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Expeditious synthesis of 'P'-protected macrocycles en route to lanthanide chelate metal complexes

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Abstract—Phosphonic acid appended tetraazacyclododecane (cyclen)-based macrocycles are attractive metal-ion chelators for diagnostic imaging and therapeutic delivery. Here, we report a novel P-protected methodology that facilitates the rapid synthesis and purification of targeted phosphonic acid bearing macrocycles. Purification of these intermediates is facile, and deprotection using neat TFA is rapid, yet mild enough to preserve the integrity of delicate peptides and/or targeting moieties.

1. Introduction

Metal complexes based on tetraazacyclododecane macrocycles are now broadly used in various biomedical imaging applications including magnetic resonance imaging (MRI) (Gd^{3+}),[1,2] positron emission tomography (PET) (^{64}Cu),[3] single-photon emission computed tomography (SPECT), (^{99}Tc, ^{177}Lu),[4,5] and luminescence imaging.[6–10] Additionally, metal chelators of this type have been successfully employed for delivering therapeutic metal isotopes such as ^{166}Ho and ^{149}Pm for tumor therapy.[5] One of the most attractive aspects of metal chelators derived from tetraazacyclododecane macrocycles, whether intended for imaging or therapy, is their metal-ion exchangeability. For example, in many cases the same chelator can be used to complex Gd^{3+} for magnetic resonance imaging, ^{64}Cu for PET, or Eu^{3+} for luminescence imaging. Complexes derived from phosphonic acid bearing chelators are particularly attractive due to their water solubility, high thermodynamic and kinetic stability, relative non-toxicity and multi-coordinate avidity towards a variety of metal ions.[3,11,12] Additionally, phosphonic acid bearing macrocycle complexes tend to possess increased lipophilic character in comparison to similar carboxylate functionalized species,[13] which can be advantageous when in vivo tissue transport is considered.

Recently, we reported the synthesis of a novel trifunctional lanthanide chelate[14] and its subsequent conjugation to a peripheral benzodiazepine receptor (PBR) ligand[7] for imaging PBR expression. By using the Eu^{3+} and the Gd^{3+} complex in concert, preliminary data suggests the PBR targeted lanthanide complex may have utility as a multi-modal imaging agent.[10]

The unique functionality of the originally reported trifunctional species departed considerably from traditional macrocycle-based chelators.[14] The tri-functional agent included a quinoline antenna for lanthanide ion sensitization, two phosphonic acid pendant arms for metal-ion chelation and a carboxylic acid pendant arm for conjugation to biological targeting moieties. Despite modest synthetic success conjugating targeting moieties to the original tri-functional species,[7] synthetic yield and ease of purification of targeted complexes was less than desired. The phosphonic acid pendant arms central to the chelator species limit conjugation efficiency and unnecessarily complicate purification of conjugates.

Given the difficulties, we experienced synthesizing and purifying the original phosphonic acid species, we

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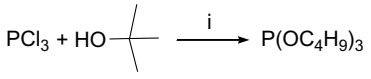
hypothesized that 'P' protected chelators would be easier to synthesize, further functionalize and subsequently purify. Additionally, 'P' protected methodology would be generally applicable to macrocyclic chemistry, and other syntheses requiring application of phosphonate groups. While various protecting groups were considered, *tert*-butyl protection was selected given that deprotection could be readily achieved using mild conditions, thereby reducing the risk to sensitive targeting conjugates.

tert-Butyl protection of phosphoric acid residues are known,[15,16] but surprisingly no examples of incorporating *tert*-butylated phosphonic acids into macrocycle synthesis were found in a thorough literature search. A desirable method yielding macrocycles bearing phosphonic acid ester pendant arms involves reaction of cyclen with paraformaldehyde and the corresponding tri-alkyl phosphite.[7,14] Two of the most common phosphites used for these reactions are the ethyl and butyl derivatives, but the corresponding phosphonate esters require either strong acid or base for removal. A much less known reagent, tri-*tert*-butyl phosphite, was reported many years ago[17] and was used to generate the corresponding phosphonate *tert*-butyl esters, we desired.

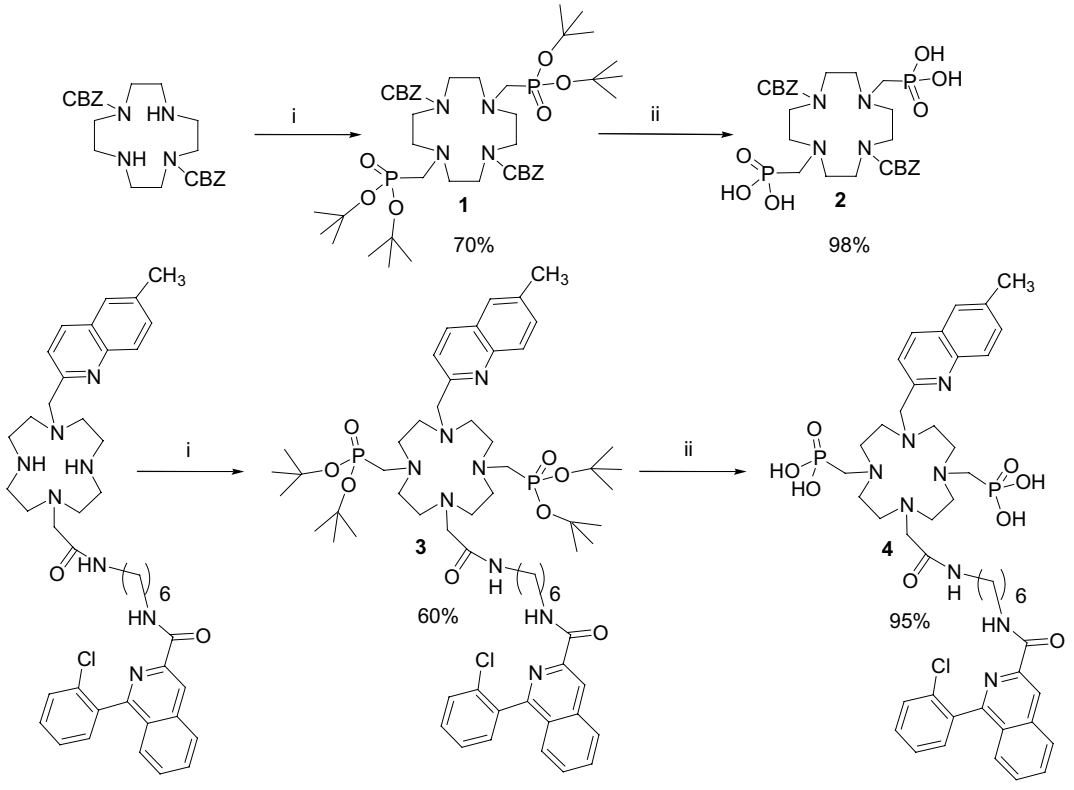


Scheme 1. Reagents and condition: (i) diethyl ether, triethylamine (TEA), 4 °C.

We set out to synthesize tri-*tert*-butyl phosphite by the reaction of phosphorous trichloride with *tert*-butyl alcohol in dry diethyl ether (Scheme 1). The reaction was heat and air sensitive, with both conditions leading to the di-*tert*-butyl phosphate species, but when stored under nitrogen at −4 °C, the reagent could be prepared in advance and stored for more than one month without significant degradation. Additionally, using ^{31}P NMR, characterization is particularly simple with the desired phosphite having a chemical shift of δ 141.39 ppm and the most common oxidized phosphate impurity having a chemical shift of δ 2.1 ppm.

With tri-*tert*-butyl phosphite in hand, we demonstrated its utility by synthesizing four macrocyclic agents **1**, **2**, **3** and **4** using the new 'P' protected methodology (Scheme 2). Compound **1**, 4,10-bis-(di-*tert*-butoxyphosphorylmethyl)-1,4,7,10 tetraazacyclododecane-1,7-dicarboxylic acid dibenzyl ester, was synthesized by alkylating 1,7 benzyloxycarbonyl tetraazacyclododecane in the 4- and 10-position using paraformaldehyde and tri-*tert*-butyl phosphite. The compound was isolated using column chromatography (silica gel) and is a useful intermediate product in macrocycle synthesis that is routinely used in our laboratory. As expected, compound **1** could be deprotected by reaction with neat trifluoroacetic acid (TFA) for 1 h at rt, yielding compound **2**.

Compounds **1** and **2** were easily synthesized using the 'P' protected methodology, but are not appended with a targeting moiety. We next wanted to apply the 'P' protected methodology to a macrocyclic intermediate that already possessed a targeting agent conjugated to it. In



Scheme 2. Reagents and conditions: (i) paraformaldehyde, THF, then tri-*tert*-butyl phosphite; (ii) TFA (neat).

practice, the targeting moiety could be a small molecule receptor ligand, peptide or antibody. Given our interest in synthesizing imaging agents targeting the peripheral benzodiazepine receptor,[7,10] we selected a macrocycle bearing an antenna for lanthanide sensitization and a PK 11195 analogue. Compound **3**, [4-[(6-{1-(2-chlorophenyl)-isoquinoline-3-carbonyl]-amino}-hexylcarbamoyl)-methyl]-7-(di-*tert*-butoxy-phosphorylmethyl)-10-(6-methyl-quinolin-2-ylmethyl)-1,4,7,10-tetraazacyclododec-1-ylmethyl]-phosphonic acid di-*tert*-butyl ester, was synthesized using paraformaldehyde and tri-*tert*-butyl phosphite and purified using column chromatography. Subsequently, the phosphonate esters were deprotected using neat TFA for 1 h at rt and compound **4** was realized in high yield (95%).

In summary, we have demonstrated the ability to generate 'P' protected macrocyclic lanthanide chelators as intermediates en route to diagnostic imaging and/or therapeutic complexes. The enabling methodology presented here will facilitate the synthesis of novel agents, improve conjugation efficiency of phosphonate acid bearing macrocycles to targeting moieties, and simplify purification of agents and intermediates.

2. Experimental details

2.1. Synthesis of tri-*tert*-butyl phosphite

Tert-butyl alcohol (37 g, 0.5 mol) and triethylamine (TEA) (55.7 g, 0.5 mol) in anhydrous ether (200 mL) were added together at 0 °C. Phosphorous trichloride (22.9 g, 0.17 mol) in ether (50 mL) was added slowly, via an addition funnel, maintaining the reaction temperature below 5 °C. After the addition was complete, the reaction mixture was allowed to stir for an additional hour at 2–5 °C. The ice bath was subsequently removed and the reaction mixture was stirred an additional 12 h at rt. Finally, the reaction mixture was filtered and the solvent was removed in vacuo, yielding a colourless oil at rt that solidified to feathery crystals upon standing at 4 °C (39.5 g, 95%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (s); ^{31}P NMR (121 MHz, $CDCl_3$) δ 141.39.

2.2. Synthesis of 4,10-bis-(di-*tert*-butoxy-phosphoryl-methyl)-1,4,7,10-tetraazacyclododecane-1,7-dicarboxylic acid dibenzyl ester 1

To a flame dried round bottom flask, 1,7-bis-benzyloxycarbonyl tetraazacyclododecane (205 mg, 0.47 mmol) was dissolved in dry tetrahydrofuran (THF) while stirring under nitrogen (10 mL). Next, paraformaldehyde (70 mg, 2.3 mmol) was added in one portion and the reaction mixture was allowed to stir at rt for 1 h. Tri-*tert*-butyl phosphite (235 mg, 0.94 mmol) was added and the reaction mixture was stirred overnight. Solvents were removed in vacuo and the desired compound was isolated via column chromatography (silica gel) eluting with a mixed solvent (eluent 100:4:1 $CHCl_3$/MeOH/NH_4OH) as a pale yellow oil that solidified upon standing (281 mg, 70% yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.48 (s, 36H), 2.90 (br s, 10H), 3.55 (br s, 8H), 3.78 (m, 6H), 5.18 (ms, 4H), 7.35 (m, 10H).

^{13}C NMR (65.4 MHz, $CDCl_3$) δ 29.91, 53.40, 58.24, 59.99, 65.29, 81.38, 126.94, 128.29, 128.50, 136.43, 140.98.

MS ($^{+}$ESI) *m*/*z* calcd (found, rel intensity): 852.47 (853.63, 100%).

2.3. Synthesis of [4-[(6-{1-(2-chloro-phenyl)-isoquinoline-3-carbonyl]-amino}-hexylcarbamoyl)-methyl]-7-(di-*tert*-butoxy-phosphorylmethyl)-10-(6-methyl-quinolin-2-yl-methyl)-1,4,7,10-tetraazacyclododec-1-ylmethyl]-phosphonic acid di-*tert*-butyl ester 3

To a flame dried round bottom flask containing 1-(2-chloro-phenyl)-isoqinoline-3-carboxylic acid (6-{2-[7-(6-methyl-quinolin-2-ylmethyl)-1,4,7,10-tetraazacyclododec-1-yl]-acetamino}-hexyl)-amide (12 mg, 16 μmol) dry THF (1 mL) was added along with paraformaldehyde (1.2 mg, 40 μmol) with stirring under nitrogen. The reaction mixture was allowed to stir for 1 h, after which tri-*tert*-butyl phosphite (8 mg, 32 μmol) was added and the reaction mixture was allowed to stir overnight. Reaction progress was monitored via silica gel TLC (eluent 20:4:1 $CHCl_3$/MeOH/NH_4OH). Solvents were removed in vacuo and the desired compound was isolated via column chromatography (silica gel) eluting with a mixed solvent (eluent 20:4:1 $CHCl_3$/MeOH/NH_4OH) as a pale yellow oil (11 mg, 60% yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20 (m, 2H), 1.27 (m, 4H), 1.38 (s, 26H), 1.43 (s, 2H), 1.46 (s, 10H), 2.44 (s, 3H), 2.56 (br s, 4H), 2.73–2.91 (br m, 16H), 3.02 (s, 2H), 3.17 (m, 2H), 3.40 (m, 2H), 3.71 (d, 2H), 3.77 (br s, 2H), 7.42 (m, 2H), 7.51 (m, 6H), 7.66 (m, 3H), 7.87 (d, 1H), 7.95 (m, 2H), 8.05 (d, 1H), 8.26 (t, 1H), 8.39 (t, 1H), 8.52–8.60 (m, 1H).

^{31}P NMR (121 MHz, $CDCl_3$) δ 17.85 (t), 19.45 (t), 19.85 (br m).

^{13}C NMR (65.4 MHz, $CDCl_3$) δ 21.43, 26.85, 27.14, 29.47, 29.83, 29.96, 30.00, 30.32, 30.37, 30.49, 30.54, 39.40, 39.55, 51.62, 51.71, 51.75, 52.93, 53.70, 60.91, 60.98, 61,16, 82.11, 82.23, 82.71, 82.85, 120.06, 120.12, 120.95, 126.84, 127.27, 127.43, 128.17, 128.36, 128.42, 128.72, 128.76, 129.72, 129.92, 130.07, 130.49, 130.71, 131.42, 132.38, 133.26, 133.46, 136.56, 137.11, 146.66, 157.33, 164.49, 172.23.

MS ($^{+}$ESI) *m*/*z* calc (found, rel intensity): 1160.61 (M+H 1161.70 and m+Na^{+} adduct 1183.69, 40%), *m*/2*z* = 580.5 (581.45, 100%).

2.4. General deprotection, compounds 2 and 4

Compounds **1** and **3** were dissolved in trifluoroacetic acid (neat) and stirred for 1 h at rt. After the reaction, the TFA was removed in vacuo and the compounds were characterized. Compound **2**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.61–3.45 (m, 20H), 4.96 and 5.08 (2s, 4H),

7.28 (m, 10H). MS ($^+$ESI) *m*/*z* calcd (found, rel intensity): 628.55 (629.37, 100%).

^{13}C NMR (65.4 MHz, d_6-DMSO) 48.68, 51.42, 55.78, 67.28, 128.66, 129.15, 129.21, 137.38, 154.84.

Compound **4**: ^{1}H NMR (300 MHz, CD_3OD) 1.16 (br m, 8H), 1.48 (br m, 2H), 2.44 (s, 3H), 2.55–3.08 (br m, 13H), 3.34–3.72 (br m, 9H), 4.32 (br s, 2H), 7.46–7.59 (m, 7H), 7.74 (m, 2H), 7.85 (s, 1H), 7.99 (m, 3H), 8.25 (d, 1H), 8.42 (m, 1H), 8.81 (br s, 1H).

^{31}P NMR (121 MHz, CD_3OD) δ 20.75 (t), 21.75 (t) MS ($^+$ESI) *m*/*z* calcd (found, rel intensity): 936.36 (937.50, 100%).

^{13}C NMR (65.4 MHz, CD_3OD) δ 21.80, 27.70, 27.78, 30.13, 30.59, 40.51, 52.03, 52.83, 54.12, 54.50, 56.29, 56.79, 58.39, 59.55, 114.68, 117.50, 121.51, 128.32, 128.75, 129.60, 129.73, 129.80, 130.07, 130.26, 130.51, 130.67, 130.84, 131.14, 131.69, 132.54, 132.61, 134.41, 138.03, 138.58, 139.04, 143.86, 159.48, 161.44, 165.20, 166.91.

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