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Aqueous Shift Reagents for High-Resolution Cation NMR Spectroscopy. 4.† DybPPPpob^{5-} ‡

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The chelate Dy(PPP)$_2^{7-}$ (where PPP^{5-} is tripolyphosphate) is a very effective shift reagent for the ^{23}Na NMR signal. However, it is also quite toxic to living animals. In addition to the effects of the equilibrium competitions of the biological metal cations Mg^{2+} and Ca^{2+} with Na^+ for Dy(PPP)$_2^{7-}$, this toxicity is most likely due to the potentially irreversible effects of ligand dissociation in vivo. In order to avoid this latter problem, two PPP moieties have been covalently linked so as to take advantage of the chelate effect. Thus, this paper reports the synthesis of the new shift reagent DybPPPpob^{5-} (where bPPPpob^{8-} is *o*-bis((3-(tripolyphosphato)propyl)oxy)benzene). In vitro ^{23}Na and ^{31}P NMR experiments show that (1) DybPPPpob^{5-} is an upfield shift reagent (like Dy(PPP)$_2^{7-}$), which shifts the ^{23}Na signal more than a third as well as Dy(PPP)$_2^{7-}$, (2) the ^{23}Na shift has a pH profile similar to that of Dy(PPP)$_2^{7-}$, (3) competition by Ca^{2+} and Mg^{2+} with Na^+ for DybPPPpob^{5-} is not as severe as it is for Dy(PPP)$_2^{7-}$ and (4) DybPPPpob^{5-} hydrolysis is catalyzed by alkaline phosphatase but neither as rapidly nor as extensively as that of Dy(PPP)$_2^{7-}$. This latter result presumably implies that an uncoordinated PPP moiety of an incompletely dissociated DybPPPpob^{5-} chelate can still act as a substrate for the enzyme. The hydrolysis is slow enough that, with proper care, DybPPPpob^{5-} may be useful as an in vivo shift reagent.

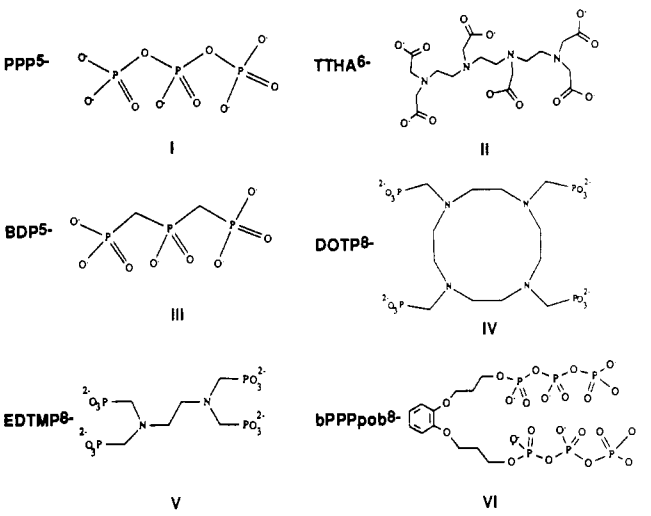
Introduction

One of the fundamental problems in the interpretation of metal cation NMR spectra from tissue is the isochronicity of the signals from different compartments. From 1981 to 1984, four groups independently introduced a number of different aqueous shift reagents (SRs) that could contribute to the solution of this problem.[2-7] All of these compounds are anionic chelates of paramagnetic lanthanide ions. These developments have been recently reviewed.[8-10]

Of all the compounds studied to date, the complex introduced by Gupta and Gupta, Dy(PPP)$_2^{7-}$ (the ligand is shown in I of Chart I), is the most effective SR.[7] However, the chemistry of Ln(PPP)$_2^{7-}$ chelates makes them susceptible to a number of deleterious reactions in vivo. Scheme I (where Ln^{3+} represents any lanthanide ion) reveals part of the situation. The process mostly responsible for causing the observed shift of the ^{23}Na NMR signal is that labeled as A. Most of the other processes are competitive and thus serve to decrease the observed shift. Evidence for the protonation equilibria, processes B and C,[7,11-14] and for the competition from other metal ions (M^{m+}), process D,[7,13-15] has been amply reported. This competition is particularly severe from divalent cations, such as the Mg^{2+} and Ca^{2+} present in physiological systems. Of course, M^{m+} can also represent a monovalent cation, such as K^+. If one is considering shifts of the ^{39}K, ^{25}Mg, or ^{43}Ca resonances, Na^+ in Scheme I should be replaced with K^+, Mg^{2+}, or Ca^{2+} and any Na^+ present would be included in the M^{m+}.

However, processes B-D, and others like them that are not shown, are reversible and could perhaps be tolerated. It is equilibria like E that probably lead to the most serious problems. (Dissociation of the protonated ligand (not shown) would be even more likely.) There is clear evidence reported for processes like E.[15-18] In each of the papers cited, the authors report larger shifts of the observed resonance if the stoichiometric molar ratio of PPP^{5-} to Ln^{3+} is greater than 2 (but, see ref 14). The fact that this shift goes through a maximum for a ratio of PPP^{5-} to Ln^{3+} between 2 and 3[16,18] rules out process F as a major contribution, although Peters and co-workers have suggested its existence in a kinetic

Chart I



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‡ Abbreviations used in this paper are as follows: PPP^{5-}, tripolyphosphate; bPPPpob^{8-}, *o*-bis((3-(tripolyphosphato)propyl)oxy)benzene; SRs, shift reagents; Ln^{3+}, any lanthanide ion; TTHA^{6-} triethylenetetraminehexaacetate; BMS, bulk magnetic susceptibility; BDP^{5-}, bis(phosphonatomethyl)phosphinate; DOTP^{8-}, tetraazacyclododecanetetrayl-*N*,*N*′,*N*″,*N*‴-tetrakis(methylene)tetrakis(phosphonate); DOTA^{4-}, 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetate; EDTMP^{8-}, ethylenediaminetetrakis(methylene)tetrakis(phosphonate); DMSO, dimethyl sulfoxide; SAX, strong anion exchange; HEPES$^-$, 4-(2-hydroxyethyl)-1-piperazineethanesulfonate; TMA$^+$, tetramethylammonium; ATP, adenosine triphosphate; EDTA^{4-}, ethylenediaminetetraacetate; DTPA^{5-}, diethylenetriaminepentaacetate.

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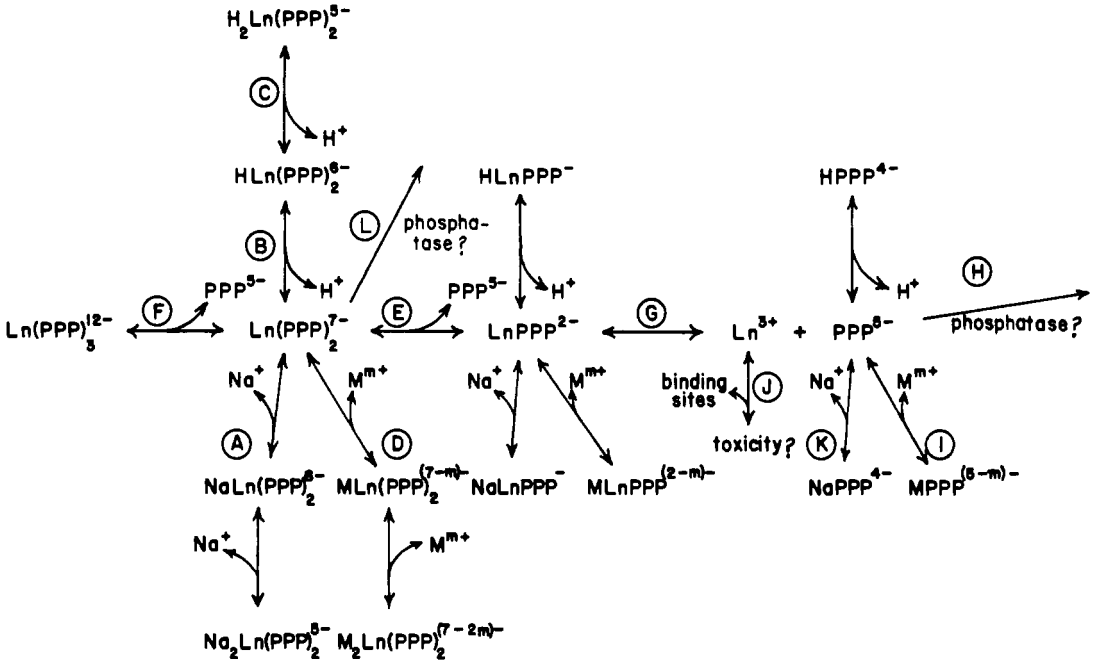
Scheme I



pathway.[19] The average lifetime of a PPP^{5-} ligand in a $Ln(PPP)_2^{7-}$ molecule is about 1 ms at 25 °C (pH not given).[19] The apparent dissociation constants for processes E and G can be estimated to be ca. $10^{-3.9}$ and 10^{-5}, respectively.[7,11,13,20,21] The most serious aspect of processes like E is that they probably lead, through processes like G, to (1) the irreversible (enzymatic?) degradation of the PPP^{5-} ligand, process H, (2) the formation and possible precipitation of M^{m+}/PPP^{5-} species, reaction I, and/or (3) the potentially irreversible binding of Ln^{3+} to macromolecular sites, process J.

The degradation of $Dy(PPP)_2^{7-}$ to inorganic phosphate in the presence of fresh rabbit kidney brush-border membranes,[22] rat muscle,[23] and *Escherichia coli*[24] has been monitored with ^{31}P NMR spectroscopy. This is probably also the source of the temporal decrease in the shift of the ^{23}Na signal observed after $Dy(PPP)_2^{7-}$ is introduced into suspensions of rabbit renal cortical proximal tubules,[15] neuroblastoma cells,[25] *E. coli*,[24] renal epithelial cells,[26] and a sample of rat muscle tissue.[23] Several investigators have also observed a disappearance of the shift of the external Na signal in suspensions of human erythrocytes[27,28] and yeast cells[29] upon lysis of the cells. This could be due to the release of intracellular phosphatases, among other things. Boulanger et al.[30] have attributed a gradual broadening of the ^{31}P resonances of human erythrocyte metabolites to the decomposition of extracellular $Dy(PPP)_2^{7-}$. Although these authors postulate that free Dy^{3+} then enters the cells, this is not proven. Apparently to avoid such problems, Wittenberg and Gupta, in a study of suspensions of myocytes, employed the laborious procedure of brief, sequential exposures of the cells to $Dy(PPP)_2^{7-}$, each followed by a thorough washing.[31]

There are many papers reporting the properties of the processes represented by equilibrium I in Scheme I.[7,13,19,32] For many years PPP^{5-} has been used to sequester Mg^{2+} and Ca^{2+} in the detergent industry. Even if process I was not irreversible in a given system, the use of $Ln(PPP)_2^{7-}$ might prove to be toxic because reversible processes D and I can serve to lower the activities of M^{m+} ions below critical levels. The apparent formation constant for process I, when M^{m+} is Ca^{2+}, is ca. 10^5,[33,34] and that for process D, when M^{m+} is Ca^{2+}, is ca. $10^{4.5}$.[13] It should be noted that in a simple in vitro situation, and low levels of Ca^{2+}, process D formally competes with only the *sum* of processes E and I, for which the overall apparent K is only ca. 10^1 when M^{m+} is Ca^{2+}. It has been directly shown, with ^{43}Ca NMR, that $Dy(PPP)_2^{7-}$ can remove Ca^{2+} from calmodulin.[13] This kind of reaction might be a particular problem with Ca^{2+} in excitable systems.

Although there have been reports of perfusion of rat[35] and frog[36] hearts with $Dy(PPP)_2^{7-}$, insufficient details of the physiological state of the tissue were given to allow the reader to assess the effects of the reactions shown in Scheme I. Burstein and Fossel do report that the frog heart rate is cut in half and that the mechanical contractions almost cease.[36] Naritomi et al. report that the administration of $Dy(PPP)_2^{7-}$ to gerbils almost always resulted in rapid cardiorespiratory arrest.[37]

In order to avoid the problems outlined above, we have used the SR $DyTTHA^{3-}$ [7] (the ligand is shown in II of Chart I) in studies of suspensions of human erythrocytes[27] and yeast cells.[38] We have also shown that it can be perfused into rat hearts[39,40]

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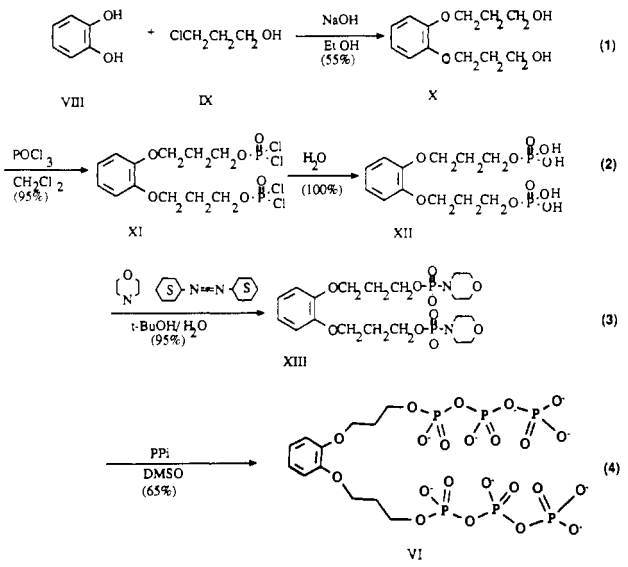
Scheme II

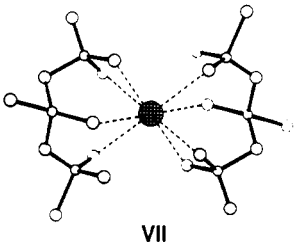


and shark rectal glands[9] with few, if any, harmful effects. Others have shown this with perfused rat mandibular glands and pancreas.[41] In confirmation, there are recent reports of the successful infusion of living animals with $DyTTHA^{3-}$.[37,42-45] This relative nontoxicity is almost certainly due to the fact that the ligand dissociation equilibrium (analogous to processes E and G in Scheme I) has a very small apparent dissociation constant, ca. 10^{-23}.[7,46]

Although $DyTTHA^{3-}$ is quite useful as a SR, the resolution that it induces in the ^{23}Na spectrum of an intact organism appears to be somewhat limited by bulk magnetic susceptibility (BMS) effects.[43,47] Thus, one would desire a compound with its low toxicity but with the greater shifting potency of the $Dy(PPP)_2^{7-}$ complex. There have been a number of clever approaches to attaining such a species. Elgavish and Elgavish have produced the $Dy(BDP)_2^{7-}$ chelate (the ligand is shown in III of Chart I), which should not be susceptible to process H in Scheme I because of the lack of high-energy P–O–P linkages.[22] Indeed, it does not appear to be hydrolyzed in tissue and, what is more, the inherent shifting potency is as large as that of $Dy(PPP)_2^{7-}$. Unfortunately, however, the protonation equilibria (analogous to processes B and C in Scheme I) are shifted in the direction of the protonated species to such an extent that the shift is severely reduced at physiological pH.[22] Sherry and co-workers have reported the $DyDOTP^{5-}$ complex (the ligand, based on $DOTA^{4-}$,[3] is shown in IV of Chart I), which should contain the hydrolysis resistance of $Dy(BDP)_2^{7-}$ and the dissociation resistance of $DyTTHA^{3-}$.[48,49] Indeed, both of these appear to be true and the shifting potency is almost as great as that of $Dy(PPP)_2^{7-}$. Unfortunately, the protonation equilibrium should be shifted in the same direction as for $Dy(BDP)_2^{7-}$ and, it turns out, that $DyDOTP^{5-}$ is more sensitive to process D when M^{m+} is Ca^{2+} and is "sequestered by muscle tissue" in some unknown manner which renders it ineffective as a SR in vivo.[48] In a similar vein, Yarmush and Chu have studied the effectiveness of $Dy(EDTMP)_2^{13-}$ (the ligand is shown in V of Chart I) as a shift reagent.[21,50] This complex produces large shifts at high pH[21] but smaller shifts at physiological pH.[50] It has not been studied in vivo.

The present paper reports our attempt to inhibit processes like E by covalently linking two PPP moieties in order to take advantage of the chelate effect. Such a chelate would provide a test for the proposition that the hydrolysis exhibited in vivo is indeed due to a process like H in Scheme I, where the free ligand is the substrate for one or more enzymatic reactions. If this were the case, the new chelate should be susceptible only to reversible processes like B–D. If, on the other hand, the bound ligand can still serve as a substrate for phosphatase enzymes in a process analogous to L in Scheme I, the new complex could also be consumed in tissue.

The ligand we have synthesized for the new chelate $DybPPPpob^{5-}$ is *o*-bis((3-(tripolyphosphato)propyl)oxy)benzene (VI, $bPPPpob^{8-}$). A 10 atom covalent link was chosen in $bPPPpob^{8-}$ with the hope of causing minimal changes in the (unknown) structure of $Ln(PPP)_2^{7-}$. A solution structure, VII,



VII

has been proposed for the latter by Nieuwenhuizen et al.[19] that is quite similar to a structure proposed by ourselves[7] (see structure XIV in the Discussion). Belton and co-workers have recently proposed a slightly different structure.[14]

The ligand synthetic scheme we devised was based on methods previously described for the preparation of nucleoside polyphosphates.[51,52] Briefly, these involved syntheses of nucleoside phosphoromorpholidates, which then reacted with phosphates or polyphosphates in anhydrous DMSO to form the desired polyphosphates. Phosphoromorpholidates have much longer storage lives[51-54] than those of the analogous phosphoimidazoles.[55]

Another potential source of toxicity in the use of $Dy(PPP)_2^{7-}$ to infuse living animals is the hyperosmolality of the infusing solution.[56] Although this is not a problem with studies of perfused tissue, analogous solutions of $DybPPPpob^{5-}$ will be less hyperosmolal.

Experimental Section

Synthesis of the bPPPpob Ligand. The overall synthetic sequence is shown in Scheme II.

General Procedures. Pyridine was dried by refluxing over and then distilling from CaH_2. Methylene chloride was dried by distilling from P_4O_{10}. Reagent-grade benzene was fractionated and a middle cut taken. Dimethyl sulfoxide was fractionally distilled in vacuo (1 mmHg) and a middle cut taken (bp 53–54 °C). All of the dried solvents were stored over 4-Å molecular sieves. Morpholine and phosphorus oxychloride were freshly distilled before use. All other chemicals were reagent-grade and

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were used without further purification.

o-Bis((3-hydroxypropyl)oxy)benzene (X). This was prepared by the method of Kyba et al.[57]

o-Bis((3-(dichlorophosphoryl)propyl)oxy)benzene (XI). Freshly distilled phosphorus oxychloride (9.18 g, 60 mmol) was dissolved in 5 mL of methylene chloride and cooled to 0 °C. A quantity of X (2.26 g, 10 mmol) in 10 mL of methylene chloride was added dropwise over 30 min to the stirred solution. A slow stream of N_2 was bubbled through the reaction mixture to remove the HCl generated. The solution was stirred at 0 °C for an 1 h and then for 1 h at room temperature. The solvent was evaporated under reduced pressure (40 mmHg at 25 °C) and the excess phosphorus oxychloride removed in vacuo (1 mmHg at 25 °C). The crude material was a brown viscous oil and was used without further purification. Isolated: 4.38 g (95%). ^{1}H NMR ($CDCl_3$): δ 6.9 (m, aromatic), 4.61, 4.58 (2 t, CH_2O–P, J_{POCH} = 9.9, J_H = 6.1 Hz), 4.14 (t, $-OCH_2-$, J_H = 5.8 Hz), 2.31 (pentet, $-CH_2-$, J_H = 5.9 Hz).

o-Bis((3-phosphatopropyl)oxy)benzene (XII). A quantity of the crude product XI (4.3 g, 9.5 mmol) was added dropwise over 10 min to 100 mL of a vigorously stirred slurry of ice and water. The stirred mixture was allowed to warm slowly to room temperature over 1 h, yielding a faintly yellow homogeneous solution. After the solution was stirred at room temperature an additional 2 h, the water was evaporated in vacuo (1 mmHg at room temperature). The remaining oil was evaporated once more with 5 mL of water and then left overnight in vacuo (1 mmHg) over P_4O_{10} at 5 °C. The product was a viscous green oil weighing 4.1 g. It was used immediately in the next step.

Bis[N,N'-dicyclohexyl-4-morpholinecarboxamidine] Salt of o-Bis((3-(4-morpholinophosphonato)propyl)oxy)benzene (XIII). A quantity of the crude product XII (4.0 g, 9.0 mmol) was dissolved in 100 mL of water, and morpholine (8.7 g, 100 mmol) was added together with 100 mL of *tert*-butyl alcohol. The solution was brought to a gentle reflux and a solution of dicyclohexylcarbodiimide (20.6 g, 100 mmol) in 200 mL of *tert*-butyl alcohol added over 3 h. Reflux was continued for 6 h and then the solution concentrated to 125 mL under reduced pressure (40 mmHg at 35 °C). The byproduct, dicyclohexylurea, was removed by filtration and washed with 50 mL of water. The combined filtrates were extracted with 3 × 40 mL of ether. The aqueous phase was evaporated to dryness in vacuo (1 mmHg at 20 °C). Residual water was removed by dissolving the product in methanol and reevaporating the solution to dryness. The product, a viscous oil, was dissolved in 20 mL of methanol and the solution added to 200 mL of dry ether, which precipitated it as an oil. The solvent was decanted and the oil triturated several times with 100 mL aliquots of dry ether, which converted it to a white powder. The product was isolated by filtration, washed with ether, and dried in vacuo (1 mmHg at room temperature). Isolated: 10.0 g. Analysis by HPLC (25 cm × 4.6 mm Whatman Partisil 10/25 SAX column; isocratic elution with 0.20 M KH_2PO_4 (pH 4.5) at 1 mL/min; UV detection at 280 nm) showed two peaks in a 1:20 ratio with retention times of 5.5 and 7.5 min. They were attributed to the mono- and bis(phosphoromorpholidates), respectively. If necessary, purification can be effected by using a column of Bio-Rad AG 1 X-8 resin (bicarbonate form) with a linear gradient of triethylammonium bicarbonate. The unpurified salt could be stored in a desiccator at −20 °C for at least 1 year with no noticeable change.

o-Bis((3-(tripolyphosphato)propyl)oxy)benzene (VI). Tetrasodium pyrophosphate decahydrate (4.61 g, 11.0 mmol) dissolved in 70 mL of water was applied to a 23 × 2.3 cm column of Bio-Rad AG 50W X-8 resin (pyridinium form) and the column eluted with 350 mL of water. The solution of the pyridinium salt of pyrophosphoric acid was concentrated to 20 mL. A solution of tri-*n*-butylamine (8.57 g, 46 mmol) in 50 mL of pyridine was added and the solution evaporated to dryness. The salt was rendered anhydrous by azeotropic drying with 5 × 30 mL of pyridine. Residual pyridine was removed by evaporating with 4 × 40 mL of benzene. All evaporations were conducted in vacuo (1 mmHg at 20 °C). The phosphoromorpholidate salt (XIII) (1.30 g, 1.1 mmol) was dried by using pyridine and benzene as above. The pyrophosphate salt was dissolved in 10 mL of DMSO with gentle warming (35 °C) and the solution added to the morpholidate. The flask was rinsed with 2 × 5 mL of DMSO and these washings also added. The reaction was kept at 30 ± 1 °C for 36 h. The reaction mixture was poured into 75 mL of ice water and applied to a 37 × 3.3 cm column of DEAE cellulose (HCO_3^- form). The column was washed with 200 mL of water and then eluted with a linear gradient of triethylammonium bicarbonate (0.01–0.40 M; 1.5 L each of initial and final buffers). Fractions of 15 mL were collected at a flow rate of 2 mL/min. The separation was monitored by UV spectroscopy at 280 nm and also by ^{31}P NMR spectroscopy. The product eluted with 0.25 M buffer. Tubes containing the product were combined, and the water was evaporated in vacuo (1 mmHg at 20 °C). Residual buffer was removed by evaporating with 4 × 20 mL of methanol. The product was dissolved in 3 mL of methanol and added dropwise to a stirred solution of sodium iodide (3.0 g, 20 mmol, excess) in 75 mL of acetone. The hexasodium salt (i.e., Na_6H_2bPPPpob) precipitated from the acetone. It was isolated by filtration, washed with acetone, and then dried in vacuo (1 mmHg at room temperature). Isolated: 667 mg (80%). ^{31}P NMR (D_2O): δ −5.00 (d), −9.75 (d), −20.90 (t). ^{1}H NMR (D_2O, CH_3CN = 1.93): δ 7.0 (m, aromatic), 4.11 (t, $-OCH_2$), 4.03 (q, $-CH_2O$–P), 2.04 (pentet, $-CH_2-$).

Chelate Formation. *TMA/HEPES Buffer Solution.* A stock solution of HEPES buffer was prepared by dissolving 9.65 g (34 mmol) of HHEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) plus 1.85 g (10.2 mmol) of TMAOH (tetramethylammonium hydroxide) in 38 mL of D_2O. The resulting solution was diluted with doubly distilled water to 250 mL. This gave a stock solution that was 40.8 mM in TMA^+-$HEPES^-$ and 95.2 mM in HHEPES. The measured pH was 6.9.

Formation of X_5DybPPPpob·3XCl (Where $X^+ = Na^+$ and TMA^+). The reaction to form the SR DybPPPpob^{5-} is that shown as eq 5, where $HEPES^-$ is the basic form of the buffer.

$$Dy^{3+} + 2HEPES^- + H_2bPPPpob^{6-} \rightarrow DybPPPpob^{5-} + 2HHEPES \quad (5)$$

The following procedure is an example of that used to monitor the formation of DybPPPpob^{5-}. A quantity, 134.4 mg (157.5 μmol), of Na_6H_2bPPPpob was dissolved in 10.0 mL of the stock TMA/HEPES buffer solution. To a 1.60-mL aliquot of this solution were sequentially added small aliquots (7–15 μL) of an unbuffered aqueous solution 460 mM in $DyCl_3$. The molar ratio of Dy^{3+} to bPPPpob^{8-} was thus increased from zero to 1.1. After addition of each aliquot, the ^{31}P{^{1}H} and ^{23}Na NMR spectra of the resulting solution were obtained. Although this chelate preparation, and most studies in this paper, were accomplished in buffered solutions, the chelate can be formed without precipitation in unbuffered solutions if the Dy^{3+} is added very slowly and with continuous stirring. Of course, two equiv of base, e.g. NaOH, is also required to produce a neutral solution in such a procedure.

For subsequent studies of the properties of DybPPPpob^{5-}, one stock solution was prepared by dissolving 175.4 mg (206 μmol) of Na_6H_2bPPPpob in 8.0 mL of the TMA/HEPES buffer solution described above. To this solution was added 421 μL of the $DyCl_3$ solution (194 μmol of Dy^{3+}). Thus, in this preparation, there was an ca. 6% excess of the H_2bPPPpob^{6-} ligand. The resulting solution was diluted with buffer solution to 10.0 mL. Since it was slightly cloudy, the solution was centrifuged and the clear supernatant was retained. Before centrifugation, the concentration of shift reagent was 19.4 mM. A similar stock solution of concentration 34.3 mM, with less excess ligand, was also prepared.

Formation of $Na_7Dy(PPP)_2$·3NaCl. Stock solutions, 15 and 16 mM, of the shift reagent $Na_7Dy(PPP)_2$·3NaCl in the TMA/HEPES buffer solution were prepared as described.[5,7] The solutions resulting from these preparations were slightly cloudy and were centrifuged.

Formation of X_3DyTTHA·3XCl. A quantity of H_6TTHA (Sigma, 76.6 mg, 0.155 mmol) was dissolved in 5.0 mL of the TMA/HEPES buffer solution. To this was added 326 μL of 460 mM $DyCl_3$. The solution was stirred at 40 °C for 20 min. The pH was raised from 3.7 to 6.7 by the addition of 300 μL of 1 M NaOH: 1.2 mL of 1 M NaCl was also added. The final solution, after dilution to 10.00 mL with the TMA/HEPES buffer solution, was 15 mM in DyTTHA^{3-} and 150 mM in Na^+.

^{23}Na Shift Potencies of Shift Reagents (Shift Titrations). Several differing volumes of each of the various stock solutions of DybPPPpob^{5-}, $Dy(PPP)_2^{7-}$, and DyTTHA^{3-} were separately diluted with the buffer solution and 1 M NaCl (also in the TMA/HEPES buffer solution), such that the total volume of each sample was 2.0 mL (20% (v/v) D_2O) and the total Na^+ concentration was approximately constant at 150 mM. The ^{23}Na NMR spectrum was obtained for each sample.

pH Dependences of the ^{23}Na Shifts Induced by Shift Reagents. *DybPPPpob^{5-}.* A 2-mL sample of an unbuffered 18.2 mM solution (20% D_2O) of Na_3H_2DybPPPpob·3NaCl was prepared that was also 42.3 mM in NaCl. The pH of this sample was lowered from 3.2 to 2.1 by the addition of one 10-μL aliquot of 1 M HCl. The pH was then increased to 10.8 by the addition of several aliquots (totalling 50 μL) of a 1 M KOH solution. The ^{23}Na and ^{31}P{^{1}H} NMR spectra were obtained after each change in pH. Additional spectra were obtained from an unbuffered 18.9 mM solution treated in an entirely analogous manner.

$Dy(PPP)_2^{7-}$. A 2-mL sample of an unbuffered 9.5 mM $Na_7Dy(PPP)_2$·3NaCl solution (20% D_2O) was prepared that was also 55 mM in NaCl. The pH of this sample was lowered from 6.8 to 3.0 by the addition of several aliquots (totalling 40 μL) of 1 M HCl. The pH was then

(57) Kyba, E. P.; Davis, R. E.; Hudson, C. W.; John, A. M.; Brown, S. B.; McPhaul, M. J.; Liu, L.-K.; Glover, A. C. *J. Am. Chem. Soc.* **1981**, *103*, 3868.

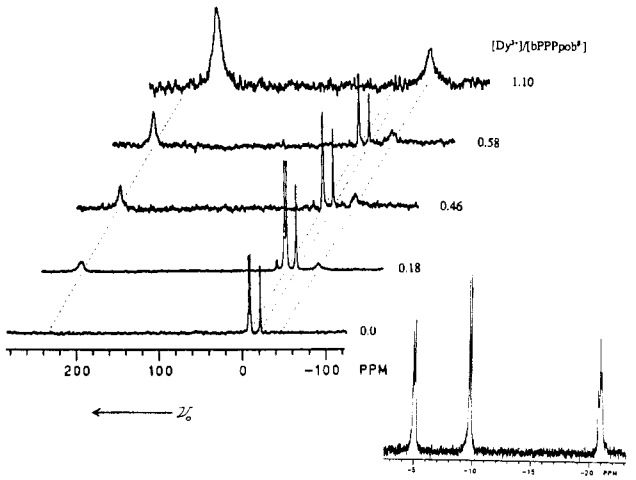


Figure 1. Stacked plot illustrating the effect on the $^{31}P\{^1H\}$ (122 MHz, 7.0 T) NMR spectrum of $H_2bPPPpob^{6-}$ as Dy^{3+} is added. To a TMA/HEPES buffered solution containing 16 mM $H_2bPPPpob^{6-}$ were added small aliquots (7–15 μL) of a 460 mM $DyCl_3$ solution. The spectra are labeled with the stoichiometric ratios of $Dy^{3+}/bPPPpob^{8-}$. The insert corresponds to the expanded spectrum for the ligand in solution without Dy^{3+}.

increased to 11.1 by the addition of several aliquots (totalling 50 μL) of a 1 M KOH solution. The ^{23}Na spectrum was obtained after each change in pH.

Ca^{2+} and Mg^{2+} Dependences of the ^{23}Na Shifts Induced by Shift Reagents. *$DybPPPpob^{5-}$.* The samples from the shift titration that had values of the ratio $DybPPPpob^{5-}$:Na^+ equal to 0.082 and 0.102 were saved. To the former were added 48-μL aliquots of a 100 mM $CaCl_2$ solution (in TMA/HEPES buffer). To the latter were added 64-μL aliquots of a 110 mM $MgCl_2$ solution (in TMA/HEPES buffer). The ^{23}Na NMR spectrum was obtained after each addition. A duplicate experiment on the Ca^{2+} competition was conducted.

$Dy(PPP)_2^{7-}$. Quantities of the 15 mM stock solution of $Dy(PPP)_2^{7-}$ were diluted with 1 M NaCl and TMA/HEPES buffer solution such that two 2-mL 150 mM Na^+ samples were produced: one being 4.20 mM in $Dy(PPP)_2^{7-}$ and one 4.95 mM in $Dy(PPP)_2^{7-}$. Aliquots (48 μL) of the $CaCl_2$ solution were added to the former, while 64-μL aliquots of the $MgCl_2$ solution were added to the latter. The ^{23}Na NMR spectrum was obtained after each addition. A duplicate experiment on the Ca^{2+} competition was conducted.

Enzymatic Hydrolyses of $DybPPPpob^{5-}$ and $Dy(PPP)_2^{7-}$. Two TMA/HEPES-buffered solutions were prepared: one was 16 mM in $Na_3(TMA)_2DybPPPpob\cdot 3NaCl$, while the other was 16 mM in $Na_7Dy(PPP)_2\cdot 3NaCl$. Volumes (100 μL) of type VII-S alkaline phosphatase solution (Sigma) were added to 2.0-mL portions of these such that each solution was 300 nM in the enzyme. $^{31}P\{^1H\}$ NMR spectra were obtained before and regularly after the enzyme additions.

Instrumentation. The pH values were recorded with a Markson combination pH/reference electrode with use of a Corning Model 112 digital pH meter.

All $^{31}P\{^1H\}$ and ^{23}Na NMR spectra were obtained with a GE NT-300(SB) spectrometer on which ^{31}P resonated at 121.6 MHz and ^{23}Na resonated at 79.4 MHz. All samples were field-frequency locked on the D_2O ^2H resonance frequency (46.1 MHz). For ^{31}P, a 5.3-μs (ca. 10° flip angle) pulse was used, followed by a 139-ms acquisition time and an additional 1.30-s delay. From 250 to 400 acquisitions were collected for each spectrum. For ^{23}Na, a 17-μs (ca. 90° flip angle) pulse was used, followed by a 256-ms acquisition time and an additional delay of 50 ms. Four acquisitions were collected for each spectrum. Proton NMR spectra were obtained on a GE QE-300 at 300 MHz and with routine instrument settings.

Results

Figure 1 depicts the $^{31}P\{^1H\}$ NMR spectra exhibited by a solution of $H_2bPPPpob^{6-}$ as Dy^{3+} is added. The spectra are labeled with the values of the stoichiometric molar ratio $Dy^{3+}/bPPPpob^{8-}$. The chemical shift scale has an arbitrary reference zero for the peak of a separate phosphoric acid sample. Since the spectrometer was always field-frequency locked on the ^2H resonance of the D_2O in the sample, the BMS shift accompanying the addition of Dy(III) is negated to the extent that D_2O suffers no hyperfine shift.[47] The three peaks at ca. −5, −9.7, and −21 ppm (expanded for the Dy-free

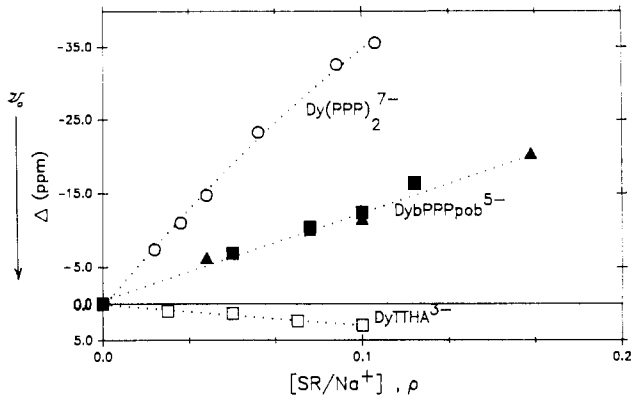


Figure 2. Isotropic hyperfine shift, Δ, of the ^{23}Na resonance (79 MHz, 7.0 T) as a function of the stoichiometric molar ratio of shift reagent to Na^+, ρ, for the following shift reagents: (■, ▲) $DybPPPpob^{5-}$, (○) $Dy(PPP)_2^{7-}$, and (□) $DyTTHA^{3-}$. For $DybPPPpob^{5-}$, two data sets are shown. Throughout each curve, the concentration of Na^+ was constant at approximately 150 mM. The aqueous solutions contained 20% (v/v) ^2H_2O, and the temperature was ca. 295 K. The dotted curves are intended merely to guide the eye.

solution), with areas approximately equal to each other, are assigned to the free ligand. The two peaks at ca. 238 and ca. −44 ppm with relative areas of ca. 4 and 2, respectively, are assigned to the bound ligand. At the highest Dy^{3+} concentration, the downfield resonance shifts slightly upfield, probably because of a lowering of the pH, even in this buffered solution (vide infra). In similar solutions, prepared from the same stocks, the pH dropped from 7.1 to 6.4 as the ratio was changed from zero to 1.02. The values of the ratio of the total area of the bound peaks to that of all of the peaks (0, 0.15, 0.46, 0.62, 1.0, from bottom to top of Figure 1) agree well with those of the stoichiometric molar ratios. This supports the assignment and demonstrates that, as expected, the formation constant for $DybPPPpob^{5-}$ is very large and, in addition, that exchange between free and bound $bPPPpob^{8-}$ is slow on the chemical shift NMR time scale. This should also be expected because even $Dy(PPP)_2^{7-}$ exhibits slow exchange in the ^{31}P spectrum because of the short time scale corresponding to the large hyperfine shifts induced by Dy(III).[14,19] As the $DybPPPpob^{5-}$ SR is formed, the ^{23}Na NMR signal is shifted upfield (data not shown). As expected, the shift increases until all the Dy^{3+} is chelated by the $bPPPpob^{8-}$ ligand. This further supports the 1:1 stoichiometry of the SR complex.

Figure 2 compares the shift potency of the $DybPPPpob^{5-}$ SR to those of the $Dy(PPP)_2^{7-}$ and $DyTTHA^{3-}$ complexes. It is a plot of the shift of the ^{23}Na resonance position (Δ) as a function of the molar ratio of SR to Na^+, ρ, while the concentration of the Na^+ is held approximately constant at 150 mM. The four data sets (two for $DybPPPpob^{5-}$) were collected from TMA/HEPES solutions prepared as similarly as possible. $DybPPPpob^{5-}$ shifts the ^{23}Na signal more than a third as well as $Dy(PPP)_2^{7-}$, in the same direction (upfield), and is more than twice as potent a SR as $DyTTHA^{3-}$, which shifts the ^{23}Na signal downfield. It should be noted that, in this case, the $Dy(PPP)_2^{7-}$ curve cannot rise above a value of $\rho = 0.10$ only because the sole countercation for the ligand used in the preparation was Na^+, i.e., Na_5PPP. In the $bPPPpob^{8-}$ and $TTHA^{6-}$ cases, the ligand was partially protonated before chelation.

Figure 3 shows the pH dependence of the $^{31}P\{^1H\}$ NMR spectrum of $DybPPPpob^{5-}$. The fact that these spectra were not obtained in sequence (pH 2.9, then pH 10.4, and then pH 7.4) suggests that the effects of pH on $DybPPPpob^{5-}$ are reversible, at least in this range. This includes what appears to be the release of some free ligand at pH 10.4. In some spectra (not shown), the downfield peak shows a small splitting into a doublet even at the higher pH values. This is presumably because of exceptionally good B_0 shimming. Figure 4 depicts the pH dependence of the ^{23}Na resonance frequency in two solutions containing $DybPPPpob^{5-}$, as well as in a solution containing $Dy(PPP)_2^{7-}$. The pH profile of the shift induced by $DybPPPpob^{5-}$ is very similar

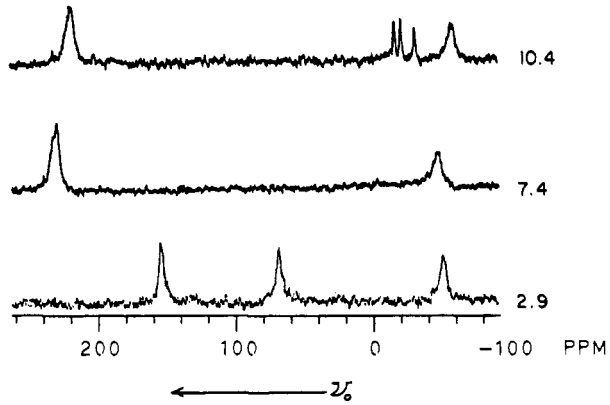


Figure 3. Effects of pH on the $^{31}P\{^1H\}$ (122 MHz, 7.0 T) NMR spectra of an unbuffered 18.2 mM $Na_3H_2DybPPPpob\cdot 3NaCl$ solution. The pH was lowered by the addition of a small aliquot of HCl (1 M) and was subsequently raised by the addition of small aliquots of KOH (1 M). (See Figure 4 for details.) The spectra are labeled with the solution pH values. The spectra were not obtained in sequence (pH 2.9, then 10.4, and then 7.4).

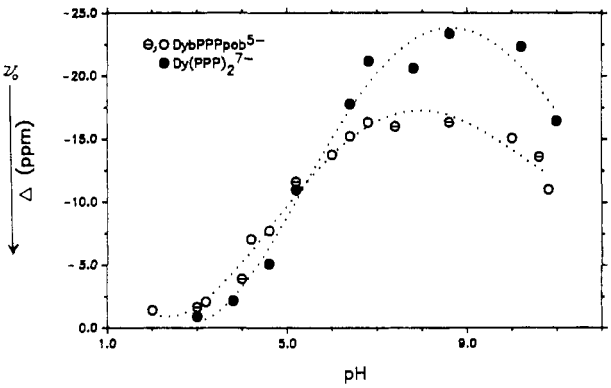


Figure 4. Effects of pH on the isotropic hyperfine shift, Δ, of the ^{23}Na resonance induced by $Na_7Dy(PPP)_2\cdot 3NaCl$ (●) and $Na_3H_2DybPPPpob\cdot 3NaCl$. The solution of the former was 9.5 mM $Na_7Dy(PPP)_2\cdot 3NaCl$ and 55 mM NaCl. There were two almost identical solutions of the latter: they were 18.2 (○) and 18.9 (Θ) mM in the shift reagent and 42.3 and 37 mM in NaCl, respectively. The pH was lowered by the addition of small aliquots of HCl (1 M) and was then raised by the addition of small aliquots of KOH (1 M). In each of the solutions, the concentration of K^+ rose to 25 mM at the highest pH value, and the concentration of Cl^- rose to 102 mM at the lowest pH value. The dotted curves are intended merely to guide the eye.

to that of $Dy(PPP)_2^{7-}$, shifted in the acid direction by perhaps one pH unit. This indicates that the protonation equilibria for $DybPPPpob^{5-}$ are very similar to those for $Dy(PPP)_2^{7-}$ (processes B and C in Scheme I).[7,14,58]

Figure 5 depicts the effects of Ca^{2+} and Mg^{2+} on the shift of ^{23}Na induced by $DybPPPpob^{5-}$, as well as on that induced by $Dy(PPP)_2^{7-}$. These indicate that the competitions of Ca^{2+} and Mg^{2+} with Na^+ for $DybPPPpob^{5-}$ are not as severe as they are for $Dy(PPP)_2^{7-}$ (process D in Scheme I).

Figure 6 depicts the time dependences of the $^{31}P\{^1H\}$ NMR spectra of solutions of $DybPPPpob^{5-}$ and $Dy(PPP)_2^{7-}$ after the injections of catalytic amounts of alkaline phosphatase. There are small upfield shifts of all peaks attendant to the addition of catalyst. These are almost certainly due to the introduction into the NMR sample of 320 mM NH_4^+, which arises from the enzyme storage solution supplied by the manufacturer. (Separate experiments with $(NH_4)_2SO_4$ also indicate that the competition from NH_4^+ can account for the dramatic decreases observed in the values of Δ upon additions of the enzyme to the SR solutions (not shown).) The small sharp peak seen growing in intensity in each experiment appears at ca. −1 ppm, the chemical shift[23,24] of orthophosphate, P_i, the product of enzymatic hydrolyses of the ligands. This indicates that the $DybPPPpob^{5-}$ complex also suffers ligand hydrolysis in the presence of alkaline phosphatase. Figure 7 depicts the time dependences of the areas of the bound ligand and P_i $^{31}P\{^1H\}$ NMR peaks after the addition of enzyme for $Dy(PPP)_2^{7-}$ as well as for $DybPPPpob^{5-}$.

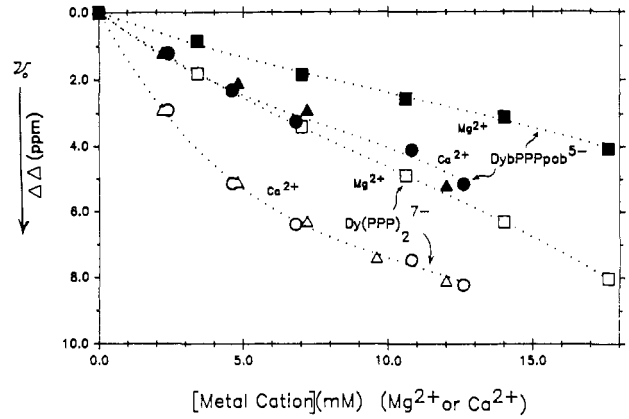


Figure 5. Effects of Ca^{2+} and Mg^{2+} on the isotropic hyperfine shifts induced in the ^{23}Na resonance by $Dy(PPP)_2^{7-}$ and $DybPPPpob^{5-}$. The change in shift, ΔΔ, is plotted against the concentration of metal ion added. In all cases, the total Na^+ concentration is 150 mM and the Ca^{2+} and Mg^{2+} are added as the Cl^- salts. The concentrations of $Dy(PPP)_2^{7-}$ are 4.20 mM and 4.95 mM for the Ca^{2+} (△, ○) and Mg^{2+} (□) experiments, respectively. The concentrations of $DybPPPpob^{5-}$ are 12.3 and 15.3 mM for the Ca^{2+} (●) and Mg^{2+} (■) experiments, respectively. The data for duplicate Ca^{2+} experiments are shown. (For $DybPPPpob^{5-}$, the SR concentration was 12.0 mM (▲).) The dotted curves are intended merely to guide the eye. In the $Dy(PPP)_2^{7-}$ solutions, cloudiness was noted above ca. 2 mM Ca^{2+}; in the $DybPPPpob^{5-}$ solutions, cloudiness was noted above ca. 5 mM Ca^{2+}. No cloudiness was evident in any of the Mg^{2+} solutions.

Discussion

As described above, the spectra in Figure 1 indicate that $DybPPPpob^{5-}$ is a rather stable chelate, both thermodynamically and kinetically. In general, the molecular structures of Ln(III) chelates in solution are quite difficult to determine and $DybPPPpob^{5-}$ is no exception. The $^{31}P\{^1H\}$ NMR spectra in Figure 1 tell us that, at physiological pH, the six phosphorus atoms in the chelate give rise to two resonances, one of degeneracy four (ca. 238 ppm) (actually, a 2:2 doublet with good shimming) and one of degeneracy two (ca. −44 ppm). The former is shifted downfield from the resonances of the free ligand: the γ doublet at ca. −5 ppm, the α doublet at ca. −10 ppm, and the β triplet at ca. −21 ppm. The latter is shifted upfield. This differs from the case for $Dy(PPP)_2^{7-}$, where there are also two peaks of relative area ratio 4:2 but both are shifted downfield from the positions of the free ligand resonances (Figure 6).[14,19,21] The hyperfine shift of a given ^{31}P signal (mostly, the difference between the resonance frequency in the chelate and that in the free ligand)[47] surely has contributions from both the contact and the pseudocontact (dipolar) mechanisms.[14,19] Only the latter has structural information, and one must go to great lengths to even attempt to extract this.[14,19] Slight structural changes could account for the differences observed between the ^{31}P NMR spectra of $DybPPPpob^{5-}$ and $Dy(PPP)_2^{7-}$. Structural changes attendant to protonation are also suggested by the pH dependence of the $^{31}P\{^1H\}$ spectrum seen in Figure 3. A decrease in the pH shifts the downfield $DybPPPpob^{5-}$ resonance upfield but also lifts half of its degeneracy. The spectrum observed at low pH values exhibits three well-split resonances, each of relative degeneracy two. These spectral changes are so large as to certainly be more than just the direct effect of protonation on the phosphorus chemical shift (no more than 5 ppm). They may reflect slight structural changes of the chelate as various phosphate groups are protonated or shifts of rapid equilibria whereby a portion of the bPPPpob ligand is uncoordinated. The apparent release of free ligand at pH 10.4 appears to be reversible because the spectrum at pH 7.4 was obtained after that at pH 10.4. This

(58) Ramasamy, R.; Espanol, M. C.; Long, K. M.; Geraldes, C. F. G. C.; Mota de Freitas, D. *Inorg. Chim. Acta*, in press.

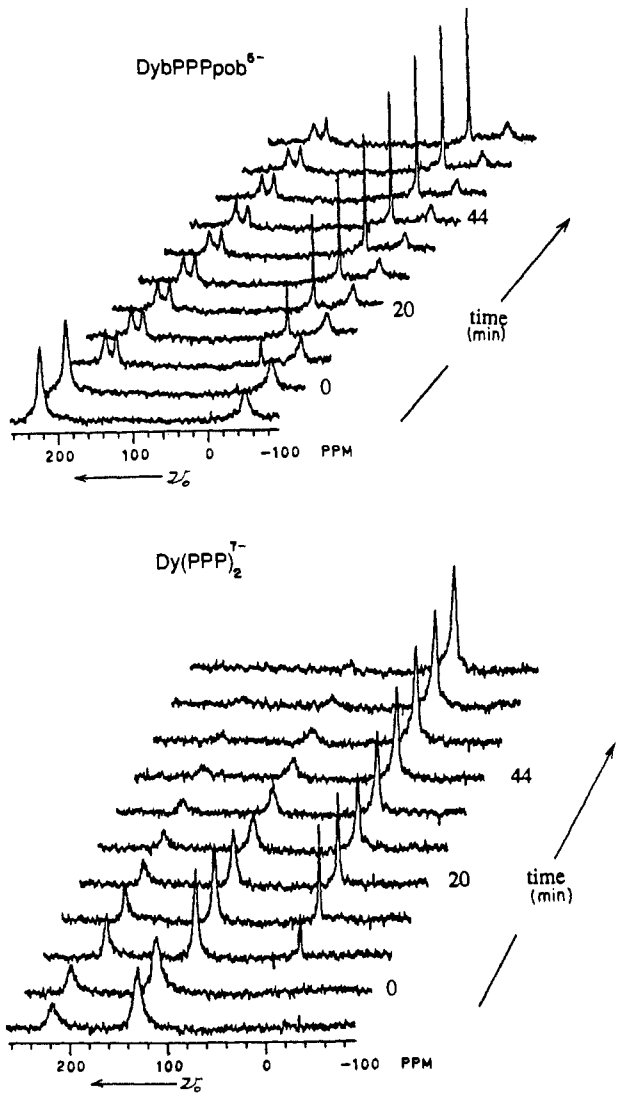


Figure 6. Stacked plots illustrating the time dependences of the $^{31}P\{^1H\}$ (122 MHz, 7.0 T) NMR spectra of 16 mM DybPPPpob^{5-} (top) and of 16 mM Dy(PPP)$_2^{7-}$ (bottom) after injections of catalytic amounts of alkaline phosphatase. The spectra are labeled with the times elapsed after each injection.

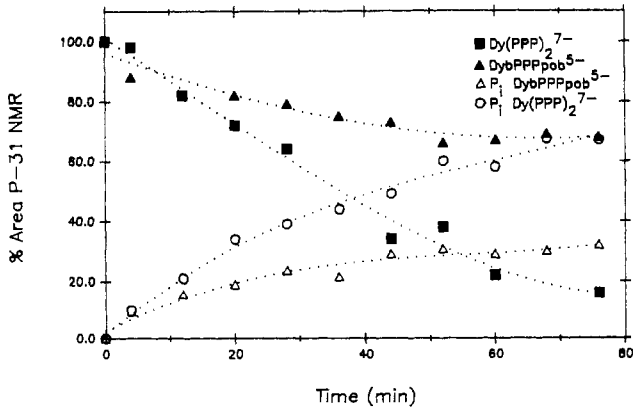
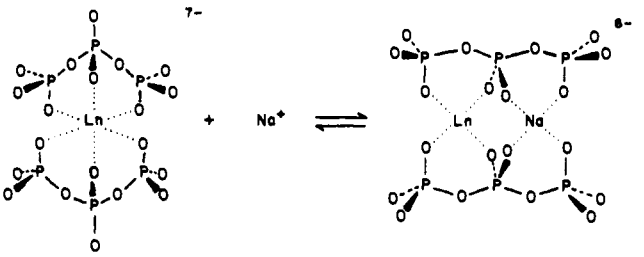


Figure 7. Time dependences of the areas of the bound ligand peaks in the $^{31}P\{^1H\}$ NMR spectra (Figure 6) of 16 mM DybPPPpob^{5-} (▲) and of 16 mM Dy(PPP)$_2^{7-}$ (■) after injections of catalytic amounts of alkaline phosphatase. Also shown are the time dependences for the peak areas observed (Figure 6) for the hydrolyzed products of Dy(PPP)$_2^{7-}$ (○) and DybPPPpob^{5-} (△) after the additions of the enzyme to the shift reagent solutions.

probably accounts for the decrease in ^{23}Na shift at high pH values (Figure 4).[7,58] However, Anson et al. do not report such a decrease even though they suggest that an irreversible hydrolysis of the Dy(PPP)$_2^{7-}$ occurs at high pH values.[14]

In any case, the ^{23}Na NMR results seen in Figures 2 and 4 indicate that whatever differences there are between the Dy(PPP)$_2^{7-}$ and DybPPPpob^{5-} structures and their changes upon protonation, the nature of the primary Na^+ binding site is apparently not affected in any way that seriously diminishes the shift effectiveness of DybPPPpob^{5-}. We have itemized the three major factors that determine the magnitude and sign of the limiting shift of a cationic resonance induced by a shift reagent.[6] It is even possible that the Na^+ binding sites and limiting shifts are identical for DybPPPpob^{5-} and Dy(PPP)$_2^{7-}$ and that the diminished observed shift caused by DybPPPpob^{5-} is simply due to a decreased *effective* binding constant because of the decreased anionic charge.[6]

We postulated a structure of the NaDy(PPP)$_2^{6-}$ adduct (XIV) in 1984:[7] Gupta has essentially concurred.[59] Anson et al. report



XIV

some contact contribution to the hyperfine shift of ^{23}Na by Ln(PPP)$_2^{7-}$ complexes, but they suggest a structure for the sodium adduct which is less perturbed from that on the left in XIV.[14] Electron spin echo studies of frozen glasses containing Na·Nd·ATP complexes have revealed nonzero scalar coupling between the ^{23}Na nucleus and the unpaired electrons on Nd(III).[60] This, and a contact shift of the ^{23}Na resonances (really, the same interaction), require a close proximity of the Na^+ binding site to the Ln^{3+} ion. Although Nieuwenhuizen et al. suggest multiple binding sites for the Na^+ ion,[19] their data do not provide evidence for this.[61] We have shown that ^{23}Na binding data for Ln(PPP)$_2^{7-}$ (where Ln = Tb^{3+}, Dy^{3+}, and Tm^{3+}) can be fitted very well by assuming only a single binding site for the Na^+ or K^+ ion.[61]

Figure 5 depicts the effects of the competition of Ca^{2+} and Mg^{2+} with Na^+ for the new SR. This is signficantly diminished when compared to the situation with Dy(PPP)$_2^{7-}$. This is possibly because reaction D but not reaction I (Scheme I) is available to DybPPPpob^{5-}. It may be that, for Dy(PPP)$_2^{7-}$, only Ca^{2+} avails itself of reaction I to any significant extent and Mg^{2+} employs reaction D. We have made Ca^{2+}-selective electrode measurements (not shown) of the decrease in Ca^{2+} activity caused by DybPPPpob^{5-}. Sufficient extra Ca^{2+} can be added so as to maintain the free Ca^{2+} level required for in vivo work: this is not the case with Dy(PPP)$_2^{7-}$.

Figures 6 and 7 show the susceptibility of the DybPPPpob^{5-} shift reagent to hydrolysis catalyzed by alkaline phosphatase. The fact that DybPPPpob^{5-} is hydrolyzed at all suggests that reaction H of Scheme I is not the only mechanism for enzyme-catalyzed hydrolysis. However, a mechanism analogous to reaction L of Scheme I is more likely for DybPPPpob^{5-} than for Dy(PPP)$_2^{7-}$. Indeed, the hydrolysis of DybPPPpob^{5-} is noticeably slower and less extensive than that of Dy(PPP)$_2^{7-}$ (Figures 6 and 7). This could mean that the hydrolysis of the PPP moiety of bPPPpob^{8-} is sufficiently kinetically hindered (by the effects of covalently linking it to a larger species) to allow DybPPPpob^{5-} to be perfused through living tissue with no ill effects. This can be effectively tested only with tissue, and we are currently studying the use of DybPPPpob^{5-} with perfused beating rat hearts. Preliminary

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experiments suggest that DybPPPpob^{5-} is slowly hydrolyzed in the heart.[1] This is more apparent at lower perfusion flow rates, where the average shift reagent residence time in the heart is longer, and may rule out the use of DybPPPpob^{5-} for ischemia studies where the perfusion flow is halted in part or all of the heart. However, when hearts are perfused at only slightly subnormal flow rates with buffer solutions that are 5.7 mM in pure DybPPPpob^{5-} (ref 1 incorrectly reports the concentration as 7 mM), they continue to beat and the extracellular sodium resonance is shifted upfield by 4 ppm.[1] This is a significant shift, which allows almost complete resolution of the intra- and extracellular signals and the determination of their relative intensities more accurately than ever before.[1] Also, the good spectral resolution allows one to observe clearly the inhomogeneity of the intracellular resonance induced by the BMS differences caused by the perfusion of the extracellular space with the paramagnetic DybPPPpob^{5-}.[1,47] This effect imparts anatomical information to the line shape of the intracellular ^{23}Na peak. Future attempts to alleviate the hydrolysis problem in vivo may include adjuvant perfusion of DybPPPpob^{5-} with a phosphatase inhibitor, such as P_i[62] or levamisole,[63] or with a free Dy^{3+} scavenger, such as EDTA^{4-}, DTPA^{5-}, or TTHA^{6-} (II).

In recent work, Sherry and co-workers have reported that TmDOTP^{5-} (IV), which induces a ^{23}Na shift equal to that of DyDOTP^{5-},[49] is not as sensitive to Ca^{2+} as DyDOTP^{5-} and can be perfused through rat hearts with impunity.[64] However, preliminary results in our laboratory indicate that the shift of the ^{23}Na signal by the TmDOTP^{5-} is quite sensitive to Ca^{2+} and that, as expected, the pH titration curve is shifted in the basic direction, by almost two pH units from that of $Dy(PPP)_2^{7-}$ (Figure 4), right into the middle of the physiological range.[65] The shift of the rat heart extracellular ^{23}Na resonance induced by TmDOTP^{5-} (2.5 ppm at 3.75 mM[64a]) is about the same as that induced by DybPPPpob^{5-} (4 ppm at 5.7 mM[1]). In comparison, at least 10 mM DyTTHA^{3-} is required to cause a 4 ppm shift in this signal.[10,39,40]

Finally, we wish to make it clear that these SRs are unlikely to produce significant perturbations of the sodium ion distribution in vivo. In a careful analysis of the relevant equilibria when DyTTHA^{3-} is present at effective concentrations in physiologic media, we have shown that no more than 2% of the extracellular Na^+ is bound to the SR.[61] Although $Dy(PPP)_2^{7-}$ has a greater affinity for the Na^+ ion,[61] it is a SR effective at much lower concentrations (e.g. <5 mM; Figure 2). Thus, under physiologic conditions, probably no more than 3% of the Na^+ would be bound to the SR. The new SR, DybPPPpob^{5-}, will, of course, be intermediate between these. Even these low percentages are not really a problem, however, because the SR can be injected wholly or partially as the Na^+ salt.[43-45]

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Solution Chemistry of Gallium and Indium 3-Hydroxy-4-pyridinone Complexes in Vitro and in Vivo

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A series of complexes of several 3-hydroxy-2-methyl-4-pyridinones with gallium(III) and indium(III) have been characterized by potentiometric (glass electrode) titration. The equilibria have been examined at 25.0 ± 0.1 °C and at an ionic strength of μ = 0.15 M (NaCl). The pyridinones have different substituents at the ring nitrogen atom: R = H, CH_3, and C_2H_5. These ligands form ML_n complexes (n = 1-3) of great stability; the overall stability constants β_3 for the 3:1 complexes are $\sim 10^{38}$ (M = Ga) and $\sim 10^{33}$ (M = In). The effective formation constants (β_{3eff}) of the various ligands for Ga^{3+} at physiological pH are close to 10^{31}. As a practical application of these data, comparative metal binding in a simple blood plasma model is simulated. This result is incorporated into the design of biodistribution experiments in mice using these ligands, their n-C_6H_{13} analogue, and *l*-mimosine. These studies show that the amount of ligand required to prevent removal of ^{67}Ga from $^{67}GaL_3$ complexes can be reliably calculated. The $^{67}GaL_3$ complexes show rapid excretion of the radionuclide through the kidneys in a rabbit.

Introduction

As part of a continuing project to detail the coordination chemistry of gallium and indium that pertains to the roles played by radioactive isotopes of these group 13 metal ions in the diagnosis of disease, we have been studying their tris(ligand) complexes containing certain bidentate monobasic ligands.[2-6] Despite differences in ionic radius, Ga and In share an almost identical aqueous coordination chemistry.[7,8] They are both found only in

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