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A Novel Lanthanide Complex with Remarkable Phosphodiester Transesterification Activity and DNA-Conjugatable Functionality

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Abstract. Encapsulated lanthanide complexes like the TCMC complexes are highly stable under physiological conditions. With the ultimate goal being an *in vivo* application of these complexes as a sequence-selective RNA/DNA cleaving agent (artificial RNAse/DNAse), kinetic stability of the complex would be a distinct advantage. We have synthesized a novel lanthanide complex with such stability and which displays high transesterification activity. The most important attribute of this compound is the nitrophenyl group which would allow further derivatization and conjugation to a DNA oligomer, thus creating a potential for the sequence selective hydrolysis of its target.

Key words: artificial enzymes, phosphodiester hydrolysis, RNA hydrolysis, lanthanide complexes

1. Introduction

A synthetic molecular construct that can cleave a DNA or RNA sequence specifically is of great interest due to its potential applications in biotechnology and gene therapy [1, 2]. The development of such an 'artificial enzyme' requires a hydrolytic unit which is capable of accelerating phosphodiester hydrolysis at near neutral pH around 37 °C and without a dependence on additives (oxidizing/reducing agents, high concentrations of metal ions, etc.) of any kind which can not be supplied *in vivo.* A number of different strategies have been employed by different groups world-wide; general acid-general base catalysis by simple amines [3] or guanidinium [4, 5] derivatives and catalysis by lanthanide [6–9] or Co^{3+}-complexes [10–13] have all proved to be promising leads. Lanthanide complexes are particularly impressive in their hydrolytic activity. In fact, certain lanthanide salts are good catalysts of RNA and phosphodiester hydrolysis in general. But to harness their activity and direct it to the desired region of the target RNA/DNA molecule, kinetically stable, yet hydrolytically active complexes that carry a 'handle', a functional group that would allow conjugation to a complementary sequence of DNA is needed. Morrow [14] reported a cyclen-derived ligand, *p*-nitrobenzyltris(carbamoylmethyl)cyclen (NBAC) (**1**), however, its complex with La^{3+} is not

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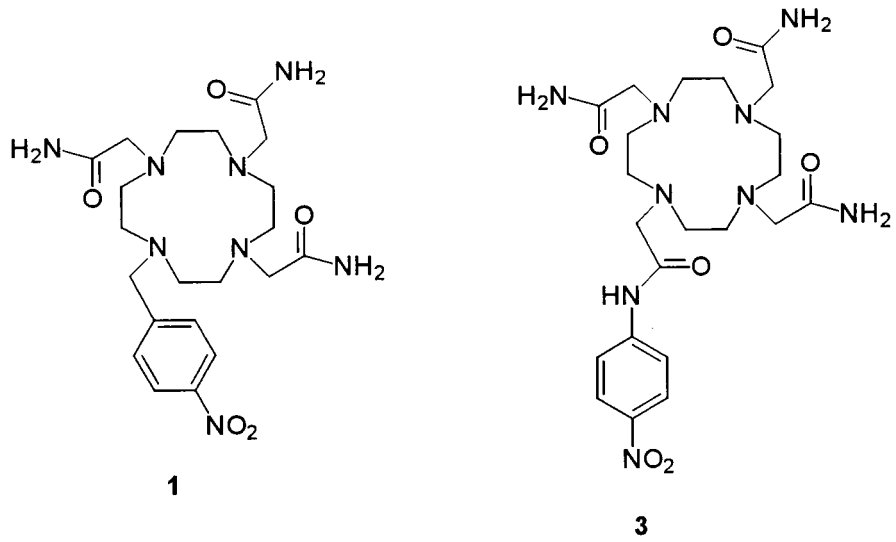


Figure 1. Structure of the heptadentate ligand NBAC (**1**) and the novel octadentate ligand, NPAC (**3**).

stable in aqueous solutions at neutral pH, because one of the donor atoms is lost, making the ligand a heptadentate rather than a octadentate ligand.

In our own approach for the synthesis of a DNA-conjugatable complex, we started with *p*-nitroaniline, converted it to a chloroacetanilide, reacted it with cyclen, converted the cyclen derivative with bromoacetamide and finally formed the complex with lanthanum. This novel octadentate ligand, *p*-nitrophenylcarbamoyl-tris(carbamoylmethyl)cyclen (NPAC) (**3**), forms very stable complexes with a number of lanthanide ions as evidenced by trapping experiments carried out with excess Cu^{2+}.

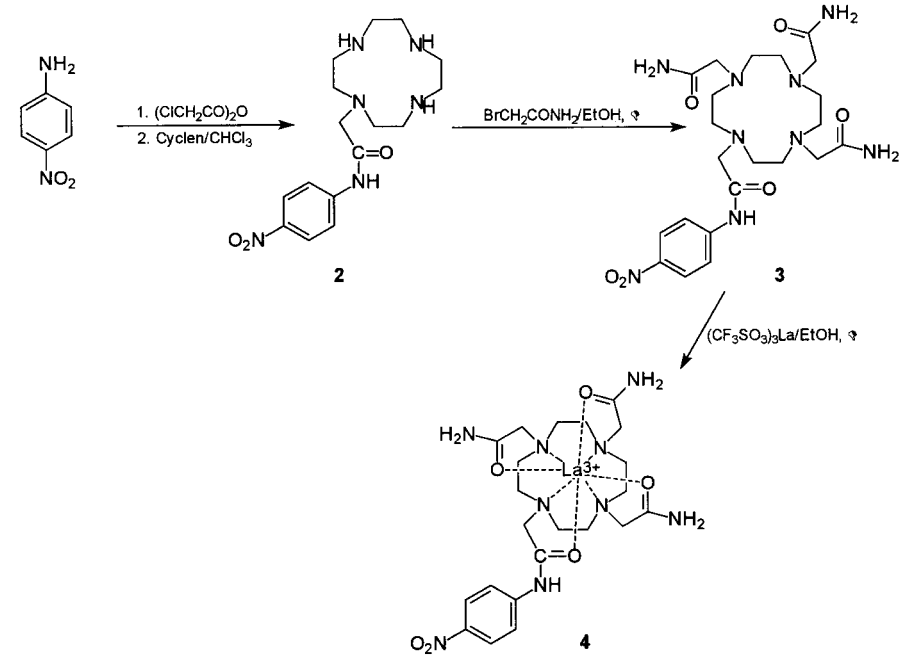
2. Experimental

2.1. MATERIALS

All reagents were purchased from commercial suppliers (Aldrich, unless otherwise noted) and used without further purification. The RNA model compound, 2-hydroxypropyl-*p*-nitrophenylphosphate was prepared according to a literature procedure [15].

2.2. CHARACTERIZATION

^{1}H-NMR and ^{13}C-NMR spectra were obtained using a Bruker Gmbh DPX-400 FT-NMR spectrometer and recorded in $CDCl_3$ or DMSO-d_6 solution with TMS as an internal reference at 400 MHz (^{1}H) and 100 MHz (^{13}C). Electron impact mass spectra were obtained using a Fisons Instruments, VG Platform II LC-MS.



Scheme 1. Synthesis of NPAC-La^{3+} (**4**).

2.3. GENERAL PROCEDURE FOR KINETICS

The hydrolysis kinetics were studied by measuring the absorbance of *p*-nitrophenolate ion at 400 nm. The absorbance spectra were recorded using a Shimadzu UV 1601 connected to a data station. In each experiment, 3 mL of the buffered solution of the complex was placed in an optical cell and the initial absorbance value measured. This was followed by the addition of a stock solution of HPNPP (40 microliter, 5 mM) and the absorbance data collected for at least 3 half lives. Pseudo-first order rate constants (k_{obs}) were obtained using a least squares program.

2.4. SYNTHESIS

2.4.1. *1-(4-nitrophenylcarbamoyl)-1,4,7,10-tetrazacyclododecane* (**2**)

2.76 g (20 mmol) *p*-nitroaniline and 3.44 g (20 mmol) chloroacetic anhydride were dissolved in 40 mL $CHCl_3$. Triethylamine (1 mL) was added, the mixture stirred at rt for 1 hour, and the product was collected by filtration. The *p*-nitrochloroacetanilide product was of satisfactory purity for use in the next step.

A portion of the anilide (7.16 mmol) obtained was reacted with cyclen (1,4,7,10-tetraazacyclododecane, 1.85 g, 10.75 mmol) in 27 mL $CHCl_3$ at rt and the solvent removed under reduced pressure. The residue was applied to a silica-gel column

and the desired product isolated using $CHCl_3$/MeOH/conc. NH_3 as the mobile phase. The yield was 1.4 g (55%). ^{1}H-NMR (DMSO-d_6, 400.1 MHz) δ 2.52–2.70 (m, 16H, —CH_2CH_2—), 3.31 (s, 2H, (—CH_2CO—), 7.99 (d, 2H, ArH), 8.23 (d, 2H, ArH) 10.5 (s, 1H, NH). ^{13}C-NMR ($CDCl_3$, 100.6 MHz) δ 46.2, 47.5, 47.8, 53.8, 59.6, 119.9, 125.7, 143.0, 146.0, 172.4. EI Mass Spectrum *m/e* 351 (M^++1).

2.4.2. *1-(4-nitrophenylcarbamoyl)-tris(carbamoyl)-1,4,7,10-tetrazacyclododecane (NPAC)* (**3**)

1-(4-nitrophenylcarbamoyl)-1,4,7,10-tetrazacyclodode cane (**2**, 2.52 mmol, 0.88 g) was suspended in 40 mL of anhydrous EtOH. To this mixture, bromoacetamide (1.18 g, 8.55 mmol) and 1.5 mL diisopropylethylamine was added. The reaction mixture was then heated under reflux for 4 hours. Upon cooling to rt, the ligand precipitated out of the solution. Further purification was achieved by recrystallizing the material from hot EtOH. Yield 1.1 g (82%). ^{1}H-NMR (DMSO-d_6, 400.1 MHz) δ 2.60–2.76 (m, 16H, —CH_2CH_2—), 2.90–3.05 (m, 6H, —CH_2CO—), 3.25–3.40 (br s, 2H), 6.70 (br s, 3H, NH), 7.49 (m, 3H, NH), 7.91 (d, 2H, ArH), 8.22 (d, 2H, ArH), 10.48 (s, 1H, NH). ^{13}C-NMR ($CDCl_3$, 100.6 MHz) δ 53.8, 58.5, 58.8, 59.5, 119.9, 125.8, 143.1, 145.7, 171.1, 173.6.

2.4.3. *NPAC-La(III) Complex* (**4**)

A solution of $(CF_3SO_3)_3$La (lanthanum triflate, 0.123 g, 0.21 mmol) was prepared and added to a stirred suspension of the ligand **3** (0.11 g, 0.21 mmol) in 75 mL EtOH. To complete the complex formation, the reaction mixture was heated at reflux for 4 hours after the dissolution of the ligand NPAC. The complex was obtained in the form of a light yellow powder (0.2 g, 90% yield). After the removal of the solvent under reduced pressure and trituration with CH_2Cl_2 and hexane. ^{1}H-NMR (DMSO-d_6, 400.1 MHz) δ 2.1–3.8 (br m, 24, —CH_2CH_2—, and —CH_2CO—), 7.92 (d, 2H, ArH), 8.22 (m, 3H, NH), 8.35 (d, 2H, ArH), 8.50 (s, 2H, NH), 8.58 (s, 1H, NH), 10.50 (s, 1H, NH). ^{13}C-NMR ($CDCl_3$, 100.6 MHz) δ 56.9, 62.1, 121.7, 126.3, 143.7, 144.6, 174.7, 177.7.

3. Results and Discussion

The hydrolysis of 2-hydroxypropyl-*p*-nitrophenylphosphate was studied under pseudo-first order conditions: the hydrolysis reaction was carried out at pH 7.4 buffer (50 mM HEPES) using 0.05 mM of the phosphodiester and 3 mM of the complex. The observed rate constant was 8.0×10^{-2} h^{-1}. The rate acceleration was approximately 700-fold, comparable to that of an unmodified TCMC (tetrakiscarbamoylmethylcyclen) complex of lanthanum. Large excesses of EDTA did not alter the hydrolysis rate, confirming that the hydrolysis is not due to free La^{3+}. The bifunctional ligand synthesized exhibits high kinetic stability in neutral aqueous solutions and is as active as the unmodified TCMC complex toward phosphodi-

ester hydrolysis. The nitrophenyl-substituent can also serve, after a reduction to an amino group, as an appropriate site to couple with appropriate oligonucleotide derivatives using standard protocols.

Work towards the development of sequence selective artificial phosphodiesterases is in progress in our laboratory.

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