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Outer-sphere and inner-sphere complexation of cations by the natural ionophore lasalocid *A*

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Contents

Abstract

The natural antibiotic lasalocid *A* has been demonstrated to adopt a pseudo-macrocyclic arrangement in both the solid and solution in which intramolecular hydrogen bonding occurs between a carboxylic group at one end of the molecule and a terminal alcohol group at the other. The cyclic structure has a lipophilic outside surface and a central hydrophilic cavity. The interaction of lasalocid with a wide range of metal ions has been reported. Complexes containing lasalocid in both its neutral and anionic forms, and exhibiting various metal : lasalocid stoichiometries, have been reported. In these, the ligand has been postulated to retain a cyclic configuration such that the outside surface of the particular complex remains hydrophobic. In a number of cases, the nature of the complex formed (including its stoichiometry) can be solvent dependent. A wide range of thermodynamic and other data for metal–ion complexation has been reported.

Lasalocid *A* has also been shown to undergo host–guest binding with a range of amine cations, and especially those of biogenic amines. Where the guest is racemic, it has sometimes proved possible to resolve this mixture through preferential adduct formation and subsequent fractional crystallisation(s), followed by regeneration of the chiral guest.

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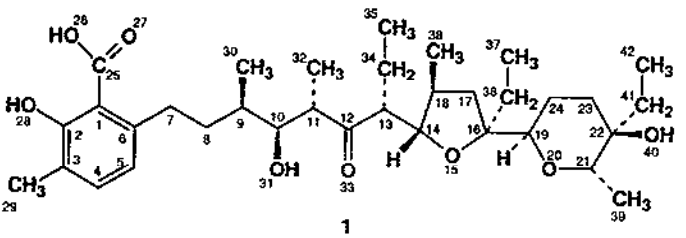
Lasalocid *A* acts as an ionophore for the transport of a range of both metal and amine cations across hydrophobic membrane types.

Outer-sphere complexes of lasalocid and inert metal ammine and amine complexes have also been reported and the crystal structure of one such product, $[Co(NH_3)_6]\cdot(Las)_3$, has been published. These results prompted a series of studies in which Las^- was used as an ionophore for the membrane transport of such complexes. Novel chiroselective and *cis/trans*-isomer selective transport of individual metal complexes has been documented.

Keywords: Lasalocid *A*; Host–guest chemistry

1. Introduction

A very large number of natural polyether antibiotics exist which are either macrocyclic or show a strong tendency to adopt a pseudo macrocyclic structure. The latter group, of which Lasalocid *A* (**1**; Las·H) (otherwise known as X-537A) is an example, also characteristically incorporate a carboxylic acid group in their structure. They are distinguished by their ability to form electrically neutral antibiotic-cation complexes.



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Lasalocid is the smallest of the carboxylate antibiotics and is produced stereospecifically by the bacterium *Streptomyces lasaliensis* [1]. It is used commercially to prevent coccocidial infection in chickens.

1.1. Objectives of this review

In this review the host-guest chemistry (including metal-binding properties) of lasalocid *A* is presented. Structural aspects of the resulting complexes, both in the solid state and in solution, are discussed. The use of this polyether carboxylic antibiotic as an ionophore for transport across artificial bulk membranes is also included. Lasalocid has been well documented to play a central role in the specific transport of cations across biomembranes; however, the role of lasalocid *A* in vivo has been well reviewed elsewhere [2–6] and will not be discussed here. Studies involving transport of species across natural biomembranes are also excluded from the present discussion.

Aspects of the complexation behaviour of lasalocid *A*, usually in conjunction with that of a range of other polyether/carboxylic acid ionophores, have been outlined in previous reviews [6–8]. Ionisation of the carboxylic acid group in these molecules

provides the means for full or partial charge neutralisation on binding to a cationic guest.

1.2. Metal-free lasalocid A

IR and Raman spectroscopic data [9,10] as well as X-ray diffraction data [10,11] for lasalocid crystallised from methanol indicate that the resulting 1:1 solvate with methanol has two distinct structures, each with a characteristic ketone stretching frequency. Lasalocid *A* adopts a pseudo-macrocyclic arrangement in each case in which there is intramolecular hydrogen bonding between the carboxylic group of the salicylic acid moiety and the terminal alcohol group at the other end of the molecule (Fig. 1). A related cyclic configuration occurs in a range of cation complexes of this antibiotic. Such an arrangement is capable of providing an internal hydrophilic environment for binding the cation while the external surface of the complex remains lipophilic. It is noted that complexation will invariably involve some conformational rearrangement of the lasalocid and, because of the small size of the cavity in this system, there may be a difficulty in the cation occupying it completely.

The structure of a 1:2 (water:lasalocid) adduct species has also been determined [12]. The latter has a 'head to head' dimeric structure, again with a lipophilic outside surface, and a central hydrophilic space which appears suitable for binding a cation. A number of NMR studies of lasalocid in its acid form have been reported [13–19], including investigations in both protic and aprotic solvents. These studies have been aimed at providing a comparison of the solution conformation of lasalocid with that observed for it (or its 5-bromo derivative) in the solid state. The results of ^{13}C NMR relaxation experiments suggest that lasalocid has a monomeric structure in both chloroform and methanol [13,14,18]; however, substantial conformational change occurs on moving between these solvents [18]. It is clear from these studies that lasalocid is capable of considerable conformational flexibility in solution.

2. Metal ion binding

Metal complexes of lasalocid sometimes, but not always, incorporate the metal cation in the Las^- cavity such that the antibiotic 'wraps around' the metal to produce

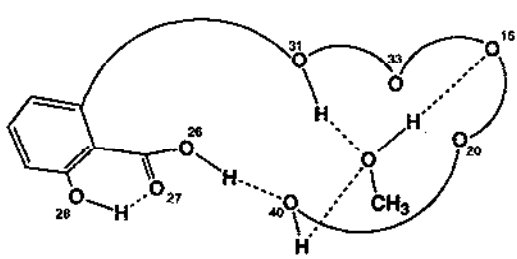


Fig. 1. The cyclic configuration of lasalocid *A* adopted in its methanol adduct.

a three-dimensional complex. As a consequence, the metal ion guest is solubilised in non-polar media (including lipid membranes). Further, the resulting complex is normally kinetically labile, an attribute essential in its biological transport role. The presence of an easily broken hydrogen bonded link in the macrocyclic ring is a feature that could assist such lability in particular instances by providing a mechanism for macrocyclic ring opening.

2.1. Metal complexes in the solid state

Crystalline lasalocid *A* complexes of a range of metal ions have been isolated. Neutral as well as charged complexes are known, both with monomeric and dimeric structures. The type of complex formed depends on the cation present and whether deprotonation of the carboxylate group of the ionophore occurs [20]. However, in most complexes one or more molecules of the deprotonated form of lasalocid *A* bind to the metal cation. It is apparent that the structures of individual solid complexes can sometimes be influenced by the polarity of the solvent and conditions of crystallisation, indicating that alternate (favourable) hydrogen bonding and localised charge neutralisation patterns are often available to these systems.

Complexes of Na^+ [11,19,21], Ba^{2+} [22–24], Ag^+ [25,26] and Tl^+ [27] have been characterised by X-ray crystallography. In nearly all of these, the ligand adopts a similar cyclic configuration, maintained by intramolecular hydrogen bonding between the carboxylic acid 'head' and the hydroxyl group 'tail'.

As observed for lasalocid itself (see earlier discussion), the metal complexed forms can exist as both monomers and dimers (the latter can involve 'head to head' or 'head to tail' coordination, defined by whether the two lasalocid molecules in the structure are aligned with the aromatic rings opposite each other, or whether these rings lie opposite the ether-containing rings).

One sodium complex, in the form of its methanol solvate, has been shown to be monomeric [11]. Dimeric 2:2 (metal:lasalocid) structures also occur in other sodium complexes [19,21] as well as in silver complexes [25,26]. The barium complex, which is also dimeric, has a 1:2 stoichiometry [22–24].

For sodium, two structures containing the 5-bromo derivative of lasalocid have been reported: both have dimeric geometries and are devoid of crystallographic symmetry, with one containing 'head to head' coordination while the other is 'head to tail' [19]. A further dimeric structure is adopted by the sodium complex of (unsubstituted) lasalocid [21] containing a sodium:lasalocid:water stoichiometry of 2:2:2. This species is considered to represent an intermediate in the monomer to dimer transition. The two sodium ions and the two water molecules are enclosed in a cavity constructed from both lasalocid anions. Six of the seven oxygens coordinated to one sodium ion are contributed by both lasalocid anions with a water occupying the seventh site. The other sodium ion is coordinated to four oxygens of a single lasalocid anion and to both water molecules. One of these waters is bound simultaneously to both sodium ions. Only one of the lasalocid anions appears to be present in the hydrogen bonded cyclic form.

One dimeric (2:2) silver complex has been shown to exhibit exact twofold symme-

try, with each silver being six-coordinated by five oxygens of one anionic molecule and a carboxylate oxygen of its pairing molecule [25] (Fig. 2). The Las^- moieties adopt the familiar cyclic configuration in this species.

The barium complex shows pseudo-twofold symmetry (Fig. 3); the metal is nine-coordinate, being bound to eight oxygen atoms of two crystallographically independent ionophores as well as to a water molecule [22–24]. The latter helps to stabilise

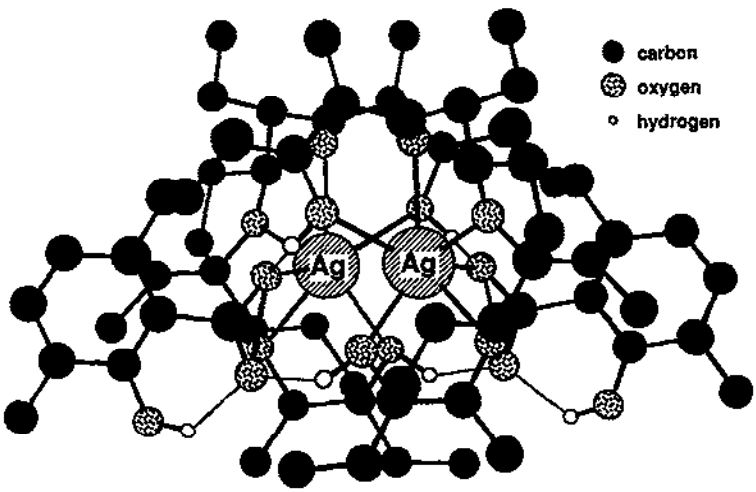


Fig. 2. The X-ray structure of one dimeric (2:2) silver complex of the lasalocid anion (Ref. [25]).

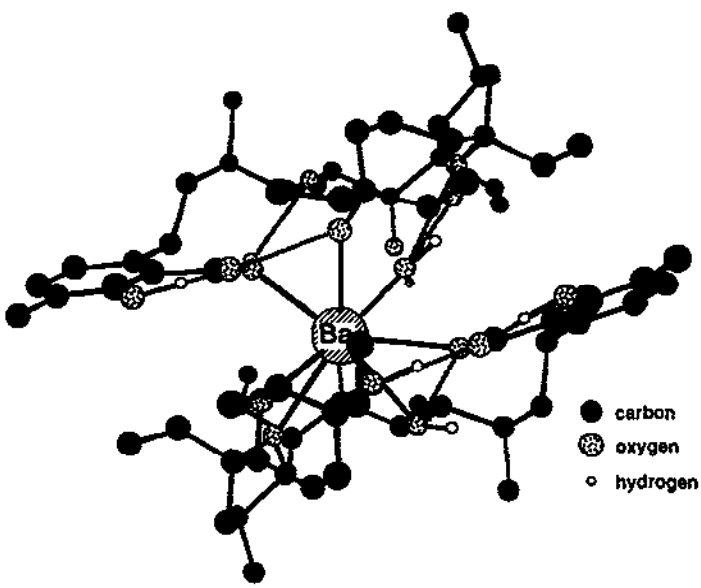


Fig. 3. The X-ray structure of the barium complex of the lasalocid anion (Ref. [22]).

the structure by forming hydrogen bonds with one of the coordinated lasalocid anions.

The structures of all the above dimeric forms consist of sandwich-type arrangements in which the cation(s) occupy the cavity between the two lasalocid molecules; there are only minor differences in the corresponding torsion angles of individual lasalocid rings across these complexes.

A solid state ^{205}Tl NMR study of a Tl(I) complex of lasalocid showed it to have nonaxial symmetry [28]. Subsequently the X-ray crystal structures of two other polymorphic thallium(I) complexes were reported [27]. The first of these was obtained from methanol and was shown to consist of a one-dimensional polymer of type $[Tl(Las)]_n$ in which the Tl(I) is coordinated on one side to five oxygens of a lasalocid anion (the carboxylate group is not coordinated), and on the other to the benzene ring of a neighbouring molecule. The second complex was obtained from a methanol/ethylene glycol solvent mixture and was shown to be a 1:1 monomer. The structure of this complex is illustrated in Fig. 4. The thallium is coordinated to six oxygens of a lasalocid anion on one side, with the other side being free of coordinated groups (but exposed to a non-polar environment of alkyl groups from neighbouring molecules). In this structure the carboxylate oxygen occupies one of the six oxygen sites around the thallium. In both the above structures the cyclic nature of the coordinated lasalocid anion is again stabilised by 'head-to-tail' $O_{26}\cdots H{-}O_{40}$ as well as by $O_{27}\cdots H{-}O_{31}$ hydrogen bonds. As usual, most of the polar oxygens are directed inwards towards the thallium ion whereas the non-polar groups are directed outwards.

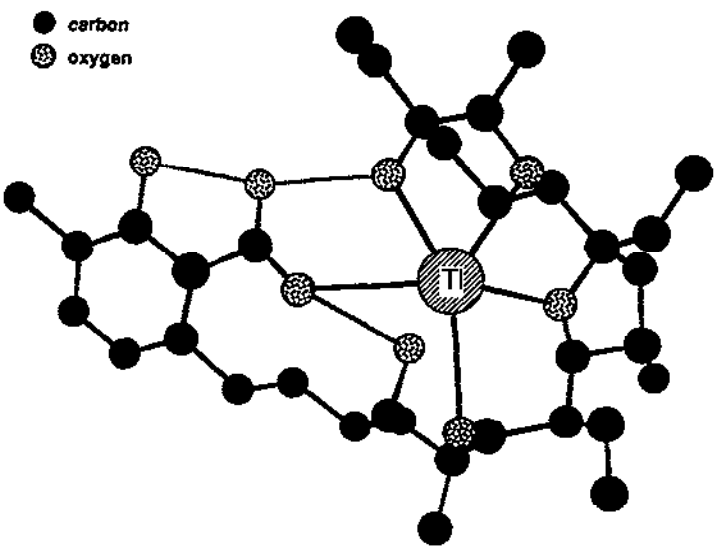


Fig. 4. The X-ray structure of the monomeric thallium complex of the lasalocid anion (Ref. [27]).

2.2. Metal binding in solution

There have been many studies of the interaction of lasalocid with metal ions in solution. In particular, Juillard et al. have published the results of a systematic study of the thermodynamic parameters associated with lasalocid complexation of metal ions in both homogeneous [29–37] and heterogeneous [38–40] media. From these and other investigations [41,42], it is clear that complexation occurs for a wide range of metal cations. It is also clear that the nature of the complex formed can be markedly influenced by the nature of the solvent present.

The interaction of alkali metal ions with lasalocid has received spasmodic attention during the past two decades, often involving NMR and circular dichroism (CD) studies. The latter technique has been claimed to be useful for distinguishing between the formation of 'inclusion' complexes with small alkali ions and 'peripheral' ion pairing with more bulky alkylammonium ions [43]. An early ^{1}H NMR investigation of the sodium complex of 5-bromolasalocid suggested that it has a dimeric structure in non-polar solvents [19]. However, ^{13}C and ^{1}H NMR studies (including relaxation studies) indicate that the sodium complex of lasalocid is monomeric in methanol [14,18,19]. As discussed already, sodium complexes crystallised from various solvents have been demonstrated to be dimeric [19,21], with a monomeric form being obtained from methanol [11]. ^{23}Na NMR studies have also been employed to probe aspects of sodium–lasalocid complex formation [44,45].

An investigation of the interaction of lithium perchlorate with lasalocid in acetonitrile using CD, ^{1}H, ^{13}C and ^{7}Li NMR has been carried out [46]. Under the conditions employed, a mixture of the monomeric 1 : 1 complex and the corresponding 1 : 2 (sandwich) complex were shown to coexist in solution.

In a more recent study, potentiometry, UV–visible spectra and calorimetry have all been used to investigate the nature and stability of a number of alkali metal complexes of lasalocid (and its 5-bromo derivative) in methanol [30]. The results were interpreted in terms of formation of a neutral 1 : 1 complex in each case (in which the carboxylate group does not play a dominant role in binding to the metal). The complexation behaviour varies in a fairly regular way with the size of the metal cation involved, suggesting that lasalocid adapts progressively to the size of the incoming metal, with no significant size selectivity being observed.

Complete assignments of the proton and carbon spectra of the potassium complex of Las^{-}, in both methanol and chloroform, have proved possible using two-dimensional NMR experiments [37]. In the chloroform case, an investigation of the effect of complex concentration variation has enabled information concerning dimerisation to be obtained.

By means of a NMR line broadening analysis, lifetimes of the 1 : 1 complexes with potassium, barium and calcium in methanol (at 25°C) were shown to be 15, 34 and 24 μs, respectively [47]. For the corresponding Li^{+} and Na^{+} complexes, the lifetimes were too short to be obtained by this method.

Dissolution of either of the two solid-state thallium complexes investigated by X-ray diffraction (see previous discussion) leads to the same thallium species in chloroform solution [27]. From its proton NMR spectrum it was concluded that

the thallium complex exists primarily as a 'head-to-tail' dimer under the conditions employed in this study [48]. The ^{13}C NMR spectrum of this complex has also been assigned [17] and, more recently, the structure of this species has been investigated further using the (^{1}H–^{1}H and ^{1}H–^{13}C) COSY technique [27].

The ^{205}Tl NMR spectrum of the thallium complex in chloroform has also been determined [48]. The chemical shift of the ^{205}Tl resonance occurs at very low field relative to those of other ionophore complexes of this ion. Overall, the chemical shifts of the latter span almost 850 ppm, indicating a very considerable sensitivity of the shifts to variation in the thallium coordination environment [48].

In other studies, comparative EXAFS investigations of the Rb^+, Tl^+ and Se^{2+} complexes of Las^- in aqueous solution confirm the inherent coordination flexibility of this ionophore [49].

The interaction of Ca^{2+} with lasalocid has also been investigated. CD spectroscopy suggests that this ion forms predominantly charged complexes of stoichiometries 1:2 and 1:1 (calcium:lasalocid) in acetonitrile, the relative amounts of which depend upon the concentration of calcium ion present [50]. In an extension of this work, the ^{1}H and ^{13}C NMR spectra of the above complexes were also investigated [51]. The results are in accordance with the calcium ion preferentially binding to one end of the lasalocid via three oxygen donors while the other end (the salicylic acid part) remains relatively free. The 1:2 species is postulated to have a sandwich structure.

The stability constants for the complexes of lasalocid with alkali and alkaline earth metal ions, as well as for Ag^+, have been determined in a range of protic and aprotic solvents [52]. The values were compared with the corresponding values for the related antibiotic, monensin. The complexes of the smaller lasalocid anion were found to be less stable, less sensitive to solvent variation and show a different selectivity pattern to those of the monensin anion. In methanol, the stabilities (log K values, $I=0$, 25°C) for the 1:1 lasalocid complexes are: Ag^+, 4.1; Li^+, 2.2; Na^+, 2.8; K^+, 3.7; Rb^+, 3.6; Cs^+, 3.7; Mg^{2+}, 3.9; Ca^{2+}, 4.7; $Sr^{2+} > 5.6$; and $Ba^{2+} > 5.6$. These values are in reasonable agreement with those obtained in prior studies (under slightly different experimental conditions) [53].

In more recent work, attention has also been given to the formation of the 1:2 (metal:ligand) complexes of the above divalent ions [29]. In this study the interaction of Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+} with lasalocid and 5-bromolasalocid (as well as with the model compounds, salicylic acid and *o*-methoxybenzoic acid) was investigated in methanol using potentiometry. In each case the stepwise formation of 1:1 and 1:2 complexes was observed with, as is usual, the first ligand binding more strongly than the second. The complex stabilities for the 5-bromo-derivative are very similar to those for lasalocid itself. Comparison of the results obtained for the 'model' acids suggests that the salicylic group of Las^- is the main binding site for these cations (and possibly the sole site in the case of Mg^{2+}).

The above study has been extended to include calorimetric data [32]. In the case of the 1:1 complexes of Mg^{2+} and Ba^{2+} the results were interpreted as reflecting the presence of an extended Las^- conformation in the magnesium complex but a

folded one in the barium complex. For the 1:2 species, the data suggest that additional oxygen sites away from the carboxylate group may also play an increased role in bonding.

The heat capacities and volumes of complexation for the formation of the 1:1 complexes with both alkali and alkaline earth ions have also been determined [35]. Different behaviour was observed for lithium and magnesium with respect to the other cations in each series; these differences are perhaps a reflection of the lasalocid having more degrees of freedom in the complexes of these two ions.

In a comparative study, lasalocid together with four other carboxylic antibiotics have each been incorporated in a PVC matrix ion-selective electrode [54,55]. All five electrodes showed a satisfactory response to divalent ions such as Ca^{2+} and Ba^{2+} although, for the lasalocid electrode, the response was not bi-Nernstian.

The dissociation constant for the 1:1 complex of Ca^{2+} in methanol has been determined [56]. Other studies have involved an investigation of the behaviour of the neutral calcium complex in chloroform [57]; crystals of this product of type $Ca(Las)_2 \cdot xCHCl_3$ ($x=1$ or 2) have been isolated from this solvent. In deutero chloroform the proton spectrum of this complex shows both narrow and broad signals, with variable temperature studies suggesting that an intramolecular exchange process involving the oxygen donors of the lasalocid anion occurs. A model for calcium binding was proposed in which both ligands are bound to the metal via O_{26} and O_{40}, with 'fluxional' binding occurring for O_{15}, O_{31} and O_{20}. It is noted that analogous behaviour was also observed for $La(Las)_3$; the chloroform solvate of this species has also been isolated [57]. In this latter case, the exchange proposed involves the on–off binding of O_{20} and O_{31} with evidence that a second exchange process involving the 'head' salicylate moiety may also be present.

The interaction of the lanthanides spanning La(III) to Lu(III) with lasalocid in acetonitrile has been investigated using CD and fluorescence spectroscopy [58]. Both 1:1 and 1:2 (cation:ionophore) complexes were observed, usually coexisting in solution. The lanthanides with larger ionic radii tend to promote formation of 1:1 species while smaller radii favour the 2:1 species. The respective complexes are less stable in methanol, with different lasalocid conformations occurring to those found in chloroform. The results emphasise the role of both ionic radii and solvent in influencing the nature of the respective cation complexes formed.

The binding of trivalent lanthanide ions to lasalocid has also been investigated in a range of different solvents [46,56,59–61]. It has generally been assumed that complexes of type $[M(Las)]^{2+}$, $M(Las)_2]^{+}$ and $[M(Las)_3]$ are formed; with the latter being promoted by non-polar solvents such as chloroform [57,61].

In methanol, Pr(III) was postulated to form complexes of all three types and approximate stepwise stability constants were reported [59]; however, in other spectroscopic studies [56] only the 1:1 and 1:2 complexes were detected. In a parallel investigation [34], potentiometric pH titration as well as ESR and UV–visible spectral studies suggested that the interaction of Gd(III) with Las^- in methanol involves all three of the above complex types. The results also suggested that the carboxylate group is the main (and possibly sole) binding site for gadolinium

in methanol. In accordance with this, it was also concluded (on the basis of NMR data) that Pr(III) binds solely to the carboxylate group of Las^- [59]. However, from the results of other work [60] (involving electronic excitation and absorption spectra of various lanthanide complexes of lasalocid) it was concluded that other lasalocid oxygen sites may also contribute to cation binding under the conditions employed.

Using ^{13}C NMR spin-lattice relaxation methods, the Las^- binding sites for Gd(III) and Mn(II) have been determined in both *N,N*-dimethylformamide, and chloroform [61]; the sites employed were observed to depend on both the cation charge and the solvent polarity. In *N,N*-dimethylformamide, Gd(III) may only bind to the anionic carboxylate 'head' of lasalocid. However, Mn(II) appears to bind to O_{20} and O_{31} in addition to this group [61]. In chloroform, Gd(III) coordinates not only to the salicylate 'head' of Las^- but also to O_{20} and O_{31} [61]; it is not certain whether O_{15} and O_{40} also bind. In fact, because of the flexibility of Las^-, it is probably best to consider the anion as being present in a number of conformations and to interpret the NMR data in terms of 'composite' binding of the individual conformations to Gd(III). Corresponding data for the Mn(II) complex indicate that the Las^- binding in this complex parallels that found for the Gd(III) analogue.

The interaction of lasalocid with a number of transition and post-transition metal ions has also been investigated. Calorimetric and potentiometric measurements have been employed to obtain free energies and enthalpies for the formation of both 1:1 and 1:2 complexes with particular divalent transition metal ions [31].

Stability constants for the complexes of Mn(II), Fe(II), Co(II), Ni(II) and Zn(II) with Las^- have been determined in methanol [31]. Both 1:1 and 1:2 complexes were observed (even though previous work involving Mn(II) [53], Co(II) [62] and Ni(II) [63] suggested that the complexes obtained are of type $[M(Las)]^+$ in methanol, but of type $[M(Las)_2]$ in aprotic media).

In a separate study the stability constants for the species $[Cu(Las)]^+$ and $[Cu(Las)_2]$ were obtained in methanol using potentiometric and polarographic means [33]. The respective values of 6.5 ($\log \beta_1$) and 10.7 ($\log \beta_2$) are higher than those observed for other first row transition metal ions and are also somewhat higher than the corresponding values for salicyclic acid. This latter observation together with ESR evidence suggest that the carboxylate group and oxygens from the lasalocid backbone participate in coordination to Cu(II).

Calorimetric studies of the interaction of Mn(II), Fe(II), Co(II), Ni(II) and Zn(II) with lasalocid and salicylic acid have been carried out in methanol [36]. Enthalpies of formation for both the 1:1 and 1:2 species were determined. Fe(II) was found to yield a stronger interaction with lasalocid than occurs with the other cations. For a particular metal, there is little difference between the thermodynamic parameters for complexation of lasalocid and the corresponding parameters for salicylic acid, suggesting that the carboxylate group of the lasalocid anion dominates the binding of the above ions.

The effect of paramagnetic Mn(II) on the ^{13}C NMR spectrum of lasalocid has enabled the individual binding sites to be assigned for the 1:2 (metal:ligand) complex

in both *N,N*-dimethylformamide and chloroform [61]. A related study involving Cu(II) has also been reported [64].

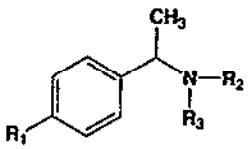
The kinetics of formation and dissociation of the Ni(II) complex of lasalocid in methanol have been investigated using the stopped-flow technique. From the ratio of the formation to dissociation rate constants, a stability constant for the Ni(II) complex in methanol of log $K = 3.7$ (± 0.2) at 25°C was obtained [65].

Juillard et al. have investigated the interaction of Cd(II), Hg(II) and Pb(II) with lasalocid as well as with the reference compounds, acetic acid and salicylic acid [66]. Free energies and enthalpies for the formation of the 1:1 and 1:2 complexes were obtained using a combination of potentiometric and calorimetric methods; the results were compared with similar data for other divalent metals obtained in previous studies by this group. Across all systems, lasalocid tends to be a much better ligand than either of the two reference compounds, a result largely reflecting enhanced entropic terms in the formation of the respective lasalocid complexes.

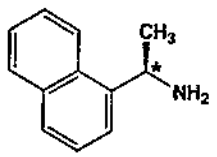
The binding of lasalocid to $TcOCl_4$ in chloroform has been investigated by fluorescence, CD, UV absorption and ^1H NMR spectroscopy [67]. The resulting $[TcO(Las)_2]^+$ has the two antibiotic ligands bound differently, with ligand exchange occurring at a rate which is approximately equal to the magnitude of the NMR chemical shift differences.

3. Amine binding

The known ability of lasalocid to act as an ionophore for the lipid membrane transport of catecholamines, together with the observation that the *R* enantiomer of norepinephrine yielded a crystalline 1:1 salt with lasalocid, led to an investigation of the possibility of using lasalocid to resolve racemic amines by fractional crystallisation [68]. Attempts were made to resolve racemic 1-amino-1-phenylethane (**2**), 1-amino-1-(4-bromophenyl)ethane (**3**), 1-dimethylamino-1-phenylethane (**4**), 1-amino-1-naphthylethane (**5**), 2-aminoheptane (**6**), tetrahydrofurfurylamine (**7**) and 1-(3′,4′-dimethoxyphenyl)propane (**8**) in the above manner. All of these, except (**7**), incorporate an asymmetric carbon directly adjacent to the primary amine. Compounds (**2**)-(**5**) were found to yield crystalline salts from CH_2Cl_2/C_6H_{14} in which the *R* isomer predominated by between six- and ten-fold over the *S* isomer. The X-ray structure of the Las^- complex of protonated *R*-(+)-1-amino-1-(4-bromophenyl)ethane (which was found to be completely resolved after three recrystallisations) shows that hydrogen bonding occurs from the $-NH_3^+$ group to O_{15}, O_{28} and O_{40} of the antibiotic. The Las^- backbone in this complex has a similar conformation to that found in the Las^- complexes of metal cations (see earlier discussion). Both steric and electronic factors were postulated to contribute to the enantioselectivity associated with this system. The simple amine, 2-amino-heptane (**6**), also showed preferential adduct formation (with the *R* isomer) whereas the preference was for the *S* isomer in the remaining two cases (**7**) and (**8**).



2 R_1, R_2, R_3 = H
3 R_1 = Br; R_2, R_3 = H
4 R_1 = H; R_2, R_3 = CH_3



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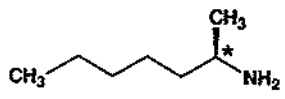

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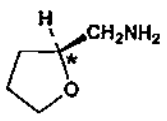

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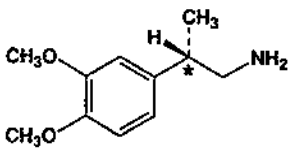

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In each of the above cases, the respective (resolved) amines can be recovered by extraction of a solution of the resolved diastereomeric complex in a non-polar solvent with dilute aqueous mineral acid; the antibiotic may also be recovered by evaporation of the organic phase.

In a subsequent study [69], the (*R*)-1-amino-1-(4-bromophenyl)ethane and (*S*)-2-amino-1-(3,4-dimethoxyphenyl)propane adducts obtained enantioselectively by co-crystallisation with lasalocid were investigated (and rationalised) using ab initio calculations.

A series of simple 1:1 adduct complexes between alkylammonium cations of type $[RNH_3]^+$ and the lasalocid anion have also been isolated [70]. NMR data indicate that binding once again involves the formation of hydrogen bonds to Las^- via its O_{15}, O_{27} and O_{40} heteroatoms.

In other work [71], it had been demonstrated that lasalocid forms 1:1 adducts with both phenethylamines and catecholamines in non-polar solvents. ^1H NMR studies (including relaxation studies) demonstrated that the lasalocid backbone conformation adopted is once again similar to that present in the lasalocid complexes of alkali and alkaline earth ions. Line shape analysis of the spectra suggested that non-polar interactions between the respective biogenic amine side chains and the lasalocid anion influence the stabilities of individual complexes in solution. Theoretical studies concerned with conformational aspects of host–guest formation by lasalocid with biogenic amines have also been reported [72].

Functional modification of lasalocid and other naturally occurring ionophores incorporating acyclic polyether backbones has been demonstrated to provide an interesting new class of chiral receptors containing uncharged terminal substituents [73]. The lasalocid benzylester derivative was obtained in greater than eighty percent yield by the cryptand (2.2.2)-promoted esterification of lasalocid with benzyl bromide [74]. Using an ion-selective electrode technique in which the modified antibiotic was incorporated in the sensing element, enantiomeric selectivity towards a number of chiral ammonium cations was observed. Interestingly, the parent lasalocid ligand was ineffectual in achieving discrimination under similar conditions.

4. Membrane transport

Lasalocid acts as a carrier for the transport of a large variety of cation types across lipophilic barriers. Monovalent, divalent and trivalent cations have all been shown to be transported across such barriers. A review of cation complexation and membrane transport by carboxylic acid ionophores, including lasalocid, has appeared [7]; the discussion encompasses a useful survey of both thermodynamic and kinetic aspects of transport behaviour.

As already discussed, the formation of a distinct complex between a cation and the anionic ionophore is characteristic of this class of antibiotic. This contrasts with some other antibiotic categories, which act by increasing the permeability of a membrane to cations by other processes.

Typically, transport experiments have been performed using a U-tube (or some derivative of a U-tube) apparatus. The organic phase (for example, chloroform containing the ionophore) is placed in the bottom of the tube such that it separates two aqueous phases, the source and receiving phases, contained in the respective 'arms' of the tube (Fig. 5). The transport behaviour involves the uptake of the cation at the aqueous source phase/membrane phase interface by the ionophore, followed by diffusion of the complexed cation to the opposite interface where it is released into the receiving phase. In many such experiments the driving force for transport is the presence of a concentration (or, more accurately, an activity) gradient between the source phase and the receiving phase.

Detailed discussions of the likely conformational dynamics of lasalocid while undergoing cation complexation/decomplexation associated with membrane transport have been presented [6,75,76]. The relative efficiencies of Las^- for transport of particular amino acid ester and metal cations across a chloroform membrane against the 'back' transport of ammonium ion are summarised in Table 1 [8,77,78]. In this system transport efficiency is associated with facile cation-exchange of an ammonium ion for the transported ion at the second chloroform/aqueous interface, with the ammonium ion then being transported back through the membrane. The concentration gradient of the ammonium ion between the two aqueous phases thus provides the 'driving force' for the anion transport in the forward direction. Liquid–liquid extraction coupled with ^{13}C NMR experiments confirmed that the various

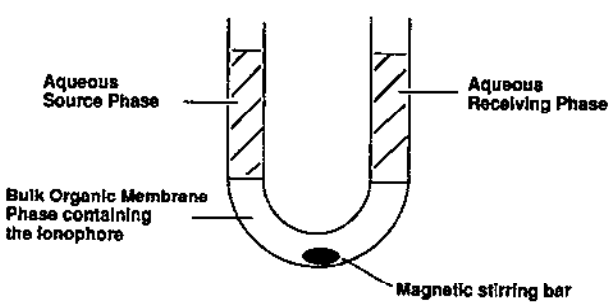

Fig. 5. A U-tube transport apparatus.

Table 1
Relative cation transport rates by lasalocid across a chloroform membrane[a,b]

Guest cation	Relative transport rate
$TrpOEt \cdot H^+$	0.76
$PheOEt \cdot H^+$	0.28
$TyrOEt \cdot H^+$	1.57
Co^{2+}	1.57
Ni^{2+}	3.33
Cu^{2+}	1.53
Zn^{2+}	1.67
Ag^+	4.84
Pb^{2+}	0.34

[a] From Ref. [77].
[b] Against a counter gradient of ammonium ions.

guest cations are incorporated in the pseudocavity of Las^- [78]. It is clear that the overall transport rate is dependent on a combination of rates associated with both guest binding and release. For maximum efficiency, both these latter processes should be fast.

Following potentiometric determination of the stabilities of the alkali and alkaline earth cation complexes of lasalocid and two other carboxylate antibiotics, the transport of calcium across a chloroform membrane by each of these ionophores was investigated [79]. An attempt was made to relate transport fluxes to the corresponding equilibrium constants but no simple correlation was observed. This is in accordance with theory since, while strong cation binding by the ionophore may enhance the extraction of the former from the source phase, too strong a binding will inhibit its loss to the receiving phase (thus inhibiting transport).

More recently, the results from solvent extraction studies (water/chloroform) using lasalocid as the extractant indicate that the alkaline earth cations are extracted as their neutral $[M(Las)_2]$ complexes [40]. Ease of extraction was found to be a function of cation size with $Mg^{2+} < Ca^{2+} < Sr^{2+} < Ba^{2+}$.

Lasalocid has been incorporated into the lipid bilayers of 'small' and 'large' vesicles composed of dipalmitoylphosphatidylcholine and egyphosphatidylcholine, respectively, for the purpose of modelling the carrier mechanism of ion transport across cell membranes [80]. Eu(III) was used as the model ion since its transport could be followed by laser-induced luminescence spectroscopy. The data implicate the presence of 1:1 and 1:2 Eu(III)/lasalocid complexes in the hydrophobic region, suggesting that both are available for transport of this ion.

^{31}P NMR has been used to monitor the transport of Pr(III) across phosphatidylcholine vesicles [81]. Transport was shown to be second-order in lasalocid in this case. When a mixture of lasalocid and monensin was employed as the ionophore, a synergistic effect was observed. A similar effect was also present when a simple crown ether carboxylic acid derivative was substituted for monersin in the above system [82].

A model for lasalocid-mediated transport of biogenic amines through lipid bilayer membranes has been proposed [83] and a correlation between amine structure and Las^- binding has proved possible [84]. Other studies involving cation transport through lipid bilayers have also been reported [85].

5. Supramolecular (outer-sphere) complexation

5.1. Outer-sphere complex formation

In pioneering work, Shaw and Everett [86] demonstrated that it is possible to isolate outer-sphere complexes of Las^- with a number of inert metal ammine and amine complexes. Crystalline complexes of stoichiometry (complex cation)$^{n+}$:$(Las^-)_n$ were obtained for the cations $[Co(NH_3)_6]^{3+}$, $[Pt(NH_3)_5Cl]^{2+}$, as well as for the △- and Λ- isomers of $[Co(1,2\text{-diaminoethane})_3]^{3+}$. The respective adducts were found to be quite soluble in solvents of low polarity such as chloroform. Spectroscopic and molecular weight data indicated that the first coordination spheres of the respective metal complexes remains intact on adduct formation and hence the lasalocid moieties were postulated to bind to the coordinated ammine and amine ligands via a network of hydrogen bonds. A subsequent X-ray structure analysis of $[Co(NH_3)_6]\cdot(Las)_3$ confirmed the outer-sphere nature of the bound Las^- moieties in this product [87] (Fig. 6). This supramolecular species consists of a $[Co(NH_3)_6]^{3+}$ core surrounded by three Las^- groups, each of which adopts the usual cyclic configuration (maintained by intra-ligand hydrogen bonds). Overall, the symmetry is approximately threefold. Two water molecules are associated with the complex and a network of hydrogen bonds involving these, the $[Co(NH_3)]^{6+}$ ion, and the oxygens belonging to the Las^- moieties are involved in maintaining the second sphere coordination. The arrangement gives the complex a hydrophobic exterior and this, together with overall charge neutralisation, accounts for its solubility in non-polar solvents.

For possible use in supramolecular photochemistry experiments, the ability or otherwise of Las^- to form supramolecular adducts with $[Cr(bpy)_3]^{3+}$, $[Cr(phen)_3]^{3+}$, $[Ru(bpy)_3]^{2+}$, and $[Pt(bpy)(NH_3)_2]^{2+}$ (bpy is 2,2-bipyridine; phen is 1,10-phenanthroline) in dichloromethane has been investigated using electronic absorption and emission spectroscopy [88]. For the first three of these it was reasoned that the flat aromatic ligands, arranged in a propeller-like fashion, might allow intercalation of the Las^- moiety in non-polar solvents, such that the resulting adduct might be held together (in part) by stacking interactions. Clear evidence for adduct formation in solution was obtained for the first and last complexes in the above list. $[Cr(bpy)_3]^{3+}$ was demonstrated to form a 1:1 species with lasalocid, while a 1:2 species (metal complex cation:lasalocid anion) was obtained with $[Pt(bpy)(NH_3)_2]^{2+}$.

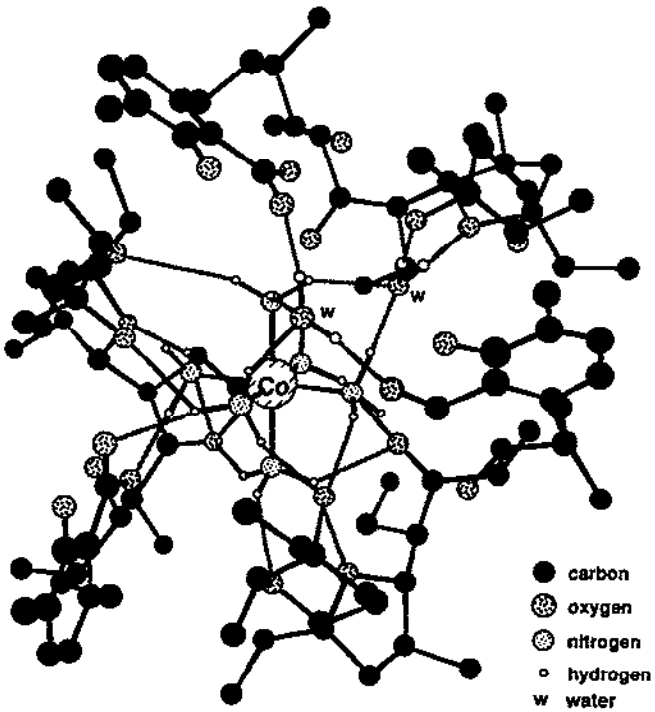

Fig. 6. The X-ray structure of the outersphere complex $[Co(NH_3)_6]\cdot(Las)_3$ (Ref. [87]).

5.2. *Membrane transport involving outer-sphere complex formation*

The ability of Las^- to form hydrophobic outer-sphere complexes with ammine and amine-ligand metal complexes prompted a series of studies in which Las^- was used as an ionophore for the membrane transport (including chiroselective transport) of particular metal complex species of the above type [89–91].

In the initial study [89], successful transport of $[Co(NH_3)_6]^{3+}$ across a chloroform/Las^- membrane was achieved using the arrangement shown in Fig. 7. Evidence

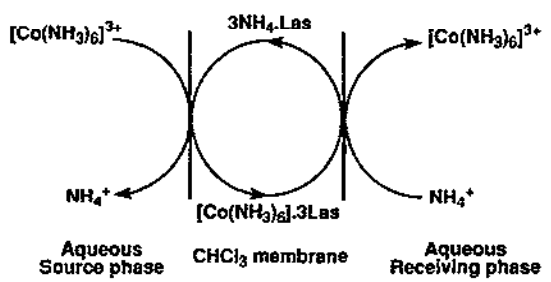

Fig. 7. Transport of $[Co(NH_3)_6]^{3+}$ by lasalocid across a chloroform membrane coupled to an ammonium ion counter gradient.

suggests that transport involves the formation of a 3:1 supramolecular complex between Las^- and the complex cation. A counter flux of ammonium ions was employed in order to promote 'uphill' transport of the metal complex; under the conditions employed, the transport of $[Co(NH_3)_6]^{3+}$ across the bulk membrane proceeded to completion. In comparative solvent extraction (water/chloroform) experiments in which the aqueous phase contained the complex in the presence of ammonium chloride, it was demonstrated that the ammonium ion acts as an effective competitive guest for the Las^- — no extraction of metal complex occurs under these conditions.

Las^- also acts as an ionophore for the transport of $[Pt(NH_3)_6]^{3+}$ and for the selective enantiomeric transport of the dissymmetric cations $[Co(1,2\text{-diaminoethane})_3]^{3+}$, *μ-cis*-$[Co(diethylenetriamine)_2]^{3+}$ and $[Co(sep)]^{3+}$ (where sep is the di-aza capped sarcophagine cage 1,3,6,8,10,13,16,19-octa-azabicyclo[6.6.6]-eicosane). Partial resolution of a racemic mixture of each of these latter complex cations (originally in the aqueous source phase) was observed in the aqueous receiving phase after the respective experiments had proceeded for several hours (see Table 2). These studies appear to be the first reported examples of enantioselective transport of intact metal complexes across a liquid membrane.

In an extension of this work, the relative transport and extraction behaviour of other Co(III) cage (sarcophagine) complexes has been investigated and the effect of cage substitution on transport behaviour determined [91]. Interestingly, along this cage series there is a tendency for Las^- to bind preferentially with complexes having a Δ configuration with respect to their C_3 axis.

In recent work, the ability of lasalocid to promote the differential transport of cis/trans isomers of amine-containing complexes across a chloroform membrane has also been documented [92]. Thus, a competitive transport experiment in which an equimolar concentration of the cis and trans diastereoisomers of the diamminebis(1,2-diaminoethane)cobalt(III) cation were present in the aqueous source phase led to enhanced transport of the trans isomer. Under the conditions employed, the final cis:trans ratio in the receiving phase was 1:3.

Apart from being a novel development in the area of host–guest chemistry, the successful transport of intact metal complexes across lipophilic membranes points

Table 2
Lasalocid A mediated transport of racemic cobalt(III) amine cations across a bulk chloroform membrane[a]

Complex	Average flux rate 10^{-7} mol h^{-1} cm^{-2}	Ratio $\Delta:\Lambda$[b] (receiving phase)
$Co(en)_3Cl_3$[c]	5.9	55:45
μ-cis-$[Co(dien)_2](ClO_4)_3$[d]	7.1	36.5:63.5
$[Co(sep)]Cl_3$[e]	8.4	58:42

[a] From Ref. [90].
[b] After 6 h.
[c] Where en is 1,2-diaminoethane.
[d] Where dien is diethylenetriamine.
[e] Where sep is the di-aza capped sarcophagine cage, 1,3,6,8,10,13,16,19-octaazabicyclo[6.6.6]-eicosane.

the way for the use of such behaviour for the practical separation and/or sensing of metal complexes and their isomers.

Acknowledgement

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References

[1] J. Berger, A.I. Rachlin, W.E. Scott, L.H. Sternbach and M.W. Goldberg, J. Am. Chem. Soc., 73 (1951) 5295.
[2] Y.A. Ovchinnikov, V.T. Ivanov and A.M. Shkrob, in Membrane-active Complexones, Elsevier, Amsterdam, 1974, p. 1.
[3] Y.A. Ovchinnikov, in Frontiers in Y.A. Ovchinnikov and M.N. Kolosov (Eds.), Bioorganic Chemistry and Molecular Biology, Elsevier, Amsterdam, 1979, Chap. 8.
[4] N.S. Poonia and A.V. Bajaj, Chem. Rev., 79 (1979) 389.
[5] B.C. Pressman, G. Painter and M. Fahim, in A.E. Martell (Ed.), Inorganic Chemistry in Biology and Medicine, ACS Symp. Ser. 140; Am. Chem. Soc., Washington, DC. 1980.
[6] G.R. Painter and B.C. Pressman, Met. Ions Biol. Syst., 19 (1985) 229.
[7] J.W. Westley (Ed.) in Polyether Antibiotics: Naturally Occurring Acid Ionophores, Vol. 1, Marcel Dekker Inc., 1982.
[8] H. Tsukube, in Y. Inoue and G.W. Gokel (Eds.), Cation Binding Macrocycles, Dekker, New York, NY, Chap. 12, 1990, pp. 497; F.G. Riddell, Chem. Britain, (1992) 533.
[9] J.M. Friedman, D.L. Rousseau, C. Shen and I.C. Paul, J. Chem. Soc., Chem. Commun., (1977) 684.
[10] J.M. Friedman, D.L. Rousseau, C. Shen, C.C. Chiang, E.N. Duesler and I.C. Paul, J. Chem. Soc., Perkin Trans. 2, (1979) 835.
[11] C.C. Chiang and I.C. Paul, Science, 196 (1977) 1441.
[12] E.C. Bissell and I.C. Paul, J. Chem. Soc., Chem. Commun., (1972) 967.
[13] D.J. Patel and C. Shen, Proc. Natl. Acad. Sci. USA, 73 (1976) 1786.
[14] C. Shen and D.J. Patel, Proc. Natl. Acad. Sci. USA, 73 (1976) 4277.
[15] M.J.O. Anteunis, Bioorg. Chem., 5 (1976) 327.
[16] H. Seto, J.W. Westley and R.G. Patcher, Antibiot., 31 (1978) 289.
[17] J.Y. Lallemand and V. Michon, J. Chem. Res. (S), (1978) 162.
[18] G.R. Painter and W.A. Gibbons, J. Chem. Soc., Perkin Trans. 2, (1986) 1151.
[19] P.G. Schmidt, A.H.J. Wang and I.C. Paul, J. Am. Chem. Soc., 96 (1974) 6189.
[20] E.N. Duesler and I.C. Paul, in J.W. Westley (Ed.), Polyether Antibiotics: Naturally Occurring Acid Ionophores, Vol. 2, Dekker, New York, 1983, Chap. 3.
[21] G.D. Smith, W.L. Duax and S. Fortier, J. Am. Chem. Soc., 100 (1978) 6725.
[22] I.-H. Suh, K. Aoki and H. Yamazaki, Acta. Cryst. C, 45 (1989) 415.
[23] S.M. Johnson, J. Herrin, S.J. Liu and I.C. Paul, J. Chem. Soc., Chem. Commun., (1970) 72.
[24] S.M. Johnson, J. Herrin, S.J. Liu and I.C. Paul, J. Am. Chem. Soc., 92 (1970) 4428.
[25] I.-H. Suh, K. Aoki and H. Yamazaki, H., Inorg. Chem., 28 (1989) 358.
[26] C.A. Maier and I.C. Paul, J. Chem. Soc., Chem. Commun., (1971) 181.
[27] K. Aoki, I.-H. Suh, H. Nagashima, J. Uzawa and H. Yamazaki, J. Am. Chem. Soc., 114 (1992) 5722.
[28] J.F.Hinton, K.R. Metz, G.L. Turner, D.L. Bennett and F.S. Millett, Magn. Reson., 64 (1985) 120.
[29] J. Juillard, C. Tissier and G. Jeminet, J. Chem. Soc., Faraday Trans. 1, 84 (1988) 951.
[30] Y. Pointud and J. Juillard, J. Chem.. Soc., Faraday Trans. 1, 84 (1988) 959.
[31] P. Laubry, C. Tissier, G, Mousset and J. Juillard, J. Chem. Soc., Faraday Trans. 1, 84 (1988) 969.
[32] Y. Pointud, E. Passelaigue and J. Juillard, J. Chem. Soc., Faraday Trans. 1, 84 (1988) 1713.

[33] P. Laubry, G. Mousset, P. Martinet, M. Tissier, C. Tissier and J. Juillard, J. Chem. Soc., Faraday Trans. 1, 84 (1988) 3175.
[34] M. Tissier, G. Mousset and J. Juillard, J. Chem. Soc., Faraday Trans. 1., 85 (1989) 1337.
[35] J. Woznicka, C. Lhermet, N. Morel-Desrosiers, J.-P. Morel and J. Juillard, J. Chem. Soc., Faraday Trans. 1, 85 (1989) 1709.
[36] Y. Pointud and J. Juillard, J. Chem. Soc., Faraday Trans., 86 (1990) 3395.
[37] R. Lyazghi, A. Cuer, G. Dauphin and J. Juillard, J. Chem. Soc., Perkin Trans. 2, (1992) 35.
[38] R. Lyazghi, M. Hebrant, M. Tissier, Y. Pointud and J. Juillard, J. Chem. Soc., Faraday Trans., 88 (1992) 1009.
[39] R. Lyazghi, Y. Pointud and J. Juillard, J. Chem. Soc., Faraday Trans. 88, (1992) 1017.
[40] R. Lyazghi, Y. Pointud, G. Dauphin and J. Juillard, J. Chem. Soc., Perkin Trans. 2, (1993) 1681.
[41] B. Ehrenberg, I.Z. Steinberg, R. Panigel and G. Navon, Biophys. Chem., 7 (1977) 217.
[42] G. Jeminet, R. Carlier and J. Simonet, J. Electroanal. Chem. Interfacial Electrochem., 147 (1983) 323.
[43] G. Painter and B.C. Pressman, Biochem. Biophys. Res. Commun., 97 (1980) 1268.
[44] E. Amat, B.G. Cox and H. Schneider, J. Magn. Reson., 71 (1987) 259.
[45] J. Grandjean and P. Laszlo, Angew. Chem., 91 (1979) 166.
[46] B.P. Shastri and K.R.K. Easwaran, Int. J. Biol. Macromol., 6 (1984) 219.
[47] C.V. Krishnan, H.L. Friedman and C.S. Springer, Biophys. Chem., 9 (1978) 23.
[48] R.W. Briggs, F.A. Elzkorn and J.F. Hinton, J. Magn. Reson., 37 (1980) 523.
[49] C. Goulon-Ginet and J. Goulon, Stud. Phys. Theor. Chem., 24 (1983) 169.
[50] C.K. Vishwanath and K.R.K. Easwaran, FEBS Letters, 153 (1983) 320.
[51] C.K. Vishwanath and K.R.K. Easwaran, J. Chem. Soc. Perkin Trans. 2, (1985) 65.
[52] B.G. Cox, N.V. Truong, J. Rzeszotarska and H. Schneider, J. Chem. Soc., Faraday Trans. 1, 80 (1984) 3275.
[53] H. Degani and H.L. Friedman, Biochemistry, 13 (1974) 5022; J. Bolte, C. Demuynck, G. Jeminet, J. Juillard and C. Tissier, Can. J. Chem., 60 (1982) 981.
[54] K. Suzuki, K. Tohda, H. Aruga, M. Matsuzoe, H. Inoue and T. Shirai, Anal. Chem., 60 (1988) 1714.
[55] K. Suzuki and K. Tohda, Trends in Anal. Chem., 12 (1993) 287.
[56] M. Albin, B.M. Cader and W.DeW. Horrocks, Inorg. Chem. 23 (1984) 3045.
[57] G.W. Everett, S.B. Parker and R.J.P. Williams, Biochemistry, 22 (1983) 6149.
[58] B.P. Shastri, M.B. Sankaram and K.R.K. Easwaran, Biochemistry, 26 (1987) 4930.
[59] S.-T. Chen and C.S. Springer, Bioinorg. Chem., 9 (1978) 101.
[60] F.S. Richardson and A. Das Gupta, J. Am. Chem. Soc., 103 (1981) 5716.
[61] D.A. Hanna, C. Yeh, J. Shaw and G.W. Everett, Biochemistry, 22 (1983) 5619.
[62] H. Degani, R.M.D. Hamilton and H.L. Friedman, Biophys. Chem., 4 (1976) 363.
[63] R. Homan and M. Eisenberg, Biochim. Biophys. Acta, 812 (1985) 485.
[64] J.Y. Lallemand, R. Rao and T. Prange, Nouv. J. Chim., 4 (1980) 315.
[65] J. Garcia-Rosas and H. Schneider, Inorg.Chim. Acta, 70 (1983) 183.
[66] M. Mimouni, Y. Pointud and J. Juillard, Bull. Soc. Chim. Fr., 131 (1994) 58.
[67] T.I.A. Gerber, W.J. Honiball and J.G.H. Du Preez, S. Afr. J. Chem., 41, (1988) 101.
[68] J.W. Westley, R.H. Evans and J.F. Blount, J. Am. Chem. Soc., 99 (1977) 6057.
[69] A. Gresh, New J. Chem., 11 (1987) 61.
[70] R.C.R. Gueco and G.W. Everett, Tetrahedron, 41 (1985) 4437.
[71] C. Shen and D. Patel, Proc. Natl. Acad. Sci., USA, 74 (1977) 4784.
[72] E.M. Popov, E.I. Mel'nik, J.F. Kinsel, I.N. Alieva, I.S. Maksumov and N.M. Godzhaev, Bioorg. Khim., 8 (1982) 1400; I.N. Alieva and N.M. Godzhaev, Stud. Biophys., 108 (1985) 125.
[73] H. Tsukube and H. Sohmiya, Supramol. Chem., 1 (1993) 297.
[74] H. Tsukube and H. Sohmiya, J. Org. Chem., 56 (1991) 875.
[75] G.R. Painter, R. Pollack and B.C. Pressman, Biochemistry, 21 (1982) 5613.
[76] V.E. Khutorskii, A.A. Kamenchuk and I.N. Alieva, Biol. Membr., 4 (1987) 756.
[77] H. Tsukube, K. Takagi, T. Higashiyama, T. Iwachido and N. Hayama, J. Chem. Soc., Chem. Commun., (1986) 448.
[78] H. Tsukube, K. Takagi, T. Higashiyama, T. Iwachido and N. Hayama, Inorg. Chem., 33 (1994) 2984.
[79] J. Bolte, C. Demuynck, G. Jeminet, J. Juillard and C. Tissier, Can. J. Chem., 60 (1982) 981.

[80] B.M. Cader and W. DeW. Horrocks, Biochem. Biophys. Res. Commun., 33 (1989) 265.
[81] J. Grandjean and P. Laszlo, Biochem. Biophys. Res. Commun., 116 (1982) 1293; Biochimie, 71 (1989) 183.
[82] R.A. Bartsch, J. Grandjean and P. Laszlo, Biochem. Biophys. Res. Commun., 117 (1983) 340.
[83] J.F. Kinsel, E.I. Melnik, S. Lindenbaum, L.A. Sternson and Y.A. Ovchinnikov, Intern. J. Pharm., 12 (1982) 97.
[84] J.F. Kinsel, E.I. Melnik, S. Lindenbaum, L.A. Sternson and Y.A. Ovchinnikov, Biochim. Biophys. Acta, 684 (1982) 233; J.F. Kinsel, E.I. Melnik, L.A. Sternson, S. Lindenbaum and Y.A. Ovchinnikov, Biochim. Biophys. Acta, 692 (1982) 377.
[85] H. Degani, Biochim. Biophys. Acta, 508 (1978) 364; B.P. Shastri, M.B. Sankaram and K.R.K. Easwaran, Biochemistry, 26 (1987) 4925; Y.N. Antonenko and L.S. Yaguzhinskii, Biochim. Biophys. Acta, 938 (1988) 125.
[86] J. Shaw and G.W. Everett, Inorg. Chem., 24 (1985) 1917.
[87] F. Takusagawa, J. Shaw and G.W. Everett, Inorg. Chem., 27 (1988) 3107.
[88] R. Ballardini, M.T. Gandolfi, M.L. Moya, L. Prodi and V. Balzani, Isr. J. Chem., 32 (1992) 47.
[89] L.F. Lindoy and G.W. Walker, J. Am. Chem. Soc., 112 (1990) 3659.
[90] P.S.K. Chia, L.F. Lindoy, G.W. Walker and G.W. Everett, J. Am. Chem. Soc., 113 (1991) 2533.
[91] P.S.K. Chia, L.F. Lindoy, G.W. Walker and G.W. Everett, Pure Appl. Chem., 65 (1993) 521.
[92] P.S.K. Chia, A.J. Leong, L.F. Lindoy and G.W. Walker, Aust. J. Chem., 48 (1995) 879.