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Facile synthesis of 1-(acetic acid)-4,7,10-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane: a reactive precursor chelating agent

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ABSTRACT

1-(Acetic acid)-4,7,10-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (DOTA-Tris-^{t}Bu ester or **1**) is a precursor chelating agent for lanthanide ions and can be efficiently labeled with various functional moieties or macromolecules to improve the targeting specificity, intracellular delivery, biocompatibility, and pharmacokinetics of resulting contrast media used in molecular imaging. This compound is commercially available but the extremely high cost seriously limits its wide utilization. Thus, we sought a convenient and inexpensive synthesis of DOTA-Tris-^{t}Bu ester that is readily adapted for use in any laboratory. The synthetic approach described here is straightforward and has an overall yield of 92%. Significantly, the product can be purified conveniently without using of a time- and labor-intensive column chromatography. Other advantages of this method, such as operational convenience, starting material availability, and atom efficiency make it very attractive to prepare DOTA-Tris-^{t}Bu ester in large quantity with a reduced cost.

Real time non-invasive medical diagnosis in living subjects has been possible with the development of diagnostic imaging techniques such as X-ray computed tomography (CT), positron emission tomography (PET), magnetic resonance imaging (MRI) and optical imaging. Contrast media play a crucial role in improving the sensitivity and resolution of images, and achieving targeting specificity at the molecular/cellular level. Among various parameters to evaluate the performance of contrast media, low toxicity tops the list. For example, lanthanide ions are widely used in MRI contrast agents,[1] radioactive tracers,[2] and optical imaging probes.[3,4] However, free lanthanide ions usually show high toxicity in vivo. To overcome this limitation, chelating agents that can coordinate metal ions tightly are widely used to minimize toxicity. In various chelating agents, polyazamacrocyclic 1,4,7,10-tetra-acetic acid-1,4,7,10-tetraazacyclododecane (DOTA) demonstrated high thermodynamic stability and kinetic inertness to a larger number of transition and lanthanide metal ions.[5] Nevertheless, small molecular DOTA chelators have their own inherent shortcomings. For example, commercial available MRI contrast agent Gd^{3+}-DOTA (Dotarem™) showed low relaxivity and extremely fast excretion rate in vivo. To increase the relaxivity, Gd^{3+}-DOTA complexes were labeled to macromolecules, such as proteins,[6] micellar aggregates,[7] dendrimers,[8] or liposomes[9] by prolonging the rotational correlation lifetime τ_R.[10] Meanwhile, to image specific cellular/molecular evens in vivo, Gd^{3+}-DOTA was conjugated to targeting domains such as antibodies,[11] peptides,[12] and biotin/avidin.[13] Additionally, Gd^{3+}-DOTA was also functionalized with polyethyl glycols (PEGs)[14] or crown ethers[15] to increase its biocompatibility and circulation lifetime in circulation system.

To efficiently label the DOTA chelate to various molecules, a reactive group for covalent binding must be introduced into this chelator. Since amine groups are prevalent in biomolecules, and the conversion of a pendant carboxylic acid of DOTA into a carboxamide does not significantly affect the stability of the labeling metal complex, a facile approach is the conjugation of DOTA derivatives with biomolecules though a physiologically stable amide bond. In this respect, regio-selective activation of carboxylic acid in DOTA to react with amines under a mild reaction condition is a feasible way to achieve this goal.

1-(Acetic acid)-4,7,10-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (DOTA-tris-^{t}Bu ester or **1**) as a reactive chelating agent has been widely used to label DOTA chelator to various biomolecules. In this molecule, three of the four secondary amines in the cyclen ring were regioselectively alkylated with *tert*-butyl acetate, and the remaining one was functionalized with an acetic acid. In this way, this compound can be directly and specifically conjugated with biomolecules by forming an amide bond without the potential intermolecular cross-linking.[16] Additionally, three pendant *tert*-butyl acetates can be easily hydrolyzed to acetic acids that are ready for complexation with metal ions.

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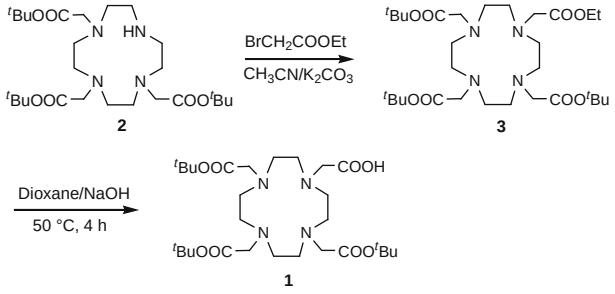
Despite the wide application potential of compound **1** in medical diagnostic imaging, to purchase the needed quantities is extremely costly, which severely limits its usage. Several synthetic procedures to prepare compound **1** were reported, however, the shortcomings include the requirement of expensive reactive resin[17] or catalyst,[18] and relatively low total yield[17,18] making them difficult to prepare **1** within a reasonable cost. *tert*-Butyl ester as a protecting group of carboxylic acids is widely used in synthetic chemistry because it is easily removed in acidic conditions, while demonstrating stability in basic surroundings. Meanwhile, alkyl esters, such as ethyl acetate, can be hydrolyzed efficiently in both acidic and basic surroundings. In this work, we present a novel synthetic method to prepare chelator **1** efficiently by taking advantage of selective hydrolysis of pedant ethyl acetate but not *tert*-butyl acetates under basic conditions. Compared with the previous methods, the advantages that include high yield, convenient purification process, ease in handling, and starting material concentration-independent yield confer the preparation of compound **1** with a reasonable price.

In a typical synthetic procedure (Scheme 1), 1.0 equiv ethyl bromoacetate in 5 mL CH_3CN was added dropwise under N_2 into a mixture of 1,4,7-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane **2**[19] and anhydrous K_2CO_3 (1.5 equiv) in 30 mL CH_3CN at 55–60 °C. The reaction was monitored by TLC until all starting material was consumed. At the end of reaction, K_2CO_3 was filtered and the solvent was removed to give 1-(ethoxycarbonylmethyl)-4,7,10-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane **3** with a yield of 98%.[20]

The selective hydrolysis of ethyl acetate in compound **3** was tested in a series of hydrolytic reaction conditions as shown in Table 1, and TLC and MS were used to monitor the reaction process. First, compound **3** was dissolved in the mixture of EtOH and aqueous solution of KOH (0.17 M, final concentration), and a product with higher polarity was detected after stirring for 4 h at 50 °C. To increase the yield, longer reaction time (up to 24 h) and higher concentration of NaOH (up to 0.5 M, final concentration) were applied. However, no obvious improvement in yield was achieved. To clarify this experimental result, a similar reaction was conducted in MeOH/H_2O. Disappointingly, an identical phenomenon was observed and the yield of product was still low. MS analysis revealed a new product 1-(methyl acetate)-4,7,10-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane **4**, which assumes the base-catalyzed trans-esterification in the methanol. To prevent the re-esterification, different solvent systems including CH_3CN/H_2O, dioxane/H_2O, and DMF/H_2O (v:v = 2:1) were tested in the presence of NaOH (0.4 M, final concentration) at 50 °C (Table 1). The experimental results showed that dioxane/H_2O was the solvent of choice, which gave the highest yield (>90%) of product **1** and no other by-products were detected by either TCL or MS. Compared with CH_3CN and DMF, the high stability of dioxane in basic solution and its excellent solubility to compound **3** are the main reasons for its optimal performance in this reaction.

In order to investigate the relationship between base concentration and the yields of **1**, comparative studies were performed in the presence of various concentrations of NaOH at 50 °C (Fig. 1). The yield of **1** was proportional to the concentrations of NaOH added in the range of 0–0.4 M, and the maximal value of 95% was obtained after a 4-h reaction in dioxane/H_2O with 0.4 M NaOH. Further increase of base did not improve the yield. The yield decreased slightly after NaOH concentration exceeded 1.0 M. It is also noteworthy that no product with higher polarity than **1** was detected even at the highest concentration of NaOH tested, which indicates that the *tert* butyl-acetate remained intact even under highly basic surroundings.

The functions of both temperature and reaction time to the yield of compound **1** under the reaction condition of dioxane/H_2O/0.4 M NaOH were also studied (Fig. 2). The hydrolytic rate of N-alkylated ethyl acetate accelerated substantially at high temperature. At room temperature, the yield of 95% was achieved after 8 h of reaction, while this yield was attained in less than 4 h at 45–50 °C. However, by-products with higher polarity than **1** were detected by TLC after 4 h of reaction when the temperature was above 80 °C. Mass spectrum analysis demonstrated that *tert*-butyl acetate began to hydrolyze at the temperature above 80 °C, and that the by-products were proved to be chelators with two or three N-alkylated acetic acids.



Scheme 1. Synthetic procedure to prepare compound **1**.

Table 1
Effect of solvents and auxiliary bases to the yield of compound **1**

Entry	Cond.[a]	Base	Yield[b] (%)	
			1	**4**
1	EtOH	CH_3ONa[c]	21	n.d.[e]
2	EtOH	NaOH[d]	56	n.d.[e]
3	MeOH	CH_3ONa[c]	15	33
4	MeOH	NaOH[d]	28	34
5	DMF	NaOH[d]	68	n.d.[e]
6	MeCN	NaOH[d]	73	n.d.[e]
7	Dioxane	NaOH[d]	96	n.d.[e]

[a] Starting material **3** with the concentration of 15 mM in selected solvent systems, 4–6 h, 50 °C.
[b] Yield of compound **1** or **4** is calculated by comparing the proton integration of –OC*H*$_2$ in **3** or –OC*H*$_3$ in **4** with CC*H*$_3$ in *tert*-butyl group from ^{1}H NMR spectra.
[c] CH_3ONa was added with catalytic dosage.
[d] NaOH was added as the aqueous solution with a final concentration of 0.4 M.
[e] Not detected.

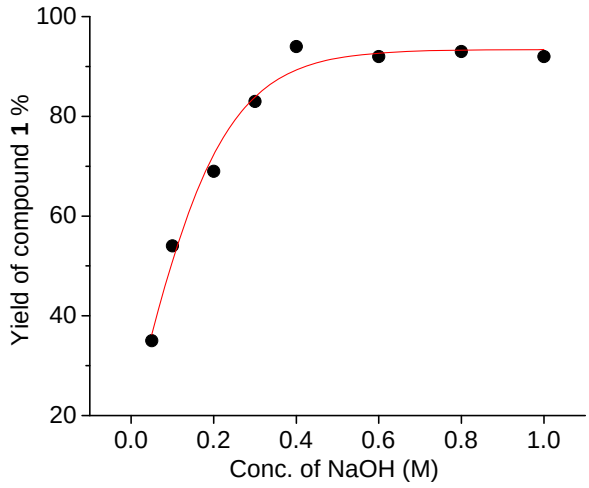


Figure 1. Plot of the yield of compound **1** as a function of concentrations of NaOH in dioxane/H_2O after reaction for 4 h at 50 °C.

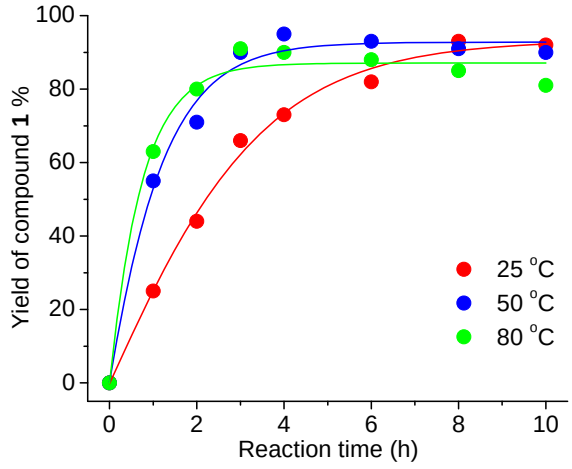


Figure 2. Plot of the yield of compound **1** as a function of temperature and reaction time in dioxane/H_2O/NaOH (0.4 M).

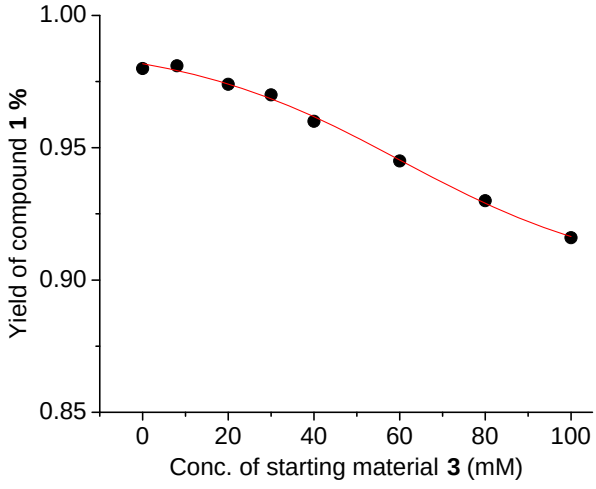


Figure 3. Plot of the yield of compound **1** as a function of the concentration of starting material **3** in dioxane/H_2O/NaOH (0.4 M) after 4 h of reaction at 50 °C.

We further investigated the relationship between the concentrations of starting material **3** and the yields of hydrolytic product **1** in dioxane/H_2O/0.4 M NaOH at 50 °C. After 4 h of reaction, the yield of **1** remained above 92% while the concentration of **3** increased from 5 to 100 mM (Fig. 3). Significantly, product **1** can be purified conveniently without the time- and labor-costly column chromatography. Simple extraction steps offered the purified compound **1** that was well characterized by ^{1}H, ^{13}C NMR and MS.[21] The solvent-friendly synthetic procedure and convenient purification strongly indicate that production of compound **1** in large scale and at reduced cost is possible.

In this study, we developed an efficient two-step synthetic procedure to prepare 1-(acetic acid)-4,7,10-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (**1**), a reactive chelating agent that is widely used in medical diagnostic and therapeutic applications. Compared with previous works, our method not only gives the highest yield, but also offers the attractive features such as operational convenience, solvent-friendliness, easy purification, and starting material availability, all of which are very helpful in reducing the production cost of this compound.

Acknowledgments

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References and notes

1. Caravan, P.; Ellison, J. J.; McMurry, T. J.; Lauffer, R. B. *Chem. Rev.* **1999**, *99*, 2293.
2. Grunberg, J.; Novak-Hofer, I.; Honer, M.; Zimmermann, K.; Knogler, K.; Blauenstein, P.; Ametamey, S.; Maecke, H. R.; Schubiger, P. A. *Clin. Cancer Res.* **2005**, *11*, 5112.
3. Parker, D. *Chem. Soc. Rev.* **2004**, *33*, 156.
4. Li, C.; Wong, W. T. *Chem. Commun.* **2002**, 2034.
5. Bianchi, A.; Calabi, L.; Giorgi, C.; Losi, P.; Mariani, P.; Paoli, P.; Rossi, P.; Valtancoli, B.; Virtuani, M. *J. Chem. Soc., Dalton Trans.* **2000**, 697.
6. Li, C.; Winnard, P. T., Jr.; Takagi, T.; Artemov, D.; Bhujwalla, Z. M. *J. Am. Chem. Soc.* **2006**, *128*, 15072.
7. Nicolle, G. M.; Toth, E.; Eisenwiener, K. P.; Macke, H. R.; Merbach, A. E. *J. Biol. Inorg. Chem.* **2002**, *7*, 757.
8. Jaszberenyi, Z.; Moriggi, L.; Schmidt, P.; Weidensteiner, C.; Kneuer, R.; Merbach, A. E.; Helm, L.; Toth, E. *J. Biol. Inorg. Chem.* **2007**, *12*, 406.
9. Kamaly, N.; Kalber, T.; Ahmad, A.; Oliver, M. H.; So, P. W.; Herlihy, A. H.; Bell, J. D.; Jorgensen, M. R.; Miller, A. D. *Bioconjugate Chem.* **2008**, *19*, 118.
10. Aime, S.; Botta, M.; Fasano, M.; Terreno, E. *Chem. Soc. Rev.* **1998**, *27*, 19.
11. Lipinski, M. J.; Amirbekian, V.; Frias, J. C.; Aguinaldo, J. G.; Mani, V.; Briley-Saebo, K. C.; Fuster, V.; Fallon, J. T.; Fisher, E. A.; Fayad, Z. A. *Magn. Reson. Med.* **2006**, *56*, 601.
12. Ke, T.; Jeong, E. K.; Wang, X.; Feng, Y.; Parker, D. L.; Lu, Z. R. *Int. J. Nanomed.* **2007**, *2*, 191.
13. Artemov, D.; Mori, N.; Ravi, R.; Bhujwalla, Z. M. *Cancer Res.* **2003**, *63*, 2723.
14. Mohs, A. M.; Wang, X.; Goodrich, K. C.; Zong, Y.; Parker, D. L.; Lu, Z. R. *Bioconjugate Chem.* **2004**, *15*, 1424.
15. Li, C.; Li, Y. X.; Law, G. L.; Man, K.; Wong, W. T.; Lei, H. *Bioconjugate Chem.* **2006**, *17*, 571.
16. Lewis, M. R.; Raubitschek, A.; Shively, J. E. *Bioconjugate Chem.* **1994**, *5*, 565.
17. Oliver, M.; Jorgensen, M. R.; Miller, A. D. *Synlett* **2004**, *3*, 453.
18. Heppeler, A.; Froidevaux, S.; Mäcke, H. R.; Jermann, E.; Béhé, M.; Powell, P.; Hennig, M. *Chem. Eur. J.* **1999**, *5*, 1974.
19. Li, C.; Wong, W. T. *Tetrahedron* **2004**, *60*, 5595.
20. *Preparation of the 1-(ethyl acetate)-4,7,10-tris(tert-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane* **3**. 1,4,7-Tris(tert-butoxycarbonyl-methyl)-1,4,7,10-tetraazacyclododecane **2** (1.0 g, 1.9 mmol) was dissolved in a mixture of 50 mL anhydrous acetonitrile and K_2CO_3 (540 mg, 3.8 mmol, 2 equiv). Then ethyl bromoacetate (317 mg, 1.9 mmol, 1.0 equiv) in 5 mL acetonitrile was added. This suspension was allowed to stir for 12 h under N_2 at 70 °C. The reaction was monitored by TLC plates. After all the starting material was consumed, crude product (light-yellow oil) was purified by flash chromatography on silica gel (dichloromethane/methane = 10/1 (v/v), T_f = 0.35) to give **3** as a light yellow foam (1.1 g, yield: 98%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.04 (q, J = 7.4 Hz, 2H), 3.78–1.65 (a set of very broad and multiple peaks with an integration corresponding to 24H), 1.36 (s, 27H), 1.16 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.7 (C), 172.4 (2 × C), 172.2 (C), 82.0 (2 × C), 81.9 (C), 59.2 (CH_2), 57.4 (CH_2), 56.2 (2 × CH_2), 55.7 (CH_2), 53.0–50.8 (4 × CH_2, a set of broad peaks), 50.4-47.4 (4 × CH_2, a set of broad peaks), 28.2 (6 × CH_3), 28.1 (3 × CH_3), 13.6 (CH_3); FAB$^+$-MS m/z 623 (M+Na)$^+$; HRFAB$^+$-MS calcd for $C_{30}H_{56}N_4O_8Na$ (M+Na)$^+$ 623.3996, found 623.3986.
21. *Preparation of the 1-(acetic acid)-4,7,10-tris(tert-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane* **1**. 1-(Ethyl acetate)-4,7,10-tris(tert-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane **3** (400 mg, 0.67 mmol, crude product without purification) was dissolved in the mixture (15 mL) of dioxane and NaOH with the ratio of 3:1 (v:v). This solution was stirred vigorously for about 4 h under N_2 at 50 °C. Dioxane was removed in vacuo and water (20 mL) was added. The mixture was extracted 3× with CH_2Cl_2 (3 × 30 mL). The organic phases were combined and further washed 2× with brine. The organic solution was pre-dried and the solvent was removed to leave a colorless glass-like solid product. This material was re-dissolved in 20 mL diethyl ether and allowed to re-crystallize by cooling the solution at 4 °C for overnight. The final product **3** was obtained as a white solid with the yield of 94%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.65–1.68 (a set of very broad and multiple peaks with an integration corresponding to 24H), 1.39 (s, 27H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.9 (C), 172.4 (2 ×), 172.1 (C), 82.0 (2 × C), 81.9 (C), 57.3 (CH_2), 56.3 (2 × CH_2), 55.6 (CH_2), 53.2–50.9 (4 × CH_2, a set of broad peaks), 50.5–47.7 (4 × CH_2, a set of broad peaks), 28.3 (6 × CH_3), 28.1 (3 × CH_3); FAB$^+$-MS m/z 595 (M+Na)$^+$; HRFAB$^+$-MS calcd for $C_{28}H_{52}N_4O_8Na$ (M+Na)$^+$ 595.3683, found 595.3668.