl)-N,N,N',N'-bis-(tetramethylene)uronium hexafluoro phosphate (HBTU) is the most attractive reagent based upon cost and availability. The latter was further supported by the fact that we were able to prepare in good yield a DOTA-bisamide derivative by coupling DOTA-bis-*tert*-butyl ester (15) to the proper amine intermediate using HBTU in DMF (16). However, under the same coupling conditions but using acetonitrile as the solvent the product was obtained in low yield (unpublished results). Unlike the previous cases where the DOTA *tert*-butyl esters reagents are soluble in common organic solvents for DOTA an organic/aqueous mixture would be preferred given the high solubility of DOTA in the later. However, in presence of a base, hydrolysis of the active ester is expected which partially explains the poor product formation previously observed when using water as solvent (11). The use of polar protic solvents such as alcohols is not recommended given the potential formation of relatively unreactive esters. On the other hand DOTA is slightly soluble in polar aprotic solvents such as DMF, but its solubility should increase considerably upon consecutive active ester formation. To test this hypothesis we coupled DOTA with a bulky commercially available *tert*-butyl (*t*-Bu) protected derivative of L-glutamic acid. DOTA was suspended in DMF followed by addition of HBTU and diisopropylethylamine (DIPEA) (4 and 6 equivalents respectively). The protected amino acid derivative was added last and the reaction was carried out at room temperature overnight. After purification the tetraamide derivative (**1**) was obtained in excellent yield (Table 1, entry **1**). The same compound was prepared using other coupling agents (PyBOP, HATU, TBTU) with or without additives (HOBt, HOAt) and or solvent (acetonitrile) under the same reaction times with compound (**1**) being obtained in excellent yields (82–90%). Only when acetonitrile was used as the solvent (**1**) was obtained in moderate yields (~60 %) regardless of the coupling agent. The latter can be explained due to changes in basicities and acidities of the participating species in acetonitrile given its poorer solvating properties and basicity than DMSO.

Several other derivatives were synthesized following the same procedure, in all cases giving the DOTA-tetraamide products in excellent yields (Table 1, entries **2–5**). Reaction of DOTA with N-methylpiperazine generated compound **5**, which otherwise would have been hard to prepare using a halide acetyl equivalent procedure given the potential quaternization of the tertiary amine. An important feature of this method when using *t*-Bu protected amino acids is that purification of the desire product is simple. Once DMF is removed from the reaction mixture most impurities can be easily removed by simple water/dichloromethane, basic water/dichloromethane sequential extractions, with the final product being collected in the organic phase owing to the hydrophobicity introduced by the *t*-Bu groups. However, if the reaction is incomplete, further standard purification procedures are needed to remove any unreacted *t*-Bu protected amino acid.

To determine if the present procedure will allow one to generate tetra-substituted DOTAM derivatives when bulky tri-peptide substituents are attached to the macrocycle, DOTA was reacted with two different tri-peptide protected sequences $\text{NH}_2\text{-ProLeuGlu(O}t\text{Bu)}_2$ and $\text{NH}_2\text{-ProLeuGly(O}t\text{Bu)}$. In both cases the tetra-substituted DOTAM compounds were obtained in good yields (Table 1, entries **6** and **7**) after purification by HPLC. Moreover, when trying to prepare these compounds using bromo (or iodo) acetyl tri-peptide reagents the desired tetra-substituted products were obtained in low yields together with a mixture of mono, di- and tri-substituted by-products. This result confirms that bulky substituents hinder further substitutions in cyclen.

Considering the quantitative and simple removal of the *t*-Bu groups as well as the commercial availability of different *t*-Bu protected amino acids or relatively simple methods to prepare them, we explored the use of *t*-Bu protected amino acids as building blocks for DOTAMR₄ peptide derivatives as shown in Scheme 1. Given that the tetra-substituted DOTAM-(GlyArgNO₂-OMe)₄ has been previously proven difficult to prepare (10), we proceeded to synthesize this compound following the protocol described in Scheme 1a–b. DOTAM(Gly-O*t*-Bu)₄ was prepared first in excellent yield (Table 1, entry **4**). After *t*-Bu removal under acidic conditions the DOTAM(Gly)₄ product was reacted with the commercially available $\text{NH}_2\text{-ArgNO}_2\text{-OMe}$ giving the tetra-substituted product (Table 1, entry **8**). Although, the yield for compound **8** is low, it is important to note that the compound obtained was a single product, unlike when acetyl halide procedures were used.

Using the methodology presented here, many other DOTAMR₄ peptide and non-peptide derivatives have been prepared in our laboratories, with several of these new compounds showing interesting properties for molecular imaging.

4. Experimental

4.1 General procedures

¹H- and ¹³C-NMR spectra were recorded on a Varian INOVA 400 MHz spectrometer. Data are reported in the following order: chemical shift in ppm (δ); multiplicities are indicated as b (broadened), s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet); coupling constants, J, are reported in Hz; integration is provided. Mass spectra (MS) were measured with a Voyager-DE PRO Biospectrometry Workstation (Applied Biosystems) [matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF)] operating in reflector mode using α-cyano-4-hydroxycinnamic acid as the matrix. HPLC purification was performed on a Waters Delta Prep system coupled to a Waters 2996 Photodiode Array Detector using a Luna 5μ C18(2) 100Å, 250 mm×30 mm, 5 microns column from Phenomenex. Typical separation procedure was carried out at room temperature with a gradient of water/acetonitrile containing 0.1% Trifluoroacetic acid (TFA) from 95/5 to 10/90 in 35 minutes detection at 215 and 250 nm unless it is stated otherwise. DOTA was prepared according to a previously reported methodology.⁽¹⁷⁾ Protected L-amino acids were purchased from Bachem Americas Inc. (Torrance, CA), Novabiochem (San Diego, CA) or Aroz Tech, LLC. (Cincinnati, OH) as the HCl salts. All other reagents were purchased from Sigma-Aldrich or Fisher Scientific (Pittsburgh, PA). All reagents and solvents were used without further purification unless otherwise noted.

4.2 Synthesis

4.2.1 $\text{NH}_2\text{-ProLeuGlu(O}t\text{Bu)}\text{O}t\text{Bu-NH}_2\text{-Glu(O}t\text{Bu)}\text{O}t\text{Bu}$ (Novabiochem) (825.7 mg, 2.8 mmol) was dissolved in ethyl acetate along with N-methylmorpholine (4 eq.). EDC (1.2 eq.), HOBt (1.2 eq.) and Cbz-ProLeuCOOH (Bachem) (1 eq.) were added to the above described solution. The reaction was left stirring at room temperature overnight. The organic

layer containing a white suspension was washed with water, brine and dried over Na_2SO_4 . The solvents were evaporated and the crude product was purified using flash chromatography over silica gel with 2% methanol in methylene chloride yielding the title compound as a colorless oil in 86% yield (1.45 g, 2.4 mmol). The Cbz-protected peptide was dissolved in ethanol and 10% of Pd(0) on carbon was added. The mixture was then placed on a Parr hydrogenation apparatus and shaken under a hydrogen pressure of 50 psi overnight. The reaction mixture was then filtered and the solvent removed to yield the free amine peptide. The tri-peptide was obtained as a hygroscopic white solid (2.2 mmol, 90%). ^1H NMR (400 MHz, CDCl_3): δ = 4.65 (1H, t, J^3 = 8 Hz), 4.42 (2H, m), 3.36 (2H, t, J^3 = 8 Hz), 2.17 (4H, m), 2.00 (2H, m), 1.82 (2H, m), 1.61 (3H, m), 1.39 (9H, s), 1.37 (9H, s), 0.84 (6H, 2 d, J^3 = 8 Hz); ^{13}C NMR (100.2 MHz, CDCl_3) δ = 172.28, 170.97, 169.87, 82.20, 80.81, 59.93, 53.49, 52.36, 47.06, 40.83, 31.70, 30.80, 28.24, 27.58, 24.94, 24.64, 23.10, 22.19.

4.2.2 NH_2 -ProLeuGlyOtBu—The tri-peptide was prepared following the procedure described above coupling Cbz-ProLeuCOOH (Bachem) with NH_2 -GlyOtBu (Bachem). The product was obtained as a hygroscopic white solid (2.2 mmol, 77% overall yield). ^1H NMR (400 MHz, CDCl_3): δ = 4.64 (1H, t, J^3 = 4.0), 4.34 (2H, m), 3.37 (2H, t, J^3 = 4.0), 2.21–2.43 (4H, m), 2.07 (2H, m), 1.87 (2H, m), 1.63 (3H, m), 1.39 (9H, s), 1.37 (9H, s), 0.85 (6H, dd, J^3 = 1.2); ^{13}C NMR (100.2 MHz, CDCl_3) δ = 172.28, 170.97, 169.87, 82.20, 80.81, 59.93, 53.49, 52.36, 47.06, 40.83, 31.70, 30.80, 28.24, 27.58, 24.94, 24.64, 23.10, 22.19.

4.2.3 General coupling procedure—DOTA (0.8 mmol) was suspended in DMF (5 mL) followed by addition of HBTU and diisopropylethylamine (DIPEA) (4 and 6 equivalents respectively). Reaction mixture was stirred for 5 minutes at room temperature with solids present all the time. The protected amino containing derivative (4 equivalents) was added last together with additional DIPEA (up to 4 equivalents) when the amino derivative comes as the HCl salt. The reaction mixture was stirred at room temperature overnight. In most cases solids were still present after the stated reaction period, the solids correspond to the urea subproduct of the reaction.

Further couplings with DOTAMR₄ intermediates (ex. Compound **8**) were repeated as above with addition of HOBT (4 equivalents) together with HBTU and DIPEA.

Purification of compounds **1–8** was carried out by removing DMF under high vacuum. Resultant residue was taken into 30 mL dichloromethane and the organic phase was washed 5× 10 mL with MilliQ grade water. Recovered organic phase was then washed 5× 10 mL with a NaOH aqueous solution (0.25 M for compounds **1–4** and **6–8** and 5 M for compound **5**). Organic phase was collected and dried with anhydrous Na_2CO_3 , filtered and solvent was removed from the filtrate by rotary evaporation. Compounds other than **4–5** were further purified by HPLC

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Figure 1.

Scheme 1.

Table 1

Characterization data for the DOTAMR₄ compounds prepared in this study.

DOTAM(R) ₄ where R is:		¹ H NMR (400 MHz, CDCl ₃ /TMS), δ, J (Hz)	¹³ C NMR (100.2 MHz, CDCl ₃ /TMS), δ	MALDI-TOF m/z (M +H) ⁺ (calcd.)	Yield %
1		7.27 (4H, d), 4.38–4.33 (4H, m), 3.71 (8H, s), 3.22 (16H, bs), 2.31 (8H, t), 2.11–1.85 (8H, m), 1.47 (36H, s), 1.44 (36H, s)	172.15, 170.65, 167.33, 82.38, 80.56, 55.25, 52.95, 50.36, 31.43, 27.67, 27.58, 27.02	1371 (1371)	88
2		4.23 (4H, s), 3.65 (8H, m), 3.50–2.50 (24H, m), 2.08 (8H, m), 1.88 (8H, m), 1.38 (36H, s)	171.36, 169.93, 80.88, 59.87, 56.62, 51.51, 49.86, 47.52, 28.97, 27.42, 24.58	1018 (1018)	81
3		7.40–7.00 (20H, m), 4.70 (4H, s), 3.20–2.35 (32H, m), 1.40 (36H, s)	171.03, 170.83, 136.72, 129.65, 129.52, 128.64, 126.96, 82.27, 59.15, 53.43, 52.62, 37.97, 28.19	1230 (M+Na) ⁺ (1230)	70
4		6.71 (4H, t, J ² = 5.2), 3.86 (8H, d, J ² = 5.2), 3.14 (8H, s), 3.14 (8H, s), 2.70–2.40 (16H, bs), 1.42 (36H, s)	168.12, 162.46, 83.74, 46.37, 39.97, 38.79, 28.22	858 (858)	90
5		(CD ₃ CN) 3.36 (24H, bs), 2.91 (16H, dt, J ² = 12.8), 2.28 (16H, dt, J ² = 8) 2.23 (12H, s)	(CD ₃ CN) 170.14, 55.15, 55.01, 54.92, 45.55, 44.43, 41.96	734 (734) 756 (M+Na) ⁺ (756)	75

DOTAM(R) ₄ , where R is:	¹ H NMR (400 MHz, CDCl ₃ /TMS), δ, J (Hz)	¹³ C NMR (100.2 MHz, CDCl ₃ /TMS), δ	MALDI-TOF m/z (M +H) ⁺ (calcd.)	Yield %
<p>6</p>	4.36 (8H, br m), 3.96–3.52 (16H, br m), 3.25 (8H, br m), 2.80–2.43 (16H, br m), 2.10–1.74 (16H, br m), 1.53 (12H, br s), 1.34 (36H, s), 0.80 (24H, br s),	172.82, 172.54, 172.26, 169.93, 82.09, 60.79, 51.69, 47.33, 47.01, 45.12, 42.15, 40.51, 40.00, 32.55, 28.42, 25.05, 23.36, 21.68	1699 (1699)	62
<p>7</p>	4.51–4.12 (16H, m), 3.71–3.48 (8H, m), 3.15–2.87 (16 H, m), 2.40–1.78 (44 H, m), 1.57 (4H, s), 1.45 (36 H, s), 0.94–0.90 (24H, m)	172.83, 172.39, 172.02, 171.32, 170.86, 81.64, 80.48, 52.84, 50.45, 49.22, 46.23, 42.90, 31.41, 28.72, 27.49, 27.23, 24.90, 22.92, 21.28	2211 (2211)	55
<p>8</p>	(D ₂ O) 4.30–4.16 (4H, m), 3.85 (8H, s), 3.58 (12H, s), 3.11 (8H, s), 2.74 (24H, bs), 1.80–1.69, 1.64–1.53 (8H, m), 1.48 (8H, bs)	(D ₂ O) 175.10, 170.62, 170.57, 162.35, 53.1, 52.63, 48.97, 46.08, 40.61, 39.20, 27.78, 24.02	1494 (1494) 1451 (M+H–NO ₂) ⁺ (1451)	20