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# Design and synthesis of an "ultrachelating" ligand based on an 18-membered ring hexaaza macrocycle

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**A new ultrachelating ligand, $H_4$PYTA (3,6,14,17,23,24-hexaazatricyclo[17.3.1.1$^{(8.12)}$]tetracosa-1(23),8,10,12(24),19,21-hexaene-3,6,14,17-tetraacetic acid), has been synthesized by tetracarboxymethylation of the parent ligand PYAN (3,6,14,17,23,24-hexaazatricyclo[17.3.1.1$^{(8.12)}$]tetracosa-1(23),8,10,12(24),19,21-hexaene). Both of these ligands are based upon the [18]aneN$_6$ macrocyclic framework in which two *trans* dimethylamino groups are replaced by 2,6-substituted pyridine. The metal binding constants for PYTA$^{4-}$ (log K$^{ML}$) were measured for sixteen metals and were found to be comparable to those for DTPA$^{5-}$ and DOTA$^{4-}$. PYTA$^{4-}$ binds well to an extraordinarily large range of metal ions from small Ga$^{3+}$ to large Pb$^{2+}$. This behavior is attributable to the flexible wrapping of the PYAN framework and the ability of PYTA$^{4-}$ to be six-coordinate with small metals and ten-coordinate with large metals.**

## INTRODUCTION

"Ultrachelating" ligands form metal complexes with unusual degrees of both thermodynamic and kinetic stability. Currently there is great interest in ultrachelating ligands for lanthanides and other heavy metal ions[1] due to the applicability of these ions in medical magnetic resonance imaging[2] and of their radionuclides in nuclear medicine as diagnostic or therapeutic agents.[3] Also, there is increasing need for chelating agents for environmental separations involving toxic heavy metals and metallic radionuclides. In the past two decades, the benchmark ultrachelating ligands, DTPA$^{5-}$ and EDTA$^{4-}$, have been joined by new families of macrocyclic aminopolycarboxylate ligands. So far, the smaller members of the macrocyclic series based on [X]aneN$_y$ with X=9 or 12 and y=3 or 4, like NOTA$^{3-}$ (1,4,7-triazacyclononane-N,N',N"-triacetate)[4,5], DOTA$^{4-}$ (1,4,7,10-tetrazacyclododecane-N,N',N",N'"-tetraacetate([6,7] and TETA$^{4-}$ (1,4,8,-11-tetraazacyclotetradecane-N,N',N",N'"-tetraacetic acid) have been studied most.[8,9] Both the thermodynamic stabilities and the kinetic labilities of the complexes have been related to features such as donor atom types, denticity, cavity size, chelate ring structure, rigidity, charge, pre-organization of the ligand, and the charge density and size of the metal ion.[10]

The larger [X]aneN$_y$ macrocycle with X=18 and y=6 has received less attention even though [18]aneN$_6$ is known to bind well to metal ions.[11] Furthermore, the 18-membered hexaaza macrocyclic ring is a logical starting point for the design of ultrachelating ligands for large metals because it is the simplest flexible ring capable of placing six donor atoms in an octahedral wrap about a metal ion.[12] Replacing two of the amine donors of [18]aneN$_6$ with two pyridine donors (see PYAN in Figure 1) created a ligand with decreased basicity, increased rigidity, and better coordinating ability than the parent [18]aneN$_6$.[13] The crystal structure of [Zn(PYAN)]$^{2+}$ revealed that the macrocycle coordinates in a helical fashion.[14]. The complex is extremely stable, log K$^{ML}$ = 21, and kinetically inert, as evidenced by the successful optical resolution of the helical enantiomers via formation of diasteriomeric salts using d-tartrate. With larger metals, PYAN untwists to form an approximately hexagonal planar ligand with additional axial ligands coordinated in some cases.[15] The stabilities of PYAN complexes can be tuned by introduction of substituents at the four positions on pyridine.[16] However, even with added substituents, the neutral PYAN ligand does not bind strongly enough to most metal ions to be classed as an ultrachelating ligand.

Carboxymethylation of the four amine nitrogen sites of PYAN produces a new ligand, $H_4$PYTA (Figure 1) that has approximately the same basicity and rigidity as PYAN but increased denticity due to the four added

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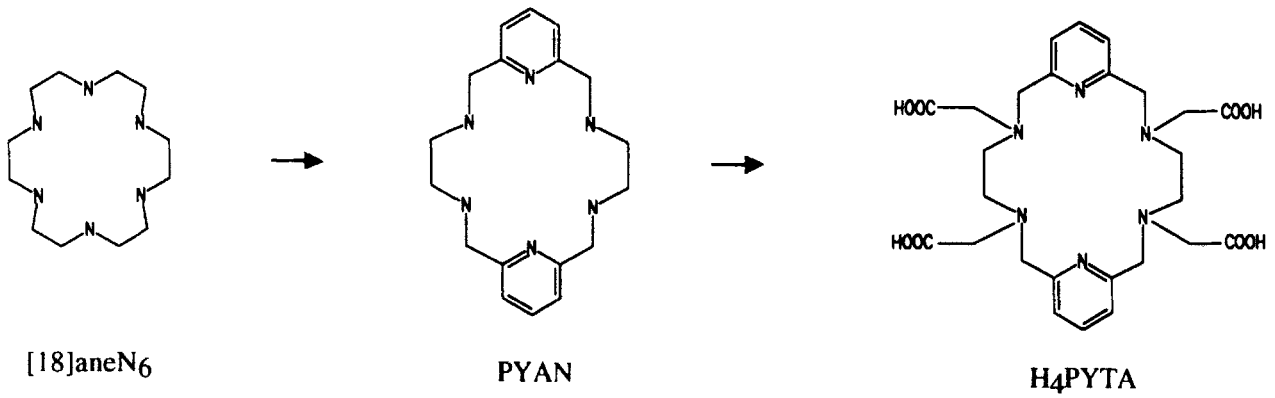


**Figure 1** Design of the ligands PYAN and $H_4$PYTA.

methylcarboxylate chelate rings. In this paper we report the synthesis, characterization, proton binding, and metal binding properties of $H_4$PYTA. The protonation constants and the stability constants will be compared with PYAN, selected organoamine ligands, and ultrachelating aminopolycarboxylate ligands.

## RESULTS

**Synthesis and characterization of $H_4$PYTA.** $H_4$PYTA was prepared by the substitution reaction of four equivalents of bromoacetate with PYAN under basic conditions as shown in Figure 2. Adjustment of the pH with formic

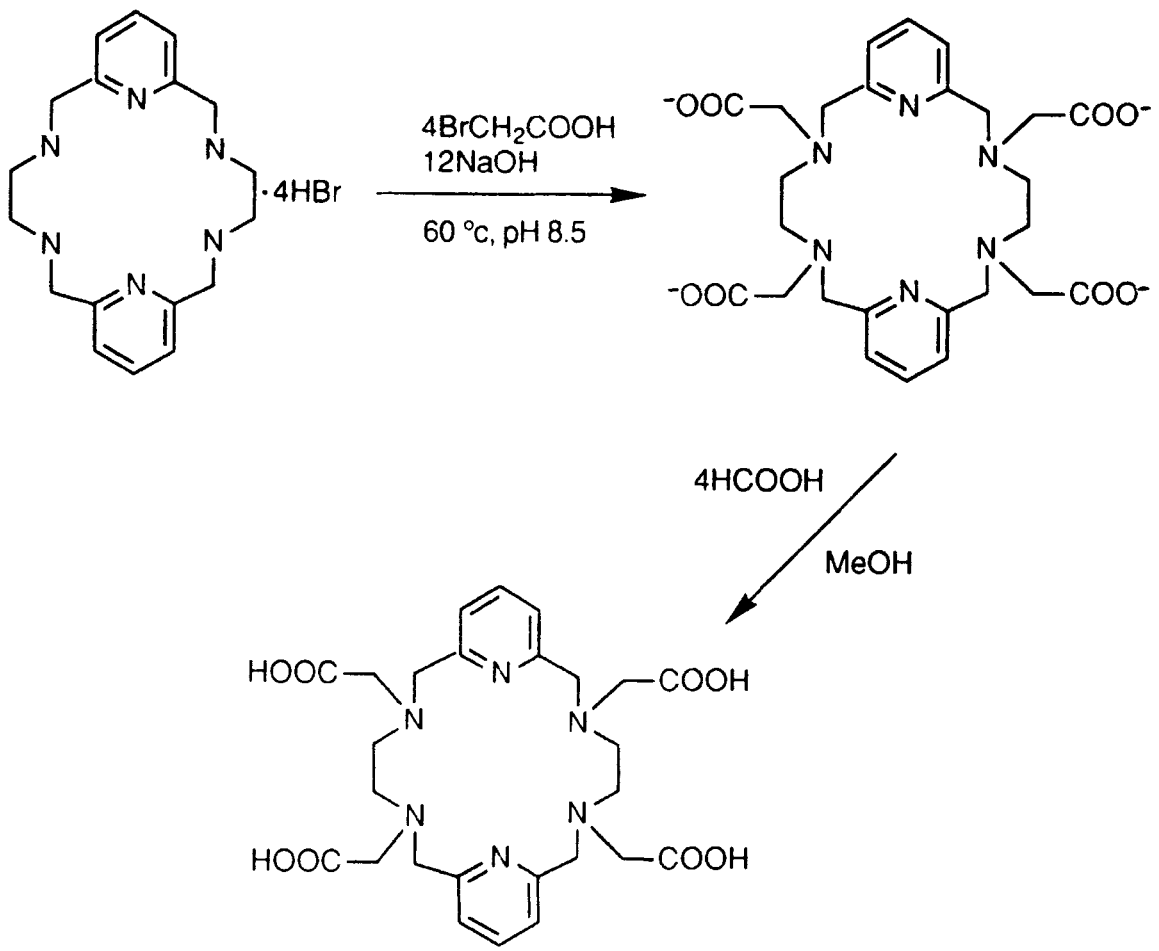


**Figure 2** Synthesis of $H_4$PYTA.

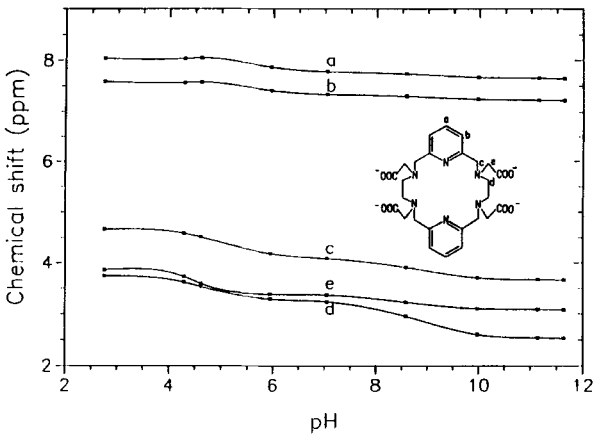


**Figure 3** Plot of the $^1H$ NMR chemical shifts of protons in $PYTA^{4-}$ versus pH of the solution. Conditions: $D_2O$ solvent with pD adjusted by additions of NaOD, 25°C.

acid afforded conditions under which the neutral tetraprotonated form of the ligand could be crystallized. The elemental analyses, infrared spectra, and NMR spectra are all consistent with formulation of the ligand as $H_4PYTA.2H_2O$. NMR assignments were made by comparison with the previously studied PYAN.4HBr.[13,16] Protons a and b (see Figure 3 and Materials and Methods Section) are assigned to the pyridine protons due to their chemical shifts and splittings as doublet and triplet,

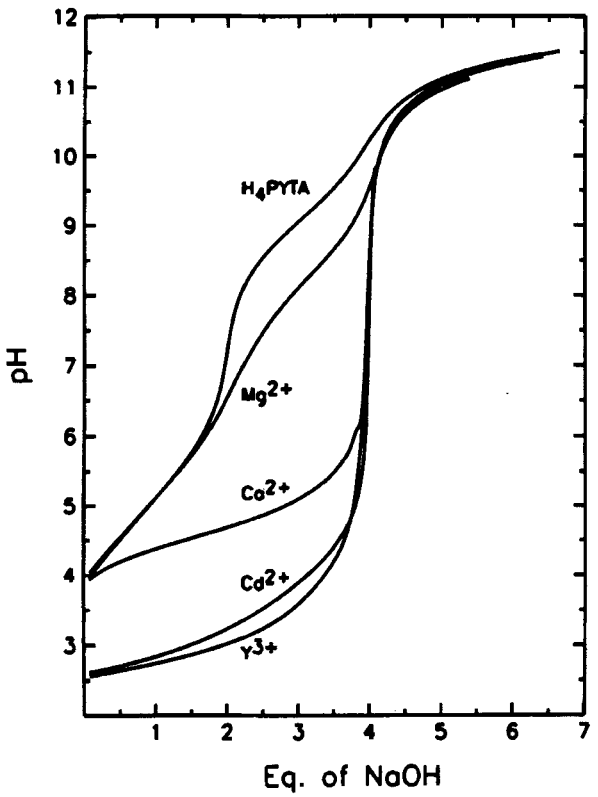


**Figure 4** Potentiometric titration curves of $H_4PYTA$ without added metal (labelled $H_4PYTA$) and with equimolar amounts of the metal ions specified. Conditions: 0.20 M KCl, 25°C.

respectively. Proton c could be assigned with confidence due to a small allylic coupling to proton b observed in the COSY spectrum. The assignments of d and e are less certain because they are so similar in chemical shift, so d is assigned by analogy to PYAN.

**Potentiometric titrations.** The curve for the potentiometric titration of aqueous $H_4PYTA$ with NaOH is shown in Figure 4. There are inflection points at 2 and 4 equivalents of base, indicating titration of protons in approximately two-proton steps. A model consisting of four protonation steps was used to fit the potentiometric titration data. Three independent data sets having approximately 85 points each gave good fits, having average deviation between the calculated and observed pH less than 0.02 pH units and resulting in the protonation constants given in Table 1. The standard deviations of the log $K_a$ values for three data sets was less than 0.1 log unit.

**pH titrations followed by NMR.** The magnitudes of the macroscopic protonation constants given in Table 1 for $H_4PYTA$ are not necessarily indicative of the sites of ligand protonation. Information regarding the microscopic protonation scheme can be obtained from the $^1H$ NMR chemical shifts of the ligand protons as a function of pH due to deshielding of methylene protons adjacent to protonated sites.[17,18] The plot of chemical shift versus pH for $H_4PYTA$ is shown in Figure 3. The region near pH 4.5-7.0 shows: (1) changes in chemical shift of all of the protons a through e, (2) greater change for methylene protons c, d and e than for pyridine ring protons a and b, (3) all protons shifted upfield. These observations are consistent with a deprotonation mechanism involving a protonated species with $H^+$ bound to amine nitrogen, pyridine nitrogen, and carboxylate oxygen atoms. The region near pH 8.0-10.5 shows changes in the chemical shift of methylene protons c, d and e, and no change of pyridine protons, thus indicating the protonation only of the four amine nitrogen atoms of the macrocycle.

**Formation Constants for Metal Complexes.** The curves for the potentiometric titrations of $H_4PYTA$ with added equimolar metal salts are shown in Figures 4 and 5. Sixteen metals were titrated with $H_4PYTA$ in this

**Table 1** Proton binding constants (log $K_n$) for Ligands in Aqueous Solution at 25°C.

| *Equilibrium Quotient* | *Ligands*[a] | | | |
|---|---|---|---|---|
| | *PYAN* | *PYTA* | *DTPA* | *DOTA* |
| [HL]/[L][H] | 9.13 | 9.37 | 10.71 | 12.09 |
| $[H_2L]/[HL][H]$ | 8.32 | 8.81 | 8.64 | 9.69 |
| $[H_3L]/[H_2L][H]$ | 6.12 | 5.80 | 4.28 | 4.47 |
| $[H_4L]/[H_3L][H]$ | 5.24 | 4.71 | 2.6 | 4.36 |
| $[H_5L]/[H_4L][H]$ | ---- | ---- | 2.0 | ---- |
| conditions | 0.1 M KCl | 0.1 M KCl | 0.1 M $R_4NCl$ | 0.1 M $R_4NCl$ |
| reference | [13, 16] | this work | [19] | [19] |

[a]Charges omitted for simplicity.

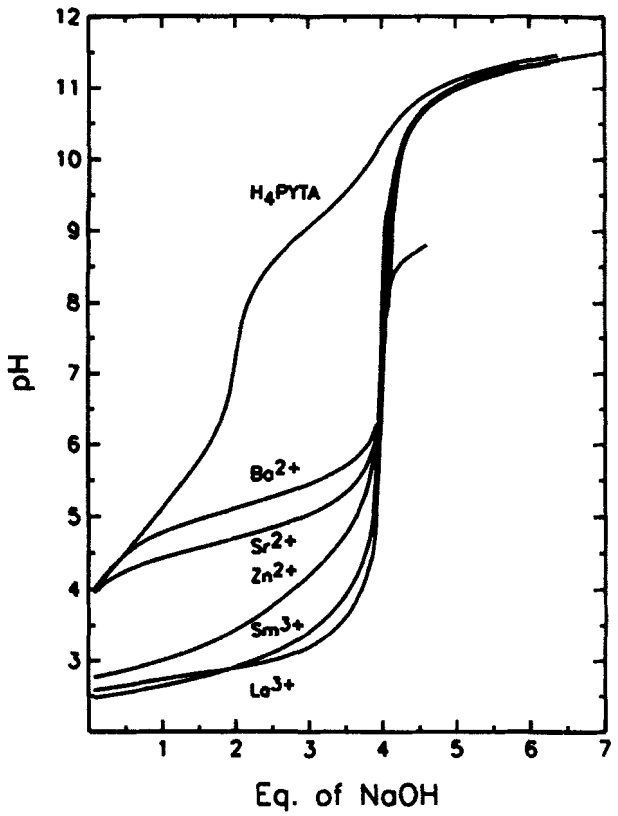


**Figure 5.** Potentiometric titration curves of $H_4$PYTA without added metal (labelled $H_4$PYTA) and with equimolar amounts of the metal ions specified. Conditions: 0.20 M KCl, 25°C.

study. In comparison with the solution of the protonated free ligand, the pH of the metal-containing solutions all are shifted to lower values by the release of protons according to the differing abilities of the metal ions to bind to the ligand. All metal-ligand curves have a break at four equivalents of NaOH added. Titrations of solutions containing metal in excess of ligand gave no indication of 2:1 binding (up to two equivalents of metal per ligand). Accordingly, the binding constants for metal complexes were evaluated from fitting the curves using a 1:1 metal:ligand model. Some titration curves demonstrated evidence of protonated species, MLH, so the protonated metal complexes with one to four protons were included in the model. In some cases the species with one or two protons were found to be significant. In no cases were the species with three or four protons found to be significant. The average deviation between the observed and calculated pH (using the program BEST, the appropriate model, and the previously determined protonation constants of $H_4$PYTA) was less than 0.03 pH units. The formation constants are listed in Table 2. The comparison values of log $K_a$ and log $K^{ML}$ shown in Tables 1 and 2 for $DTPA^{5-}$ and $DOTA^{4-}$ were obtained from the NIST *Standard Database of stability constants.*[19]

**Spectrophotometric titrations.** If a ligand forms complexes too slowly, or the stability constants of its complexes are too large(log $K^{ML}$ over about 18), the potentiometric titration method is not useful for accurately determining the stability constants of the complexes. Sherry and co-workers[14] developed a spectrophotometric titration method that allows for the determination of stability constants for the lanthanide complexes of $NOTA^{3-}$, $DOTA^{4-}$, and $DTPA^{5-}$ where log $K^{ML}$ is over 20. In this method, initial titrations of La(III), Gd(III), and Lu(III) with Arsenazo-III in 0.01 M acetate buffer and 0.1 M

**Table 2** Equilibrium constants (log $K^{ML}$) for Metal - Ligand Binding in aqueous solution (0.10 M KCl, 25 °C.[a]

| *Metal Ion* | *Radius* | *Equilibrium Quotient* | *Ligands*[b] | | | |
|---|---|---|---|---|---|---|
| | | | PYAN | PYTA | DTPA | DOTA |
| $Ga^{3+}$ | 0.76 | [ML]/[M][L] | ---- | [20] | 24.3 | 21.3 |
| $Mg^{2+}$ | 0.86 | [ML]/[M][L] | 2.6 | 3.2 | 9.34 | 11.9 |
| $Cu^{2+}$ | 0.87 | [ML]/[M][L] | [25] | 18.6 | 21.4 | 22.2 |
| | | [MLH]/[ML][H] | ---- | 4.0 | 4.8 | 4.4 |
| | | $[MLH_2]/[MLH][H]$ | ---- | 3.5 | 3.0 | 3.7 |
| $Zn^{2+}$ | 0.88 | [ML]/[M][L] | [21] | 18.1 | 18.3 | 21.1 |
| | | [MLH]/[ML][H] | ---- | 4.7 | 5.5 | 4.2 |
| | | $[MLH_2]/[MLH][H]$ | ---- | 3.5 | 3.1 | 3.8 |
| $Sc^{3+}$ | 0.89 | [ML]/[M][L] | 11.2 | [20] | [24.4] | ---- |
| | | [MLH]/[ML][H] | ---- | 5.4 | ---- | ---- |
| | | $[MLH_2]/[MLH][H]$ | ---- | 3.2 | ---- | ---- |
| $Mn^{2+}$ | 0.97 | [ML]/[M][L] | 15.1 | 15.1 | 15.5 | 20.2 |
| | | [MLH]/[ML][H] | ---- | 5.5 | 4.4 | 4.2 |
| $Y^{3+}$ | 1.04 | [ML]/[M][L] | 7.1 | [20] | 22.1 | ---- |
| | | [MLH]/[ML][H] | ---- | 4.2 | ---- | ---- |
| | | $[MLH_2]/[MLH][H]$ | ---- | 2.4 | ---- | ---- |
| $Cd^{2+}$ | 1.09 | [ML]/[M][L] | [20] | 19.8 | 19.0 | 21.3 |
| | | [MLH]/[ML][H] | ---- | 4.1 | 4.2 | 4.4 |
| | | $[MLH_2]/[MLH][H]$ | ---- | 3.3 | 3.3 | 3.0 |
| $Ca^{2+}$ | 1.14 | [ML]/[M][L] | 4.4 | 13.6 | 10.8 | 17.2 |
| | | [MLH]/[ML][H] | ---- | 5.1 | 6.1 | 3.6 |
| | | $[MLH_2]/[MLH][H]$ | ---- | 2.8 | ---- | 4.2 |
| $Lu^{3+}$ | 1.12 | [ML]/[M][L] | 7.8 | 21.7[c] | 22.4 | 25.4 |
| $Gd^{3+}$ | 1.19 | [ML]/[M][L] | 8.1 | 21.7[c] | 22.3 | 24.7 |
| $Sm^{3+}$ | 1.22 | [ML]/[M][L] | 9.0 | [22] | 22.3 | 23.0 |
| $La^{3+}$ | 1.30 | [ML]/[M][L] | 7.4 | 22.1[c] | 19.5 | 22.9 |
| $Sr^{2+}$ | 1.40 | [ML]/[M][L] | 2.8 | 12.7 | 9.7 | 15.2 |
| $Ba^{2+}$ | 1.56 | [ML]/[M][L] | <2 | 11.0 | 8.8 | 12.9 |
| $Pb^{2+}$ | 1.54 | [ML]/[M][L] | [21] | 17.7 | 18.7 | 22.7 |
| | | [MLH]/[ML][H] | 4.1 | 5.0 | 4.5 | 3.9 |
| | | $[MLH_2]/[MLH][H]$ | 4.1 | 4.1 | ---- | 3.3 |

[a]See reference 19 and references therein for $DTPA^{5-}$ and $DOTA^{4-}$ constants.
[b]Charges omitted for simplicity.
[c]Determined by spectrophotometric titration.

**Table 3** Conditional Stability Constants ($K^{ML}$ conditional) for lanthanide metals with $PYTA^{4-}$ and $DTPA^{5-}$ in aqueous solution at pH 3.95 and 25° C (0.1 M KCl).

| *Metal/Ligand* | *PYTA*[a] | *DTPA*[a] |
|---|---|---|
| $La^{3+}$ | 1.9 (0.10) × $10^9$ | 2.1 (0.35) × $10^8$ |
| $Gd^{3+}$ | 8.4 (0.07) × $10^8$ | 2.0 (0.08) × $10^{10}$ |
| $Lu^{3+}$ | 7.3 (0.48) × $10^8$ | 8.1 (0.61) × $10^9$ |

[a]The number in parentheses is the uncertainty in the preceeding number.

NaCl provide the conditional stability constants for lanthanide-Arsenazo complexes used in all later competition reactions. Then the conditional stability constants are determined at pH 3.95 for $PYTA^{4-}$ (or $DTPA^{5-}$ as a check) by starting with a known total concentration of Ln(III) and Arsenazo-III, and titrating that solution with the competing ligand directly. The conditional stability constants at pH 3.95 for Ln(III) with $PYTA^{4-}$ or $DTPA^{5-}$ are listed in Table 3. Finally, the thermodynamic stability constants can be calculated from the conditional stability constants by the equation[20]

$$K^{ML}(\text{therm.}) = K^{ML}(\text{cond.})(1 + K_1[H] + K_1K_2[H]^2 + K_1K_2\ldots K_n[H]^n)$$

Where $K_1$, $K_2\ldots K_n$ are the stepwise protonation constants for ligands, and [H] is the concentration of $H^+$ at the pH of the experiment. The thermodynamic stability constants calculated from the pH 3.95 conditional stability constants and the protonation constants are listed in Table 2.

## DISCUSSION

**Synthesis of $H_4PYTA$.** It is important to control the reaction conditions for the reaction shown in Figure 2, including the reaction pH, equivalent ratio of reactants, reaction temperature, and reaction time. First, heat removal is necessary when neutralizing PYAN.4HBr with NaOH because reaction temperatures exceeding 80°C cause a decrease in yield of $H_4PYTA$. It is also important to rigorously control the pH during the reaction period after addition of $BrCH_2COOH$, because the multiple equilibra in the reaction system (equations 1–8 below) are pH dependent.

(1) $BrCH_2COO^- + OH^- = HOCH_2COO^- + Br^-$
(2) $H_4L^{4+} + OH^- = H_3L^{3+} + H_2O$ (L = PYAN)
(3) $H_3L^{3+} + OH^- = H_2L^{2+} + H_2O$
(4) $H_2L^{2+} + OH^- = HL^+ + H_2O$
(5) $HL^+ + OH^- = L + H_2O$
(6) $H_2L^{2+} + 2BrCH_2COO^- = H_2L(CH_2COO^-)_2 + 2Br^-$
(7) $HL^+ + 3BrCH_2COO^- = HL(CH_2COO^-)_3 + 3Br^-$
(8) $L + 4BrCH_2COO^- = L(CH_2COO^-)_4 + 4Br^-$

From the species distribution curve[13,16] of PYAN.4HBr, it appears that pH 11 is best for the desired reaction, because at this pH the species L (equilibrium 5 above) is predominant, $HL^+$ has only a small concentration, and almost no other species are present. But the yield of $H_4PYTA$ is only 45% at this pH. Possibly, the side reaction (1) is dominant when the pH is 11. The highest yield is obtained when the pH is controlled near 8.5. The reaction temperature is important also, since the yield decreases when the temperature is higher or lower than 60°C during the carboxymethylation reaction period. The maximum yield of 70% was obtained under the conditions described below in the Materials and Methods Section.

**Protonation of $PYTA^{4-}$.** It has been shown in several $^1H$ NMR investigations[17,21] that the sequence of protonation is the same for the anions of all linear polyaminopolyacetic acids containing from two to six nitrogen atoms: The amino groups are successively protonated before a proton is attached on any of the carboxylate groups. In contrast, the $^1H$ NMR and potentiometric investigation of $NOTA^{3-}$ shows that one nitrogen atom is less basic than at least one of its carboxylate groups.[4] This property is shared by other cyclic polyazapolycarboxylates. The $^1H$ NMR and potentiometric investigations[22] of $DOTA^{4-}$ and $TETA^{4-}$ show that log $K_1$ and log $K_2$ correspond to the protonation of two nitrogen atoms located *trans* to each other in the macrocyclic ring. The two following constants, log $K_3$ and log $K_4$, correspond to protonation of the two carboxylate groups which are not adjacent to the nitrogen atom already bearing a proton. The $^1H$ NMR and potentiometric investigation of $PYTA^{4-}$ shows that log $K_1$ and log $K_2$ (pH range 8-10) correspond to the protonation of amine nitrogen atoms of macrocycle, most likely by protons shared between the nitrogen atoms of each diaminoethane moiety forming five-membered hydrogen-bound rings. This hypothesis is consistent with the basicity of the sites, with the larger shift observed for protons d relative to protons c and e, and with the behavior of the parent ligand, PYAN[13,16] (Table 1). In the pH range 4-7, corresponding to log $K_3$ and log $K_4$, the NMR shifts indicate that protonation involves the four nitrogen atoms of the macrocycle, the four carboxylate groups, and the two pyridine nitrogen atoms. The log $K_a$'s for these protonations are close to that of acetic acid (log $K_a$ = 4.8) Thus the triply and quadrupally protonated forms are consistent with the protons having multiple interactions with carboxylate oxygen and both types of nitrogen. From Table 1 it is seen that first two log $K_a$'s for $PYTA^{4-}$ are higher than those for parent ligand PYAN while the second two are lower than those of PYAN. This fact suggests that the first two protons are further stabilized by interaction with the negatively charged ligand, whereas the second two protons are slightly destabilized relative to PYAN by their multiple interactions which include carboxylate oxygens and pyridine nitrogens.

**Metal binding equilibrium constants.** For small metal ions (ionic radius less than 1 angstrom) PYAN and $PYTA^{4-}$ bind in most cases with similar equilibrium constants. This behavior is suggestive of six-coordination with both ligands bound through the nitrogen atoms. In the cases of $Cu^{2+}$ and $Zn^{2+}$, $PYTA^{4-}$ binds weaker than PYAN; thus, the carboxylate groups may contribute to repulsive intraligand interactions. Preliminary character-

ization data on isolated complexes (IR stretches of C=O groups and NMR chemical shifts) suggest that the carboxylate arms are not coordinated.[23] On the other hand, for large metal ions (ionic radius greater than 1.1 Å) $PYTA^{4-}$ consistently binds stronger than PYAN by 9–15 orders of magnitude. Furthermore, preliminary characterization data on lanthanide $PYTA^{4-}$ complexes (C=O infrared stretches and NMR chemical shifts) and the crystal structure of NaGdPYTA[24] reveal that in these cases the carboxylate groups are coordinated to give a ten coordinate complex. Thus, for the lanthanide complexes, the decreasing stability constants for $PYTA^{4-}$ across the series may be related to the decreasing size of the metal ion and the resulting crowding of the ten ligating atoms as the ligand changes conformation to accommodate metal ion size. In contrast, the stability constants for $[Ln(DTPA)]^-$ increase across the series presumably due to increased ionic attractions of the smaller lanthanide ions for the pentacarboxylate triamine ligand. Similar arguments apply to $DOTA^{4-}$. Two very interesting cases are $Sc^{3+}$ and $Y^{3+}$ which from the increases in stability constants between PYAN and $PYTA^{4-}$ of 9 and 13 orders of magnitude, respectively, seem to be indicative of ten coordination in the $PYTA^{4-}$ complex. Thus, isolation and characterization of these complexes is necessary in order to understand their behavior.

The protonation behavior of the $PYTA^{4-}$ complexes seems to parallel the protonation behavior of $DTPA^{5-}$ and $DOTA^{4-}$ complexes. As could be expected for a ten coordinate ligand, $PYTA^{4-}$ binds equally or better than $DTPA^{5-}$ for the largest metal ions, the lanthanides, and $Ba^{2+}$, $Sr^{2+}$, and $Pb^{2+}$.

For the transition metal ions, $Cu^{2+}$, $Zn^{2+}$, and $Cd^{2+}$, the binding constants of all four ligands are remarkably similar. Thus, for these metals the energetics of binding through covalent interaction with the nitrogenous donors must be similar to the energetics of binding to carboxylate ligands. In addition the properties of the macrocyclic cavity or lack thereof seem to make little difference. All four ligands provide an excellent coordination environment for these metals. For $Mn^{2+}$, PYAN, $PYTA^{4-}$ and $DTPA^{5-}$ bind equivalently, but $DOTA^{4-}$ binds five orders of magnitude more strongly. This seems to imply that the rigid cavity of $DOTA^{4-}$ contributes strongly to the binding in this case.

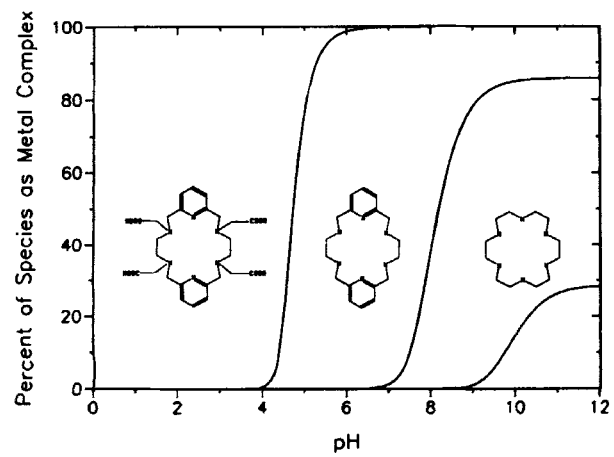


**Figure 6** Plot of the percent of species as metal complex versus pH for $Ca^{2+}$ and ligands $[18]aneN_6$, PYAN or PYTA. Conditions: ligand and $Ca^{2+}$ each 2 mM.

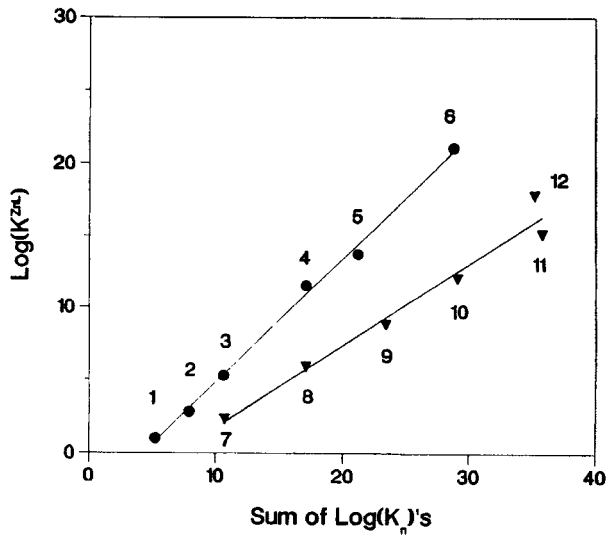


**Figure 7.** Plot of sum of log protonation constants (log $K_a$'s) versus log stability constant for the $Zn^{2+}$ complex (log $K^{ZnL}$) for pyridylamine ligands 1–6 and polyamine ligands 7–12. The ligands and references for the thermodynamic data are: 1, pyridine[19]; 2, bis(2-pyridyl)methane; 3, 2-aminomethylpyridine (AMP)[32]; 4, N,N'-bis(2-aminomethylpyridyl) ethylenediamine[32]; 5,bis(2-pyridyl)-2,6,10-triazanonane[33]; 6,PYAN; 7,ethylamine[19]; 8,ethylenediamine[19]; 9,diethylenetriamine[19]; 10,triethylenetetraamine[19]; 11,tetraethylenepentaamine[19]; 12,$[18]aneN_6$[19].

The pattern for $Sc^{3+}$, $Y^{3+}$, and the lanthanides is that $PYTA^{4-}$, $DTPA^{5-}$ and $DOTA^{4-}$ bind similarly and 13 to 14 orders of magnitude more strongly than PYAN. Thus, the role of additional carboxylate containing chelate rings toward these metal ions is clear. The trend in binding to $PYTA^{4-}$, $DTPA^{5-}$, and $DOTA^{4-}$ shows that as the metal ion becomes large, these ligands bind to within an order of magnitude of each other with log $K^{ML}$ about 22-23.

The largest amount of variation in binding among the ligands occurs for the alkaline earth ions $Mg^{2+}$, $Ca^{2+}$, $Sr^{2+}$, and $Ba^{2+}$, where variation in log $K^{ML}$ among the four ligands is 8.7, 6.4, 5.5, and 4.1 log units, respectively. The same trend in log $K^{ML}$ is seen in each case with $Ca^{2+} > Sr^{2+} > Ba^{2+} >> Mg^{2+}$. For $Mg^{2+}$, PYAN and $PYTA^{4-}$ bind equivalently but $DTPA^{5-}$ and $DOTA^{4-}$ bind much more strongly. This behavior reflects the preference of $Mg^{2+}$ and other hard metal ions for carboxylate over amine or pyridine donor and the fact that $PYTA^{4-}$ possibly cannot coordinate to the small $Mg^{2+}$ ion with the four carboxylate groups and a subset of the nitrogen donor atoms.

Figure 6 shows the calculated percent of calcium bound to ligand as a function of pH for solutions containing 2 mM each of $Ca^{2+}$ and ligand for each of the three ligands, [18]ane$N_6$, PYAN, and $PYTA^{4-}$. Under these conditions, [18]ane$N_6$ binds to a maximum of 28% of Ca and the percent bound falls rapidly below pH 11 due to the onset of proton competition. PYAN binds a maximum of 85% of Ca and succumbs to proton competition between pH 7 and 8. In contrast, $PYTA^{4-}$ binds 100% of Ca at the maximum, and proton competition does not interfere above a pH of about 6. These curves demonstrate the improvement in [18]ane$N_6$ by substitution of improved ligands (pyridine with its lower basicity, rigidity, and higher dipole moment than amine) and of charged ligands (carboxylate) and of larger numbers of chelate rings. For the macrocyclic amines, [18]ane$N_6$ and PYAN, there is good correlation of the total basicity (sum of log $K_a$'s) and stability of the $Zn^{2+}$ complex (log $K^{ZnL}$) with linear polyamine or polypyridylamine homologs as shown in Figure 7. Thus, the stability and proton competition behavior of these macrocycles are approximately typical of that expected based on their donor atom types and denticity. There are no significant indications of either positive or negative steric effects for these two macrocyclic ligands.

**Indications of kinetic stability.** Few studies of kinetic stability on PYAN and $PYTA^{4-}$ complexes have been carried out to date, but there are nonetheless some very important indications of unusual kinetic stability in the complexes of these ligands. First, the $[Zn(PYAN)]^{2+}$ complex does not exchange its NH protons with $D_2O$ in neutral solution over a 3 week time scale.[14] Secondly, the helical isomers of this complex can be resolved into delta and lambda optical isomers. These isomers retain optical activity for at least 11 months in neutral aqueous solution.[14] Third, $[Gd(PYTA)]^-$ has low toxicity in mouse, comparable to $[Gd(DTPA)]^{2-}$ and $[Gd(DOTA)]^-$ and does not exchange Gd with $Cu^{2+}$ or $Ca^{2+}$ in neutral aqueous solution.[25] $[Gd(PYTA)]^-$ also does not exchange Gd with Arsenazo III under neutral pH conditions. The complexes of PYAN and $PYTA^{4-}$ are nonfluctional (rigid) on the NMR timescale.

## CONCLUSIONS

The thermodynamic stability constants for $PYTA^{4-}$ with sixteen metals have been measured in aqueous solution and are large, typically approximating those for $DTPA^{5-}$ and $DOTA^{4-}$. Furthermore, $PYTA^{4-}$ is tentatively assigned two binding modes: ten-coordinate for large metal ions (ionic radius greater than 1.0 Å) and six-coordinate with the four carboxylate arms free for small metal ions. The complexes of $PYTA^{4-}$ and PYAN exhibit kinetic inertness. Thus, the [18]ane$N_6$ framework serves as an excellent platform for the design of ultrachelating ligands by adding pyridine groups within the macrocyclic ring and pendant carboxymethyl groups on the amine nitrogen atoms.

## MATERIALS AND METHODS

**Preparations.** Starting materials were obtained from Aldrich Chemical Co. (lanthanide salts) or Fisher Scientific Co. and were used without further purification. The ligand PYAN.4HBr was prepared as described by Rothermel, *et al.*[13,16]

**$H_4$PYTA.3,6,14,17,23,24-hexaazatricyclo[17.3.1.1$^{(8.12)}$]tetracosa-(23),8,10,12(24),19,21-hexaene-3,6,14,17-tetraacetic acid dihydrate ($H_4PYTA.2H_2O$).** To a stirred suspension of PYAN.4HBr.$H_2O$(7.15 g, 0.011 mol in 20 mL $H_2O$) was added a solution of NaOH(1.76 g, 0.044 mol in 15 mL $H_2O$) slowly while keeping the temperature near 35°C by means of a water bath. The mixture was stirred while solid $BrCH_2COOH$( 3.056 g, 0.022 mol) was added. The reaction mixture was connected to a pH controller (Cole Parmer model 5652-10) and syringe pump (Sage Instruments model 341B) to maintain the pH near 8.5 by continuous addition of NaOH solution and a heating mantle to maintain the temperature at 60°C for the duration of the reaction. After 4 hours another 0.011 mol (1.53 g) of solid $BrCH_2COOH$ was added and after another 4 hrs. the final 0.011 mol (1.53 g) of solid $BrCH_2COOH$ was added. During these additions the pH was maintained at 8.5 and the temperature was 60°C. After about 24 hours total, the consumption of NaOH solution ceased and the reaction was stopped. A total of 14.5 mL of NaOH solution (3.52 g/15 mL) was consumed. The pH of the reaction mixture was then adjusted to 2.0 by addition of 48% HBr. The salt and excess reagents were removed from the product by cation exchange chromatography (Dowex 50*8–200). The chromatographic column (5 cm diameter by 13 cm height, acid form) was prepared by washing with water until the eluate was about pH 4.5. The reaction mixture was loaded onto the column and was washed with water until the eluate no longer tested positive for halide ions and the pH was about 4.5. The ligand was eluted from the column with 0.5 M aqueous $NH_3$. The fractions with pH 4.2–6.5 were collected and were evaporated to dryness under reduced pressure. The solid was dissolved in 25 mL $H_2O$ and formic acid was added to adjust the pH to 3.00. The mixture was evaporated to dryness under reduced pressure and the resulting solid was dissolved in 20 mL MeOH and stirred with heating at about 45°C, cooled to room temperature overnight to obtain very fine white crystals. The crystals were collected and stored in a vacuum desiccator. Yield: 4.8 g (70%). Recrystallization: The product from above (4.8 g) was dissolved in 20 mL MeOH at about 45°C, filtered, cooled, then placed in a refrigerator overnight to obtain pure white crystals

which were collected and stored in a vacuum desiccator (3.90 g, 81% recovery). $^1$H NMR ($D_2O$, reference DSS at 0.00 ppm): δ = 3.66 (s, 4 H), 3.76 (s, 4 H), 4.63 (s, 4 H), 7.64 (d, J = 7.8 Hz, 2 H), 8.10 (t, J = 7.8 Hz, 1 H); $^{13}$C NMR ($D_2O$, proton decoupled, dioxane ref. at 66.5 ppm): δ = 51.4, 56.2, 57.9, 125.2, 141.4, 150.7, 172.3. IR ($cm^{-1}$): $V_{OH}$ 3450; $V_{CH}$ 2950; $V_{C=O}$ 1640; $V_{C=N}$ 1598; $V_{CN}$ 1450. Anal. Calcd. for $C_{26}H_{34}N_6O_8.2H_2O$: C, 52.52; H, 6.44; N, 14.13. Found: C, 52.27; H, 5.95; N, 14.07.

**Characterization.** Infrared Spectra were obtained from samples prepared as KBr pellets using a Mattson 4020 FT-IR with a Dell 210 computer system. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. $^1$H NMR and $^{13}$C NMR Spectra were obtained from a Varian VXR-200 NMR spectrometer on samples dissolved in $D_2O$ or $CDCl_3$. UV/VIS spectra for spectrophotometric titrations were obtained using a Hewlett Packard 8452A diode array spectrophotometer with a Zenith data system. pH measurements for potentiometric titrations were obtained in a jacketed cell at 25°C using a Mettler DG111-SC electrode, Mettler DL-21 titrator, and a Brinkmann RM6 constant temperature circulator. The titration data were recorded automatically using Mettler TS2 titration software on a Gateway 486 computer.

**pH titrations followed by $^1$H NMR spectra.** The pH titrations of $H_4$PYTA were followed by $^1$H NMR spectra recorded on a Varian VXR 200 MHz spectrometer at an ambient temperature 24.6±0.1°C. A solution of $H_4$PYTA or at a concentration of 100 mM was prepared with $D_2O$ as the solvent. KCl (0.5 M) was added to maintain constant ionic strength. NaOD in $D_2O$ (2.5 M) was added to adjust the pD. The pD of the solution was measured with a semimicro combination electrode (Aldrich Chemical Co.) which was previously standardized with aqueous buffer standards and then equilibrated in $D_2O$. The pH was then calculated from the measured pD by using the following relationship:[26] pH = pD - 0.40.

**Potentiometric titrations.** Titrations of each ligand were performed using approximately 0.1 mmol dissolved in 35 mL of $CO_2$-free, deionized $H_2O$. KCl (0.2 M) was employed to maintain constant ionic strength. Other supporting electrolytes such as $NaNO_3$ or NaCl gave the same log $K_a$ values as KCl. Nitrogen gas was bubbled through the solution for 20 minutes before the titration began and then an atmosphere of $N_2$ was kept above the solution of titration vessel during the titration period. The solution was maintained at 25 ± 0.1°C and the pH was measured using a glass combination electrode (Mettler DG-111-SC) standardized at pH 4.00 and 7.00 (Ingold buffers). The solution was titrated with 0.1 M NaOH which had been previously standardized against potassium hydrogen phthalate. The $LnCl_3$ solutions (metal salts were purchased as 99.9+% purity) were standardized by titration with EDTA using xylenol orange as indicator.[27] Metal solutions were added by volumetric pipet to an equimolar quantity of ligand and titrated. The protonation and formation constants were determined from three separate potentiometric titrations by fitting titration data using a model for the equilibria present in the system and the program BEST.[28]

**Spectrophotometric titrations.** The stability constants of the lanthanide complexes of $PYTA^{4-}$ were determined spectrophotometrically by competition with the chelometric indicator dye, Arsenazo-III, using the method developed by Sherry.[29,30] This dye forms well-defined 1:1 and 1:2 Gd(III): arsenazo(III) complexes at pH 4.0 which absorb strongly at 660 nm ($\varepsilon_1$ = 35000, $\varepsilon_2$ = 50000 Liters/mol-cm, respectively) while the uncomplexed dye absorbed little at 660 nm ($\varepsilon$= 650 Liter/mol-cm).[31] Initial titrations of lanthanide solutions with Arsenazo-III in 0.01 M acetate buffer, 0.1 M NaCl provided the conditional stability constants used in all later competition reactions. A pH 3.95 conditional stability constant may be determined for $PYTA^{4-}$ (or DTPA) by starting with a known total concentration of lanthanide and Arsenazo-III, titrating this solution with $PYTA^{4-}$ (or $DTPA^{5-}$) directly. The decrease in absorbance is a measure of the ability of the added ligand to displace Arsenazo-III. The data were used with the computer program written by Cacheris[29,30] to calculate the conditional stability constants. The method was checked by determining the conditional stability of the $DTPA^{5-}$ complexes which gave good agreement with literature values.



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