NMR and Luminescence Binding Studies of Ytterbium, Thulium, and Europium Macrocyclic Complexes with Phosphorus(V) Oxy Anions

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Dedicated to Professor *André Merbach* on the occasion of his 65th birthday

The binding of a series of phosphate anions to coordinatively unsaturated macrocyclic complexes of Yb, Tm, and Eu was studied by ^{1}H-NMR, emission and circularly polarized luminescence (CPL) spectroscopy. Each ternary adduct was distinguished by its spectral profile. With *O*-phosphorylated amino acids and peptides, chemoselective ligation of the phosphate moiety was favored by Eu over chelation involving the terminal amino group, which was competitive for Tm and Yb. A preference for binding *O*-phosphono-L-tyrosine sites was found with various Eu complexes, and was signalled by ratiometric changes in metal-based emission and CPL spectra.

Introduction. – The quest for the chemoselective signaling of reversible anion binding in aqueous media has been an improvement driver in the development of supramolecular chemistry [1]. In devising appropriate synthetic receptors that may serve as analytical probes, particular consideration needs to be given to the target binding affinity and the manner in which selectivity over competing anionic species, or related electron-rich interferents, is to be achieved. Given the high free energy of hydration of the common oxy anions, a large electrostatic contribution to binding is essential. This may arise either from the use of a positively charged receptor or by devising a system in which multiple H-bond donors are incorporated, positioned appropriately to interact with the H-bond-accepting atoms of the target anion. In addition, given the differing size/geometry of anions, complementarity of size and shape must be engineered into the receptor design [2].

The family of anions based on pentavalent phosphorus constitutes probably the most important class of anions in nature. The *O*-phosphorylated proteins and peptides control many cellular processes, including differentiation, development, and proliferation [3]. Their interaction with essential kinase and phosphatase enzymes requires adenosine 5′-(tetrahydrogen triphosphate) (ATP) – the essential bioactive species in chemical-energy distribution. There are many phosphorylated signaling molecules (secondary messengers), such as adenosine cyclic 3′,5′-(hydrogen phosphate) (AMP) and inositol 1,4,5-(hexahydrogen triphosphate). Furthermore, phosphates play an essential structural role in bone (apatite) and in ferritin. Such systems are well-suited to being studied by ^{31}P-NMR spectroscopy, as each P^V oxy anion is distinguished by its chemical shift. This allows the time-dependence of the concentration of abundant phosphorylated species in cells to be monitored. However, magnetic resonance lacks

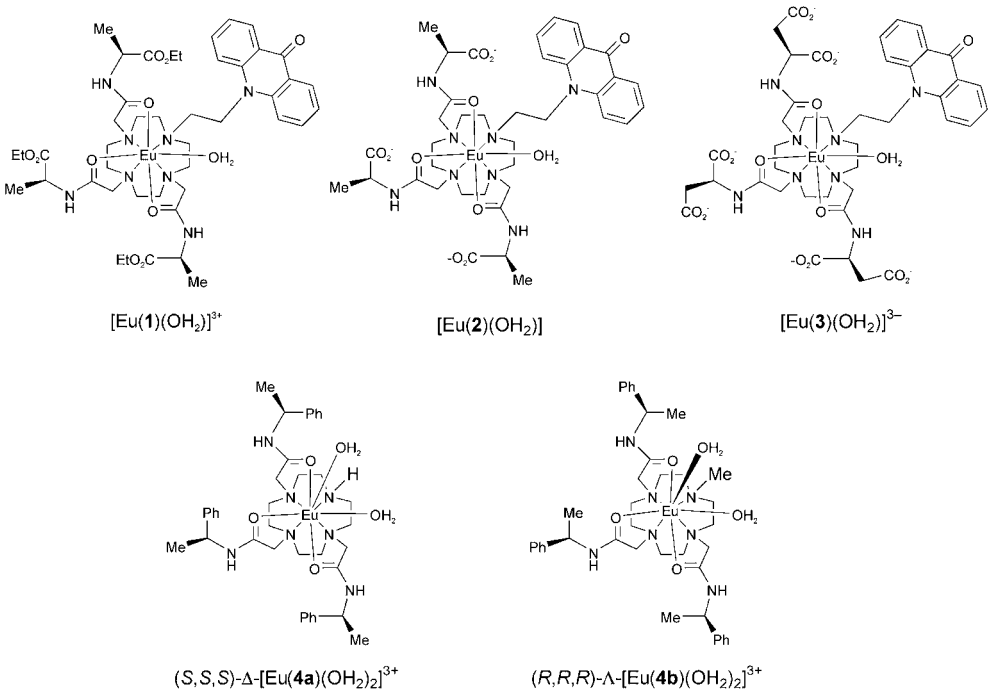
the required spatial resolution to distinguish intra/inter-cellular events, notwithstanding the use of extracellular NMR shift reagents. Moreover, whilst the extracellular concentration of species such as ATP/ADP (ADP = adenosine 5′-(trihydrogen diphosphate)) and inorganic phosphate can be determined by certain bioluminescence assays [4], noninvasive optical methods have not been defined that will allow the direct monitoring, in real time, of the spatial distribution of essential phosphorylated species.

With this background in mind, we have begun to explore the binding in solution of a range of phosphate anions, from simple examples such as inorganic phosphate (HPO_4^{2-}), D-glucose 6-phosphate ((Glc-6-P)$^{2-}$), or *O*-phosphono-L-tyrosine ((*O*-P-Tyr)$^{2-}$) to more complex systems such as *O*-phosphorylated peptides (phospho = phosphono = $(HO)_2P(=O)$- or ionized forms = P). The longer-term objective is challenging; it seeks the definition of selective nontoxic luminescent, intracellular probes for essential bioactive species/sites. An example might be to address signaling the behavior of certain *O*-phosphono-L-tyrosine sites in a protein, in real time, *via* live-cell imaging.

The synthetic receptors for such *O*-phosphorylated species that we have chosen to explore are chiral lanthanide (Ln) complexes based on a macrocyclic heptadentate ligand. A typical series of complexes, $[Ln(\mathbf{1})OH_2)]^{3+}$, $[Ln(\mathbf{2})(OH_2)]$, and $[Ln(\mathbf{3})(OH_2)]^{3-}$ combine modulation of electrostatic affinity through variation of overall complex charge with metal or ligand-based permutation. Thus, anion affinity is controlled by permuting the Ln ion and follows the charge-density change ($Yb \approx Tm > Tb > Gd > Eu >> Ce$). Innumerable ligand variations may be envisaged, of which variation of the substituent at the fourth ring N-atom and of the nature of the amide pendant arm moiety are most apparent. Preliminary work has revealed that inorganic phosphate binds reversibly to simple cationic complexes, such as $[Eu(\mathbf{4a})(OH_2)_2]^{3+}$, with an affinity of $> 7 \times 10^4$ M^{-1} (298 K, pH 7.4, 0.1M NaCl), and that considerable selectivity for this anion is conserved in the presence of protein and endogenous anions [5][6].

An advantage of using the paramagnetic lanthanide complexes as receptors is that they not only allow the development of luminescent probes but also facilitate structural analysis, especially of bound phosphate species by NMR spectroscopy. Indeed, recent work highlighting the relationship between the coordination environment of such Eu and Yb complexes and their spectral emission and NMR properties has established a reliable means of determining complex solution structure [7–9]. It was shown that in 9-coordinate systems adopting a square-antiprismatic geometry, the salient second-order crystal-field coefficient, B_O^2, which determines the paramagnetic dipolar NMR shift, is particularly sensitive to the polarizability of the 'axial' donor [9]. For example, adducts of $[Ln(\mathbf{4a})(OH_2)_2]^{3+}$ with HPO_4^{2-} and F^- give species retaining an axial H_2O molecule, while malonate, oxalate, or carbonate give chelated structures with a polarizable O-atom of the ligand in the axial site. Dipolar ^{1}H-NMR shifts of selected ligand resonances in these two sets of ytterbium complexes differ by over 70 ppm [8].

^{1}H-NMR Studies of Phosphate-Anion Binding. – The binding of a series of anions to the trifluoromethanesulfonate (= triflate) salts of the model complexes $[Yb(\mathbf{4a})(OH_2)_2]^{3+}$ and $[Tm(\mathbf{4a})(OH_2)_2]^{3+}$ was examined by ^{1}H-NMR spectroscopy (500 MHz, 295 K). Typically, to a solution of the complex (4 mM) was added the given



anion in up to a fivefold excess maintaining the pH at 7.4, and the NMR spectra were recorded. Under these conditions, independent titrations revealed that the resultant ternary adducts have 1:1 stoichiometry. These complexes are in slow exchange on the NMR timescale with the diaqua precursor complex.

Values of the limiting ligand ^{1}H-NMR shifts for the fully bound species were recorded. In particular, the mean shift (average of 4) of the most-shifted macrocyclic ring methylen proton was calculated. This 'pseudo-axial' proton resonates to highest frequency in each ternary adduct, and its shift is determined purely by the pseudo-contact contribution [8][9]. Representative spectra (*Figs. 1* and *2*) for the adducts from $[Tm(\mathbf{4a})(OH_2)_2]^{3+}$ and $[Yb(\mathbf{4a})(OH_2)_2]^{3+}$ highlight the sensitivity of this resonance to the local coordination environment. In the NMR spectra of the D-glucose 6-phosphate ((Glc-6-P)$^{2-}$) adducts[1]), the mean shift of the axial ring proton resonance is at δ(H) 254 and 87 in the Tm and Yb system, respectively, and similar limiting mean δ(H)s were measured for AMP^{2-} (adenosine 5′-(monophosphate)), HPO_4^{2-} (pH 7.4), and for the *O*-phosphorylated amino acids L-serine, L-threonine, and L-tyrosine (*Table*). With these *O*-phosphorylated amino acids, however, a minor species was evident (typically in ratio 1:3), resonating at lower frequency, by $\Delta\delta$ *ca.* 50 for Yb and *ca.* 150 for Tm. This species is the amino acid chelate, with the amino N-atom capping the square-antiprism and a carboxylate O-atom in a similar coordination site to the

[1]) The partial resolution of the highest-frequency resonances of the Yb, Tm, and Eu adducts with D-glucose 6-phosphate may be tentatively related either to the presence of D-glucopyranose/D-glucofuranose adducts or to formation of α,β-D-glucopyranose or -glucofuranose epimers.

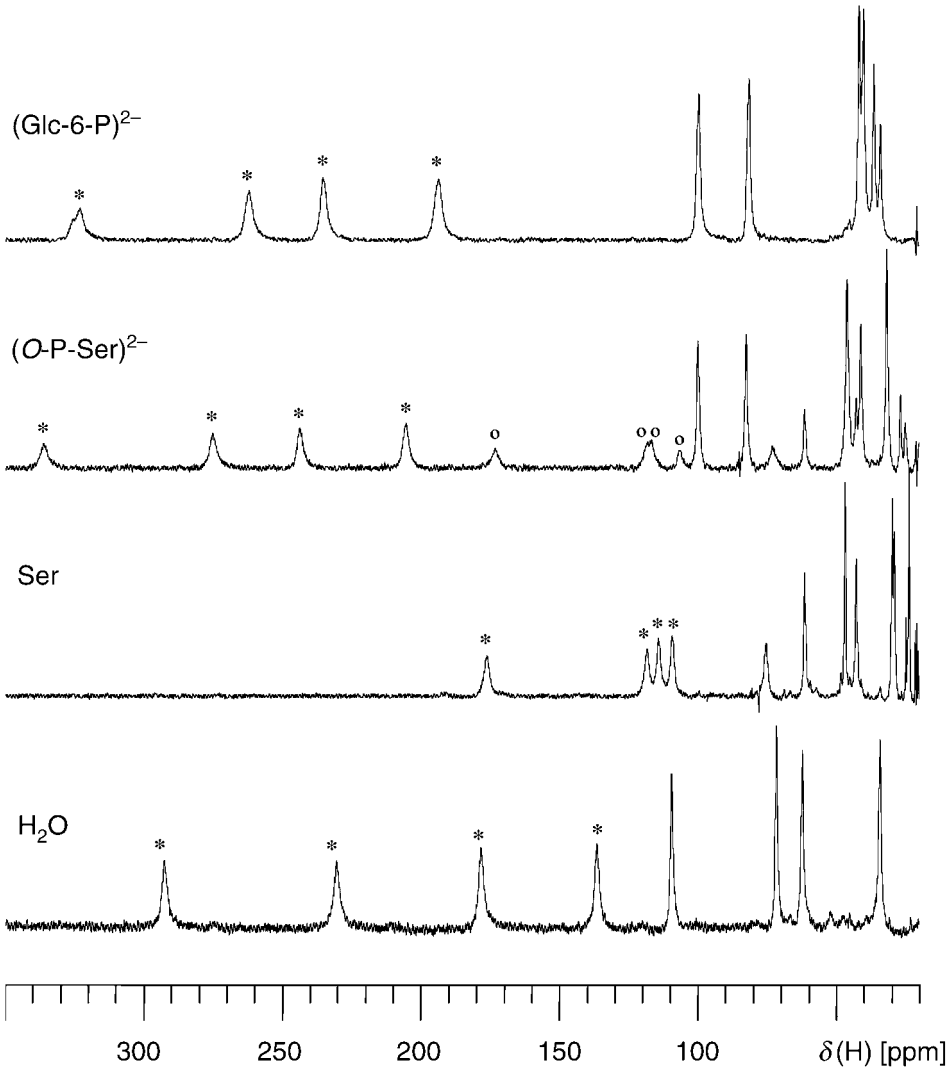


Fig. 1. *^{1}H-NMR Spectra* (500 MHz, 295 K, pH 7.4, D_2O; 4 mM [Tm(**4a**)$(OH_2)_2]^{3+}$, 20 mM anion) *of ternary adducts with the given anion, highlighting the high-frequency spectral region*. Asterisks (*) or circles (○) indicate each of the most-shifted axial ring methylene resonances. Glc-6-P = D-Glucose 6-(dihydrogen phosphate), *O*-P-Ser = *O*-phosphono-L-serine, ser = L-serine.

three ligand amide carbonyl O-atoms. Chelation of such amino acids to these Yb complexes has previously been established by X-ray analyses of eight different amino acid ternary adducts of [Yb(**4a**)$(OH_2)_2]^{3+}$ [8]. Thus, in the adducts of the *O*-phosphorylated amino acids with [Ln(**4a**)$(OH_2)_2]^{3+}$ (Ln = Yb, Tm), phosphate coordination competes with amino acid chelation. Confirmation of this hypothesis was provided by examining the corresponding adducts with the *N*-acetyl-*O*-phosphono-

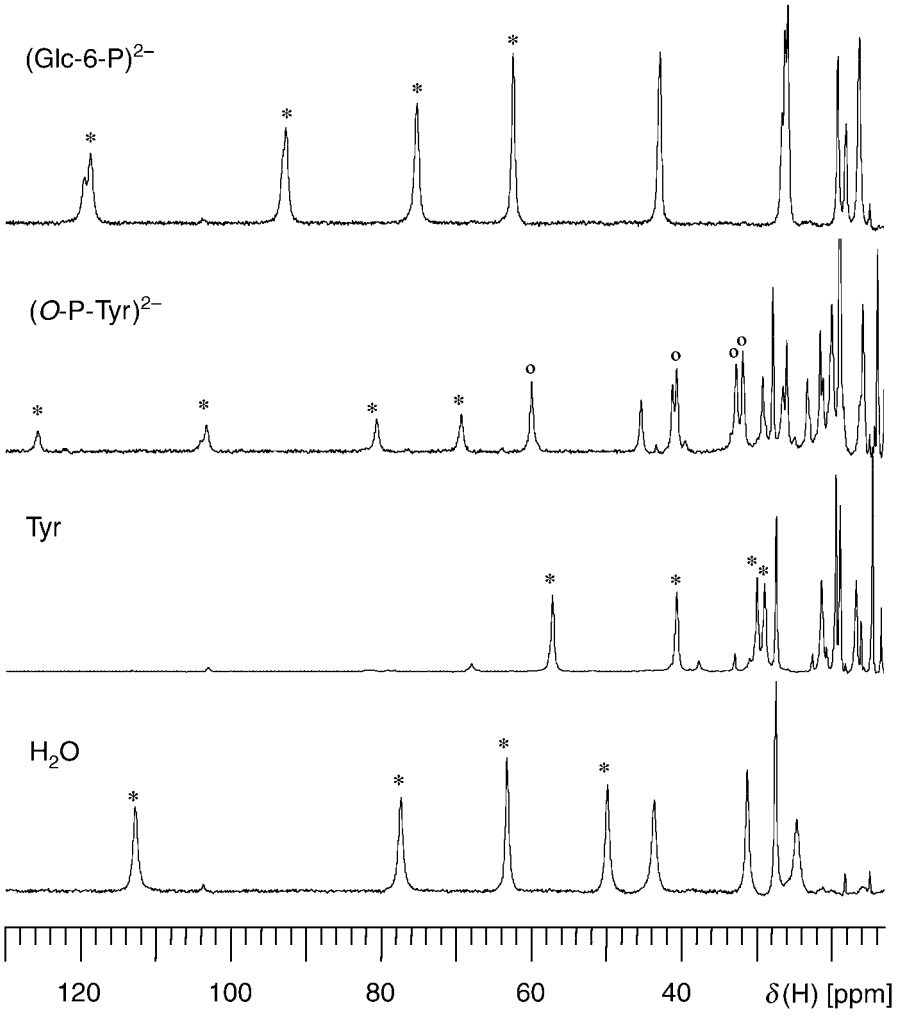


Fig. 2. *^{1}H-NMR Spectra* (500 MHz, 295 K, pH 7.4, D_2O; 4 mM [Yb(**4a**)$(OH_2)_2]^{3+}$, 20 mM anion) *of the anion adducts of glucose 6-phosphate* $(G\text{-}6\text{-}P)^{2-}$, *O-phosphono-*L*-tyrosine anion* $(O\text{-}P\text{-}Tyr)^{2-}$), L*-tyrosine* (Tyr), *and triflate* (unbound), *showing the most paramagnetically shifted resonances.* Asterisks (*) indicate the axial ring methylene CH resonance, and the circles (○) refer to the H–N(4) resonances of the *N*,*O*-amino acid chelates.

L-tyrosine anion $(N\text{-Ac-}O\text{-P-Tyr})^{2-}$. In each case, the limiting shifts were within ± 2 ppm of the phosphono-bound species, and only one species was observed. Addition of a solution of human serum albumin (0.35 mM) to the ternary phosphate adducts caused no change in spectral form over a period of a week, indicative of some chemoselectivity for the phosphate anion. Interestingly, addition of 10 equiv. of $(O\text{-P-Ser})^{2-}$ to a solution containing 1 equiv. of [Yb(**4a**)$(OH_2)_2]^{3+}$ and 5 equiv. of $(O\text{-P-Tyr})^{2-}$ gave rise to a ^{1}H-NMR spectrum with ≥ 80% of the $(O\text{-P-Tyr})^{2-}$-bound species. The spectrum did

Table. *Mean Chemical Shifts*[a]) (295 K, pH 7.4, 500 MHz) *of the Most-Shifted Axial Ring Proton, H–N(4), in Ternary Anion Adducts*

Anion[b])	$[Yb(\mathbf{4a})(OH_2)_2]^{3+}$	$[Yb(\mathbf{4b})(OH_2)_2]^{3+}$	$[Tm(\mathbf{4a})(OH_2)_2]^{3+}$	$[Tm(\mathbf{4b})(OH_2)_2]^{3+}$
$CF_3SO_3^-$	75	101	210	313
HPO_4^{2-}	100	100	247	349
$(O\text{-P-Ser})^{2-}$	91 (45)	54 (107)	265 (125)	161 (360)
$(O\text{-P-Thr})^{2-}$	92 (43)	57 (109)	268 (135)	171 (365)
$(O\text{-P-Tyr})^{2-}$	95 (40)	53 (110)	281 (120)	158 (373)
$(O\text{-P-}N\text{-Ac-Tyr})^{2-}$	92	124	274	360
$(Gl\text{-}6\text{-}P)^{2-}$	87	105	255	356
AMP^{2-}[c])	88	108	258	362
F^-	99	114	299	430
$Lactate^-$	71	89	212	265
AcO^-	69	76	207	231
Ser	44	55	130	165
Thr	44	58	137	171
Tyr	39	51	122	152
CO_3^{2-}	27	32	78	94

[a]) Values in parentheses refer to the minor species. [b]) With triflate as the counter-ion, there is no bound anion; for phosphate-anion adducts and F^-, H_2O molecule occupies the capping site in the nine-coordinate adduct [8]; with OAc^-, Ser, Thr, Tyr, and CO_3^{2-} a chelated structure is adopted with no bound H_2O molecules. [c]) $cAMP^-$ showed no significant binding to the aqua complexes studied here.

not change over a period of 5 days at room temperature, consistent with some preference for binding $(O\text{-P-Tyr})^{2-}$ over $(O\text{-P-Ser})^{2-}$.

An analysis of the average NMR shift of the axial proton 'probe' resonance in the anion adducts of $[Yb(\mathbf{4a})(OH_2)_2]^{3+}/[Tm(\mathbf{4a})(OH_2)_2]^{3+}$ revealed a clear correlation (*Fig. 3*). The linear relationship obtained is consistent with the dominance of the pseudo-contact (dipolar) contribution in determining the paramagnetic shift. Moreover, the magnitude of the variation of the chemical shift of this probe resonance through this series (δ^{mean} *ca.* +78 for the carbonate chelate of $[Tm(\mathbf{4a})(OH_2)_2]^{3+}$, compared to δ^{mean} +281 for the $(O\text{-P-Tyr})^{2-}$ adduct), confirms the observations made with the related ytterbium complexes.

Parallel NMR studies were undertaken with the corresponding series of *N*-methylated complexes, *i.e.*, with $[Tm(\mathbf{4b})(OH_2)_2]^{3+}$ and $[Yb(\mathbf{4b})(OH_2)_2]^{3+}$ (see *Table* and *Fig. 4*). In each of these series, the dominant solution species with the phosphorylated amino acids was the amino acid chelate typically in a ratio of 4:1 *vs.* the phosphate-bound adduct. This reversal of chemoselectivity with respect to complexes of **4a** (H–N(4) *vs.* Me–N(4)) is likely to be associated with the reduced free energy of hydration of the *N*-methylated series of complexes, that may tend to favor the more 'closed' chelated structures.

The europium complexes of **4a** and **4b** were studied in the original observations of anion binding with 'coordinatively unsaturated' lanthanide complexes [5]. They bind anions less strongly than the Yb and Tm analogues, due to the lower charge density at the metal center. For example, with amino acids, over the whole pH regime (4 → 10), only a weak association of the carboxylate group was observed and there was no evidence for *N*,*O*-chelation [5][8]. Addition of a fivefold excess of $(Glc\text{-}6\text{-}P)^{2-}$, HPO_4^{2-},

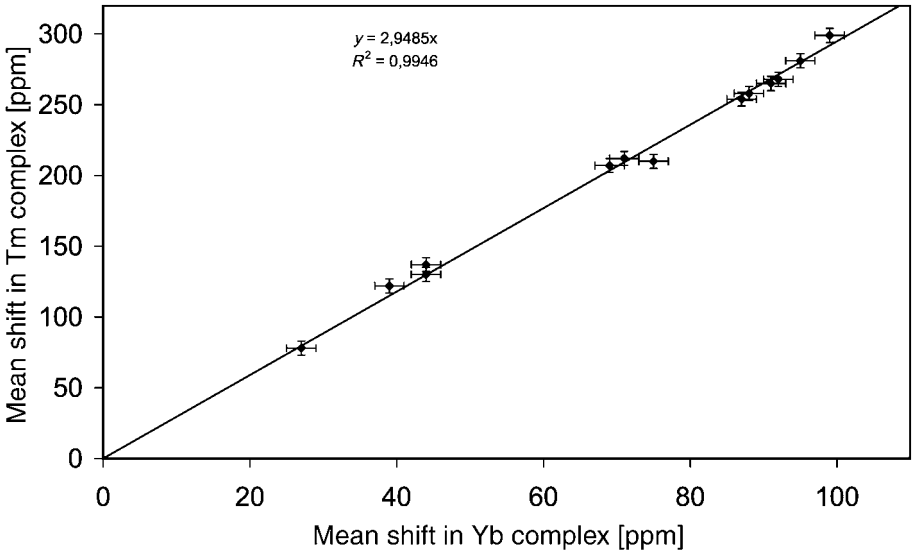


Fig. 3. *Correlation of the mean ^{1}H-NMR shift* (500 MHz, 295 K, D_2O) *for the most-shifted axial ring probe resonance, in anion adducts with [Tm(**4a**)$(OH_2)_2]^{3+}$ and [Yb(**4a**)$(OH_2)_2]^{3+}$*

and (*O*-P-Tyr)$^{2-}$ at pH 7.4 gave one discernible solution species with a very similar spectral profile for the paramagnetically shifted proton resonances, *i.e.*, the most-shifted axial ring proton resonated at $\delta(H)$ +20 ($\pm$2) in each case. In a separate experiment, [Eu(**4a**)$(OH_2)_2$] was added to 4 equiv. of the phosphorylated hexapeptide (Gly-Ala-Pro-Tyr(*O*-P)-Lys-Phe)$^{2-}$ and the ^{1}H-NMR spectrum was also very similar in form ($\delta(H) > 10$ and $\delta(H) < 0$) to that obtained with the simple phosphate anions discussed above, with one dominant solution species (*Fig. 5*). Such behavior is consistent with chemoselective ligation of the phosphate O-atom in every case, and suggests that phosphate anion affinity and selectivity may be controlled by lanthanide-ion permutation. Accordingly, further studies were undertaken examining luminescent europium complexes, based on [Eu(**4b**)$(OH_2)_2$], bearing an 'antenna' chromophore. The series of complexes [Eu(**1**)$(OH_2)]^{3+}$, [Eu(**2**)(OH_2)], and [Eu(**3**)$(OH_2)]^{3-}$ was examined [10].

Luminescence Studies of Anion Binding. – The europium emission spectra of the series of complexes [Eu(**1**–**3**)$(OH_2)]^{3+}$ containing in the ligand the *N*-ethylacridin-9(10*H*)-one moiety were recorded (295 K, 0.1M MOPS (= morpholine-4-propanesulfonic acid), pH 7.4, 0.1 mM complex) in the presence of 10 equiv. of added phosphate anion, following excitation of the sensitizing chromophore at 410 nm. A representative *Job* plot, for the binding of AMP^{2-}, confirms the 1:1 stoichiometry of these complexes (*Fig. 6*). The observation of a clear maximum at $x = 0.5$ (where $x = [AMP^{2-}]/([AMP^{2-}] + [[Eu(\mathbf{1})(OH_2)]^{3+}])$) is consistent with the formation of a 1:1 complex, and the symmetry of the plot also suggests that there are no significant 1:2 or 2:1 complexes present. The emission spectra obtained were very similar in form for the three sets of Eu complexes, and each phosphate-anion adduct spectrum was subtly

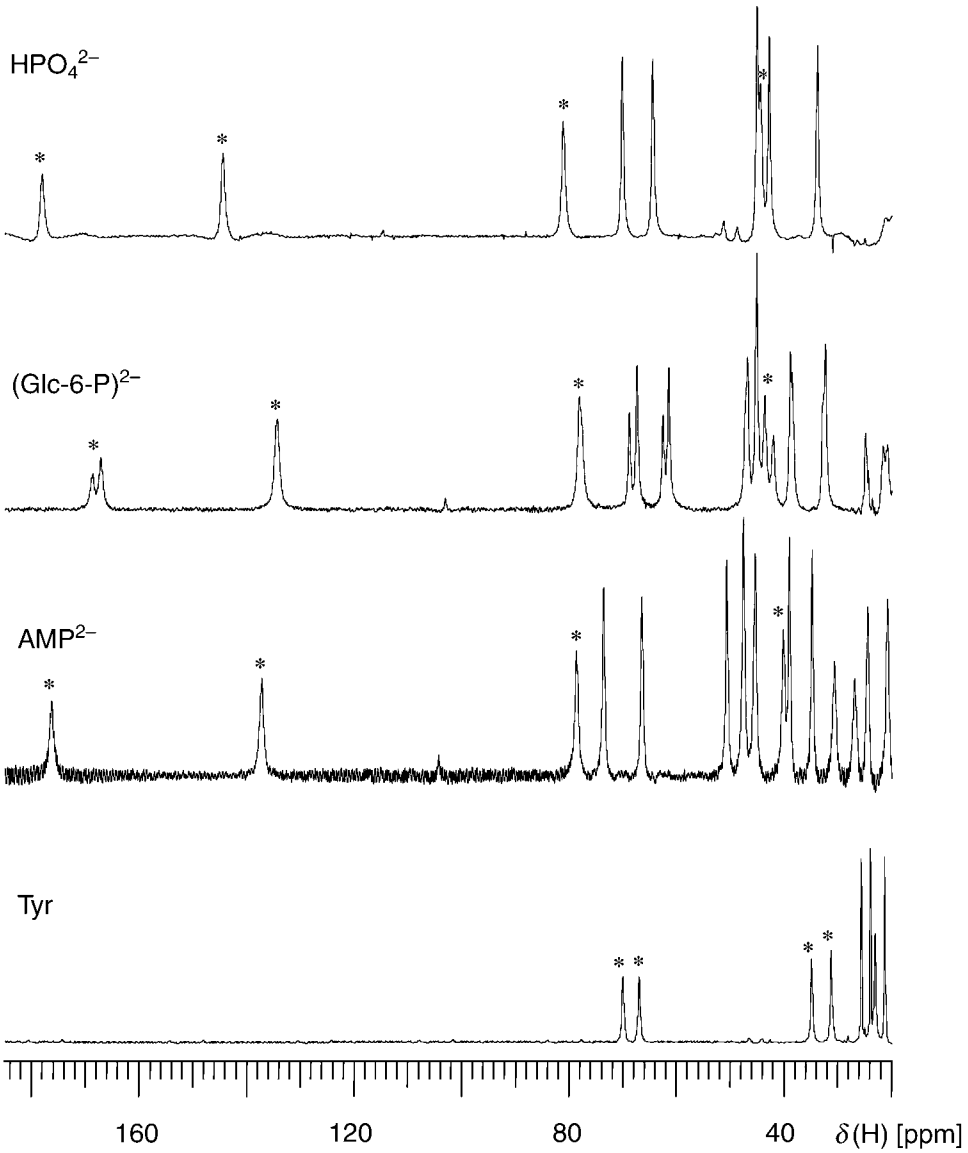


Fig. 4. *^{1}H-NMR Spectra* (500 MHz, 295 K, pH 7.4, D_2O) *for selected anion adducts of [Yb(***4b***)$(OH_2)_2$]$^{3+}$ showing the most paramagnetically shifted resonances*

different in the relative intensity and form of the MD-allowed $\Delta J = 1$ manifold (3 components) and the hypersensitive ED-allowed $\Delta J = 2$ transition manifold. In particular, emission for the (*O*-P-Tyr)$^{2-}$-bound adduct was distinctive from the (*O*-P-Ser)$^{2-}$ species (and the (*O*-P-Thr)$^{2-}$ analogue) and was characterized by a relatively more intense transition at 590 nm and a $\Delta J = 2/\Delta J = 1$ intensity ratio that was 50%

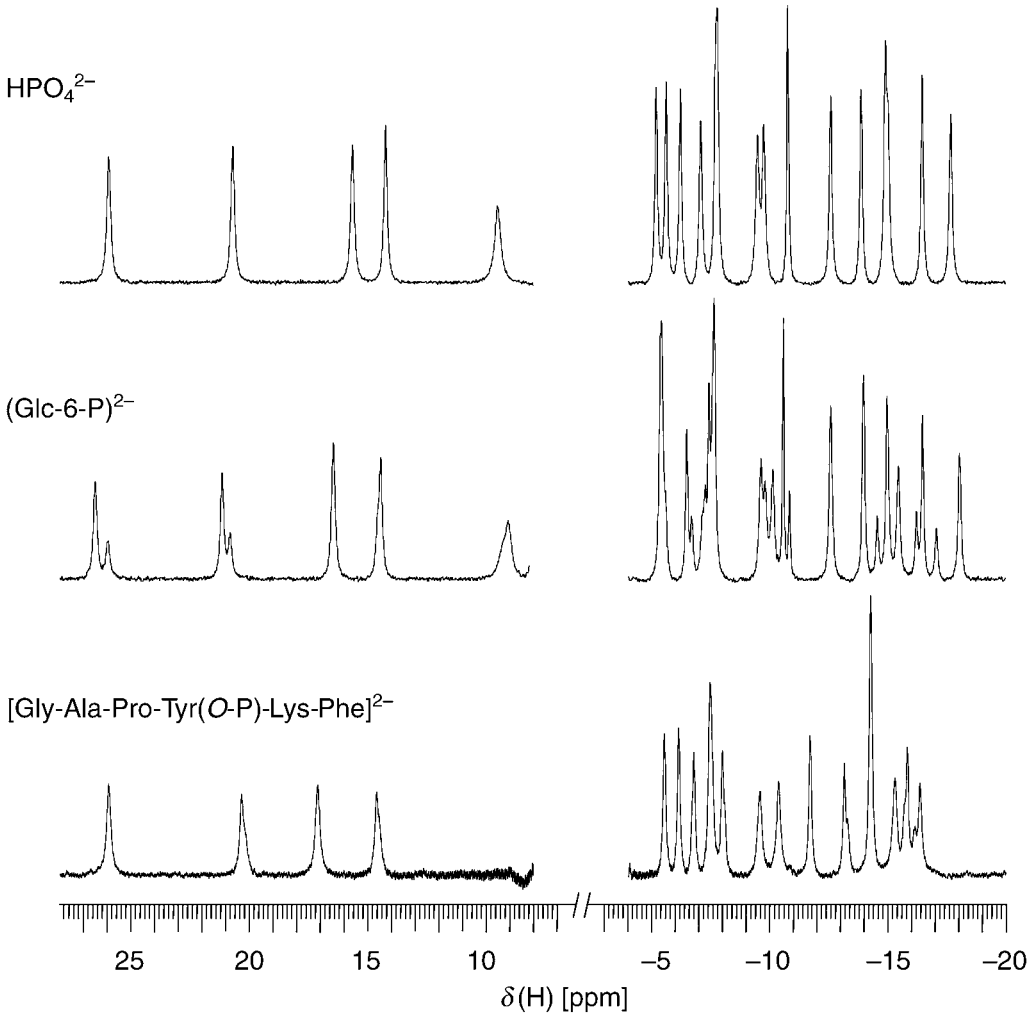


Fig. 5. *^{1}H-NMR Spectra* (500 MHz, 295 K, D_2O; 2 mM complex, 10 mM anion), *for the ternary anion complexes of [Eu(***4a***)$(OH_2)_2]^{3+}$ with HPO_4^{2-}* (upper), *$(Glc\text{-}6\text{-}P)^{2-}$* (center: splitting at higher frequency relates to isomeric saccharide complexes[1])) *and the mono-*O*-phosphorylated peptide Gly-Ala-Pro-Tyr(*O*-P)-Lys-Phe, revealing the common phosphate-anion ligation*

lower than for other phosphate anion adducts (*Fig. 7*). Addition of a 100-fold excess of $cAMP^-$ gave rise to no significant change in the spectrum of the parent aqua species, whereas addition of AMP^{2-} gave a spectrum that was near-identical to that obtained with HPO_4^{2-} and $(Glc\text{-}6\text{-}P)^{2-}$.

The distinctive behavior of the $(O\text{-}P\text{-}Tyr)^{2-}$ adduct was probed further by examining the emission spectra of the adducts of [Eu(**2**)(OH_2)] with three *O*-phosphorylated hexapeptides, Gly-Ser(*O*-P)-Pro-Tyr(*O*-P)-Lys-Phe, Gly-Ala-Pro-Tyr(*O*-P)-Lys-Phe and Gly-Ser(*O*-P)-Pro-Phe-Lys-Phe. The former two spectra were

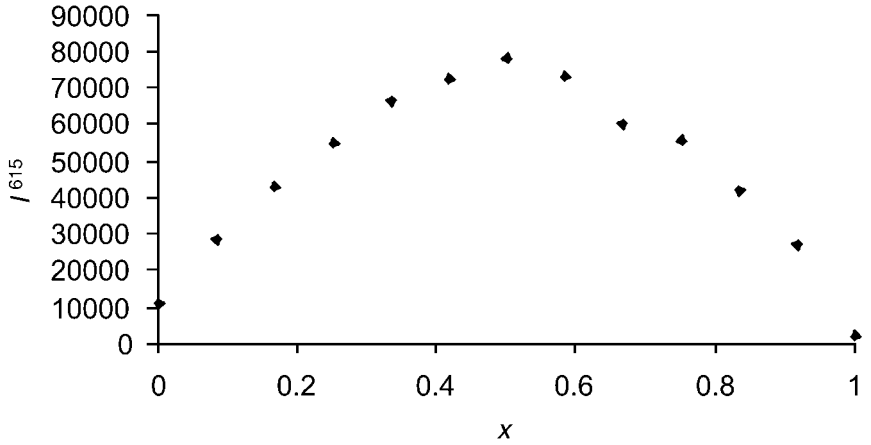


Fig. 6. *Job plot establishing 1:1 binding stoichiometry for the association of* AMP^{2-} *with* $[Eu(\mathbf{1})(OH_2)]^{3+}$ (pH 7.4, 0.1M MOPS). $x = [AMP^{2-}]/([AMP^{2-}] + [[Eu(\mathbf{1})(OH_2)]^{3+}])$.

identical ($\Delta J = 2/\Delta J = 1$ ratio 2:5) and the latter was distinctly different [6] with a 3.6:1 $\Delta J = 2/\Delta J = 1$ band intensity ratio. Such behavior is consistent with selective ligation at the $(O$-P-Tyr$)^{2-}$ site as had been suggested by the parallel NMR studies discussed above. The relative affinity of $[Eu(\mathbf{2})(OH_2)]$ for $(O$-P-Ser$)^{2-}$ and $(O$-P-Tyr$)^{2-}$ was measured (295 K, pH 7.4, 0.1M MOPS, 5 mM $NaHCO_3$) by plotting the change in the ratio of the 616/595 nm bands as a function of added phosphate anion and calculating an apparent affinity constant for a 1:1 equilibrium process. Iterative data fitting gave values of $\log K = 2.7$ (± 0.1) and 4.2 (± 0.2), respectively, consistent with the preferential binding of the $(O$-P-Tyr$)^{2-}$ anion. The origins of this chemoselectivity are intriguing but may relate to the lesser free energy of hydration of free $(O$-P-Tyr$)^{2-}$ in aqueous media.

Circularly Polarized Luminescence (CPL) Emission Signaling of Phosphate-Anion Binding. – CPL Spectroscopy is the emission analogue of circular dichroism and is well-suited to report on the local chirality of the lanthanide ion in appropriate chiral complexes [11][12]. CPL Spectra for $[Eu(\mathbf{4a})(OH_2)_2]^{3+}$ and $[Eu(\mathbf{4b})(OH_2)_2]^{3+}$ were recorded in the presence of a tenfold excess of HPO_4^{2-}, (Glc-6-P)$^{2-}$, (O-P-Tyr)$^{2-}$, (N-Ac-O-P-Tyr)$^{2-}$, and (Gly-Ala-Pro-Tyr(O-P)-Lys-Phe)$^{2-}$. Particular attention was paid to the analysis of the $^5D_0$-$^7F_1$ transition, following excitation at the maximum of the $^5D_0$-$^7F_0$ transition at 579.4 nm. Typically g_{lum} values of ± 0.06 were observed for the most polarized transitions. With $[Eu(\mathbf{4a})(OH_2)_2]$, CPL spectra for HPO_4^{2-} and (Glc-6-P)$^{2-}$ adducts were almost identical and were distinctively different from those with the other three phosphate anions examined (*Fig. 8*). More marked differences were observed with complexes of (R,R,R)-Λ-$[Eu(\mathbf{4b})(OH_2)_2]$ and (S,S,S)-Δ-$[Eu(\mathbf{4b})(OH_2)_2]$. In each case, three transitions were observed (the shoulders in spectra with $(O$-P-Tyr$)^{2-}$ and HPO_4^{2-} in particular are artifacts), consistent with the presence of one major solution species that had been revealed by ^{1}H-NMR studies. Almost mirror-image spectra were obtained for the stereoisomeric series of Δ and Λ complexes, and the ternary adducts

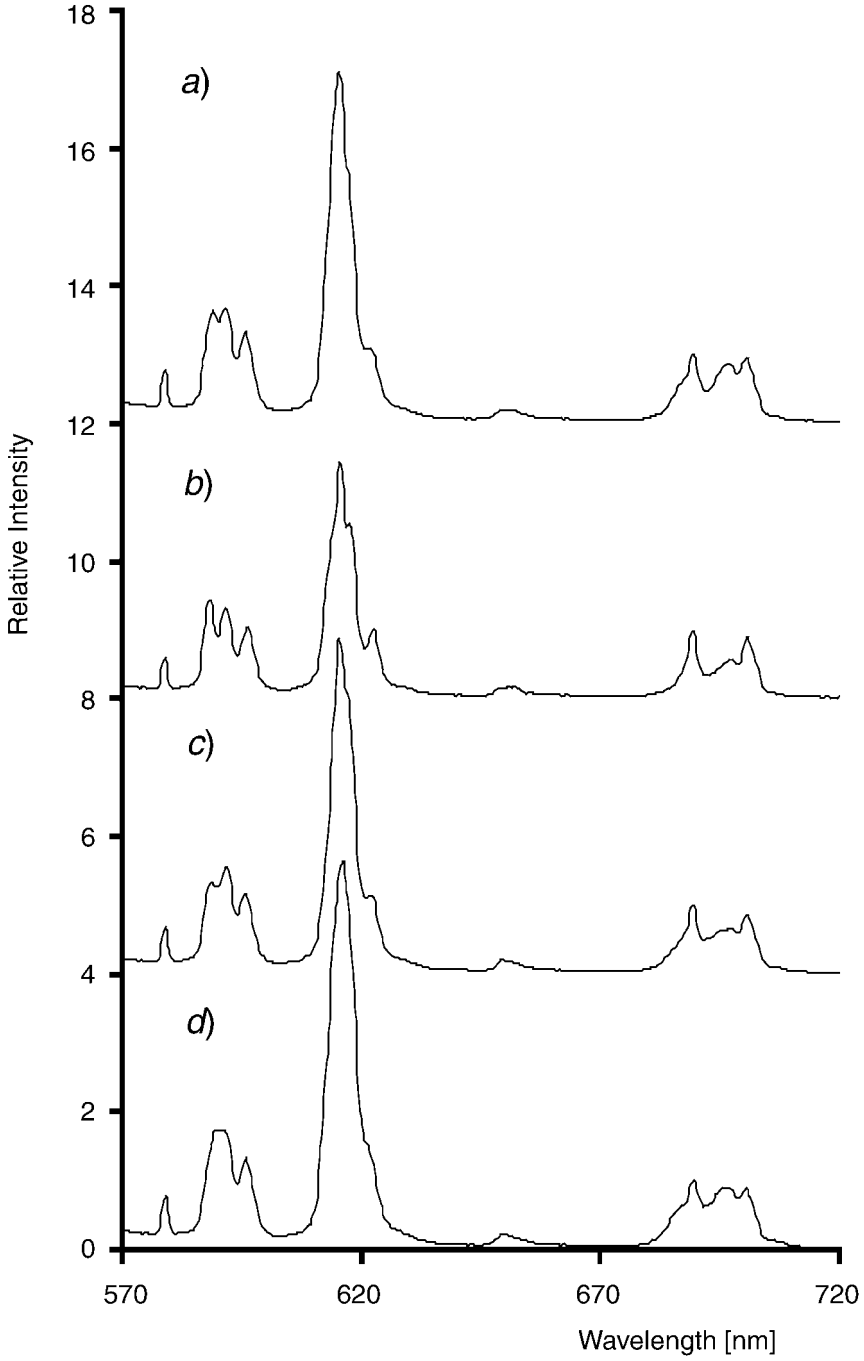


Fig. 7. *Europium emission spectra for [Eu(**2**)(OH$_2$)]* (0.1 mM) *in the presence of 1 mM added phosphate anion* (pH 7.4, 295 K, 0.1M MOPS). a) (*O*-P-Ser)$^{2-}$, b) (*O*-P-Tyr)$^{2-}$, c) (Glc-6-P)$^{2-}$, and d) HPO_4^{2-}.

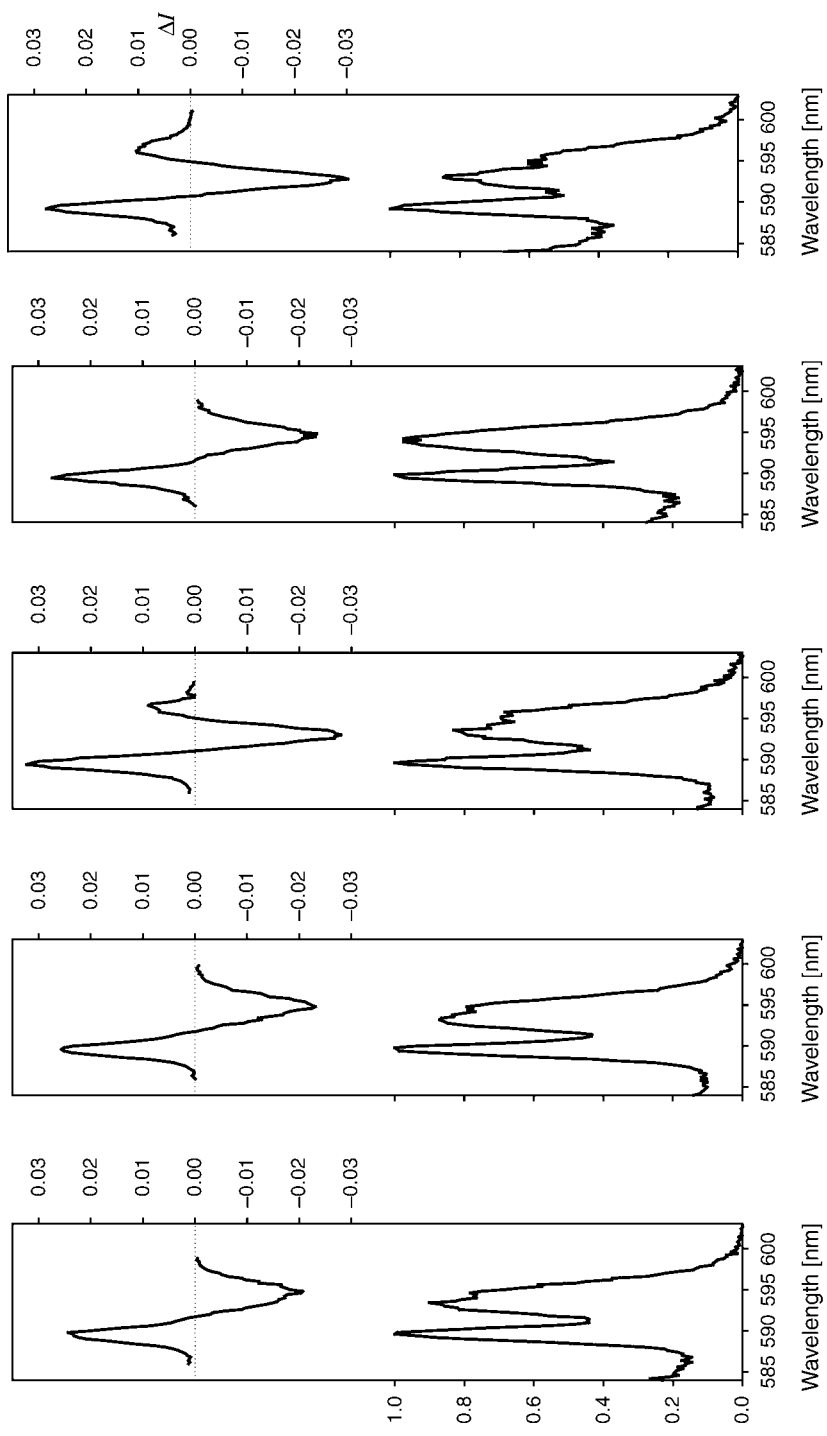


Fig. 8. *CPL Spectra of the* $\Delta J = 1$ *transitions for* $[Eu(\mathbf{4a})(OH_2)_2]^{3+}$ *in the presence of 10 equiv. of the given phosphate anions* (293 K, pH 7.4, 0.1M MOPS)

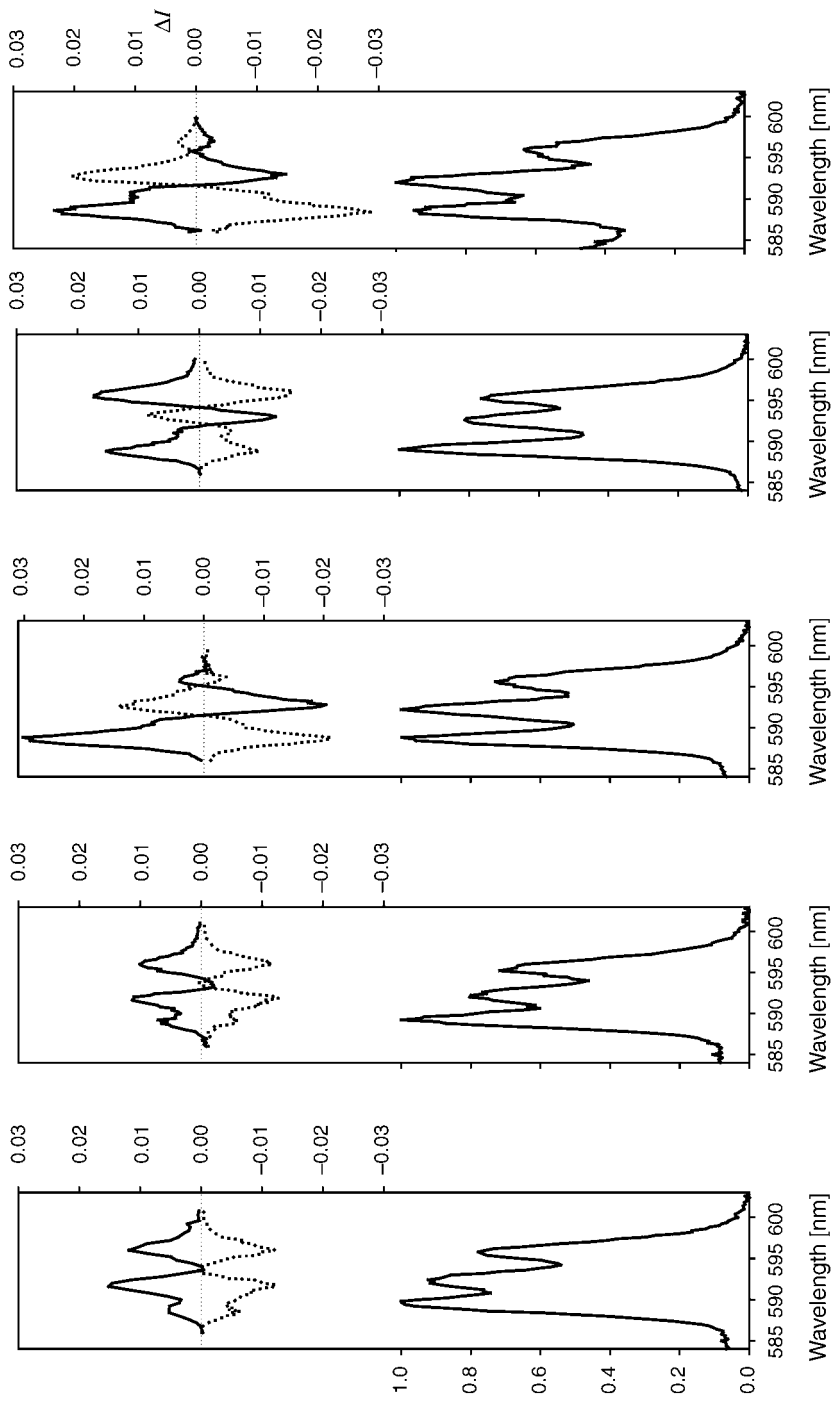


Fig. 9. *CPL Spectra for* (R,R,R)-*Λ-[Eu*(**4b**)*(OH$_2$)$_2$]$^{3+}$ (bold) *and* (S,S,S)-*Λ-[Eu*(**4b**)*(OH$_2$)$_2$]$^{3+}$ (dashed) *in the presence of 10 equiv. of the given phosphate anions* (0.1M MOPS, pH 7.4, 293 K)

with HPO_4^{2-} and $(Glc\text{-}6\text{-}P)^{2-}$ were near identical in overall form. However, the similar spectra for $(N\text{-}Ac\text{-}O\text{-}P\text{-}Tyr)^{2-}$ and the *O*-phosphorylated hexapeptide (Gly-Ala-Pro-Tyr(*O*-P)-Lys-Phe)$^{2-}$ differed clearly from that due to $(O\text{-}P\text{-}Tyr)^{2-}$ (*Fig. 9*), notwithstanding their very similar ^{1}H-NMR spectral profiles. In particular, the sign of the transition at 592 nm was positive for (R,R,R)-Λ-[Eu(**4b**)$(OH_2)_2$]/$(N\text{-}Ac\text{-}O\text{-}P\text{-}Tyr)^{2-}$ but negative for (R,R,R)-Λ-[Eu(**4b**)$(OH_2)_2$]/$(O\text{-}P\text{-}Tyr)^{2-}$. The origins of this difference are not clear at present.

Conclusions. – ^{1}H-NMR Spectroscopy offers a convenient means of assessing the structure of the adducts of various phosphate anions. Affinity is greatest for complexes of Yb and Tm, for which N-terminal ligation is a competitive binding process, with simple amino acids and peptides [8]. With Eu analogues, phosphato ligation is the preferred binding mode, and, amongst the three *O*-phosphorylated amino acids, a marked selectivity for *O*-phosphono-L-tyrosine residues was discovered (*ca.* 30:1). Selective binding at a $(O\text{-}P\text{-}Tyr)^{2-}$ site to a Eu-center may be distinguished by emission spectroscopy. This work suggests that appropriately functionalized lanthanide complexes related to the series studied here may prove to be valuable probes for *O*-phosphorylated peptides/proteins – either aiding in NMR solution structural analysis (relaxation/shift behavior) [13] or serving themselves as optical probes with the potential for monitoring in real time, *O*-phosphorylation bursts at tyrosine sites, of particular interest to our understanding of cell biology and signal-transduction processes.

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Experimental Part

General details for lanthanide-complex synthesis, instrumentation for NMR, and luminescence methods are reported elsewhere [5][8][9][10].

The circularly polarized luminescence and total luminescence spectra were recorded with an instrument described previously [14], operating in a differential photon-counting mode. Excitation and emission measurements for Eu^{III} complexes were accomplished by using a *Coherent 599* tunable dye laser (0.03 nm resolution) with a *Coherent Innova-70* Ar ion laser as a pump source. The laser dye used in all measurements was rhodamine 6G dissolved in ethylene glycol. Calibration of the emission monochromator (and subsequently the dye-laser wavelength) was accomplished by passing scattered light from a low-power He–Ne laser through the detection system. The error in the dye-laser wavelength is assumed to equal the resolution of the emission monochromator (0.1 nm). The optical detection system consisted of a focusing lens, long-pass filter, and 0.22-m monochromator. The emitted light was detected by a cooled *EMI 9558B* photo-multiplier tube operating in photon-counting mode.

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