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Synthesis and Phosphodiester Transesterification Activity of the La^{3+}-Complex of a Novel Functionalized Octadentate Ligand

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Abstract: Various lanthanide complexes have been shown to promote phosphodiester hydrolysis. Distribution of the available coordination sites of the lanthanides between the ligand and the labile water molecules has proved to be critical in determining the activity and stability of the complex in water at neutral pH. Since the final goal is to conjugate such hydrolytically active molecules to DNA oligomers, the design must include a functionality that can be made reactive in standard conjugation protocols. To that end, we have synthesized a nitrophenyl-derivatized TCMC-La^{3+} complex, and it is as active as the unmodified TCMC-La^{3+} in the transesterification of an RNA model substrate.

Design and synthesis of artificial systems accelerating phosphodiester hydrolysis under near physiological conditions is an important goal. Implications in biotechnology, and gene therapy in connection with anti-sense techniques is the primary reason for this interest,[1] but we should not dismiss the challenge involved as a motive: The half-life for the hydrolysis of a phosphodiester bond in DNA at pH 7.0 and 25 °C, is more than 200 Million years.[2] To accelerate this hydrolysis, various metal ions, complexed or free have been used. Simple amines[3] or guanidinium[4] derivatives were also shown to accelerate transesterification of RNA or model phosphodiester compounds. The highest rate accelerations have been observed with lanthanides[5] and Co^{3+}-complexes.[6] The latter approach has been hampered with product inhibition, as substitutionally inert phosphate complexes in most cases remove any chances for true catalytic behavior.[7] On the other hand, lanthanides with their strong Lewis acid properties[8] and flexible coordination geometry[9] have proved to be highly useful in this context. One of the challenges involved in designing a hydrolytically active lanthanide complex is to find a stable complex which does not have reduced Lewis acidity. These are essentially antagonistic goals, and finding the right balance is not easy. Tetrakis(carbamoylmethyl)cyclen (TCMC) complex of La^{+3} (**1**) is one example of such careful considerations.[10] It has been shown to be stable under many conditions and at the same time highly active in facilitating transesterification of phosphodiester model compounds. Unlike DOTA complex **2** , there are no negatively charged carboxylates to reduce Lewis acidity, but carbonyl oxygen coordination

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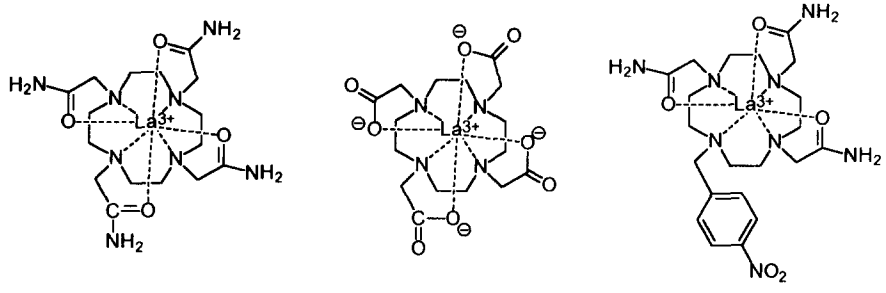
provides a stable encapsulation. One of the final goals of this design is to be able to tether hydrolytically active group to DNA oligomers and mediate sequence-specific RNA cleavage. To that end La^{3+} complexes with functional groups which can be converted to DNA-conjugatable forms are highly desirable. A Eu^{3+}-analog of the TCMC derivative **3** was reported,[11] but a donor atom in the complex is lost in that approach, resulting in reduced stability for the La^{3+} complex.



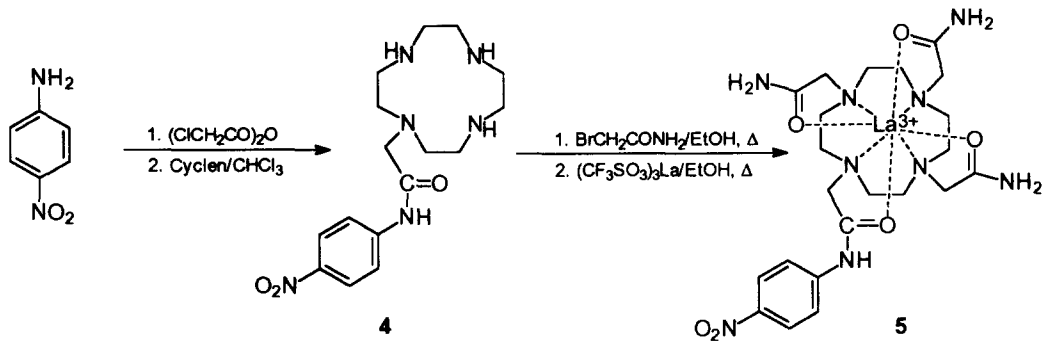
1 2 3

Figure 1. Structure of the lanthanide complexes TCMC-La^{+3} (**1**), DOTA-La^{3+} (**2**) and the nitrobenzyl derivative of TCMC, NBAC-La^{3+} (**3**)

We now report a short synthesis for the compound **5**, which is both stable under metal exchange conditions with Cu^{2+} and more active than the parent TCMC complex **1**.

The target complex has been synthesized starting from p-nitroaniline. p-Nitrochloroacetanilide was prepared by the reaction of p-nitroaniline with chloroacetic anhydride in $CHCl_3$, in the presence of triethylamine. Cyclen was selectively monofunctionalized adapting a previously reported procedure[12] and purified by silica-gel column chromatography using $CHCl_3$/MeOH/NH_3 (12:4:1). Compound **4** was then treated with bromoacetamide and Et_3N in refluxing ethanol to yield the ligand (4-nitrophenylcarbamoyl)-tris(carbamoyl)-1,4,7,10-tetrazacyclododecane (NPAC). Lanthanide complex **5** was prepared by refluxing an ethanolic solution of lanthanum triflate and the ligand for 4 hours, and was isolated as the triflate salt. The NPAC-La^{3+} (**5**) complex prepared in this way, does not dissociate in water at pH 7.4 as evidenced by trapping experiments carried out with excess Cu^{2+}. Compound **5** promotes rapid transesterification of 2-hydroxypropyl-p-nitrophenylphosphate (HPNPP, **6**), a widely used model compound for RNA hydrolysis. Transesterifications were studied by following the release of p-nitrophenolate at 400 nm. Data were collected for a duration of at least 5 half-lives. At pH 7.4 (buffered with 50 mM HEPES) and 25 °C, when 3 mM aqueous solution of the NPAC complex was used, a pseudo-first order rate constant of 8.0×10^{-2} h^{-1} was obtained, which is comparable to the pseudo-first order rate constant obtained[10] in the presence of 1 mM TCMC-La^{3+} (5.8×10^{-2} h^{-1}) at pH 7.4 and 37 °C.

Use of 1 mM EDTA did not result in any appreciable decrease in the hydrolysis rate, so the hydrolysis is only due to the complexed La^{3+}.



Scheme 1. Synthesis of NPAC-La^{3+} (**5**).

A plot of k_{obs} as a function of NPAC-La^{3+} concentration yields a second order rate constant of $8x10^{-3}$ M^{-1} s^{-1}. Thus, we have synthesized a bifunctional lanthanide complex with high stability in neutral aqueous solutions and at the same time as active as the unmodified TCMC complex in phosphodiester hydrolysis. The nitro-substituent can be reduced to an amino group, which can be reacted with an aminoalkyl oligodeoxynucleotides following either isothiocyanate[13] or iodoacetamide[14] protocols.

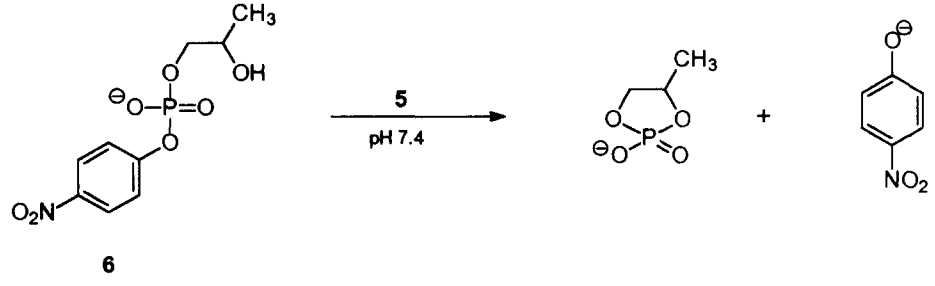


Figure 2. Tranesterification reaction of HPNPP (**6**) .

In summary, the kinetics data demonstrate that we were successful in designing and synthesizing an encapsulated lanthanide complex with a nitrophenyl-attachment, without sacrificing either the transesterification activity or the 8th donor atom of the parent TCMC ligand. As a result, the kinetic stability of the La^{3+}-complex reported here is expected to be higher than that of a previously reported[11] Eu^{3+}-compex similar to the compound **3**, and this may prove to be critical in potential *in vivo* applications. Our work in the synthesis of artificial ribonucleases by attaching hydrolytically active lanthanide complexes to various molecular recognition units is in progress.

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