280. Anion Coordination Chemistry: Polyguanidinium Salts as Anion Complexones

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Dedicated to the memory of Professor *Gerold Schwarzenbach*

(3.X.79)

Summary

The synthesis of a series of polyguanidinium salts of potential interest as *anion complexones* is described. Among the various synthetic methods investigated, the polyguanidinium salts were found to be most conveniently prepared from polyamines *via* polynitroguanidine intermediates. The complexation of phosphate and carboxylate anions by these complexones and by related polyammonium salts were studied by analysis of pH-metric titration data. The ligands studied form relatively stable complexes (log $K_s = 2.0$–4.0 for PO_4^{3-} in water) which also present good selectivities in some cases. Both the stability and the selectivity of complexation are primarily governed by electrostatic forces and thus depend on charge accumulation in the interacting species; structural effects are also observed. Since the binding is primarily electrostatic, polyammonium salts form more stable complexes (at a given charge) than do polyguanidinium salts. However, whereas the complexation properties of the latter are independent of pH, the complexes of the former are observed only in the limited ranges of pH where both the protonated polyamine and the anion of interest can coexist. The polycationic ligands may, in principle, form chelate type anion complexes. Comparison with the corresponding single binding sites reveals an increase in complexation constant of about two or three orders of magnitude; this may be considered as a thermodynamic indication of a chelate effect for the polydentate ligands (by analogy with the well known effects displayed by cation complexones); however, structural data on the formation of chelate 'rings' are not yet available. The nature of the complexes and the prospects of anion complexones in various fields are discussed.

1. Introduction. – Since *Gerold Schwarzenbach* and his school introduced the polycarboxylate complexones [1] [2] (EDTA, nitrilotriacetic acid, *etc.*), the coordination chemistry of these very efficient complexing agents has spread into widely diverse areas of pure and applied chemistry. Together with acyclic poly-

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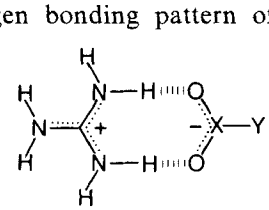
amines, they form chelate complexes [2] of varied stabilities and selectivities with a broad variety of metal cations and define a major area of cation coordination chemistry.

In marked contrast, the coordination chemistry of anions has received comparatively little attention and only scattered examples of anion binding were noted until the recent development of macropolycyclic ligands capable of forming highly stable and selective anion cryptates [3–6][2]). It thus appears highly desirable to search for a class of substances which would be relatively simple to prepare, but which would nevertheless display marked anion complexation properties. We now wish to report our studies on some *anion complexones*, an entry to the field of *anion chelates* in the general domain of anion coordination chemistry. Like the well known cation complexones, such substances may also have an impact in various areas of pure and applied chemistry.

The basic idea was to replace the amine or carboxylate functions of cation complexones by an equivalent group which would be a potential anion binding site, *i.e.* a site bearing a positive charge and capable of forming ionic hydrogen bonds ($A^+-H\ldots X^-$) with anions. The salts of polyamines are potential candidates, but, due to the generally low pK_a's for full protonation, anion complexation by *polyammonium* salts would be restricted to acidic solutions. However, the biological polyamines (putrescine, spermidine, spermine), where the amine functions are distant enough to be protonated at physiological pH, bind to nucleic acids and play an important role in nucleic acid and protein synthesis and in cell growth [8].

The *guanidinium group* presents several interesting features: i) It remains protonated over a much wider range of pH than does the ammonium group due to its much higher pK (13.5 for guanidinium itself [9]); ii) It may form characteristic pairs of zwitterionic hydrogen bonds $N-H^+\ldots X^-$ which provide binding strength by their charge and structural organization by their arrangement as seen from the crystal structures of many guanidinium salts [10] [11]. For instance, the binding in methylguanidinium dihydrogenphosphate ($CH_3NCH(NH_2)NH_2H_2PO_4$) [11a] involves a bidentate hydrogen bonding pattern of the following general type:



A similar arrangement is present in carboxylate salts [10] [12]; iii) Finally, the guanidinium group of arginyl residues in proteins has an important function in maintaining protein tertiary structure *via* internal 'salt bridges' with carboxylate groups, as well as in binding and recognition of anionic substrates by enzymes, receptor sites and antibodies [12–14].

We have thus synthesized a series of potential anion complexones consisting of a framework modelled on known cation complexones and containing guanidinium groups as the positively charged binding sites. We have undertaken a study of the

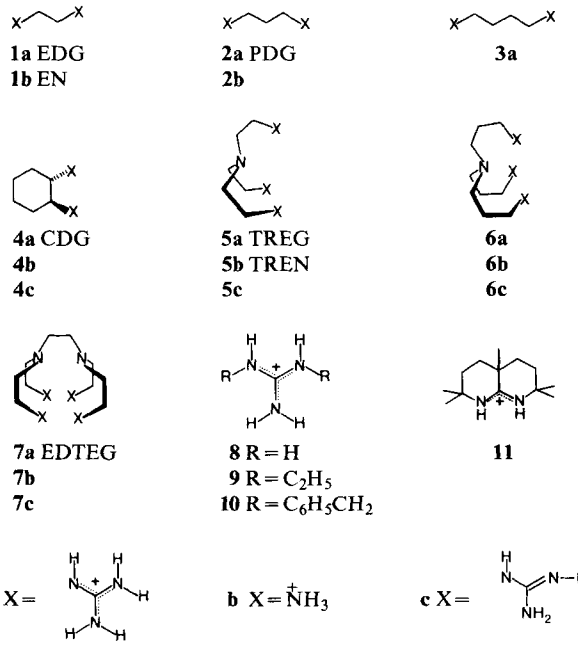
[2]) Lipophilic cation – lipophilic anion associations have also been described [7].

complexation of various common anions by these ligands as well as by the corresponding analogues bearing primary ammonium groups as binding sites. The synthesis and some anion binding properties of macrocyclic guanidinium salts have been described elsewhere [15].

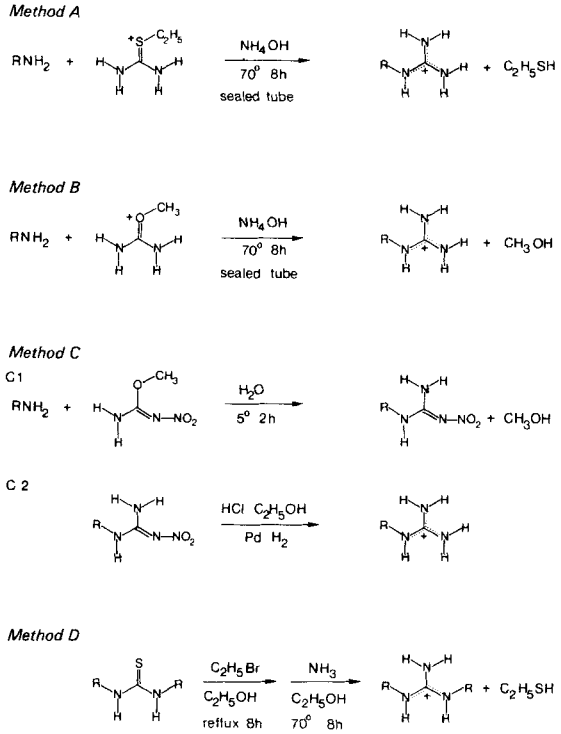
2. Synthesis of the polyguanidinium salts. – The compounds prepared are shown in *Scheme 1*. The ammonium salts were either commercially available or prepared by literature methods (**4b** [16], **6b** [17], **7b** [18]). Apart from guanidinium chloride itself (**8**) and the naturally occurring diguanidinium salt, arcaïne (**3a**) the members of the guanidinium series (**a**) were prepared from the corresponding amines (**1**–**7**) or thioureas (**9**, **10**). Several methods for the conversion of primary amines into monosubstituted guanidinium salts have been described. The principal methods employed throughout this study are shown in *Scheme 2*.

Method A, the reaction of a primary amine in basic solution with *S*-ethylthiouronium bromide, to give the guanidinium salt with loss of ethylmercaptan, has been the most throughly described [19–21]. At ambient temperature in open systems the yields were generally poor (~30%) especially if the product could not be crystallized from the reaction mixture [21]. However, at 70° in a sealed tube with the reagent in excess, the yields were generally good (~90% per guanidinium group).

Scheme 1. *Structures and trivial names of the anion complexones studied:* EDG, *e*thylene*di*guanidinium; PDG, *p*ropylene *di*guanidinium; CDG, *c*yclohexyl*di*guanidinium; TREG, *t*ris(*e*thyleneguanidinium) amine; EDTEG, *e*thylene*di*amine *t*etrakis(*e*thyleneguanidinium); CDTEG, *c*yclohexyl*di*amine*t*etrakis (*e*thyleneguanidinium). These trivial names are modelled on those of cation complexones in en, TREN, EDTA. This scheme indicates only the structural formulae; the exact degree of protonation of ammonium and guanidinium groups will be specified in the discussion. Counter anions are omitted throughout for clarity.



Scheme 2. *General methods for the synthesis of polyguanidinium salts.* Counter anions are omitted throughout for clarity.



The major by-products are urea and guanidinium bromide resulting from hydrolysis and ammoniolysis of the reagent respectively; these two contaminants could be removed by ion exchange chromatography (see Experimental Part).

Method B, using *O-methylisouronium* sulfate as reagent [22] [23], under conditions similar to those used with method A, gave similarly acceptable yields (~85–90% per guanidinium) together with the corresponding by-products. This method does, however, avoid the inconvenience of ethyl mercaptan, since methanol is formed.

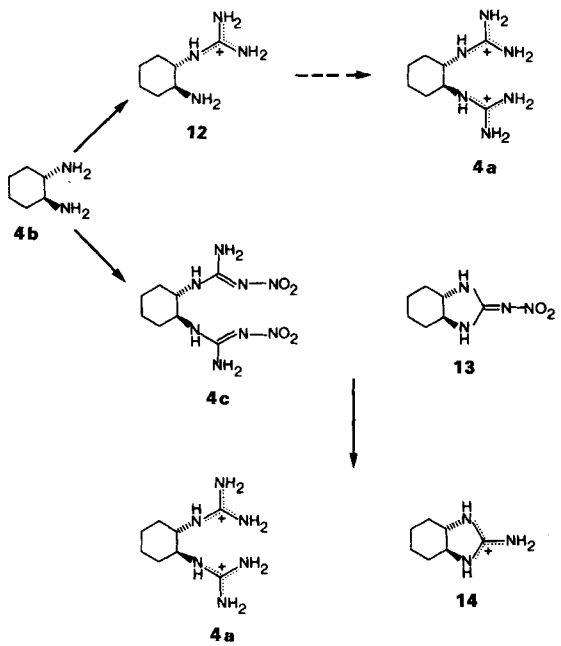
Both these methods proved to be of limited generality for the synthesis of polyguanidinium salts since the reactivity of the primary amines remaining in intermediate products diminishes rapidly with the increasing number of positively charged guanidinium groups introduced. The rate of reaction for the introduction of the final guanidinium group thus becomes slow compared to decomposition of the reagent by water or ammonia and mixtures of polyammonium-polyguanidinium salts were obtained. Such mixtures were difficult to separate except in the (fortunate) case where the desired product could be selectively crystallized from the mixture. Ion exchange chromatography of these mixtures was used to provide products of sufficiently high purity for subsequent physical measurements but the yields of isolated pure products were poor (~ 10%).

The method of choice for the preparation of polyguanidinium salts is that shown in *Scheme 2-Method C*. The reagent, 2-methyl-1-nitro-isourea, prepared by nitration of *O*-methylisouronium sulfate [24], reacted readily with polyamines in water to precipitate the corresponding poly-nitroguanidines, which were purified by recrystallization. The corresponding polyguanidinium salts were then prepared by hydrogenolysis of the nitroguanidines in acidic solution. Overall yields are not as high as with *methods A* and *B* (~ 80% per guanidinium group for the two steps) but the products were more easily isolated and purified than with the previous two methods. Thus the polyguanidinium salt EDTEG (**7a**), free of primary amine impurities, was prepared in yields comparable to those obtained with simpler compounds.

Method D (Scheme 2) represents a general synthesis of *N,N'*-disubstituted guanidinium salts as previously reported for the synthesis of guanidinium containing macrocycles [15]. Disubstituted thioureas were readily alkylated by ethylbromide and the resultant *S*-ethyl-isothiouronium salts were then sealed with ammoniacal ethanol and heated at 70° for 8 h. The guanidinium salts were isolated and purified either by recrystallization or by ion exchange chromatography. The method is generally applicable to polyguanidinium salts and yields are excellent (> 90% per guanidinium).

The synthesis of compound **4a** is outlined in *Scheme 3*. By either *method A* or *B*, even under forcing conditions with large excesses of reagent, the only product isolated was the monoammonium monoguanidinium salt **12**. Only small amounts

Scheme 3. *Synthesis of* trans-*1,2-diguanidinocyclohexane* **4a**



(< 5%) of the desired diguanidinium salt (CDG **4a**) were produced indicating that introduction of the second guanidinium group was a very unfavourable process under the reaction conditions. The inhibition is probably primarily electrostatic and not steric, since **4b** reacted readily with 2-methyl-1-nitro-isourea to give the desired *bis*-nitroguanidine **4c** albeit contamined with the cyclic nitroguanidine **13**. The proportion of **4c** in the mixture could be increased by ensuring that the reagent, 2-methyl-1-nitro-isourea was always present in large excess throughout the course of the reaction. Cyclization, however, always remained a significant (~ 10%) side reaction[3]). Compound **4c** could be purified by fractional crystallization but purification was more easily effected after reduction of the mixture of **4c** and **13** to **4a** and the aminoimidazolium salt **14**. Fractional crystallization of the picrate salts of **4a** and **14** gave **4a** as the more insoluble picrate.

3. Determination of stability constants. – 3.1. *Treatment of pH-metric data.* The concentration stability constants [26] were determined from an analysis of the pH-metric titration curves of various weak acids ($HA^{(n-1)-}$) in the presence and in the absence of ligands (L^{m+}) capable of anion complexation [27] [28]. The fundamental equilibria considered are given by equations (1) and (2) which respectively define the acidity constant of the weak acid ($HA^{(n-1)-}$) and the stability constant for formation of a complex (A^{n-}, L^{m+}) between the conjugate base of the weak acid (A^{n-}) and the ligand (L^{m+})

$$HA^{(n-1)-} \rightleftharpoons A^{n-} + H^+ \qquad K_{aHA(n-1)-} = \frac{[H^+][A^{n-}]}{[HA^{(n-1)-}]} \tag{1}$$

$$L^{m+} + A^{n-} \rightleftharpoons (A^{n-}, L^{m+}) \qquad K_s = \frac{[A^{n-}, L^{m+}]}{[L^{m+}][A^{n-}]} \tag{2}$$

Further equilibria may also occur in our systems such as:

protonation of the ligand L^{m+},

$$HL^{(m+1)+} \rightleftharpoons L^{m+} + H^+ \qquad K_{aHL(m+1)+} = \frac{[H^+][L^{m+}]}{[HL^{(m+1)+}]} \tag{3}$$

complexation of $HA^{(n-1)-}$ by L^{m+},

$$HA^{(n-1)-} + L^{m+} \rightleftharpoons (HA^{(n-1)-}, L^{m+}) \qquad K'_s = \frac{[(HA^{(n-1)-}, L^{m+})]}{[HA^{(n-1)-}][L^{m+}]} \tag{4}$$

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[3]) Facile cyclization of 2-amino-1-guanidinium salts to 2-aminoimidazolium salts even in acidic media has been noted in other systems. Similarly, 1,2-*bis*-isothiouronium salts on treatment with ammonia give only poor yields of 1,2-diguanidinium salts; cyclization was the important competing reaction [25].

and additional complexation equilibria between protonated forms of the anion and ligand, for example:

$$HA^{(n-1)-} + HL^{(m+1)+} \rightleftharpoons (HA^{(n-1)-}, HL^{(m+1)+}) \qquad K''_s = \frac{[(HA^{(n-1)-}, HL^{(m+1)+})]}{[HA^{(n-1)-}][HL^{(m+1)+}]} \quad (5)$$

All the complexation equilibria considered can be generalized as given in equation (6), which then defines a series of overall complexation constants β_{alh} defined by equation (7).

$$aA^{n-} + lL^{m+} + hH^{+} \rightleftharpoons (aA^{n-}, lL^{m+}, hH^{+}) \quad (6)$$

$$\beta_{alh} = \frac{[(aA^{n-}, lL^{m+}, hH^{+})]}{[A^{n-}]^a [L^{m+}]^l [H^{+}]^h} \quad (7)$$

The integers a, l, and h are respectively the number (including zero) of anions (A^{n-}), ligands (L^{m+}) and protons bound in a given complex. When $l = 0$, the β_{a0h} are simply the cumulative acidity constants for A^{n-} which can easily be converted to the conventional stepwise acidity constants. Similarly, when $a = 0$, the β_{0lh} are the cumulative acidity constants for L^{m+}.

The stability constants for complex formation can be derived from the value of β_{alh}. The simplest case is that described by (2) where $\beta_{110} = K_s$. In cases where the complex formed contains protons ($h \neq 0$), the value of a stability constant can also be derived. With reference to (4), the value of K'_s may be determined, given a cumulative complexation constant (in this case β_{111}). Rearrangement is given in (8)

$$\beta_{111} = \frac{[(A^{n-}, L^{m+}, H^{+})]}{[A^{n-}][L^{m+}][H^{+}]} = \frac{[HA^{(n-1)-}]}{[A^{n-}][H^{+}]} \cdot \frac{[(HA^{(n-1)-}, L^{m+})]}{[HA^{(n-1)-}][L^{m+}]} = \frac{1}{K_{aHA(n-1)-}} \cdot K'_s \quad (8)$$

gives $K'_s = (K_{aHA(n-1)-})(\beta_{111})$. Since the acidity constant $K_{aHA(n-1)-}$ is known, K'_s can be calculated. The more complicated case, described by (5), produces

$$\beta_{112} = \frac{[(A^{n-}, L^{m+}, 2\,H^{+})]}{[A^{n-}][L^{m+}][H^{+}]^2} = \frac{[HA^{(n-1)-}]}{[A^{n-}][H^{+}]} \cdot \frac{[HL^{(m+1)+}]}{[L^{m+}][H^{+}]} \cdot \frac{[(HA^{(n-1)-}, HL^{(m+1)+})]}{[HA^{(n-1)-}][HL^{(m+1)+}]}$$

$$= \frac{1}{K_{aHA(n-1)-}} \cdot \frac{1}{K_{aHL(m+1)+}} \cdot K''_s \quad (9)$$

whence $K''_s = (K_{aHA(n-1)-})(K_{aHL(m+1)+})(\beta_{112})$.

It must be noted that the rearrangement of β_{112} as outlined in equation (9) is not the only possibility. An alternative equilibrium satisfying the same stoichiometry is given by (10). In this case, a stability constant K'''_s can be calculated by (12).

$$A^{n-} + H_2L^{(m+2)+} \rightleftharpoons (A^{n-}, H_2L^{(m+2)+}) \qquad K_s''' = \frac{[(A^{n-}, H_2L^{(m+2)+})]}{[A^{n-}][H_2L^{(m+2)+}]} \tag{10}$$

$$\beta_{112} = \frac{[(A^{n-}, L^{m+}, 2\,H^+)]}{[A^{n-}][L^{m+}][H^+]^2} = \frac{[HL^{(m+1)+}]}{[L^{m+}][H^+]} \cdot \frac{[HL_2^{(m+2)+}]}{[H_2L^{(m+1)+}][H^+]} \cdot \frac{[(A^{n-}, H_2L^{(m+2)+})]}{[A^{n-}][H_2L^{(m+2)+}]}$$

$$= \frac{1}{K_{aHl(m+1)+}} \cdot \frac{1}{K_{aH2L(m+2)+}} \cdot K_s''' \tag{11}$$

$$K_s''' = (K_{aHL(m+1)+})(K_{aH2L(m+2)+})(\beta_{112}). \tag{12}$$

Unambiguous distinction between these two alternatives and others which satisfy the same stoichiometry is not possible based on the pH-metric titration results alone. Consideration of the relative acidity constants of the species involved together with the range of pH in which the complex has a maximum concentration can usually indicate which, of the several potential processes, will be the predominant one. Subsequent spectroscopic investigation of the complexes involved could also aid and confirm the interpretation of the complexation constants and the species involved. In any event, in all cases we will implicitly or explicitly define the manner in which the reported stability constant was obtained from the cumulative complexation constant. The information presented allows a calculation of the initial value of β_{alh} and thus the examination of other interpretations not specifically discussed. This point will be considered further in the discussion to follow.

3.2. *Titration methods.* For the determination of each stability constant, three types of measurements have been performed: i) titration of the anion alone in the presence of a non complexing cation (Me_4N^+) to maintain a constant ionic strength, permitting a determination of the acidity constants of the anion under the experimental conditions; ii) titration of the ligand alone, in the presence of a non-complexed anion (Cl^-, see discussion) to maintain a constant ionic strength, permitting a determination of the acidity constants of the ligand under the experimental conditions; iii) titration of the anion plus the ligand at various concentrations, in the presence of added salt (Me_4NCl) to maintain a constant ionic strength.

The pK_a values of the anion and ligand were calculated from the titrations i) and ii) above by an iterative computer program designed to calculate β_{alh} for generalized species as defined in equation (7) [29]. The third type of titration curve was then analyzed using these pK_a's as invariable constants and a set of β_{alh} as variable constants. The latter were iterated by the program to reproduce the experimental curve obtained from titrations of type iii) above. Stability constants were then calculated as described above. Further discussion of titration and computing procedures will be found in the experimental section.

4. Results. 4.1. *Acidity constants.* Stepwise acidity constants for the anions and ligands considered are given in *Table 1*. Values for two solvent systems, water (W) and methanol:water 9:1 (M/W) are given, together with literature values where available. Agreement with literature values is good; departures arise due to differences in experimental conditions (temperature, ionic strength, background salt). The acidity constants reported in *Table 1* refer only to protonation of amino groups. All guanidinium groups are assumed to remain fully protonated throughout the range of pH and concentration studied. This assumption is reasonable as shown by the following observations: i) Attempts to determine the pK_a's of the guanidinium groups by direct titration with concentrated NaOH solutions were unsuccess-

Table 1. *Stepwise acidity constants (pK_n) of the ligands and anions studied*[a])

Ligand/Anion	Solvent	pK_1	pK_2	pK_3	pK_4
1b[b])	W	9.84	7.02	–	–
1b	M/W	11.12	7.52	–	–
2b[b])	W	10.32	8.67	–	–
2b	M/W	11.94	9.26	–	–
4[c])	W	9.60	6.48	–	–
5a	W	2.48	–	–	–
5a	M/W	< 2.0	–	–	–
5b[d])	W	9.89	9.34	8.22	< 2.0
5b	M/W	14.00	11.25	9.54	< 2.0
6a	W	6.86	–	–	–
6b[e])	W	10.52	9.97	9.13	5.62
7a	W	4.14	< 2.0	–	–
7a	M/W	< 2.0	< 2.0	–	–
7b[f])[g])	W	9.92	9.39	8.72	8.16
7b[f])	M/W	11.97	10.55	10.00	8.42
12	W	8.05	–	–	–
PO_4^{3-}[h])	W	10.95	6.76	2.33	–
PO_4^{3-}[i])	M/W	12.10	4.81	< 2.0	–
$P_2O_7^{4-}$[j])	W	8.63	6.19	–	–
$P_3O_{10}^{5-}$[k])	W	8.33	5.79	–	–
maleate^{2-}	M/W	12.07	< 2.0	–	–
fumarate^{2-}	M/W	7.44	5.23	–	–
oxalate^{2-}	M/W	7.64	2.22	–	–
acetate$^-$	M/W	7.73	–	–	–

a) Experimental conditions: $2.5 \cdot 10^{-3}$M solutions of ligand (fully protonated) as the chloride salt or of anion (fully deprotonated) as the sodium salt were made to an ionic strength of 0.1M with Me_4NCl. Ligand solutions were titrated with 0.2M NaOH; anion solutions were titrated with 0.2M HCl. Temperature 25.0 ± 0.1°; W = water; M/W = methanol/water 9:1.

b) $pK_1 = 9.96$, $pK_2 = 7.10$ ($NaClO_4$, 25°, corrected to 0.1M ionic strength) [63].

c) $pK_1 = 9.89$, $pK_2 = 6.72$ (0.1M KCl, 25°) [64].

d) $pK_1 = 10.29$, $pK_2 = 9.59$, $pK_3 = 8.56$ (0.1M KCl, 20°) [65].

e) $pK_1 = 10.51$, $pK_2 = 9.82$, $pK_3 = 9.13$, $pK_4 = 5.61$ (0.1M KCl, 25°) [16].

f) pK_5, $pK_6 < 2.0$.

g) $pK_1 = 10.10$, $pK_2 = 9.60$, $pK_3 = 9.00$, $pK_4 = 8.44$, $pK_5 = 1.33$ (0.1M KNO_3, 25°) [66].

h) $pK_1 = 11.10$, $pK_2 = 6.61$, $pK_3 = 2.36$ (0.1M Me_4NBr, 25°) [67].

i) Me_4N^+ salt used.

j) $pK_1 = 8.93$, $pK_2 = 6.13$ (0.1M Me_4NCl, 25°) [68].

k) $pK_1 = 8.73$, $pK_2 = 6.00$ (0.1M Me_4NCl, 25°) [68].

Table 2. *Stability constants,* log K_s, *for complex formation with phosphate anions in aqueous solution at 25°* (K_s in 1 mol^{-1})[a]

Ligand[b]	PO_4^{3-}	HPO_4^{2-}	$P_2O_7^{4-}$	$HP_2O_7^{3-}$	$H_2P_2O_7^{2-}$
1a^{2+}	2.3	<	2.5	1.3	<
1b^{2+}	*	1.8	*	2.6	1.9
1b^{+}	<	0.4	1.5	2.5	*
2a^{2+}	1.8	<	2.2	1.4	<
2b^{2+}	*	2.1	3.3	2.6	2.6
2b^{+}	<	1.8	0.6	*	*
3a^{2+}	1.1	<	-	-	-
4a^{2+}	2.4	1.2	2.7	2.0	<
4b^{2+}[c]	*	3.6	-	-	-
4b^{+}	3.4	2.9	-	-	-
5a^{3+}[d]	3.5	1.9	4.3	3.0	<
5b^{3+}[e][f]	*	3.3	5.6	3.8	2.7
5b^{2+}	<	2.3	3.1	*	*
6a^{4+}	*	<	*	3.1	1.3
6a^{3+}	2.1	1.8	2.4	2.0	*
6b^{3+}[g]	*	2.6	-	-	-
6b^{2+}	<	2.0	-	-	-
7a^{4+}[h]	3.1	1.9	4.1	2.6	<
7b^{4+}	*	2.1	4.8	3.2	<
7b^{3+}	<	2.0	3.9	*	*
8^{+}[i]	0.4	<	-	-	-
8^{+}[j]	3.3	1.5	-	-	-
9^{+}	0.8	<	1.4	1.2	<
9^{+}[j]	2.6	1.2	-	-	*
10^{+}[j]	3.1	1.4	-	-	-
11^{+}[j]	3.0	1.4	-	-	-
Li^{+}[k]		0.72[l]	2.39[m]	1.03[m]	
Na^{+}[k]		0.60[l]	1.00[m]	<	
K^{+}[k]		0.49[l]	0.80[m]	<	
Mg^{2+}[k]		1.88[n]	5.41[m]	2.34[m]	
Ca^{2+}[k]		1.70[n]	5.55[m]	2.28[m]	

[a] Experimental conditions: see Experimental Section; ionic strength 0.1M with Me_4NCl, 25°, solvent = water except as noted[j]. The sign < indicates that a complex of the given stoichiometry was not necessary to fit the data, thus weaker than would be detectible by this technique. The sign * indicates that a complex of the same stoichiometry is assigned elsewhere in the same ligand/phosphate anion group.

[b] The total charge on the ligand indicates for ammonium ligands the degree of protonation of the primary amines.

[c] log K_s: **4b**$^{2+}$/$H_2Po_4^-$ = 3.4.

[d] log K_s: **5a**$^{3+}$/$P_3O_{10}^{5-}$ = 4.3; **5a**$^{3+}$/$HP_3O_{10}^{4-}$ = 3.

[e] log K_s: **5b**$^{3+}$/$H_2PO_4^-$ = 2.1

[f] log K_s: **5b**$^{3+}$/$P_3O_{10}^{5-}$ = 6.3; **5b**$^{3+}$/$HP_3O_{10}^{4-}$ = 4.2; **5b**$^{2+}$/$H_2P_3O_{10}^{3-}$ = 2.8.

[g] log K_s: **6b**$^{3+}$/$H_2PO_4^-$ = 1.6.

[h] log K_s: **7a**$^{4+}$/$P_3O_{10}^{5-}$ = 3.2.

[i] Ionic strength = 1.0M, Me_4NCl, 25° [32].

[j] Solvent: methanol/water 9:1.

[k] log K_s: $Li^+/HP_3O_{10}^{4-}$ = 2.87, $Li^+/H_2P_3O_{10}^{3-}$ = 0.88, $Na^+/HP_3O_{10}^{4-}$ = 1.64, $Na^+/H_2P_3O_{10}^{3-}$ = 0.77, $K^+/HP_3O_{10}^{4-}$ = 1.39, $Mg^{2+}/HP_3O_{10}^{4-}$ = 5.83, $Mg^{2+}/H_2P_3O_{10}^{3-}$ = 2.13, $Ca^{2+}/HP_3O_{10}^{4-}$ = 5.44, $Ca^{2+}/H_2P_3O_{10}^{3-}$ = 3.04 (ionic strength = 0.1M Me_4NCl, 25°) [36].

[l] Ionic strength 0.2M ($(nPr)_4NCl$, 25°) [30].

[m] Ionic strength 0.2M ($(nPr)_4NCl$, 25°) [37].

[n] Ionic strength 0.1M (Me_4NCl, 25°) [27].

ful [25]; ii) The proton and ^{13}C-NMR. spectra of ligands containing only guanidinium groups (*e.g.* **1a**, **2a**) are independent of hydroxide (deuteroxide) ion concentration up to 0.5 M. Similar observations which apply to other guanidinium containing ligands after deprotonation of amine functional groups are accounted for[4]); iii) The charge of the guanidinium group is more delocalized than that of the ammonium group, thus the electrostatic effect of a guanidinium group on protonation equilibria at adjacent sites is expected to be less marked than that of an ammonium group. This is clearly shown by comparing compounds **4b** and **12**; pK_2 for the former is 6.48 while the pK_a of the ammonium group of the latter is 8.05. The substitution of an ammonium group by a guanidinium group significantly reduces electrostatic interactions. For polyguanidinium species, the first and final pK_a's would thus be expected to differ much less than for the corresponding polyammonium species. Since guanidines are more basic than amines, full protonation of the former can be retained even at high pH. The charge on the ligand will be indicated for each species whenever desirable for the sake of clarity and ease of reading.

Table 3. *Stability constants* (log K_s), *for complex formation with carboxylate anions in methanol/water* 9:1 *at* 25° (K_s in 1 mol^{-1})[a])

Ligand[b])	Acetate	Maleate dianion	Maleate monoanion	Fumarate dianion	Fumarate monoanion
1a^{2+}[c])	2.2	4.2	<	ppt.	-
1b^{2+}	-	*	3.4	ppt.	-
1b^{+}	-	4.5	3.3	-	-
2a^{2+}	1.8	<	2.2	1.4	<
2b^{2+}	-	*	3.6	*	2.2[e])
2b^{+}	-	4.3	3.6	<	4.6[e])
3a^{2+}	-	3.2	<	2.1	1.5
4a^{2+}	-	3.9	<	3.0	1.4
5a^{3+}	2.4	4.4	<	2.5	1.6
5b^{3+}	-	*	<	3.3	2.3
5b^{2+}	-	3.9	2.9	3.0	*
5b^{+}	-	2.5	*	2.9	*
7a^{4+}	2.7	5.1	<	3.5	2.4
7b^{4+}	3.7	7.1	<	6.0	<
7b^{3+}	<	5.8	*	4.6	*
10^{+}[f])	1.4	1.9	<	0.9	<
11^{+}	1.7	2.1	<	1.8	1.2
12^{+}	1.2	-	-	-	-

[a]) Experimental conditions, see Experimental Part. The symbols *, < and - are as defined for *Table 2*, footnote [a]).

[b]) The charge is defined as in *Table 2*, footnote [b]).

[c]) Log K_s **1a**$^{2+}$/oxalate^{2-} = 2.9.

[d]) Log K_s **2a**$^{2+}$/oxalate^{2-} = 1.6.

[e]) These values are less reliable, due to precipitation of insoluble salts during the titration; log $K_s \pm 0.5$.

[f]) Log K_s **10**$^{+}$/oxalate^{2-}) = 1.2.

[4]) Free guanidines can be observed in CD_3OD using $NaOCD_3$ as the base. Deprotonation shifts of 0.25 ppm upfield were observed in the proton NMR. spectrum of **1a**. This compares to a deprotonation shift of 0.8 ppm for **1b**.

4.2. *Stability constants.* Stability constants for complex formation with phosphate anions are given in *Table 2*; they refer to water solutions except for monopositive ligands which were measured in methanol:water 9:1. Data for the complexation of phosphates by alkali metal and alkaline-earth cations are included for comparison purposes. Stability constants for complex formation with carboxylate anions are given in *Table 3.* (methanol:water 9:1 solutions). In all cases the constants are relative to ~0.1 M chloride anion (~20-fold excess) (see below).

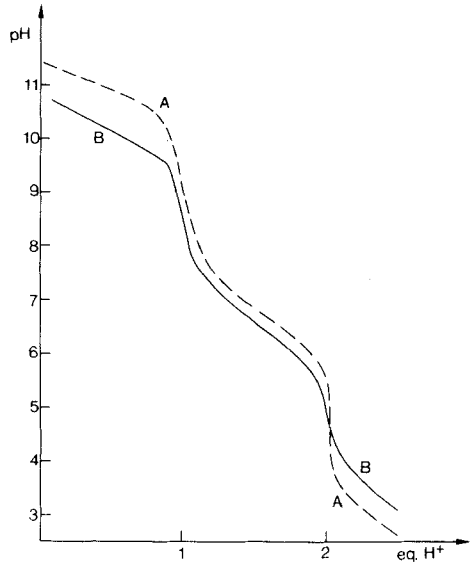

Fig. 1. *Titration curves* for A: $5 \cdot 10^{-3}$M PO_4^{3-} (---) and B: $5 \cdot 10^{-3}$M PO_4^{3-} and $5 \cdot 10^{-3}$M TREG **5a** (——) in aqueous solution at 25°

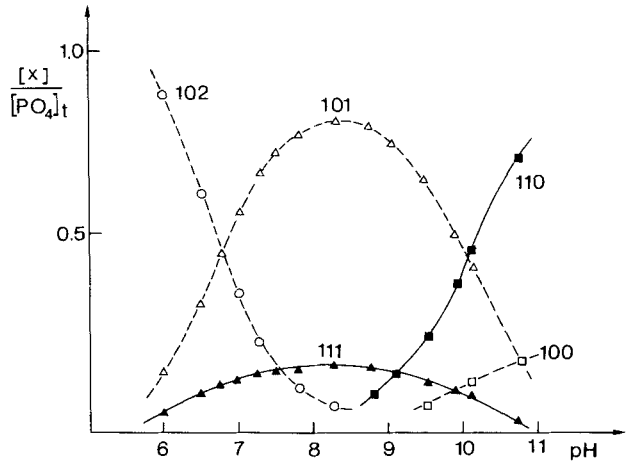

Fig. 2. *Distribution curves of species x in solution for the complexation of phosphate anions by ligand TREG* (**5a**). The curves give $[x]/[PO_4]_{total}$ as a function of pH, as calculated from the titration curves of *Figure 1.* The stoichiometry (a1h) of each species is indicated as defined in the text. The broken lines refer to free phosphate anions; the solid lines refer to complexes of phosphate and ligand; $100 = PO_4^{3-}$ (--□--); $101 = HPO_4^{2-}$ (--△--); $102 = H_2PO_4^-$ (--○--); $110 = PO_4^{3-}$, $\mathbf{5a}^{3+}$ (—■—); 111 HPO_4^{2-}, $\mathbf{5a}^{3+}$ (—▲—).

The analysis of titration curves involving guanidinium ligands is usually straight forward. A typical experiment is shown in *Figure 1* for the case of TREG (**5a**) with orthophosphate. The calculated distribution curves for this example, showing the relative concentrations of various species as a function of pH are given in *Figure 2*. The titration curves indicate complexes with PO_4^{3-} and with HPO_4^{2-} since the curves in the presence of ligand are shifted to lower pH as expected for a simple system described by equations (1) and (2) [27] [30]. Cumulative association constants for complexes having the stoichiometries (110) (1 phosphate, 1 ligand, no dissociable protons) and (111) (1 phosphate, 1 ligand, 1 dissociable proton) were thus calculated giving the results in *Figure 2*. The value of log β_{110} (3.50) is log K_s for a complex of $TREG^{3+}$ with PO_4^{3-}. The value of log β_{111} (12.90) is the sum of pK_1 (PO_4^{3-}) = 10.95 and log K_s for the complex of $TREG^{3+}$ and HPO_4^{2-} (1.95). Analysis of the titration curves of other guanidinium containing ligands was accomplished along the same lines. The association constants of the guanidinium cation **8** with various oxyanions have been reported in the literature [31] [32].

The analysis of the titration curves of ligands containing primary ammonium groups is more complex. *Figure 3* shows the titration curves for TREN **5b**, orthophosphate and an equimolar mixture of the two species. The horizontal axis is given as the number of equivalents of added acid. The origin is defined as a solution containing **5b** as the free base and fully deprotonated phosphate (PO_4^{3-}). The slight inflexion points at 1 and 4 equivalents suggest the points where the phosphate is being titrated; the long buffer region between 1 and 4 equivalents would thus represent protonation of **5b**. The calculated distribution curves for this system are given in *Figure 4*. Inspection of the pH ranges where various complexes exhibit maximal concentrations suggests that the complex (115) involves $H_2PO_4^-$ and the complex (114) contains HPO_4^{2-}; the nature of the complex (113) is not clear. The interpretation of these distribution curves is facilitated by

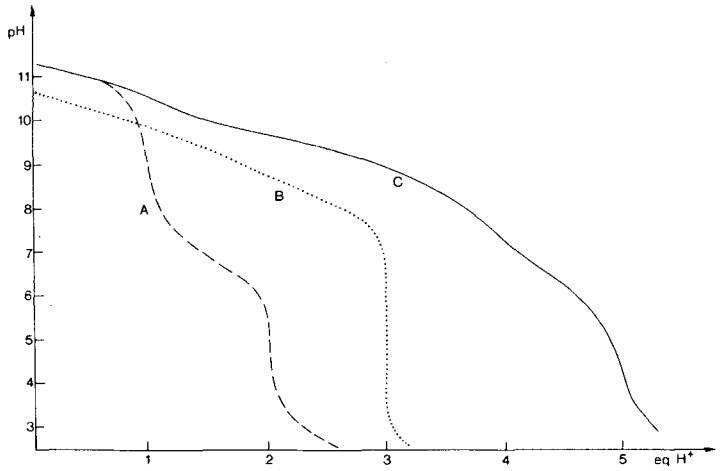

Fig. 3. *Titration curves for:* A: $5 \cdot 10^{-3}$M PO_4^{3-} (----); B: $5 \cdot 10^{-3}$M TREN **5b** (.....); C: $5 \cdot 10^{-3}$M PO_4^{3-} and $5 \cdot 10^{-3}$M TREN **5b** (——), in aqueous solution at 25°.

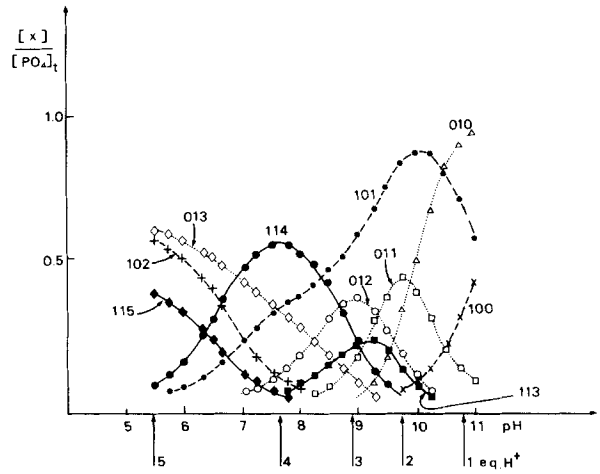

Fig. 4. *Distribution curves of species x in solution for the complexation of phosphate anions by TREN* **5b** *cations.* The curves give $[x]/[PO_4]_{total}$ plotted as a function of pH (and of the number of equivalents of added mineral acid) as calculated from the titration curves of *Figure 3.* Species are defined by stoichiometry number as defined in the text. The broken lines refer to free phosphate species; the dotted lines refer to free ligand species; and the solid lines refer to complexes. $100 = PO_4^{3-}$ (– – × – –); $101 = HPO_4^{2-}$ (– – • – –); $102 = H_2PO_4^-$ (– – + – –); $010 = \mathbf{5b}^{\circ}$ (·····△·····); $011 = \mathbf{5b}^{+}$(·····□·····); $012 = \mathbf{5b}^{2+}$ (·····○·····); $013 = \mathbf{5b}^{3+}$ (·····◇·····); $113 = PO_4^{3-}/\mathbf{5b}/3\ H^+$ (——■——); $114 = PO_4^{3-}/\mathbf{5b}/4\ H^+$ (——●——); $115 = PO_4^{3-}/5b/5\ H^+$ (——◆——).

considering the ^{13}C-NMR. protonation shifts of the carbon atoms *α* to the tertiary nitrogen of ligand **5b** in the absence and presence of equimolar orthophosphate *(Fig. 5)*[5]). In the absence of phosphate, protonation of **5b** produces a regular upfield shift. In the presence of equimolar phosphate, addition of the first equivalent of acid produces a modest upfield shift, whereas the following three equivalents cause upfield shifts similar to those observed with **5b** alone. Finally, the fifth equivalent of acid again induces only a modest upfield shift. The ^{13}C-NMR. spectra of all the ligands prepared in general, and of **5b** in particular, are independent of counter anion (at constant pH) [25]. The observed protonation shifts reflect primarily the degree of protonation of the ligand and are not due to changes in the proportions of free and complexed **5b**. Therefore between 0 and 1 equivalents, PO_4^{3-} is the principal species being protonated to give HPO_4^{2-}, between 1 and 4 equivalents, mainly protonation of **5b** occurs giving successively $\mathbf{5b}^+$, $\mathbf{5b}^{2+}$ and $\mathbf{5b}^{3+}$, and between 4 and 5 equivalents, the main process is protonation of HPO_4^{2-} to give $H_2PO_4^-$. The complex (115) which has a maximal concentration around 5 equivalents thus involves $H_2PO_4^-$ and $\mathbf{5b}^{3+}$; the complexes (114) and (113) are complexes of HPO_4^{2-} with $\mathbf{5b}^{3+}$ and $\mathbf{5b}^{2+}$ respectively. The stability constants found in *Table 2* were calculated accordingly. In other cases (with other ligands) when two or more interpretations of the processes represented by a given

[5]) The shifts of the carbon atoms *α* to the primary ammonium groups are much smaller (25 Hz total range) than those displayed in *Figure 5.* These smaller shifts are significant in that they exhibit the same tendencies as, hence serve to confirm the conclusions drawn from, the larger shifts.

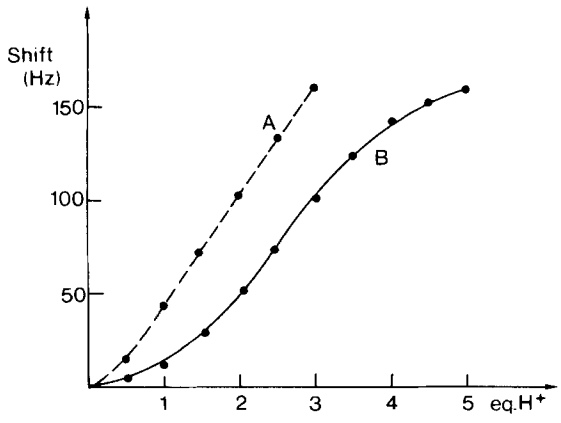

Fig. 5. *^{13}C-NMR. protonation shifts of carbon α to the tertiary nitrogen of TREN* **5b** *vs* the number of equivalents of added mineral acid for A: TREN alone (----); B: TREN and PO_4^{3-} (——).

stoichiometry are possible, similar analysis have been undertaken and the results obtained are those reported in *Tables 2* and *3*. We have been unable to determine association constants of stoichiometries ligand/anion $\neq 1$; such complexes are expected to be less stable and, if present were not required to fit the data even for experiments run at much higher concentrations (0.1 M) of ligand and of anion [25].

5. Discussion. – 5.1. *Stabilities and selectivities of anion complexation.* The stability constants reported permit a number of general conclusions to be drawn concerning the complexation of anionic species by the ligands studied.

a) *Electrostatic charge-charge interaction* is the *dominant factor* governing both the stability and selectivity of complexation. The strongest complexes are thus formed between the *species of highest charge density.* For example, among various protonated forms of a given phosphate anion, the most highly charged are the most strongly complexed; thus with ligand $\mathbf{5b}^{3+}$ $\log K_s$ $P_2O_7^{4-} > \log K_s$ $HP_2P_7^{3-} > \log K_s$ $H_2P_2O_7^{2-}$. Similarly, increasing the positive charge of the ligand increases the stability constants with a given anion; considering any anion, $\log K_s$ increases along the series monoguanidinium (**8**–**11**) < diguanidinium (**1a**–**4a**) < triguanidinium (**5a**). Among ligands of the same charge and binding group, increasing the distance between the positively charged sites decreases the electrostatic interactions with the anion, hence decreases the stability constant; for instance with PO_4^{3-} and the series of diguanidinium species, $\log K_s$ **1a** > $\log K_s$ **2a** > $\log K_s$ **3a**. Similar trends have been noted previously with respect to complexation of phosphates by alkali metals and alkaline earth cations [30] and by the parent guanidinium cation [32]. Complexes involving monocharged ligands or anions are observable only in solvents of lower dielectric constant such as aqueous methanol.

b) *Comparison between guanidinium and ammonium groups* as positively charged binding sites shows that in all cases where direct matching is possible (among species of same charge), the complexes of ammonium bearing ligands are about an order of magnitude more stable than those of the corresponding guanidinium ligands; consider $P_2O_7^{4-}$ with $\mathbf{2a}^{2+}/\mathbf{2b}^{2+}$, $\mathbf{5a}^{3+}/\mathbf{5b}^{3+}$, *etc.* This again reflects the

dominance of the electrostatic interactions. Since the charge on the guanidinium group is much more delocalized than on the ammonium group, charge-charge attractions are expected to be more important for the latter. This is apparently so despite the fact that the guanidinium group has a suitable geometry for forming *two* linear H-bonds with a bent XO_2^- center as represented in the structure depicted above.

Some complexes of biologically important phosphates with salts of naturally occurring polyamines (putrescine, spermine, spermidine) have been studied by NMR. [33] and crystallography [34], and their stability constants have been measured by an anion exchange method [35]. The stability constants (in the pH 7 region) ranged from log $K_s = 1.6$ for the complex of diprotonated putrescine $^+H_3N(CH_2)_4NH_3^+$ with AMP^{2-}, to log $K_s \sim 3.9$ for the complex of tetraprotonated spermine $^+H_3N(CH_2)_3NH_2^+(CH_2)_4NH_2^+(CH_2)_3NH_3^+$ with ATP^{4-}. These log K_s values fall within the ranges expected for interactions between species of similar charges as measured with the ligands of this study.

It must be noted however that the complexation of an anion by a polyammonium salt occurs only in limited ranges of pH where the protonated amine and the anion in question can coexist. This restriction is of importance with respect to the complexation of PO_4^{3-} (and other basic anions). Neither the polyammonium salts investigated, nor alkaline-earth cations can be presumed to form complexes in solution with this anion; the former are not sufficiently protonated while the latter form insoluble precipitates [27] [36] [37] in basic solutions. Since the degree of protonation of (hence the complexation of anions by) polyguanidinium salts is independent of pH, they are more generally applicable as anion complexones than polyammonium salts.

c) *The anion binding selectivities* of the ligands rest on electrostatic interactions; the more highly charged anion is selectively complexed by a given ligand. Among equally charged anions, *structural effects* play a role *(Table 3)*. Thus the selectivity for maleate dianion over fumarate dianion reaches 90 for $TREG^{3+}$ $\mathbf{5a}^{3+}$, in favour of the anion of higher local charge density.

Appreciable selectivities are found among monoanions. Control experiments showed that log K_s for chloride binding by EDG ($\mathbf{1a}^{2+}$) and TREN ($\mathbf{5b}^{3+}$) are respectively < 0.3 and < 1.7[6]). Thus, the selectivity of EDG for acetate over chloride is > 75 and may result from the paired hydrogen bonding scheme observed in guanidinium carboxylate crystal structures [10] [12].

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[6]) All stability constants reported here were determined relative to a large (20-fold) excess of chloride anion; in general, when measured at two or more anion concentrations (hence two or more anion/chloride ratios), they were virtually independent of chloride concentration (within the limits of the experiment). No association could be detected between $TREN^{3+}$ ($\mathbf{5b}^{3+}$) and chloride anion in methanol/water 9:1 at pH 4.0 using a chloride specific ion electrode technique [25]. A complex having $K_s > 50$ (log $K_s > 1.7$) would have been observable under the conditions of the experiment (ligand concentration, chloride concentration). Similarly an attempt to determine chloride complex of EDG ($\mathbf{1a}^{2+}$) by competition with acetate in methanol/water 9:1 was also unsuccessful; the apparent K_s for the complexation of acetate was independent of chloride concentration from $2.5 \cdot 10^{-4}$ to $9.5 \cdot 10^{-2}$ M showing that a complex of chloride with EDG must have log $K_s < 0.3$.

It is probable that more extensive studies with a variety of different anions would reveal many other structural effects.

d) Since the ligands studied here bear several binding sites, the observed anion complexation may present features analogous to the *chelate effect* for cation complexation by polycarboxylate and polyamine complexones [2] [38]. Two main aspects may be considered: thermodynamic, an increase in stability of the complexes, and structural, the formation of chelate rings.

From the reported results it follows that the polyguanidinium and polyammonium ligands display thermodynamic features of a *chelate effect for anion complexation.* A strict comparison would require knowledge of the stepwise stability constants for the parent guanidinium and ammonium cations; these have only been determined for guanidinium itself with different anions and are very small [32]. However, the stabilities of the PO_4^{3-} complexes of EDG (**1a**), TREG (**5a**) and EDTEG (**7a**) are higher than for diethylguanidinium (**9**) by factors of about 10^2 to 10^3. On the other hand, factors of > 10 and $\sim 10^{10}$ are found for the complexation of Ca^{2+} by succinate and EDTA respectively compared to acetate [39].

The stability increase observed for anion complexation appears much smaller than the large effects which are achieved for cation chelation by the usual cation complexones. This may arise from a number of differences between the two complexation processes: i) Since complexation competes with solvation, and since anions are more strongly hydrated than cations of the same size [40], the complexation of anions appears more difficult than that of cations. In the absence of very stable complexes, high chelate effects appear unlikely; ii) The ligands which give very stable cation complexes generally contain binding sites which come into direct contact with the bound cation; orbital overlap between the bound ion and the binding site may contribute to the complexation process (especially for transition metal ions) [2] [38]. The ionic hydrogen-bonding scheme envisaged for anion complexation is primarily of electrostatic nature [41] [42] and precludes any such close contact or orbital overlap[7]); iii) Strong cation binding by polydentate ligands usually involves formation of the favourable 5-membered chelate rings [2] [38]. Comparison of the structural fragments of EDTA and EDTEG reveals that such small chelate rings cannot form with the latter. In fact, since two methylene groups are present between the amine nitrogen atom and the charged site, EDTEG resembles ethylenediamine-*N, N, N', N'*-tetrapropionate more closely than EDTA. The cation complexes of the former are at least seven orders of magnitude less stable than the corresponding EDTA complexes and also exhibit a 'chelate' effect of only ~ 30 for Mg^{2+} when compared to acetate [39]. Similar considerations hold for other cation ligands structurally comparable to the present anion complexones and bearing carboxylates in the place of guanidinium groups (acetate, succinate, *β β'β''*-nitrilotripropionate, *etc.*); indeed, any chelate effect observed, *e.g.* with

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[7]) Sites with vacant orbitals, like boron containing groups, would bind anions directly. However organic ligands of this type are usually quite reactive, especially towards hydrolysis. In general terms, species like $PtCl_6^{2-}$ or hemin chloride may also be considered as anion complexes in which the binding site is a metal cation.

Ca^{2+}, is < 10. In comparison, oxalate dianion is bound about 50 times more strongly by EDG than by diethylguanidinium **9**.

It thus appears that the anion complexones described here present the thermodynamic features of polydentate chelating ligands. Whether the anion complexes formed also show the structural chelate features must await crystal structure determinations. It may be noted however that in the binding of spermine to transfer-RNA, one polyamine molecule curls around a phosphate group [34a]. Furthermore, the ethylenediammonium group exists in a *gauche* type chelating conformation in crystalline ethylenediammonium sulfate [43a] and citrate [43b]; similarly, the TREN unit forms a chelating structure towards a chloride anion in the crystals of TREN-trihydrochloride [44]. In addition, molecular models indicate that TREG (**5a**) and EDTEG (**7a**) are able to hold the phosphate and diphosphate polyanions in a chelating structure making use of all guanidinium sites.

e) Finally, many important biological molecules bear anionic groups which are recognized and bound by receptor sites [12–14]. Polycationic arginine rich polypeptides and proteins (the protamines and histones) bind to nucleic acids and play a major role in chromatin and chromosome structure and in nuclear function [45–47][8]). These interactions being mainly electrostatic, biological receptor sites probably make use of two other factors for increasing binding strength of anions: desolvation of the cationic binding groups and a low local dielectric constant inside a protein cavity.

6. Conclusions and prospects. – Polyguanidinium and polyammonium anion complexones represent a step in developing an anion coordination chemistry, yielding relatively stable anion complexes which display anion chelate effect and structural selectivity features. The polyguanidinium ligands, although they bind less strongly, are of special interest because of their pH independence. A more detailed structural and thermodynamic analysis of these anion complexes and of anion chelation requires the determination of crystal structures and of the enthalpies and entropies of complexation. Comparison with the corresponding features of cation chelation should be very instructive for the general understaning of complexation processes. The design and synthesis of other types of polyguanidinium anion ligands should be of interest in various areas of pure and applied fields of chemistry. They may incorporate structural units in which the guanidinium group is held *rigidly* (as in bicyclic systems derived from compound **11** or from imidazo[1,2-a]imidazole) or surrounded by *lipophilic* groups, or held inside a molecular *cavity* in order to inhibit solvation and to enhance substrate recognition ('*crypto*'-sites) *etc.*

In addition to their basic interest as new types of receptor molecules for the selective binding of anionic substrates, such systems may display interesting chemical properties and lead to practical applications in analytical chemistry, separation science, *etc.* Thus, modification of the anion reactivity may occur on complexation (see for instance [49]). Synthetic molecular catalysts may be

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[8]) Salmine, a protamine polypeptide containing 21 arginine residues, has been shown to display six high affinity sites ($\log K_s > 3$) for anions [48].

designed which would perform reactions on bound anionic substrates, as has been demonstrated with cation binding molecules (see [50] and ref. therein). Complexation of the anion may also affect the reactivity of the associated cation in a similar manner to the effect of cation complexation on anion reactivity which has been extensively studied in recent years. The remarkable chloride/carboxylate selectivities observed suggest that lipophilic anion complexones would be of interest as selective carriers for anion transport and anion specific electrodes. Polymers bearing polyguanidinium units (derived from EDG or TREG) may be of use as pH independent anion exchange resins of stronger binding capacity than the quaternary ammonium salts, and as materials for membranes, electrodes, batteries, *etc.*

Finally, it may be possible to design selective complexing agents for various biologically important anionic molecules such as polycarboxylic acids or AMP, ADP and ATP for instance. Highly cationic synthetic molecules and polymers might have interesting biological effects by interfering with natural guanidinium salts (like arginine, creatine, streptidine [52], marine toxins [53] *etc.*) and polycationic species (such as the protamines, histones and chalones [45–48] [51]) as well as with anion transport systems in membranes [54]. There is little doubt that like cation complexation, the development of highly stable and selective receptor molecules for anionic substrates may have a broad impact on both pure and applied fields of chemistry.

The authors wish to thank Prof. *Eschenmoser* for the gift of compound **11** [55], the *Süddeutsche Kalkstickstoffwerke AG* for a gift of *O*-methylisouronium sulfate and *P. Plumeré* for the synthesis of compound **4b**. One of us *(T. Fyles)* thanks the *National Research Council of Canada* for a post-doctoral fellowship (1977–1979).

Experimental Part

The ^{1}H-NMR. spectra were measured on a *Varian* A 60 spectrometer at 60 MHz. Chemical shifts for spectra in DMSO-d_6 are referenced to internal tetramethylsilane (TMS). Spectra in D_2O are referenced to internal sodium trimethylsilylpropanesulfonate (TPS). The ^{13}C-NMR. spectra were measured on a *Varian* XL-100 spectrometer at 25.1 MHz operating in the *Fourier* Transform mode with complete proton decoupling. Chemical shifts in DMSO-d_6 are given in ppm down field from internal TMS. Chemical shifts in D_2O, using *t*-butyl alcohol as internal standard, are given in ppm downfield from internal TPS ($\delta_{TPSint.} = \delta_{tBuOHint.} + 32.1$ ppm). The microanalyses were performed by the *Service Central de Microanalyse* of the *C.N.R.S.*

Commercially available amines were purified by recrystallization of their hydrochloride salts and the salts were dried at high vacuum (HV.) before use. Arcaïne sulfate (1,4-diguanidinobutane dihydrogen sulfate) (**3a**) was obtained commercially, converted to the chloride salt by ion exchange using *Dowex* 1×8 Cl^- anion exchange resin, recrystallized and dried at HV. before use. Tetramethylammonium chloride was recrystallized and dried at HV. before use. Other commercially obtained starting materials were used as received.

General procedure for ion exchange chromatography. The chromatographic column used for routine ion exchange purification of salts was prepared from a slurry of 125 g *Amberlite* CG-50(H) (100–200 mesh) weak acid cation exchange resin in 500 ml of 0.001M HCl. The freshly prepared column was subjected to three washing cycles consisting of 500 ml each of 0.05M HCl and 0.001M HCl in order to pack the column and to eliminate small particles. The resultant bed (3.5×30 cm) allowed a gravity flow rate of ~3 ml/min and thereafter all solutions were drawn onto the column by a closed siphon system at the gravity flow rate. The mixed salts to be separated (0.2–2.0 g) were converted to a

mixture of hydroxides (and free amines) by passing down a column of *Dowex* 1×8 OH^- stongly basic anion exchange resin which was washed with water until the column effluent was neutral to litmus. The basic solution was then concentrated (if necessary) to a total volume of 50–100 ml on a rotary evaporator at room temperature. Fractions of 150 drops ($\sim$10 ml) were collected using an LKB *Ultrorack* fraction collector. Column flow was started and the mixture of salts was drawn onto the column, followed by 100 ml of a solution of 0.001M HCl. A pH gradient was created from 200 ml each of 0.001M HCl and 0.05M HCl connected by a liquid bridge. The pH was monitored at the column inlet and plotted as a function of fraction number. At the end of the gradient, sufficient 0.05M HCl was passed to bring the pH at the column outlet to the same value as the input pH. The pH of each fraction was recorded and the input/output pH *vs* fraction number curves were superimposed by correcting for column volume. Product containing fractions were those having a higher pH than the input value at that fraction. Products were identified by evaporation of single or adjacent fractions followed by NMR. examination. The column was regenerated by washing with 100 ml of 0.1M HCl followed by reequilibration with 0.001M HCl (250–300 ml of solution).

Preparation of polyammonium salts. – (±)trans-*1,2-Diaminocyclohexane dihydrochloride* (**4b**). Compound **4b** was prepared from cyclohexane by stereospecific *trans* ring-opening of cyclohexenaziridine using azide ion followed by reduction of the aminoazide to the diamine [16]. The product was purified by recrystallisation of the dihydrochloride salt and was identical to an authentic sample by a mixed melting point determination; m.p. 330° (330° [16]); overall yield from cyclohexene 37%. – NMR., see *Table 4.*

Tris*(3-aminopropyl)amine tetrahydrochloride* (**6b**). This compound was prepared by diborane THF reduction of 3,3′,3″-nitrilotripropionamide followed by acidic hydrolysis with 6M HCl at reflux for 24 h. The product was isolated by chloroform extraction of a strongly basic solution of the crude hydrolysis product. Purification by ion-exchange chromatography (general method) followed by recrystallisation from methanol gave **6b**·4 HCl as hygroscopic crystals m.p. 227–229° (227° [17]); yield 85%. – NMR., see *Table 4.*

N,N,N′,N′-*Tetrakis(2-aminoethyl)-1,2-diaminoethane hexahydrochloride* (**7b**). This compound was prepared by a procedure somewhat modified from that of *Schwarzenbach et al.* [18]. Aziridine benzenesulfonate (4 equivalents) prepared from hydroxyethylamine [56], reacted with ethylenediamine to give **7b**·tetrabenzenesulfonate. The sulfonate groups were removed in 40% HBr-solution/acetic acid/phenol [57] and the compound was isolated by passing the crude product down a column of *Dowex* 1×8 OH^- anion exchange resin and evaporating to dryness. Recrystallization from 20% conc. hydrochloric acid in ethanol gave **7b**·6 HCl m.p. 240°; yield 60%; NMR., see *Table 4.*

$C_{10}H_{34}N_6Cl_6$ (451.13) Calc. C 26.62 H 7.60 N 18.63% Found C 26.58 H 7.63 N 18.71%

Syntheses of guanidinium salts. – *Method A* [19–21] (CAUTION: Ethylmercaptan is evolved; use a good fume hood). In a small volume pressure reactor were placed 20 milli-equiv. of a poly-primary amine (free base or hydrochloride salt), 2.25 g of *S*-ethylisothiouronium bromide [21] (25 milli-equivalents) and 10 ml of concentrated ammonium hydroxide solution. The reactor was sealed and the mixture was heated at 60° for 8 h; longer heating times result in no further reaction as the reagent is consumed within the first 8 h. The reactor was cooled to room temperature and unsealed in a fume hood. The mixture was diluted with 50 ml of water and reheated to 30–35° in a current of N_2 to drive off ethylmercaptan and ammonia. The mixture was then evaporated to dryness at 25–30°. The polyguanidinium salt was isolated by ion exchange chromatography (general method). The urea and guanidinium halide by-products are eluted well before the product polyguanidinium salts using the chromatographic system described. The following substances were prepared by this method.

1,2-Diguanidinoethane dihydrochloride (**1a**) in 81% yield from **1b**, isolated by ion exchange chromatography and recrystallized from 2-propanol; m.p. 223–225° (225° [58]). – NMR., see *Table 4.*

(±)trans-*1-Guanidino-2-aminocyclohexane dihydrochloride* (**12**) in 81% yield from **4b**, isolated by ion exchange chromatography and recrystallisation from 1-octanol/ethanol; m.p. >260°. – NMR., see *Table 4.*

$C_7H_{16}Cl_2N_4$ (162.25) Calc. C 36.69 H 7.92 N 24.45% Found C 36.12 H 7.82 N 24.33%

Method B. In a small volume pressure reactor were placed 20 milli-equiv. of a polyprimary amine (free base or hydrochloride salt), 3.08 g of *O*-methylisouronium sulfate [23] and 10 ml of conc.

NH_3-solution. The reactor was sealed and the mixture was heated to 60° for 8 h; the reagent is completely consumed within this period of heating. The reactor was cooled to room temperature, unsealed and the mixture evaporated to dryness at 25–30°. The polyguanidinium salt was isolated from the mixture by direct recrystallization of the sulfate or by ion exchange chromatography (general method). The following substances were prepared by this method.

1,2-Diguanidinoethane dihydrochloride (**1a**) in 85% yield from **1b**, isolated by ion exchange chromatography and recrystallization, identical to the previously obtained sample.

1,3-Diguanidinopropane dihydrochloride (**2a**), from **2b** in 58% yield, isolated by crystallization of the sulfate and converted to the chloride salt by anion exchange (*Dowex* 1×8 Cl^- anion exchange resin). Recrystallization from ethanol gave **2a** · 2 HCl; m.p. 200–201° (197–200° [59]). – NMR., see *Table 4.*

Tris(2-guanidinoethyl)amine tetrahydrochloride (**5a**), in 80% yield from **5b**, isolated by recrystallization of the sulfate; m.p. > 260°.

$C_9H_{28}N_{10}O_8S_2$ (468.5) Calc. C 23.07 H 6.02 N 29.90% Found C 23.28 H 5.91 N 29.86%

Compound **5a** was converted to the chloride salt by anion exchange (*Dowex* 1×8 Cl^- anion exchange resin) followed by recrystallization from 5% conc. HCl.-solution in 2-propanol to give **5a** · 4 HCl as white hygroscopic crystals; m.p. 187–188°. – NMR., see *Table 4.*

Method C. a) Preparation of polynitroguanides. To a vigorously stirred solution of 20 milli-equiv. of a polyprimary amine in 50 ml of distilled water cooled to 5°, was added in one portion 4.7 g of 2-methyl-1-nitro-isourea [24] (40 milli-equiv.). The mixture was rapidly stirred for 2 h at 0–5°, the precipitate was isolated by filtration and washed with two 15 ml portions of cold water. The polynitroguanides were purified by recrystallization from a suitable polar solvent (water, alcohols) before the subsequent step. The following substances were prepared by this method:

Tris(2-(nitroguanidino)ethyl)amine (**5c**) from **5b** in 79% yield as a white powder from water; m.p. 160°. – NMR., see *Table 4.*

$C_9H_{21}N_{13}O_6$ (407.35) Calc. C 26.53 H 5.19 N 44.70% Found C 26.41 H 5.10 N 44.63%

Tris(3-(nitroguanidino)propyl)amine (**6c**), from **6b** in 77% yield, as a white powder from water, m.p. 188–189°. – NMR., see *Table 4.*

$C_{12}H_{27}N_{13}O_6$ (449.43) Calc. C 32.06 H 6.05 N 40.51% Found C 32.12 H 6.10 N 40.47%

N,N,N′,N′-*Tetrakis(2-(nitroguanidino-)ethyl)amine* (**7c**), from **7b** in 65% yield, as a white solid from DMF/water and was used without further purification for the subsequent reaction.

b) Hydrogenolysis of poly nitroguanidines. A suspension of 10 milli-equiv. of a polynitroguanidine and 200 mg of 10% Pd/C in 50 ml of ethanol/water/conc. HCl-solution 2:2:1 was stirred under 1 atm. of hydrogen. Hydrogen uptake was typically slow and the reaction was stopped only after 750 ml of hydrogen (> 30 milli-equiv.) had been consumed (16–24 h). The catalyst was removed by filtration, washed throughly with distilled water and the filtrate was evaporated to dryness. The polyguanidinium salts were isolated by exchange chromatography. The principal by-product, presumed from the stoichiometry of reduction to be hydroxylamine, is easily removed by the general method of ion exchange chromatography described above. The bulk of this contaminant is lost during the concentration at reduced pressure of the basic solution of the polyguanidinium salt. By this method, the following substances were prepared:

Tris(2-guanidinoethyl)amine tetrahydrochloride (**5a**), from **5c** in 82% yield, isolated and purified by ion exchange chromatography. The product was identical to previously obtained samples.

Tris(3-guanidinopropyl)amine tetrahydrochloride (**6a**), from **6c** in 75% yield, isolated as the tripicrate salt and purified by recrystallization at pH 9 in water; m.p. 96–100° (dec).

$C_{30}H_{39}N_{19}O_{21}$ (1001.74) Calc. C 35.96 H 3.92 N 26.56% Found C 35.83 H 4.01 N 26.47%

The chloride salt was prepared from the picrate and conc. HCl-solution by extraction of liberated picric acid into chloroform. The aqueous phase was evaporated; the residue was dried over KOH under HV. to give **6a** · 4 HCl as white hygroscopic crystals; m.p. 60–65°. – NMR., see *Table 4.*

N,N,N′,N′-*Tetrakis(2-guanidinoethyl)-1,2-diaminoethane hexahydrochloride* (**7a**) from **7c** in 57% yield, isolated by ion exchange chromatography as a white powder; m.p. 240°. – NMR., see *Table 4.*

$C_{14}H_{42}Cl_6N_{14}$ (619,29) Calc. C 27.15 H 6.84 N 31.66% Found C 26.93 H 7.00 N 31.51%

Method D. Preparation of N,N'-disubstituted guanidinium salts (Ethyl mercaptan evolved). A solution (or suspension) of 20 milli-equiv. of an *N,N'*-disubstituted thiourea in 60 ml of abs. ethanol/ethyl bromide 1:1 was allowed to reflux overnight. The resultant pale yellow solution was evaporated to a thick gum and the last traces of ethylbromide were removed by pumping the gum at HV. for 2 h. The crude isothiouronium bromide salt was taken up in 20 ml of abs. ethanol and added to 100 ml of a saturated solution of ammonia in abs. ethanol in a medium pressure reactor. The reactor was sealed, heated to 70° for 8 h, cooled to room temperature, unsealed and reheated to 30–35° in a current of air or nitrogen to drive off ethyl mercaptan and ammonia. The solution was then evaporated to dryness under reduced pressure at 25–30° and the crude guanidinium salt was taken up in 50 ml of distilled water. The aqueous solution was washed with two 50 ml portions of chloroform to remove sulfur containing impurities and was then evaporated to dryness. The product was purified by ion exchange chromatography and/or recrystallization. Compounds **9** and **10** were prepared by this method.

N,N'-*Diethylguanidine hydrochloride* (**9**), in 88% yield from *N,N'*-diethylthiourea, purified by recrystallization of the chloride salt from 2-propanol/acetone, as large hygroscopic crystals; m.p. 108°. The nitrate salt was prepared from the chloride by treatment with $AgNO_3$ in water, removal of the precipitated AgCl by filtration, evaporation to dryness followed by recrystallization from 2-propanol; m.p. 168–170° (170° [60]). – NMR., see *Table 4.*

N,N'-*Dibenzylguanidine hydrochloride* (**10**), in 95% yield from *N,N'*-dibenzylthiourea, isolated by recrystallization of the bromide salt from water; m.p. 150°. The chloride salt was prepared by ion

Table 4. *(^1H-) and (^{13}C-) NMR. spectral data of the compounds prepared*[a])

Compound	Solvent (pH)	Chemical shift (multiplicity, J(Hz)), assignment[b])
(**1a**)	D_2O(7)	3.50(*s*), CH_2G^+
1a	D_2O (7)	39.5, CH_2G^+
(**2a**)	D_2O (7)	3.53 (*t*, 7), CH_2G^+; 2.11 (*qi*, 7) $CH_2CH_2G^+$
2a	D_2O (7)	39.6 CH_2G^+; 24.7 $CH_2CH_2G^+$
3a	D_2O (7)	39.6 CH_2G^+; 25.0 $CH_2CH_2G^+$
4a	D_2O (2)	157.8, G^+; 57.2, CHG^+; 32.8, CH_2CHG^+; 25.2, $CH_2CH_2CHG^+$
4b	D_2O (2)	54.6, $CHNH_3^+$; 31.6, $CH_2CHNH_3^+$; 25.1, $CH_2CH_2CHNH_3^+$
4c	DMSO d_6	159.2, GNO_2; 53.5, $CHGNO_2$; 31.7, CH_2CHGNO_2; 23.8, $CH_2CH_2CHGNO_2$
(**5a**)	D_2O (2)	3.75 (*m*, *AA'XX'*), $^+HNCH_2CH_2G^+$
(**5a**)	D_2O (8)	3.41 (*t*, *AA'*), CH_2G^+; 2.83 (*t*, *XX'*), CH_2N
5a	D_2O (2)	159.4, G^+; 54.6, CH_2N; 38.4, CH_2G^+
5a	D_2O (10)	159.3, G^+; 54.5, CH_2N; 38.5, CH_2G^+
5b	D_2O (2)	52.3, CH_2N; 38.9, $CH_2NH_3^+$
5b	D_2O (11)	59.3, CH_2N; 40.8, CH_2NH_2
6a	D_2O (2)	159.1, G^+; 52.9, CH_2N; 40.6, CH_2G^+; 25.3, $CH_2CH_2G^+$
6b	D_2O (7)	51.6, CH_2N; 38.0, $CH_2NH_3^+$; 23.1, CH_2CH_2N
6b	D_2O (11)	52.0, CH_2N; 40.6, CH_2NH_2; 29.7, CH_2CH_2N
6c	DMSO d_6	159.5, GNO_2; 50.7, CH_2N; 39.3, CH_2GNO_2; 25.7, $CH_2CH_2GNO_2$
7a	D_2O (1)	159.5, G^+; 54.6, $CH_2CH_2G^+$; 51.3, CH_2N; 39.3, CH_2G^+
7b	D_2O (0)	52.5, $CH_2CH_2NH_3^+$; 51.5, NCH_2CH_2N; 38.4, $CH_2NH_3^+$
(**9**)	D_2O	3.50 (*qi*, 7.5), CH_2; 1.1 (*t*, 7.5), CH_3
(**10**)	DMSO d_6	8.1 (*t*, 10), CH_2NH; 7.8 (br), NH_2; 7.3 (*s*), $C_6H_5^-$; 5.2 (*d*, 10), CH_2NH
12	D_2O (2)	158, G^+; 56.1, CH–N; 34.0 (br.), CH_2CHN; 26.0, 25.6, CH_2CH_2CH–N
13	DMSO d_6	165.1, NH–C(NH)=NNO_2; 62.1, CH–N; 28.9, CH_2CH–N; 23.7, CH_2CH_2CHN
14	D_2O (2)	164.1, NH–C(NH)=NH_2^+; 64.9, CHN; 30.5, CH_2CHN; 25.1, CH_2CH_2CH–N

[a]) Compound numbers in parentheses, *e.g.* (**1a**) ..., indicate ^{1}H-NMR. data; otherwise ^{13}C-data; *s:* singlet; *d:* doublet; *t:* triplet; *qi:* quintet; *m:* multiplet; br: broad.

[b]) G^+ = –NH–C(NH_2)=NH_2^+; GNO_2 = –NH–C(NH_2)=NNO_2.

exchange (*Dowex* 1×8 Cl^- anion exchange resin) followed by recrystallization from water; m.p. 192–194° (193–195° [61]). – NMR., see *Table 4.*

(±)-trans-*1,2-Diguanidinocyclohexane* (**4a**). A solution of *trans*-1,2-diaminocyclohexane was prepared from 1.87 g of **4b** dihydrochloride (20 milli-equiv.) and 5.0 ml of a solution of 4N NaOH (20 milli-equiv.), made up to a total volume of 20.0 ml with distilled water. Aliquots of 4.0 ml each of the solution together with 1.0 g portions of 2-methyl-1-nitro-isourea were added every 15–20 min with vigorous stirring to 30 ml of distilled water at 0–5°. Stirring was continued at 0–5° for 2 h after the last addition, the precipitate was isolated by filtration and washed with two 15 ml portions of cold water. The ^{13}C-NMR. showed the product obtained to be a 9:1 mixture of the desired product and another product presumed to be a cyclic nitroguanidine (**13**) (see *Table 4* for NMR. spectral properties of these compounds). Hydrogenolysis of this crude product by *method C* above, gave (by ^{13}C-NMR. analysis) a 9:1 mixture of **4a** and **14**. The crude product was taken up in 30 ml of distilled water an a solution of lithium picrate was added until no more precipitate was formed. The aqueous phase was decanted and the yellow gum was taken up in the minimum amount of boiling water (~200 ml). Cooling to room temperature gave a fine yellow powder which was isolated by filtration and the recrystallization process was repeated a second time to give **4a**-dipicrate; m.p. >260° (dec).

$C_{20}H_{24}N_{12}O_{14}$ (656.47) Calc. C 36.58 H 3.68 N 25.60% Found C 36.38 H 3.71 N 25.49%

The chloride salt of **4a** was prepared from the dipicrate by addition of conc. HCl-solution, followed by extraction of liberated picric acid into chloroform. Ion exchange chromatography of this product gave 0.82 g **4a**·2 HCl (30% yield from **4b**) as a very hygroscopic white powder; m.p. 130–135°. – NMR., see *Table 4.*

pH-Metric measurements. The apparatus used and methods for calibration in methanol/water solutions have been previously described [62]; the reference electrode bridge contained 0.1M Me_4NCl for the present study. Ligand chloride salts in their fully protonated forms and other commercially available anhydrous salts were dried at HV. before use. Hydrated salts (*e.g.* $Na_3PO_4 \cdot 10\,H_2O$) of the highest purity available were used as received. New stock solutions of fully protonated ligands and of fully deprotonated anions (as sodium salts) were prepared daily. All measurements were performed in thermostated cell at 25.0±0.1° under nitrogen atmosphere. The pK_a's of the ligands were determined by titrations with 0.2N NaOH of a solution containing typically $5 \cdot 10^{-3}$–$1 \cdot 10^{-2}$M ligand with the ionic strength made to 0.1M with Me_4NCl. The pK_a's of the anions were determined by titrations with 0.2N HCl of a solution containing typically $5 \cdot 10^{-3}$M anion with the ionic strength made to 0.1M with Me_4NCl. Stability constants were determined by titrations with 0.2N HCl of solution containing: $5 \cdot 10^{-3}$–$1 \cdot 10^{-2}$M ligand plus n equivalents of NaOH to deprotonate the n equiv. of ammonium groups present in the ligand; $5 \cdot 10^{-3}$M anion; Me_4NCl to make the ionic strength 0.1M. For the purposes of calculation, pK_a's were determined for each concentration of ligand or anion considered.

Data from all titrations were analyzed by the computer program SCO 76 [29]. The fit of the calculated curve with the experimental curve was assumed to be adequate if: a) the standard deviation in titre calculated was less than ±5 μl (less than 3 μl in most cases) *and* b) the same values were obtained for all adjusted constants from the analysis of titrations at different ligand/anion ratios when calculated individually and when calculated together. Only the stoichiometries necessary to produce an adequate fit as defined above were retained; stoichiometries which failed to improve the fit or resulted in a poorer fit were rejected. The error limits were about ±0.2 for $\log K_s$ in all cases.

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