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Selective $^{226}Ra^{2+}$ Ionophores Provided by Self-Assembly of Guanosine and Isoguanosine Derivatives

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Abstract: The self-assembled guanosine (G **1**)-based hexadecamers and isoguanosine (isoG **2**)-based decamers are excellent $^{226}Ra^{2+}$ selective ionophores even in the presence of excess alkali (Na^+, K^+, Rb^+, and Cs^+) and alkaline earth (Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+}) cations over the pH range 3−11. G **1** requires additional picrate anions to provide a neutral assembly, whereas the isoG **2** assembly extracts $^{226}Ra^{2+}$ cations without any such additives. Both G **1**−picrate and isoG **2** assemblies show $^{226}Ra^{2+}$ extraction even at a 0.35×10^6 fold excess of Na^+, K^+, Rb^+, Cs^+, Mg^{2+}, or Ca^{2+} (10^{-2} M) to $^{226}Ra^{2+}$ (2.9×10^{-8} M) and at a 100-fold salt to ionophore excess. In the case of the G **1**−picrate assembly, more competition was observed from Sr^{2+} and Ba^{2+}, as extraction of $^{226}Ra^{2+}$ ceased at an $M^{2+}/^{226}Ra^{2+}$ ratio of 10^6 and 10^4, respectively. With the isoG **2** assembly, $^{226}Ra^{2+}$ extraction also occurred at a $Sr^{2+}/^{226}Ra^{2+}$ ratio of 10^6 but ceased at a 10^6 excess of Ba^{2+}. The results clearly demonstrate the power of molecular self-assembly for the construction of highly selective ionophores.

Introduction

Inhalation or ingestion of Ra^{2+}, a notorious bone seeker, can cause cancer and severe developmental or immunological problems. The toxicity of $^{226}Ra^{2+}$ is not solely due to its gamma and alpha particle emission, as the daughter nuclides, radon-222, pollonium-218, and lead-214, also contribute to in vivo radiotoxicity.[1] As a naturally occurring radioactive material (NORM),[2] Ra^{2+} is a subsurface pollutant in, e.g., ores, oil and gas, and drinking water. In the industries utilizing these subsurface products, Ra^{2+} salts precipitate in production pipes as scales and sludges, while the nonprecipitated Ra^{2+} cations are released into the environment with the "produced water". The resulting "technologically enhanced NORM" (TENORM)[3] wastes increase this radionuclide's health risks.

The TENORM waste contains a large excess of alkali and alkaline earth cations relative to Ra^{2+}.[4] Therefore, Ra^{2+} separation from more abundant cations requires selective methods. Precipitation,[5] absorption,[6−8] and extraction[9−12] techniques have all been used for the selective removal and/or detection of Ra^{2+}. The few Ra^{2+} extraction procedures use crown-ether-like compounds with ionizable groups that allow for overall neutrality of the resulting salt−crown complex. The synergistic cation extraction by a crown ether and a lipophilic carboxylate pair,[10] or extraction using crown ethers that are functionalized with acidic side chains,[11,12] render anion coextraction unnecessary. However, the drawbacks to these crown ethers as extractants include the considerable effort often required for their synthesis and the limitation that they function only over a limited pH range,[11] since cation extraction depends on the ionization state of the acidic groups.

Nature uses molecular self-assembly to form many receptors and would also provide a powerful tool for the formation of selective ionophores.[13] Guanosine-rich nucleic acids are well-known to coordinate alkali and alkaline earth cations using the G-quartet motif.[14,15] Synthetic guanosine derivatives also form highly organized and well characterized structures in the presence of a range of monovalent and divalent cations.[16] As

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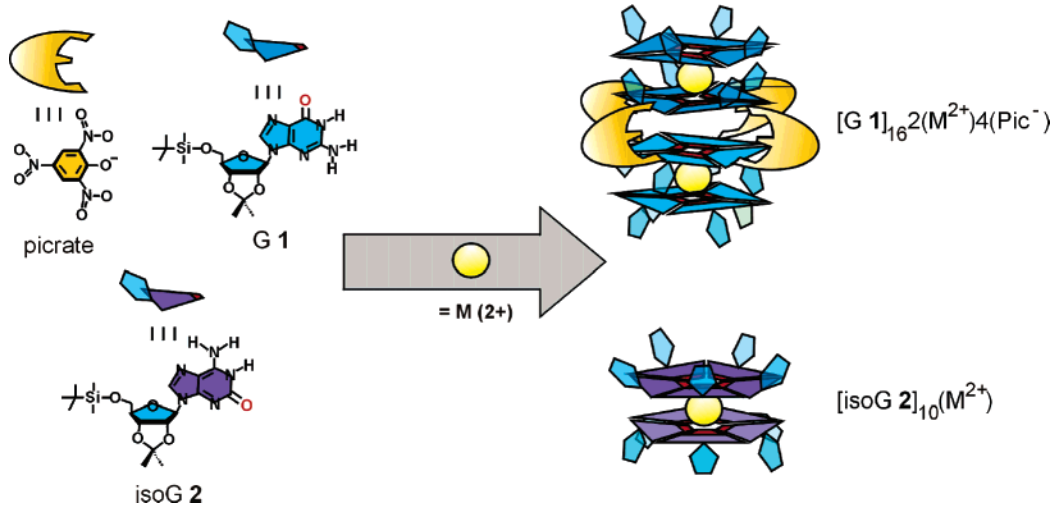
(1) Toxicological Profile for Radium. *Agency for Toxic Substances and Disease Registry*; U.S. Public Health Service in collaboration with U.S. Environmental Protection Agency: 1990.
(2) Eisenbud, M.; Gesell, T. F. *Environmental Radioactivity − From Natural, Industrial, and Military Sources*, 4th ed.; Academic Press: New York, 1997.
(3) Cohen, S. et. al. *Diffuse NORM Wastes − Waste Characterization and Preliminary Risk Assessment.* U. S. Environmental Protection Agency Office of Radiation and Indoor Air: Washington, D. C., 1993.
(4) Wiegand, J.; Feige, S. *Origin of Radium in High-Mineralized Waters*; IAEA: Vienna, 2002.
(5) Curie, M. S. *Century Magazine* **1904**, 461−466.
(6) Moon, D. S.; Burnett, W. C.; Nour, S.; Horwitz, E. P.; Bond, A. *Appl. Radiat. Isot.* **2003**, *59*, 255−262.
(7) Purkl, S.; Eisenhauer, A. *J. Radioanal. Nucl. Chem.* **2003**, *256*, 473−480.
(8) Komarneni, S.; Kozai, N.; Paulus, W. J. *Nature* **2001**, *410*, 771.
(9) McDowell, W. J.; Arndsten, B. A.; Case, G. N. *Solvent Extr. Ion Exch.* **1989**, *7*, 377−393.
(10) Dietz, M. L.; Chiarizia, R.; Horwitz, E. P. *Anal. Chem.* **1997**, *69*, 3028−3037.
(11) Chen, X. Y.; Ji, M.; Fisher, D. R.; Wai, C. M. *Inorg. Chem.* **1999**, *38*, 5449−5452.
(12) Beklemishev, M. K.; Elshani, S.; Wai, C. M. *Anal. Chem.* **1994**, *66*, 3521−3527.
(13) Whitesides, G. M.; Grzybowski, B. *Science* **2002**, *295*, 2418−2421.
(14) Laughlan, G.; Murchie, A. I. H.; Norman, D. G.; Moore, M. H.; Moody, P. C. E.; Lilley, D. M. J.; Luisi, B. *Science* **1994**, *265*, 520−524.
(15) Deng, J.; Xiong, Y.; Sundaralingam, M. *PNAS* **2001**, *98*, 13665−13670.

Scheme 1. Cation Induced Self-Assembly of Guanosine (G **1**) Giving an 8:1 Ionophore−Cation Stoichiometry and Isoguanosine (isoG **2**) Giving a 10:1 Stoichiometry[a]



[a] These stoichiometries are based on their Ba^{2+} and Cs^{+} complexes, respectively.[18,19]

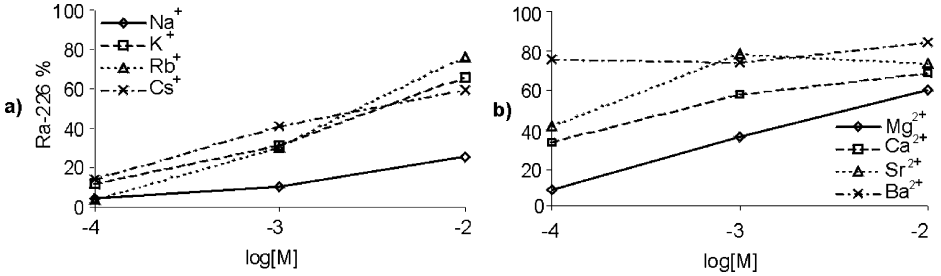


Figure 1. $^{226}Ra^{2+}$ (%) present in aqueous solutions with varying concentrations of alkali(ne earth) cations at blank conditions. Different salt concentrations $M^n(NO_3)_n$ [(a) M = Na^+, K^+, Rb^+, and Cs^+; (b) M = Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+}] and fixed $^{226}Ra^{2+}$ (2.9×10^{-8} M) concentrations were used.

schematically depicted in Scheme 1, X-ray crystallography has shown that the guanosine (G **1**) derivative forms noncovalent assemblies that are based on (G **1**)$_8$−$M^{+(+)}$ octamers, while the isoguanosine (isoG **2**) derivative forms decamers in the presence of a templating cation. The G **1** assembly has a relatively high K^+, Sr^{2+}, and Ba^{2+} affinity,[17,18] whereas the isoG **2** assembly shows strongest affinity for the larger Cs^+ and Ba^{2+} cations.[19,20] This cation binding selectivity is attributed to the geometry of the hydrogen bond donors and acceptors: G **1** forms tetramers, whereas isoG **2** prefers to form hydrogen-bonded pentamers in the presence of the cation template. In addition, recent calculations on a series of (isoG)$_n$−M^+ complexes support our experimental observations that (isoG)$_5$−M^+ pentamers are favored over the (isoG)$_4$−M^+ tetramers by the alkali metal cations larger than Li^+.[21] The macrocycle cavity size of these self-assembled ionophores determines the cation binding selectivity.

Our goal is to develop Ra^{2+}-selective ionophores. In separations of Group I and II cations, selectivity is often controlled by the ionophore cavity size. A self-assembled receptor with multiple oxygen donors and a cavity that fits the Ra^{2+} coordination sphere is a logical start for identifying *Ra^{2+} ionophores*. Since G **1**−picrate and isoG **2** assemblies were shown to possess high affinities for cations similar to Ra^{2+}, we reasoned they might be excellent candidates for Ra^{2+} coordination.

Here, we report on the extremely high $^{226}Ra^{2+}$ extraction selectivity of assemblies formed by the lipophilic nucleosides G **1** and isoG **2**, even in the presence of alkali and alkaline earth cations and over a wide pH range.

Results and Discussion

Precipitation of $^{226}Ra^{2+}$. During blank $^{226}Ra^{2+}$ extraction experiments performed in the presence of varying concentrations of alkali and alkaline earth metal nitrates, a deficit in detected $^{226}Ra^{2+}$ cations, as compared to the actual amount added, was observed. Experiments showed a decrease of $^{226}Ra^{2+}$ cations in the aqueous phase (Figure 1); however, $^{226}Ra^{2+}$ cations could not be detected in the organic phase. This suggests that under these extraction conditions $^{226}Ra^{2+}$ salts precipitate on the glass wall of the vials used, which would be a form of "scale/sludge formation" similar to that causing contamination of production pipes in industry.[2] To obtain insight into the influence of the different competing cations and their abundance on this "precipitation" of $^{226}Ra^{2+}$ salts, the deficiency starting from the same $^{226}Ra^{2+}$ concentration (2.9×10^{-8} M) was monitored in

(16) Davis, J. T. *Angew. Chem., Int. Ed.* **2004**, *43*, 668−698.
(17) Forman, S. L.; Fettinger, J. C.; Pieraccini, S.; Gottarelli, G.; Davis, J. T. *J. Am. Chem. Soc.* **2000**, *122*, 4060−4067.
(18) Shi, X. D.; Mullaugh, K. M.; Fettinger, J. C.; Jiang, Y.; Hofstadler, S. A.; Davis, J. T. *J. Am. Chem. Soc.* **2003**, *125*, 10830−10841.
(19) (a) Cai, M. M.; Marlow, A. L.; Fettinger, J. C.; Fabris, D.; Haverlock, T. J.; Moyer, B. A.; Davis, J. T. *Angew. Chem., Int. Ed.* **2000**, *39*, 1283−1288. (b) Shi, X.; Fettinger, J. C.; Cai, M. M.; Davis, J. T. *Angew. Chem., Int. Ed.*, **2000**, *39*, 3124−3127.
(20) Lee, S. C.; Lamb, J. D.; Cai, M. M.; Davis, J. T. *J. Inclusion Phenom.* **2001**, *40*, 51−57. The $^{226}Ra^{2+}$ selectivity of the isoG **2** assembly is in agreement with competitive liquid membrane experiments, showing that the isoG **2** assembly transports Ba^{2+} nitrate across supported liquid membranes much faster than it does the corresponding Na^+ and K^+ nitrate salts.
(21) Meyer, M.; Suhnel, J. *J. Phys. Chem. A* **2003**, *107*, 1025−1031.

the presence of varying concentrations of different salts (M = Na^+, K^+, Rb^+, Cs^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+}).

Monovalent alkali cations (Figure 1a) gave rise to large deficiencies of $^{226}Ra^{2+}$ cations in solutions with low concentrations of alkali cations ($<10^{-3}$ M). In particular, the presence of the smallest and hardest cation (Na^+) caused a significant deficiency of $^{226}Ra^{2+}$ cations, even at 10^{-2} M. The other cations (K^+, Rb^+, and Cs^+) gave smaller deficiencies at concentrations of 10^{-2} M and a highly similar $^{226}Ra^{2+}$ "precipitation" behavior.

In the case of the alkaline earth cations (Figure 1b), deficiencies of detected $^{226}Ra^{2+}$ cations were significantly lower than those observed with the alkali cations. This may be a result of the similar chemistry of the alkaline earth cations, including $^{226}Ra^{2+}$. As a result Ba^{2+}, the cation most similar to $^{226}Ra^{2+}$, caused the smallest deficiency of $^{226}Ra^{2+}$ cations. Nevertheless, the smaller and harder alkaline earth cations, viz. Mg^{2+}, Ca^{2+}, and to a lesser extent Sr^{2+}, showed significant "precipitation" of $^{226}Ra^{2+}$ at the lower salt concentrations (Figure 1b (log[M] < -2)). At salt concentrations of 10^{-2} M the "precipitation" of $^{226}Ra^{2+}$ salts significantly decreased in all cases.

The "deficiency" data (Figure 1) suggest that, in extraction experiments (vide infra), the actual amount of $^{226}Ra^{2+}$ cations present in solution can be somewhat lower than the amount (2.9×10^{-8} M) of $^{226}Ra^{2+}$ cations added. However, we have found that the assemblies formed by the nucleosides G **1** and isoG **2** not only provide excellent $^{226}Ra^{2+}$ selective ionophores but also for the greater part reduce the amount of $^{226}Ra^{2+}$ salt precipitates.

Noncompetitive $^{226}Ra^{2+}$ Extraction Experiments. To evaluate the $^{226}Ra^{2+}$ extraction ability of G **1** and isoG **2** assemblies, liquid−liquid extractions were first performed under noncompetitive conditions. In each experiment, the aqueous phase contained the same concentration of added $^{226}Ra^{2+}$ cations ($^{226}Ra_{add}{}^{2+} = 2.9 \times 10^{-8}$ M) and the organic phase contained 10^{-4} M of self-assembled ionophore, assuming complete formation of the (G **1**)$_8$−M^{2+} and the (isoG **2**)$_{10}$−M^{2+} complexes. The G **1** assembly only extracted $^{226}Ra^{2+}$ cations when picrate anions were present in the aqueous phase (100% extraction). In marked contrast, the isoG **2** assembly extracted 100% of the $^{226}Ra^{2+}$ cations, both with and without picrate anions in the aqueous phase. These extraction results are consistent with previous findings that additional "stabilizing" picrate anions are needed for the formation of neutral G-quadruplex structures using G **1**,[17,18] whereas cation-templated formation of the isoG **2** decamer is known to be anion independent.[19,20]

Binding Potential of G 1−Picrate and isoG 2 Assemblies for Divalent Alkaline Earth Cations. To study whether G **1**−picrate and isoG **2** assemblies have a binding affinity for alkaline earth cations (Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+}), the relative extraction ability of G **1**−picrate and isoG **2** assemblies under noncompetitive conditions, using nonradioactive nitrate salts (10^{-4} M), were determined with inductively coupled plasma mass spectrometry (ICP-MS). The results are summarized in Table 1.

The G **1**−picrate assembly shows high relative extraction abilities for Sr^{2+} and Ba^{2+}, compared to Ca^{2+} and Mg^{2+} cations. In the case of the isoG **2** assembly in general a lower extraction ability is observed, which may be due to the lack of a lipophilic anion such as picrate to facilitate partitioning of metal ions into the organic phase. However, the isoG **2** assembly still allows about 50% extraction of Ca^{2+} and Ba^{2+} cations under these conditions.

Table 1. Extraction Percentages of Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+} Cations by G **1**−Picrate and isoG **2** Assemblies, at an Equimolar Self-Assembled Ionophore to Cation Concentration (10^{-4} M), Determined by ICP-MS[a]

ionophore	Mg^{2+} (%)	Ca^{2+} (%)	Sr^{2+} (%)	Ba^{2+} [b] (%)
G **1**−picrate	2	39	90	84
isoG **2**	1	57	32	48

[a] In all cases metal nitrate salts were used. [b] Extraction percentages were verified under identical conditions with a ^{133}Ba tracer.

Competitive $^{226}Ra^{2+}$ Extraction Experiments in the Presence of Alkaline Earth Cations. To determine whether $^{226}Ra^{2+}$ would be extracted in competition with Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+}, extractions were performed under the same conditions as those used for the ICP-MS analyzed extractions (Table 1). For these $^{226}Ra^{2+}$ extraction experiments, a ratio of $^{226}Ra^{2+}$ cations to competing alkaline earth cations of 2.9×10^{-8} M to 10^{-4} M was used.

After adding $^{226}Ra^{2+}$ cations, competitive extraction experiments with G **1**−picrate and isoG **2** assemblies revealed high $^{226}Ra^{2+}$ selectivities with all four competing cations. This means that both nucleosides are able to selectively extract $^{226}Ra^{2+}$ from a 3.5×10^3-fold excess of Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+} cations (Figure 2; log([M^{2+}]/[I]) = 0).

To determine the range in which G**1**−picrate and isoG **2** assemblies are effective $^{226}Ra^{2+}$ ionophores, competition experiments with the single cations (Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+}) were carried out. The smallest of the alkaline earth cations Mg^{2+} showed high $^{226}Ra^{2+}/Mg^{2+}$ extraction selectivities for both the G **1**−picrate and isoG **2** assemblies. At a 10^6-fold excess of Mg^{2+} to $^{226}Ra^{2+}$ cations (salt to ionophore ratio of 240), 63% of the $^{226}Ra^{2+}$ cations were still extracted (Figure 2a; log([Mg^{2+}]/[I]) = 2.4) by the G **1**−picrate assembly. However, the isoG **2** assembly (without added picrate) did not show any interference in its ^{226}Ra extraction percentage by Mg^{2+}, which is a common cation in saline waters containing Ra^{2+}.[4] The isoG **2** assembly can quantitatively remove $^{226}Ra^{2+}$ cations from solutions containing more than a million-fold excess of Mg^{2+} cations, and the G **1**−picrate assembly maintains a significant $^{226}Ra^{2+}$ selectivity under identical conditions.

At a $Ca^{2+}/^{226}Ra^{2+}$ ratio of 10^6, 45% and 25% of the $^{226}Ra^{2+}$ cations were still extracted by both the G **1**−picrate and isoG **2** assemblies, respectively (Figure 2b; log([Ca^{2+}]/[I]) = 2.4). These comparative extraction data show that assemblies of both the lipophilic nucleosides G **1** and isoG **2** are able to selectively remove $^{226}Ra^{2+}$ from a million-fold excess of Ca^{2+} cations.

In the presence of Sr^{2+} cations, the isoG **2** assembly shows a remarkable $^{226}Ra^{2+}$ cation selectivity (53%; $Sr^{2+}/^{226}Ra^{2+} = 10^6$), but the G **1**−picrate assembly fails to extract $^{226}Ra^{2+}$ above a ratio of 10^5 (Figure 2c). This results in a more than 100-fold higher selectivity in the case of the isoG **2** assembly, allowing for $^{226}Ra^{2+}$ extraction at a million-fold excess of competing Sr^{2+} cations.

In the case of Ba^{2+}, the cation most similar in size to Ra^{2+},[22] the $^{226}Ra^{2+}/Ba^{2+}$ extraction selectivities are significantly lower for both ionophores (Figure 2d). Whereas the G **1**−picrate

(22) At concentrations > 10^{-2} M the total detected amount of $^{226}Ra^{2+}$ cations was almost equal to that originally added ($^{226}Ra_{add}{}^{2+}$), which means that there are no losses due to $^{226}Ra^{2+}$ salt precipitation.

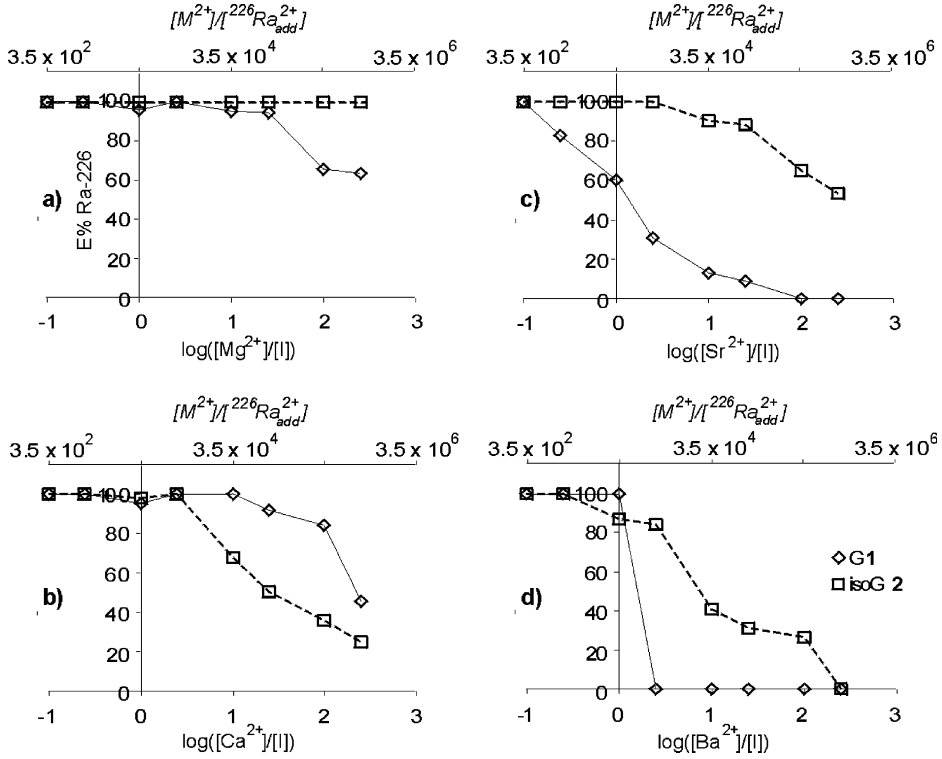
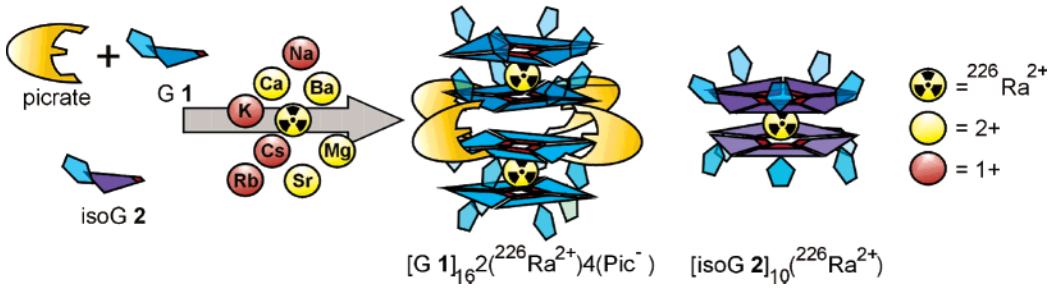


Figure 2. $^{226}Ra^{2+}$ extraction percentages recorded for different ratios of ionophore (I) to $M(NO_3)_2$ (M = Mg^{2+} (a), Ca^{2+} (b), Sr^{2+} (c), or Ba^{2+} (d)), using fixed ionophore ([(G **1**)$_8$ + 2(Pic^-)] and [(isoG **2**)$_{10}$]; 10^{-4} M) and $^{226}Ra^{2+}$ (2.9×10^{-8} M) concentrations. The log([M^{2+}]/[I]) values correlate directly with [M^{2+}]/[$^{226}Ra_{add}^{2+}$] values; 0 = 3.5×10^3, 1 = 3.5×10^4, 2 = 3.5×10^5, and 2.4 = 0.8×10^6.[22]

Scheme 2. Selective $^{226}Ra^{2+}$-Induced Assembly Formation of G **1** and isoG **2** in the Presence of Monovalent Alkali and Divalent Alkaline Earth Cations



assembly shows a sharp drop as soon as the Ba^{2+} concentration exceeds that of [(G **1**)$_8$] (at a $Ba^{2+}/^{226}Ra^{2+}$ ratio of 10^4; Figure 2d; log([Ba^{2+}]/[I]) = 0), the isoG **2** assembly continues extracting $^{226}Ra^{2+}$ up to a ratio of 10^6. The ability to differentiate between the similar chemical properties and sizes of Ba^{2+} (1.35−1.61 Å) and Ra^{2+} (1.48−1.70 Å)[23] cations suggests that the self-assembled ionophores prepared from isoG **2** are excellent $^{226}Ra^{2+}/Ba^{2+}$ selective ionophores.

In comparing the Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, and $^{226}Ra^{2+}$ selectivities and the relative extraction ability of the G **1**−picrate assembly, a trend related to the cation size can be observed. The G **1**−picrate assembly has the highest affinity for Sr^{2+} and Ba^{2+} (Table 1), which is in direct agreement with previous findings that G **1**-based octamers have the optimal coordination sphere for Sr^{2+} and Ba^{2+} cations.[18] Therefore, the lowest $^{226}Ra^{2+}$ selectivities were observed in competition experiments with these cations (Figure 2c and d). In the case of Mg^{2+} and Ca^{2+} hardly any affinity of the G **1**−picrate assembly was observed (Table 1); as a consequence, very high $^{226}Ra^{2+}$ selectivities by the G **1**−picrate assembly were obtained for these cations (Figure 2a and b). While the isoG **2** assembly shows relatively low extraction abilities for the alkaline earth cations (Table 1), it has impressive $^{226}Ra^{2+}$ selectivities in their presence (Figure 2; Scheme 2). This indicates that the isoG **2** decamer has a preference for binding the "largest" $^{226}Ra^{2+}$ (1.48−1.70 Å) cations, over the smaller alkaline earth cations (0.52−1.61 Å). This is consistent with the isoG **2** assembly being a Cs^+ (1.67−1.88 Å)[23] selective ionophore in the presence of the smaller alkali cations.[24]

In addition to the high selectivities reported in the presence of the other alkaline earth cations, the presence of G **1** and isoG **2** in the organic phase greatly reduced the deficit of detectable $^{226}Ra^{2+}$ cations, as compared to that reported for the blank experiments (see Figure 1b). With Mg^{2+} cations the presence of G **1** and isoG **2** still allows for the detection of nearly all the $^{226}Ra^{2+}$ cations (>80%) at 10^{-4} M, which is a considerable improvement to the 10% of $^{226}Ra^{2+}$ detected under blank

(23) Shannon, R. D. *Acta Crystallogr. A* **1976**, *32*, 751−767.

(24) Davis, J. T.; Tirumala, S. K.; Marlow, A. L. *J. Am. Chem. Soc.* **1997**, *119*, 5271−5272.

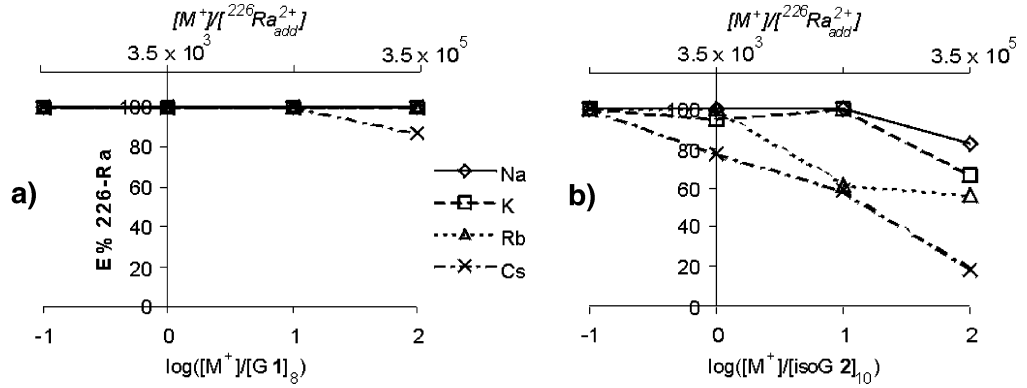


Figure 3. $^{226}Ra^{2+}$ extraction percentages recorded for different ratios of ionophore (I) to $M(NO_3)$ ($M = Na^+$, K^+, Rb^+, and Cs^+), using fixed ionophore ($[(G\ \mathbf{1})_8 + 2(Pic^-)]$ (a) and $[(isoG\ \mathbf{2})_{10}]$ (b); 10^{-4} M) and $^{226}Ra^{2+}$ (2.9×10^{-8} M) concentrations. The log($[M^+]/[I]$) values correlate directly with $[M^+]/[^{226}Ra_{add}{}^{2+}]$ values; $0 = 3.5 \times 10^3$, $1 = 3.5 \times 10^4$, $2 = 3.5 \times 10^5$, and $2.4 = 0.8 \times 10^6$.[25]

conditions. IsoG **2** continues to show a near quantitative presence of $^{226}Ra^{2+}$ cations with Ca^{2+}, Sr^{2+}, and Ba^{2+} cations. In the case of G **1**, the presence of Ca^{2+} and Sr^{2+} cations gave similar amounts of detected $^{226}Ra^{2+}$ cations as was reported for the blank experiments, while the presence of Ba^{2+} cations gave near quantitative amounts (>80%) of detected $^{226}Ra^{2+}$ cations. These findings suggest that precipitation of $^{226}Ra^{2+}$ salts is significantly prevented by the nucleosides used. The G **1**−picrate assembly and, in particular, the isoG **2** assembly not only are very selective $^{226}Ra^{2+}$ ionophores but also can prevent "^{226}Ra-scale formation" from solutions containing an excess of alkaline earth cations (for details see Supporting Information).

Competitive $^{226}Ra^{2+}$ Extraction Experiments in the Presence of Alkali Cations. Since Ra^{2+}-containing waters also contain relatively high concentrations of alkali cations,[4] and previous work[17,19] has shown that both the G **1**−picrate and isoG **2** assemblies have high affinities for some of these cations, competitive extraction experiments with alkali cations were performed (Figure 3).

For both self-assembled systems of the nucleosides, G **1** and isoG **2**, the $^{226}Ra^{2+}$ extraction efficiency does not seem to be perturbed by the presence of Na^+, even at a $Na^+/^{226}Ra_{add}{}^{2+}$ ratio of 0.35×10^6; the isoG **2** assembly showed only a slight drop in $^{226}Ra^{2+}$ extraction at this point. Furthermore, both nucleosides extracted significant percentages of $^{226}Ra^{2+}$ cations in the presence of high concentrations of competing K^+, Rb^+, and Cs^+ cations. Figure 3 shows that impressive $^{226}Ra^{2+}$ extraction percentages were obtained for both the G **1**−picrate assembly [K^+ (54%), Rb^+ (64%), Cs^+ (87%)] and the isoG **2** assembly [K^+ (66%), Rb^+ (56%), Cs^+ (18%)], even at $M^+/^{226}Ra_{add}{}^{2+}$ ratios of 0.35×10^6. Since the lipophilic G **1**−picrate assembly was shown to have a high K^+ affinity,[17] and the isoG **2** assembly, a high Cs^+ affinity,[20,24] it is understandable that these particular alkali cations interfere the most with $^{226}Ra^{2+}$ extraction.

In accordance with its high $^{226}Ra^{2+}$ selectivity in the presence of alkali cations, the G **1**−picrate assembly shows, in all cases, near quantitative percentages of detected $^{226}Ra^{2+}$ cations, which is a significant improvement to the amounts found under blank conditions (see Figure 1a). The isoG **2** assembly, however, still shows deficiencies of $^{226}Ra^{2+}$ cations in the presence of Na^+, K^+, and, in particular, Cs^+ cations. As the isoG **2** assembly has its $^{226}Ra^{2+}$ extraction considerably interfered with by the presence of Cs^+ cations, the loss of $^{226}Ra^{2+}$ cations can be attributed to its lower $^{226}Ra^{2+}$ selectivity in the presence of these cations (for details see Supporting Information).

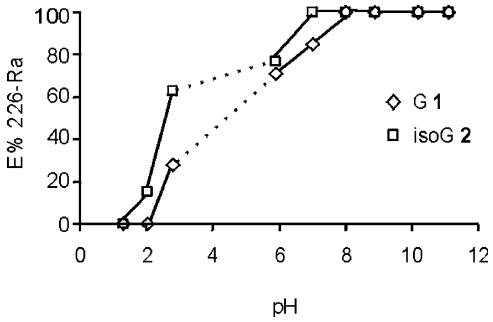


Figure 4. Influence of the pH on the $^{226}Ra^{2+}$ extraction of G **1**−picrate and isoG **2** assemblies.[26]

Extraction of $^{226}Ra^{2+}$ by G **1**−picrate and isoG **2** assemblies occurs even in the presence of a 0.35×10^6-fold excess of monovalent alkali cations. Additionally, $^{226}Ra^{2+}$ "precipitation" is significantly prevented by the presence of both G **1** and isoG **2**. Clearly, the noncovalent assemblies formed by G **1** and isoG **2** are much more favorable at binding divalent $^{226}Ra^{2+}$ cations than monovalent alkali cations (Scheme 2).

Determination of the Effective pH Range. The pH profiles for $^{226}Ra^{2+}$ extraction by G **1**−picrate and isoG **2** assemblies, obtained under noncompetitive conditions, indicate that both these nucleosides are effective $^{226}Ra^{2+}$ extractants between pH 3 and pH 11 (Figure 4).

Between pH 2 and pH 8 the extraction efficiency shows an upward trend for both the G **1**−picrate and the isoG **2** assemblies, and above pH 8 the $^{226}Ra^{2+}$ extraction becomes quantitative. This significantly improves the effective pH region compared to that reported by Chen et al. for self-neutralizing systems (pH 6−11) viz. *p-tert*-butylcalix[4]arene-crown-6-dicarboxylic acid, *p-tert*-butylcalix[4]arene-crown-6-dihydroxamic acid, a 17-crown-5 ether with one pendant carboxylic acid group, and an acyclic polyether with two pendant carboxylic acid groups.[11] To verify the results obtained with the low $^{226}Ra^{2+}$ concentrations used ($[I]/[^{226}Ra^{2+}] = 3.5 \times 10^3$), extraction experiments were performed using a 4×10^2 excess of Ba^{2+} nitrate and 2 equiv of LiPic in the case of G **1**. These experiments were monitored by ^{1}H NMR spectroscopy for the formation of the corresponding G **1**−picrate and isoG **2** assemblies. The hexadecameric assembly of G **1**, $[G\ \mathbf{1}]_{16}\cdot 2Ba^{2+}\cdot 4Pic^-$, was shown to be stable between pH 2 and 11. Below

pH 2, the picrate ($pK_a = 0.38$ in water)[27] peak and signals for $[G\ \mathbf{1}]_{16}\cdot 2Ba^{2+}\cdot 4Pic^-$ broaden and NMR signals for "free" G **1** are observed. Above pH 11, NMR signals for the G-quadruplex $[G\ \mathbf{1}]_{16}\cdot 2Ba^{2+}\cdot 4Pic^-$ could no longer be observed.[28] Characteristic NMR signals for the isoG **2** decamer, $[isoG\ \mathbf{2}]_{10}\cdot Ba^{2+}$, were also observed between pH 3 and 11. Below pH 3, an additional NH signal appeared and only partial complex formation was observed, while, above pH 11, the characteristic NH1 signals of the decamer $[isoG\ \mathbf{2}]_{10}\cdot Ba^{2+}$ were no longer present.[29] Assembly formation is clearly influenced by the presence of charges on the nucleoside monomers, as generated by protonation or deprotonation of the NH groups. Nevertheless, the effective pH range covers a broad region, making assemblies of G **1** and isoG **2** widely applicable ionophores.

Conclusions

The noncovalent assemblies formed by G **1** and isoG **2** show high $^{226}Ra^{2+}$ selectivities in the presence of divalent alkaline earth cations. Where in the case of G **1** the presence of a lipophilic anion is required before giving a high $^{226}Ra^{2+}$ selectivity in the case of Mg^{2+} and Ca^{2+}, isoG **2** gives high selectivities in the presence of Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+}, without any additional additives.[30] Furthermore, a remarkable $^{226}Ra^{2+}$ preference in the presence of monovalent alkali cations, "precipitation preventing/dissolving" characteristics, and a broad effective pH range were observed for both G **1** and isoG **2**. The ability to quantitatively and selectively extract a highly toxic radioisotope from a solution containing a million-fold excess of other cations shows the power and potential of using molecular self-assembly to construct highly selective receptors.

Experimental Section

Materials. The preparation of G **1**[17] and isoG **2**[31] was according to 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^+ (≥99.5%) and Rb^+ (p.a.) were purchased from Fluka Chemie, and those of 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. $^{133}Ba^{2+}$ stock solutions were purchased from Isotope Products Europe Blaseg GmbH. $^{226}Ra^{2+}$ stock solutions were purchased from AEA Technology QSA GmbH; ^{226}Ra was used due to its long half-life ($t_{1/2} = 1.6 \times 10^3$ y). **(Note: ^{226}Ra has a very high radiotoxicity and should be handled with care and under radionuclear supervision.)**

Solutions. All basic experiments were performed using an aqueous phase with pH 8.9 (tris-HCl buffer) and an organic phase containing 10^{-4} M of ionophore in CH_2Cl_2. The ionophore concentrations of the organic solutions were based on the amount of "free" ionophore needed to complex one alkaline earth cation [$(G\ \mathbf{1})_8$, $(isoG\ \mathbf{2})_{10}$]. The different nitrate salt concentrations were obtained by diluting stock solutions to the required concentration. From a 10 μg Ba^{2+}/mL carrier containing stock solution of $^{133}Ba^{2+}$ in 0.1 M HCl, a dilution of 45.18 kBq/g in water was made. From a carrier free stock solution of $^{226}Ra^{2+}$ in 0.5 M HCl, a dilution of 1.2 kBq/g (1.4×10^{-6} M) in 0.1 M HNO_3 was made.

General Extraction Procedures. Equal volumes (1.0 mL) 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. In the case of the G **1** extraction experiments 2 molar equiv, compared to $[(G\ \mathbf{1})_8]$, of LiPic were added to allow for full extraction. After extraction, the solutions were disengaged by centrifugation (1600 rpm for 5 min), and aliquots (0.5 mL) 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%.

ICP-MS Monitored Extraction Procedures. The solvent of the aliquot taken from the organic phase was evaporated, and the residue dissolved in 0.5 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).[32]

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

Noncompetitive ICP-MS (Table 1). In the noncompetitive extraction experiments the salt concentrations ($M(NO_3)_2$; M = Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+}) were equal to those of the ionophores (10^{-4} M) and were verified by their mass balances.

^{133}Ba Tracer Experiments (Table 1). In $^{133}Ba^{2+}$ extraction experiments the exact conditions were used as described above for the ICP-MS experiments. But in this case, 10 μL of ^{133}Ba tracer (452 Bq) were added. The gamma activity was determined using a NaI scintillation counter. The obtained extraction percentage is defined as 100% times the ratio of $^{133}Ba^{2+}$ activity in the organic phase (A_o) and the total $^{133}Ba^{2+}$ activity ($A_o + A_{aq}$) (eq 2).

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

$^{226}Ra^{2+}$ Extraction Procedures. In the case of the $^{226}Ra^{2+}$ extraction experiments, to the aqueous phase 20 μL of $^{226}Ra^{2+}$ tracer (240 Bq) were added. The gamma activity was determined with a Ge(Li) scintillation counter. The obtained extraction percentage is defined as 100% times the ratio of $^{226}Ra^{2+}$ activity in the organic phase (A_o) and the total $^{226}Ra^{2+}$ activity ($A_o + A_{aq}$) (eq 2).

Noncompetitive $^{226}Ra^{2+}$ Extractions. In the noncompetitive extraction experiments the aqueous phase only consisted of $^{226}Ra^{2+}$ cations in 1 mL of pH 8.9 tris-HCl buffer. To determine the influence of picrate anions on the extraction behavior of the G **1** and isoG **2** assemblies, additional experiments were performed in which 2 molar equiv of LiPic (2×10^{-4} M) were added to the aqueous phases.

Competitive $^{226}Ra^{2+}$ Extraction Curves (Single Competing Cation; Figures 2 and 3). In the competitive extraction experiments, aqueous phase pH 8.9 (tris-HCl), the ratio of competing $M^n(NO_3)_n$ (M = Na^+, K^+, Rb^+, Cs^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+}) salt concentrations compared to a fixed ionophore concentration (1 mL; 10^{-4} M) was altered to provide competing cation-concentration-dependent extraction curves.

Precipitation Experiments (Figure 1). Extraction experiments were performed as described above, but in this case the detectable amount

(25) At concentrations of 10^{-2} M the total detected amount of $^{226}Ra^{2+}$ cations with G **1** was almost equal to that originally added ($^{226}Ra^{2+}{}_{add}$), whereas isoG **2** still showed $^{226}Ra^{2+}$ salt precipitation.

(26) Due to the competitive nature of the available buffers between pH 3 and 6, the $^{226}Ra^{2+}$ extraction percentages could not be accurately determined in this region. Therefore, the results are extrapolated from those obtained at pH 3 and 6.

(27) Botoshansky, M.; Herbstein, F. H.; Kapon, M. *Acta Crystallogr. B* **1994**, *32*, 191−200.

(28) Rogstad, K. N.; Jang, Y. H.; Sowers, L. C.; Goddard, W. A. *Chem. Res. Toxicol.* **2003**, *16*, 1455−1462. The loss of the G **1** complex stability below pH 2 and above pH 11 appears to match the guanosine pK_a values (NH1 = 9.65 and NH7/NH3 = 3.2) determined in water.

(29) Jang, Y. H.; Goddard, W. A.; Noyes, K. T.; Sowers, S. H.; Chung, D. S. *J. Phys. Chem. B* **2003**, *107*, 344−357. The loss of the isoG **2** complex stability below pH 3 and above pH 11 appears to match the isoguanosine pK_a values (NH1 = 11.1 and NH3 = 3.8) determined in water.

(30) A direct comparison with known radium ionophores was not made, due to the different testing methods used. In our case the extraction of $^{226}Ra^{2+}$ cations was performed from a large *excess of competing* cations using a *deficiency* of ionophore, while in refs 9 and 11 the extractions have been carried out using an *excess of ionophore* to competing cations.

(31) Davis, J. T.; Tirumala, S.; Jenssen, J. R.; Radler, E.; Fabris, D. *J. Org. Chem.* **1995**, *60*, 4167−4176.

(32) The isotope ^{43}Ca was corrected for the doubly charged interference of the isotope ^{88}Sr.

of $^{226}Ra^{2+}$ tracer was determined. The organic phase contained dichloromethane; no ionophore was present. The $^{226}Ra^{2+}$ percentage is defined as 100% times the ratio of $^{226}Ra^{2+}$ detected in the aqueous phase (A_{aq}) and the amount of $^{226}Ra^{2+}$ added (A_{add}) (eq 3).

$$Ra\% = 100\%(A_{aq}/A_{add}) \quad (3)$$

Extraction vs pH Curves (Figure 4). Experiments were performed using a fixed ionophore (G **1**)$_8$ or (isoG **2**)$_{10}$ concentration of 10^{-4} M and a fixed $^{226}Ra^{2+}$ concentration (2.9×10^{-8} M). The pH values were set using different buffer solutions: pH 1−3, glycerol−LiCl−HCl (G **1**) and HCl (isoG **2**);[33] pH 6−7, imidazol−HCl; pH 8−10, tris-HCl; and pH 10−13, tris-$(CH_3)_3$NOH.

^{1}H NMR Monitored Extraction Experiments. Using the same buffer solutions as mentioned above, solid-phase extraction experiments of $Ba(NO_3)_2$ (0.1 g; 3.8×10^{-4} mol) were performed with a 1 mL buffer, containing aqueous phase, and a 1.0 mL organic phase, containing 10^{-3} M of ionophore.

(33) Below pH 3, G **1** seemed to have its $^{226}Ra^{2+}$ extraction interfered by HCl, whereas isoG **2** had its $^{226}Ra^{2+}$ extraction interfered by glycerol−HCl−LiCl.

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Supporting Information Available: Overall $^{226}Ra^{2+}$ percentages present in solutions with varying concentrations of alkali-(ne earth) cations, after extraction with G **1** and isoG **2**, are given. This material is available free of charge via the Internet at http://pubs.acs.org.

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