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Carboxylic Groups as Cofactors in the Lanthanide-Catalyzed Hydrolysis of Phosphate Esters. Stabilities of Europium(III) Complexes with Aza-benzo-15-crown-5 Ether Derivatives and Their Catalytic Activity vs Bis(*p*-nitrophenyl)phosphate and DNA

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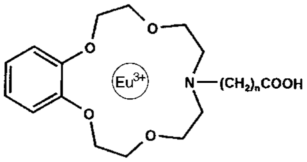
ABSTRACT



The stability of europium(III) complexes with benzocrown ether derivatives 2–4 containing carboxylic functions with methylene spacers of different length was found to vary surprisingly little. In sharp contrast, the hydrolytic activity of these catalysts against bis(*p*-nitrophenyl)phosphate (BNPP) varied by a factor of 30. The results show for the first time the efficiency of the carboxylic group as cofactor in phosphate ester hydrolysis, which until now was established only in some enzmyes. Other than with BNPP, the hydrolysis with plasmid DNA (nicking experiments) showed no rate enhancement.

Hydrolytic cleavage of biocidic phosphate esters and of nucleic acids is of much current interest, in particular after the re-discovery[1] of highly charged metal cations as effective catalysts.[2] We have directed our efforts in this field mostly toward the development of stable yet active metal complexes and of ligands,[3] which as in related metalloenzymes can further enhance the efficiency of these artificial nucleases, which are still several powers of magnitude below that of natural counterparts.

The macrocycles used in the present study were prepared from benzoaza-15-crown-5 ether by a two-step synthesis. Alkylation reaction of the initial crown ether with the ethyl

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(1) For earlier reports, see: Bamann, E., Trapmann, H. *Adv. Enzymol.* **1959**, *21*, 169.

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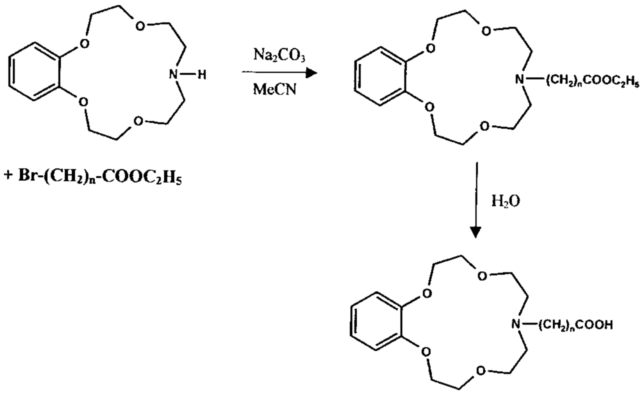
ester of bromoalkanoic acids followed by hydrolysis of amino acid ethyl ester by refluxing in water gave **2**−**4** with yields up to 90−95% (Scheme 1).

Scheme 1



2, n = 1, 91 %
3, n = 3, 92 %
4, n = 5, 94 %

The benzocrown ether derivatives **2**−**4** contain carboxylic functions, which also should serve to enhance the stability of corresponding lanthanide complexes. Potentiometric measurements indicate that without such pendent COOH groups (**1**) the stability in water is very low and does not even allow the evaluation of K with Eu^{3+} in water. This is somewhat unexpected, as the presence of nitrogen in crown ethers generally increases the affinities, and the parent all-oxa-benzo-15-crown-5 ether complexes have constants around $\log K = 5$ in water.[4] Usually one observes a steady increase of $\log K$ values with the number of pendent COOH groups,[5] although less regular behavior can occur if the ion size does not match the ionophore.[6]

The presence of the pendent COOH groups does lead to enhanced binding of Eu^{3+}, however, with little dependence on the spacer methylene groups (Table 1). This is again surprising in view of the large difference reported[7] e.g. for $\log K$ values of 1,10-diaza-18-crown-6 ethers with N-substituents either R = CH_2COOH ($\log K = 12.0$) or R = $(CH_2)_2COOH$ ($\log K = 7.4$). One might expect that entropic disadvantage associated with the complexes containing more flexible bonds should lower the affinities. Recently it has been shown, however, that the change of free complexation energy in other complexes is less than 1.5 kJ per mol and per one rotable single bond.[8]

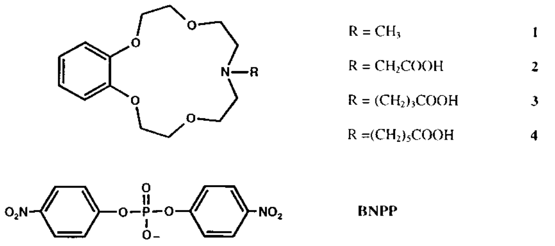
Table 1. Protonation and Complexation Constants of Eu^{3+} Salts with *N*-Carboxyalkyl Benzoaza-15-crown-5 Ethers[a]

ligand		log K	percent of $Eu(ClO_4)_3$ as[e] EuL^{2+}	Eu^{3+}
2[b]	$L^- + H^+ = LH$	$\log K_1 = 8.96 \pm 0.02$		
	$L^- + 2H^+ = LH_2^+$	$\log K_2 = 11.36 \pm 0.02$	12.3	87.7
	$L^- + Eu^{3+} = (LEu)^{2+}$	$\log K = 3.45 \pm 0.04$		
3[c]	$L^- + H^+ = LH$	$\log K_1 = 9.08 \pm 0.02$		
	$L^- + 2H^+ = LH_2^+$	$\log K_2 = 13.06 \pm 0.02$	16.7	83.3
	$L^- + Eu^{3+} = (LEu)^{2+}$	$\log K = 3.77 \pm 0.04$		
4[d]	$L^- + H^+ = LH$	$\log K_1 = 9.47 \pm 0.02$		
	$L^- + 2H^+ = LH_2^+$	$\log K_2 = 13.88 \pm 0.02$	15.2	84.8
	$L^- + Eu^{3+} = (LEu)^{2+}$	$\log K = 4.10 \pm 0.04$		

[a] Stability constants at 20.0 °C calculated from titration data with crown ether + $HClO_4$ and crown ether + $Eu(ClO_4)_3$ + $HClO_4$, respectively; in aqueous solution with NaOH in the range pH 2.300−9.500 for crown ethers alone and pH 2.300−7.200 for complexes (up to hydrolysis of $Eu(ClO_4)_3$). All equilibrium calculations were performed by using the computer program Hyperquad.[10] No complexation detectable with *N*-methylbenzoaza-15crown-5 ether **1**. [b] L = $Eu(ClO_4)_3$ = $HClO_4$ = 4.6×10^{-3} M; NaOH 0.05 M. [c] L = 6.4×10^{-3} M; $Eu(ClO_4)_3 = 8 \times 10^{-3}$ M; $HClO_4 = 1.06 \times 10^{-2}$ M; NaOH 0.0995 M. [d] L = 4.5×10^{-3} M; $Eu(ClO_4)_3 = 4.5 \times 10^{-3}$ M; $HClO_4 = 6.4 \times 10^{-3}$ M; NaOH 0.05 M.

In contrast to the affinity constants, the catalytic acitivity of the Eu^{3+} complexes on the BNPP hydrolysis, which was measured as reported previously,[3] changes dramatically with the length of the spacer, with the longest side chain (**4**, R = $(CH_2)_5COOH$) being 30 times higher than that with **1**, R = CH_3 (Table 2). The affinity data in Table 1 secured that under the applied concentrations the complexation degree with Eu^{3+} varies only between 77% and 88% and cannot be responsible for the differences in catalytic activity. Thus, we observe not only a substantial activity increase due to the introduction of carboxylic cofactors but a higher catalytic efficiency with

Table 2. Rate Constants of Hydrolysis with Eu^{3+} Complexes



ligand	BNPP[a] $k_{obs} \times 10^4$ [s^{-1}]	DNA[b] $k_{obs} \times 10^4$ [s^{-1}]
1	0.36	0.53
2	0.79	0.68
3	3.08	0.58
4	11.30	0.62

[a] [ligand] = 5×10^{-3} M, [Eu^{3+}] = 5×10^{-3} M in 0.01 M EPPS, pH = 7.0, T = 50 °C, [BNPP] = 3.76×10^{-5} M. [b] [ligand] = 5×10^{-3} M, [Eu^{3+}] = 5×10^{-3} M in 0.01 M EPPS, pH = 7.0, T = 37 °C, incubation time 2 h, [DNA] = 1.9×10^{-5} M (bp); rates for Eu^{3+} alone: k_{obs}(BNPP) = 1.67×10^{-4} s^{-1}; k_{obs}(DNA) = 0.59×10^{-4} s^{-1}.

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longer, more flexible side chains. This is in line with the abovementioned small disadvantage of flexible bonds in the formation of supramolecular complexes; it is also reminiscent of highly efficient enzymes such as alkaline phosphatase[9] which also contain cofactors such as carboxylic groups as part of amino acids which are rather loosely embedded in their active center.

Whereas the presence of carboxylic groups in Eu^{3+} complexes with, e.g., **4**, R = $(CH_2)_5COOH$, leads for the BNPP hydrolysis to the highest efficiency increase by ligand modification which until now is observed, the effect of these cofactors on double-stranded DNA hydrolysis is marginal. The rates, determined as previously described[3] by plasmid DNA nicking experiments, show actually a small decrease of efficiency (Table 2). Obviously, the binding affinity decrease of the new complexes to the negatively charged DNA groove counteracts the favorable catalytic effect on the phosphate group cleavage itself. The introduction of additional groups in the metal complexes, such as polyammonium centers or intercalators, should remedy this situation. Such studies are in progress and should further contribute to the development of, e.g., artificial restriction enzymes.

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