Available online at www.sciencedirect.com
SCIENCE DIRECT®
Inorganica Chimica Acta 359 (2006) 1779–1785
Inorganica Chimica Acta
www.elsevier.com/locate/ica

Non-covalent (iso)guanosine-based ionophores for alkali(ne earth) cations

Fijs W.B. van Leeuwen [a], Jeffery T. Davis [b], Willem Verboom [a,*], David N. Reinhoudt [*,a]

[a] *Laboratory of Supramolecular Chemistry and Technology, MESA+ Institute for Nanotechnology, University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands*
[b] *Department of Chemistry and Biochemistry, University of Maryland, College Park, MD 20742, USA*

Received 18 May 2005; accepted 13 June 2005
Available online 11 August 2005

Dedicated to Professor Gerard van Koten.

Abstract

Different (iso)guanosine-based self-assembled ionophores give distinctly different results in extraction experiments with alkali(ne earth) cations. A lipophilic guanosine derivative gives good extraction results for K^+, Rb^+, Ca^{2+}, Sr^{2+}, and Ba^{2+} and in competition experiments it clearly favors the divalent Sr^{2+} (and Ba^{2+}) cations. 1,3-Alternate calix[4]arene tetraguanosine hardly shows any improvement in the extraction percentages compared to its reference compound 1,3-alternate calix[4]arene tetraamide. This indicates that one G-quartet does not provide efficient cation complexation under these conditions. In the case of the lipophilic isoguanosine derivative there is a cation size dependent affinity for the monovalent cations ($Cs^+ \gg Rb^+ \gg K^+$), but not for the divalent cations ($Ca^{2+} > Ba^{2+} > Sr^{2+} > Mg^{2+}$). In competition experiments the isoguanosine derivative, unlike guanosine, does not discriminate between monovalent and divalent cations, giving an almost equal extraction of Cs^+ and Ba^{2+}.

Keywords: (Iso)guanosine; Ionophores; Alkali metals; Alkaline earth metals; Self-assembly

1. Introduction

In general, ionophores for alkali(ne earth) cations are covalent compounds, such as, e.g., (calix[4])crown ethers [1]. In many cases, these covalent ionophores are based on the ''made to fit'' principle to introduce selectivity for one particular cation. This often requires a considerable synthetic effort. However, noncovalent synthesis has been shown to provide a useful alternative in the field of molecular recognition [2,3]. The preparation of ionophores via noncovalent synthesis is a logical step, as in Nature (iso)guanosine residues in nucleic acids form noncovalent tetraplex structures in the presence of a templating alkali(ne earth) metal ion [4,5]. Recently, synthetic (iso)guanosine derivatives have also shown to selectively bind group I and II cations via noncovalent self-assembly [6–8].

Three different lipophilic guanosine derivatives have been developed by the Davis group: lipophilic guanosine residues (Chart 1; G **1** [7]), guanosine residues attached to a calix[4]arene platform in the 1,3-alternate conformation (cG **2** [10]), and isoguanosine residues (isoG **3** [9]). During self-assembly, G **1** forms hexadecameric structures stabilized by lipophilic picrate anions [6], while cG **2** gives tetrameric guanosine assemblies, stabilized by water [10]. On the other hand, isoG **3** forms decamers, merely stabilized by cation complexation [9,11]. The complexation behavior of these assemblies has only been studied for a number of alkali(ne earth) cations [6,8]. In this paper, we present the results of a comprehensive binding study towards both alkaline

* Corresponding author. Tel.: +31 53 4892977; fax: +31 53 4894645.
E-mail address: w.verboom@utwente.nl (W. Verboom).

0020-1693/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ica.2005.06.047

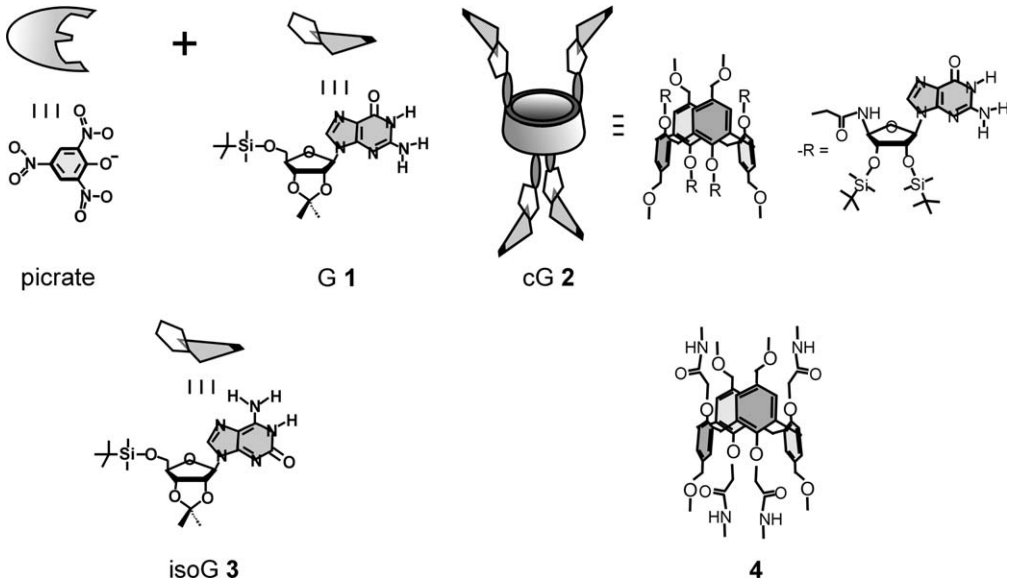

Chart 1.

and alkaline earth cations. The distinct difference in (iso)G-stacking of G **1**, cG **2**, and isoG **3** gives the unique opportunity to directly compare the cation binding affinity/selectivity of a single tetraplex (cG **2**), a G-quadruplex ((G **1**)$_8$), and a isoG-pentaplex ((isoG **3**)$_{10}$) [12].

2. Experimental

2.1. Materials

The preparation of G **1** [7], cG **2** [10], isoG **3** [6], and **4** [13] was according to the literature procedures. The acids (concentrated HCl and HNO_3) and CH_2Cl_2 were of p.a. grade and used as received. The nitrate salts of K^+ ($\geqslant$99.5%), Rb^+ (p.a.) were purchased from Fluka Chemie and Na^+ (p.a.), Cs^+ (99%), Mg^{2+}(p.a.), Ca^{2+} (p.a.), Sr^{2+} (p.a.), and Ba^{2+} (p.a.) were purchased from Acrôs Organics. ^{22}Na and $^{90}Sr^{2+}$ isotope solutions were purchased from Amersham, UK. $^{137}Cs^+$ and $^{133}Ba^{2+}$ isotope solutions were obtained from Isotope Products Europe Blaseg, GmbH.

2.2. Solutions

All basic experiments were performed using an aqueous phase with pH 8.9 (Tris–HNO_3 buffer) and an organic phase containing 10^{-4} M of ionophore, (G **1**)$_8$ and (cG **2**)$_2$ in CH_2Cl_2. The different nitrate salt concentrations were obtained by diluting 10^{-2} M stock solutions to the required concentration. From a carrier free stock solution of $^{22}Na^+$, a dilution of 2.0 kBq/g in 0.1 M $NaClO_4$ was prepared. From a CsCl carrier containing stock solution of $^{137}Cs^+$, a dilution of 9.8 kBq/g in 0.1 M HCl was made. From a carrier free stock solution of $^{90}Sr^{2+}$, a dilution of 2.5 MBq/g in 0.1 M HNO_3 was prepared. From a 10 μg Ba^{2+}/ml carrier containing stock solution of $^{133}Ba^{2+}$ in 0.1 M HCl, a dilution of 45.2 kBq/g in water was made.

2.3. General extraction procedures

Equal volumes (1.0 ml for the tracer experiments and 2 ml for the inductively coupled plasma mass spectroscopy, ICP-MS monitored experiments) of the organic and aqueous solutions were transferred into a screw cap vial with a volume of 4 ml. The samples were shaken (1500 rpm) at ambient temperatures (22–24 °C) for 1 h to ensure complete settling of the two-phase equilibration. After extraction, the solutions were disengaged by centrifugation (1600 rpm for 5 min) and aliquots (0.5 ml for the tracer experiments and 1 ml for the ICP-MS monitored experiments) of the organic and aqueous phases were pipetted out. Experiments were performed in duplicate; average values are reported, with an estimated error of 10–15%.

2.4. ICP-MS monitored extraction procedures

The solvent of the aliquot taken from the organic phase was evaporated and the residue destructed in 1 ml of concentrated HNO_3. The cation concentrations were measured on a Perkin Elmer Sciex Elan 6000 ICP-MS instrument, using a Cross flow nebulizer. The extraction percentage is defined as 100% times the ratio of cation concentration in the organic phase ([M_o]) and the added cation concentration ([M_{add}]) (Eq. (1)).

$$E\% = 100\%([M_o]/[M_{add}]) \quad (1)$$

2.5. Non-competitive K^+, Rb^+, and Ca^{2+} ICP-MS (Tables 1 and 2)

In the non-competitive extraction experiments, the $M^n(NO_3)_n$ ($M = Na^+$, K^+, Mg^{2+} or Ca^{2+}) salt concentrations (10^{-4} M) were equal to that of the ionophore concentration (10^{-4} M).

2.6. Competitive K^+, Rb^+, and Ca^{2+} ICP-MS (Tables 1 and 2)

The competitive extraction experiments were performed in either: mixed MNO_3 ($M = Na^+$, K^+, Rb^+, and Cs^+) solutions, mixed $M(NO_3)_2$ ($M = Mg^{2+}$, Ca^{2+}, Sr^{2+}, and Ba^{2+}) solutions, or mixed $M^n(NO_3)_n$ ($M = Na^+$, K^+, Rb^+, Cs^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+}) solutions. In each case, the individual salt concentrations (10^{-4} M) were equal to that of the ionophore concentration (10^{-4} M).

2.7. Different Ca^{2+} salts ICP-MS (Table 3)

In the non-competitive extraction experiments, the salt concentrations of $Ca(A)_2$ ($A = NO_3^-, Cl^-, I^-$, ClO_4^-, or SCN^-) salts were twice that of the ionophore concentration (10^{-4} M), viz. 2×10^{-4} M. This excess was used to drive the extraction to the organic phase. Furthermore, the aqueous phase was not buffered, as Tris–HCl or Tris–HNO_3 buffers would both give excesses of competing anions, Cl^- and NO_3^-, respectively.

2.8. Tracer monitored extraction procedures

The extraction percentages were determined using the appropriate tracer in either: individual $M^n(NO_3)_n$ ($M = Na^+$, Cs^+, Sr^{2+}, or Ba^{2+}) solutions, mixed MNO_3 ($M = Na^+$, K^+, Rb^+, and Cs^+) solutions, mixed $M(NO_3)_2$ ($M = Mg^{2+}$, Ca^{2+}, Sr^{2+}, and Ba^{2+}) solutions, or mixed $M^n(NO_3)_n$ ($M = Na^+$, K^+, Rb^+, Cs^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+}) solutions. In each case, the individual salt concentrations (10^{-4} M) were equal to that of the ionophore concentration (10^{-4} M). The obtained extraction percentage is defined as 100% times the ratio of activity in the organic phase (A_o) and the total activity ($A_o + A_{aq}$) (Eq. (2)).

$$E\% = 100\%(A_o/(A_o + A_{aq})) \quad (2)$$

2.9. ^{22}Na tracer experiments (Tables 1 and 2)

The Na^+ extraction percentages were determined by adding a solution of 20 μl of $^{22}Na^+$ tracer (41 Bq) in 0.1 M of $NaClO_4$. The gamma-activity was determined using a NaI scintillation counter. Since the extractions were performed with a 20-fold excess of [Na^+] to [(cG **2**)$_2$], and the maximal $^{22}Na^+$ extraction percentage is 5%, the obtained extraction percentages (Eq. (2)) were multiplied by 20 to give a more realistic representation of the Na^+ affinity.

2.10. ^{137}Cs tracer experiments (Tables 1 and 2)

The Cs^+ extraction percentages were determined using 20 μl of $^{137}Cs^+$ tracer (196 Bq). The gamma-activity was determined using a NaI scintillation counter.

2.11. $^{90}Sr^{2+}$ tracer experiments (Tables 1 and 2)

The Sr^{2+} extraction percentages were determined using 2.5 μl of $^{90}Sr^{2+}$ tracer (616 Bq). The activity was determined using a liquid scintillation counter to detect the Cherencov radiation.

2.12. $^{133}Ba^{2+}$ tracer experiments (Tables 1 and 2)

The Sr^{2+} extraction percentages were determined using 2.5 μl of $^{133}Ba^{2+}$ tracer (452 Bq). The gamma-activity was determined using a NaI scintillation counter.

2.13. MS-sample preparation and measurements

Solid phase extraction of a 10-fold excess of $Ca(NO_3)_2$ salt, by 10^{-3} M of [(cG **2**)$_2$] in water saturated CH_2Cl_2 yielded the [$Ca^{2+} \cdot 2$(cG **2**) $\cdot 2NO_3^-$] complex. Laser desorption ionization mass spectrometry was performed using a modified MALDI-TOF instrument, Voyager RP-DE, Perceptive Biosystems/Applied Biosystems, equipped with delayed extraction.

2.14. CD-sample preparation and measurements

Using the same buffer solutions as mentioned above, extraction experiments with a 1 ml aqueous phase containing $M(NO_3)_n$ ($M^{n+} = Ca^{2+}$, Sr^{2+}, Ba^{2+}, and Cs^+; 10^{-3} mol) were performed with a 1.0 ml organic phase, containing 10^{-3} M ionophore. After phase separation, the organic phase was measured using a JACSO J-715 spectropolarimeter with a cell-width of 0.01 cm.

3. Results and discussion

3.1. Alkali(ne earth) affinities of G **1**, cG **2**, and isoG **3**

To determine the relative extraction abilities of the (iso)guanosine residues **1**–**3** in the alkali(ne earth) series, non-competitive extraction experiments were performed with both alkali and alkaline earth cations (Table 1). In G **1** assemblies, the lipophilic picrate anions strongly bind at sites remote from the cation [3,14]. In cG **2** assemblies, the cations could interact with an anion

Table 1
Extraction percentages (E%) of alkali(ne earth) cations, under non-competitive conditions and at an equimolar [L][a]/[M] ratio (pH 8.9 Tris–HCl buffer) determined with tracers and with inductively coupled plasma mass spectroscopy (ICP-MS)

	Na^{+}[b]	K^{+}	Rb^{+}	Cs^{+}	Mg^{2+}	Ca^{2+}	Sr^{2+}	Ba^{2+}
G **1** + picrate	0	24	22	0	2	39	90	84
cG **2**	0	6	0	0	1	7	1	2
IsoG **3**	52	17	32	87	1	57	32	48

[a] [L] is based on the amount of single units needed for the complexation of one (alkaline earth) cation by $(G\ \mathbf{1})_8$, $(cG\ \mathbf{2})_2$, and $(isoG\ \mathbf{3})_{10}$ [8,10].

[b] $[Na^{+}] = 2 \times 10^{-3}$ M and the extraction percentages of Na^{+} were determined from extraction experiments, in which $[Na^{+}]$ was $20 \times [L]$ and the E% values reported are obtained by multiplying the actual E% by 20.

bound in the same cavity, while in the case of isoG **3** assemblies, neither anion binding nor an anion influence on the extraction data is expected [9]. Although the difference in anion binding does not allow for a direct comparison between the extraction percentages of the three self-assembled ionophores, we argue that a comparison can be made based on trends observed for the cation affinty/selectivities[1], as these should only be caused by the metal complexation of the (iso)guanosine units.

Table 1 shows that G **1**-assemblies have a limited extraction affinity as no more than 24% of the cations were extracted. The highest affinity is found for K^{+}, together with Rb^{+} cations, both very similar in size, 1.37–1.64 and 1.52–1.83 Å, respectively [15]. The affinity of G **1** is in agreement with the Eisenman series [16] of G-quartets and the stabilities reported by Williamson for a variety of oligonucleotide quadruplexes, both giving the following order in metal ion affinity $K^{+} > Rb^{+} > Na^{+} > Cs^{+}$ [17]. Using cG **2** only gives minor extraction of K^{+} cations (6%), while it does not give extraction of the other alkali cations under these conditions. Since the reference compound for cG **2**, 1,3-alternate calix[4]arene tetraamide **4**, only gave 3% K^{+} extraction, this indicates that the presence of one G-quartet induces (a small) increase in the cation affinity. IsoG **3** very clearly shows high affinities for alkali cations, in particular Cs^{+}, a result that agrees with previous studies describing the Cs^{+} selectivity of this compound [18]. However, surprisingly, the smaller Na^{+} cations are also effectively extracted by isoG **3**. Since in the case of Na^{+}, the $[Na^{+}]/[(isoG\ \mathbf{3})_{10}]$ ratio is 20, these results cannot be directly compared to those obtained with K^{+} and Rb^{+}, which are both extracted in a $[M^{+}]/[(isoG\ \mathbf{3})_{10}]$ ratio of 1:1.

As expected with alkaline earth cations, a much more distinct difference in affinity was observed between G **1**, cG **2**, and isoG **3** (Table 1). G **1** assemblies clearly have the largest affinity for Sr^{2+} and Ba^{2+} cations in the alkaline earth series, which is in agreement with the previously reported trend, $Sr^{2+} > Ba^{2+} > Ca^{2+} > Mg^{2+}$ [14]. Furthermore, near quantitative complex formation is observed, which is significantly higher than the ~25% found in the case of the alkali cations. Since the extraction ability significantly decreases for Ca^{2+} and Mg^{2+}, it seems that the quadruplex-cavity does not provide the right environment for the complexation of alkaline earth cations with a radius of 1.34 Å [15] or smaller. In the case of cG **2**, only minor extraction (<8%) is observed. The fact that the Ca^{2+} extraction percentage in the case of reference compound **4** is more than twice as high (17%) suggests that in the case of a cG **2** assembly, the cation binding by the amide groups is hampered by the presence of the guanosine units. IsoG **3** shows the highest affinity for Ca^{2+}, followed by that of Ba^{2+}, Sr^{2+}, and Mg^{2+}, in that order. Since the coordination cavity of isoG **3** is larger than that of G **1**, the highest extraction affinity was expected for the relatively large Ba^{2+} cations. The fact that Ca^{2+} is extracted more efficiently than Ba^{2+} may indicate a different coordination mode than that reported in Fig. 1. For instance, the increased efficiency for binding Ca^{2+} by isoG **3** could also reflect outer-sphere coordination of a hydrated Ca^{2+} ion by the isoG **3** decamer.

3.2. Selectivities of G *1*, cG *2*, and isoG *3*

To determine the selectivity of the three (iso)guanosine derivatives, G **1**, cG **2**, and isoG **3** in the alkali and alkaline earth series, competition experiments were performed. The results are summarized in Table 2.

Surprisingly, under competitive conditions for the alkali cations G **1** clearly favors K^{+}, while under non-competitive conditions G **1** binds K^{+} and Rb^{+} in a nearly equal manner (Table 1). The results obtained

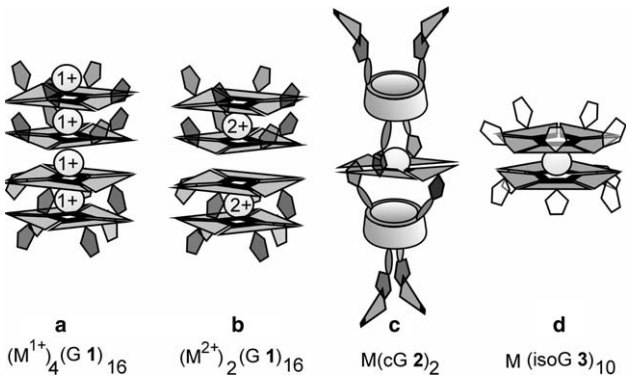

Fig. 1. Complex stoichiometries suggested for both alkali and alkaline earth cations. In the case of G **1**, two different binding modes are observed: four alkali cations are bound by the hexadecameric assembly (a), while only two alkaline earth cations are bound by the same assembly type (b). cG **2** has a tetrameric assembly type with both cation types (c), and isoG **3** is expected to form a decameric assembly with both cation types (d) [10,14,18].

[1] Comparison in affinities/selectivities of different non-covalent cation complexes has been previously reported for G **1** and its isoguanosine derivative isoG **3** [4].

Table 2
E% obtained from mixtures containing alkali and alkaline earth cations at equimolar [L][a]/[M_{single}] ratios (pH 8.9 Tris–HCl buffer) determined with both tracers and ICP-MS

	Na[b]; K; Rb; Cs	Mg; Ca; Sr; Ba	Na[b]; K; Rb; Cs; Mg; Ca; Sr; Ba
G **1**	0; 22; 7; 0	2; 8; 46; 44	0; 0; 0; 0; 1; 0; 53; 34
cG **2**	0; 2; 0; 0	3; 9; 1; 1	0; 0; 0; 0; 2; 5; 1; 1
IsoG **3**	5; 6; 28; 76	2; 51; 37; 30	12; 0; 0; 43; 2; 20; 0; 48
Total (%)	118	120	125

[a] [L] is based on the amount of single units needed for the complexation of one (alkaline earth) cation by (G **1**)$_8$, (cG **2**)$_2$, and (isoG **3**)$_{10}$.
[b] Only for the samples in which the Na^+ extraction percentage was determined [Na^+] was 2×10^{-3} M and corrected therefore (Table 1). In the determination of the extraction percentages of the other cations, a [Na^+] of 10^{-4} M was used.

suggest that under these conditions the total extraction percentage of alkali cations is limited to a maximum of approximately 25–30%, bringing out the K^+ selectivity. In the case of cG **2**, only a minor amount (<2%) of K^+ is extracted. IsoG **3** gives the expected selectivity, namely $Cs^+ \gg Rb^+ \gg K^+ > Na^+$ [18]. In the alkaline earth series, G **1** assemblies clearly favor Sr^{2+}, followed by Ba^{2+} cations, while for cG **2**, a minor Ca^{2+} selectivity can be observed. IsoG **3** shows a slightly different trend in the competition experiments as reported above for its cation affinity, namely $Ca^{2+} > Sr^{2+} > Ba^{2+} \gg Mg^{2+}$. Finally, competition experiments with both alkali and alkaline earth cations showed a clear preference for the latter with both G **1** and cG **2**, a result that is surprising as the single G-quartets, based on the complex stoichiometries in Fig. 1, are expected to favor the binding of monovalent cations. With isoG **3**, the largest cations give the highest extraction percentages, namely Cs^+ (43%) and Ba^{2+} (48%).

The selectivities of G **1**, cG **2**, and isoG **3** are in line with the affinities described in Table 1. In the case of G **1** and cG **2**, there is an increased affinity for alkaline earth over alkali cations. However, for isoG **3**, no distinct difference between the extraction of monovalent and divalent cations is observed.

3.3. Complex stoichiometries

With G **1**, the complex stoichiometries have previously been determined for the alkali(ne earth) cations. However, for cG **2** and isoG **3**, the stoichiometry determinations are limited to the alkali cations.

In the case of cG **2**, the Ca^{2+} complex stoichiometry was determined. Although the extraction results of cG **2** (see above) suggest that the amide groups on the calix[4]arene platform are responsible for the Ca^{2+} extraction, molecular modeling studies performed by Louit et al. [19] indicate that Ca^{2+} cations can form complexes with a single G-quartet. They found that Ca^{2+} gives a planar geometry binding in the internal cavity of a G-quartet. To determine the complex stoichiometry, Maldi-MS spectra were taken of the [Ca · cG **2**] complex obtained after solid phase extraction of an excess of $Ca(NO_3)_2$ salt in water saturated CH_2Cl_2 ([cG **2**] = 10^{-3} M). The single units of cG **2** (Mw = 2972 d) should form a dimeric assembly with Ca^{2+} [$Ca^{2+} \cdot 2$(cG **2**)$\cdot 2NO_3^-$], resulting in a molecular mass (*m*/*z*) of 6082. In the obtained Maldi-MS spectrum (Fig. 2), two complex peaks are present next to that of free cG **2** (2978.6 d): one at *m*/*z* = 3016.5 belonging to [($Ca^{2+} \cdot 2$(cG **2**) · NO_3^-)$^+$]$^{2+}$ (Mw = 3015 d) and one at *m*/*z* = 6035.8 belonging to [$Ca^{2+} \cdot 2$(cG **2**) · NO_3^-]$^+$ (Mw = 6030 d). This result provides proof of dimeric cG **2** assemblies binding Ca^{2+} cations together with NO_3^- anions. In addition, it gives the first MS data for dimeric cG **2** assemblies, however, it does not exclude the binding of Ca^{2+} to the amide groups.

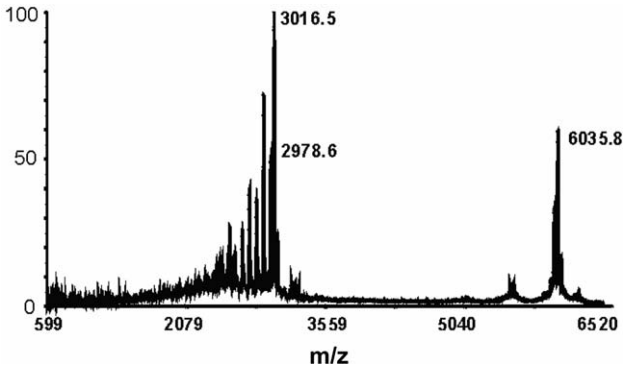

Fig. 2. Maldi-MS spectra (positive mode) of the [$Ca^{2+} \cdot 2$(cG **2**)] complex.

The fact that the total extraction percentages reported for isoG **3** in Table 2 are higher than 100% suggests that the complex stoichiometry of some cations is not identical to that of the [$Cs^+ \cdot$ (isoG **3**)$_{10}$] complex previously described [9]. According to Meyer and Sühnel [20], isoguanosine allows for the formation of tetrads rather than pentads in the presence of small metal ions, which may explain the increased extraction percentages observed. Due to the chiral ribose units of isoG **3**, the type of assembly formed can be studied using circular dichroism (CD) spectroscopy, as different assembly types are expected to give different CD curves. CD spectroscopy shows that the complexes formed with isoG **3** and Ca^{2+}, Sr^{2+}, or Ba^{2+} all give (nearly) identical CD curves (Fig. 3). Since these curves resemble that of the

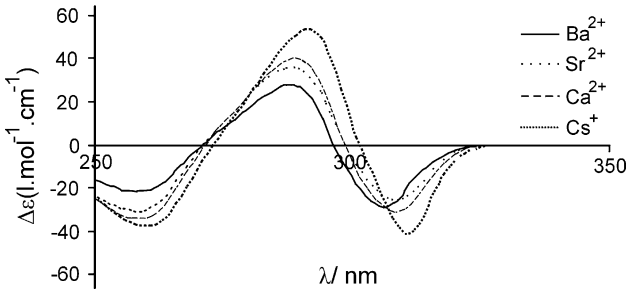

Fig. 3. CD curves of metal complexes with isoG **3** ($M^{n+} = Ca^{2+}$, Sr^{2+}, Ba^{2+}, and Cs^{+}) in CH_2Cl_2 at 25 °C.

Cs^+ complex [11], this result suggests that pentad-based assemblies of isoG **3** are formed with all four cations.

Previously, the different metal binding modes of hexadecameric G **1** assemblies with alkali and alkaline earth cations have been assigned via X-ray crystal structures (for schematic representations see Fig. 1) [14]. However, the CD curves of the guanosine complexes with K^+ and Ba^{2+} are identical [21], proving that the CD signal is not dependent on the number of cations bound by the assembly, but only on the assembly type itself. Consequently, the increased extraction percentages of isoG **3** (Table 2) may be caused by the complexation of two cations to one isoG **3** decamer, in which one metal ion may be bound between the two isoG **3** pentet layers and one metal ion on top of the upper layer, giving a $[2M \cdot (\text{isoG } \mathbf{3})_{10}]$ complex.

3.4. Anion influence on the Ca^{2+} extraction capability of cG 2

Previously, the anion influence on the metal ion complexation/extraction of G **1** and isoG **3** has extensively been studied [9,14]. With G **1**, the co-complexation of a large lipophilic anion such as picrate is essential for the formation of metal ion complexes, while in the case of isoG **3** the complexation of metal ions appears to be anion independent. In the case of cG **2**, previous results suggest that it binds both a cation and an anion in a ditopic manner wherein the Na^+ complexation is dependent on the anion used, e.g., Cl^- or Br^- [13]. To investigate the possible influence of anions on the Ca^{2+} extraction of cG **2** [10], extraction experiments were performed with $Ca(NO_3)_2$, $CaCl_2$, CaI_2, $Ca(ClO_4)_2$, and $Ca(SCN)_2$ salts (Table 3).

The anions used have different shapes, which may influence the anion binding ability of the amide moiety on the calix[4]arene platform [10,13]; Cl^- and I^- are spherical, SCN^- is linear and NO_3^- and ClO_4^- are planar. In this series, the softness of the anions also differs; it increases from $NO_3^- > Cl^- > ClO_4^- > I^- > SCN^-$ [22]. The extraction data in Table 3 show that with a 2-fold excess of Ca^{2+} cations in demi-water, the loading percentages of the organic phase are significantly improved compared to those shown in Table 1, namely 7% versus 15%. Furthermore, the softest anions ClO_4^-, I^-, and SCN^- increase the extraction percentage by about one third, compared to NO_3^- and Cl^-. Apparently, only the softness of the anion influences the complex formation, and not the anion shape, which may indicate that the anions are not (selectively) bound by the amide moieties.

Table 3
Complex formation, % $[Ca^{2+} \cdot 2(\text{cG } \mathbf{2})]$ of cG **2** with Ca^{2+} salts, with $[Ca^{2+}]$ twice that of $[\text{cG } \mathbf{2}_2]$

Salt	$[Ca^{2+} \cdot 2(\text{cG } \mathbf{2})]$
$Ca(NO_3)_2$	15
$CaCl_2$	11
CaI_2	18
$Ca(ClO_4)_2$	17
$Ca(SCN)_2$	20

4. Conclusions

Both G **1** and isoG **3** provide excellent ionophores for alkali(ne earth) cations, where G **1** is clearly selective for the divalent cation Sr^{2+} under competitive conditions, isoG **3** has a high affinity for most alkali(ne earth) cations tested and under competitive conditions favors the largest cations Cs^+ and Ba^{2+}, independent of their charge. The assemblies formed by cG **2**, however, are not efficient ionophores, under the conditions used. In general, the hexadecameric G **1** assemblies, with their well defined coordination cavities and binding modes, provide the most distinct and predictable mode of selectivity.

Acknowledgments

This research is supported by the Technology Foundation STW, Applied Science Division of NWO and the Technology Program of the Ministry of Economic Affairs and by the US Department of Energy (JD). We gratefully acknowledge the Fuels, Actinides and Isotopes (FAI) Department at the Nuclear Research & Consultancy Group (NRG) in the Netherlands for providing the radio-nuclear facilities and Mrs. T. Tomasberger for her support. We thank C.J.H. Miermans from the Information and Measurement Technology Laboratory for Inorganic Analysis (IMLA) Department at the Institute for Inland Water Management and Waste Water Treatment (RIZA) for performing the ICP-MS measurements.

References

[1] G.W. Gokel, W.M. Leevy, M.E. Weber, Chem. Rev. 104 (2004) 2723.

[2] G.M. Whitesides, B. Grzybowski, Science 295 (2002) 2418.
[3] D.N. Reinhoudt, M. Crego-Calama, Science 295 (2002) 2403.
[4] G. Laughlan, A.I.H. Murchie, D.G. Norman, M.H. Moore, P.C.E. Moody, D.M.J. Lilley, Science 265 (1994) 520.
[5] J. Deng, Y. Xiong, M. Sundaralingam, Proc. Natl. Acad. Sci. USA 98 (2001) 13665.
[6] J.T. Davis, Angew. Chem. Int. Ed. 43 (2004) 668.
[7] S.L. Forman, J.C. Fettinger, S. Pieraccini, G. Gottareli, J.T. Davis, J. Am. Chem. Soc. 122 (2000) 4060.
[8] F.W.B. van Leeuwen, W. Verboom, X. Shi, J.T. Davis, D.N. Reinhoudt, J. Am. Chem. Soc. 50 (2004) 16575.
[9] M.M. Cai, A.L. Marlow, J.C. Fettinger, D. Fabris, T.J. Haverlock, B.A. Moyer, J.T. Davis, Angew. Chem. Int. Ed. 39 (2000) 1283.
[10] F.W. Kotch, V. Sidorov, Y.-F. Lam, K.J. Kayser, H. Li, M.S. Kaucher, J.T. Davis, J. Am. Chem. Soc. 125 (2003) 15140.
[11] X. Shi, J.C. Fettinger, M.M. Cai, J.T. Davis, Angew. Chem. Int. Ed. 39 (2000) 3124.
[12] T.P. Whaley, in: J.C. Bailar, H.J. Emeléus, Sir R. Nylom, A.F. Trotman-Dickenson (Eds.), Comprehensive Inorganic Chemistry, vol. 1, 1st ed., Pergamon Press, New York, 1973, p. 369.
[13] V. Sidorov, F.W. Kotch, G. Abdrakhmanova, R. Mizani, J.C. Fettinger, J.T. Davis, J. Am. Chem. Soc. 124 (2002) 2267.
[14] X.D. Shi, K.M. Mullaugh, J.C. Fettinger, Y. Jiang, S.A. Hofstadler, J.T. Davis, J. Am. Chem. Soc. 125 (2003) 10830.
[15] R.D. Shannon, Acta Crystallogr., Sect. A 32 (1976) 751.
[16] G. Eisenman, Biophysics 2 (1962) 259.
[17] J.R. Williamson, Annu. Rev. Biophys. Biomol. Struct. 23 (1994) 703.
[18] S.C. Lee, J.D. Lamb, M.M. Cai, J.T. Davis, J. Inclusion Phenom. 40 (2001) 51.
[19] G. Louit, A. Hocquet, M. Ghomi, M. Meyer, M. Sühnel, J. Phys. Chem. Commun. 6 (2003) 1.
[20] M. Meyer, J. Sühnel, J. Phys. Chem. A 107 (2003) 1025.
[21] X. Shi, J.C. Fettinger, J.T. Davis, J. Am. Chem. Soc. 123 (2001) 6738.
[22] U. Olsher, M.G. Hankins, Y.D. Kim, R.A. Bartsch, J. Am. Chem. Soc. 115 (1993) 3370.