Supplementary Material for Organic & Biomolecular Chemistry

Electronic supplementary information

Thiacalix[4]arene derivatives as radium ionophores: a study on the requirements for $^{226}Ra^{2+}$ extraction

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2D ^{1}H NMR Spectra

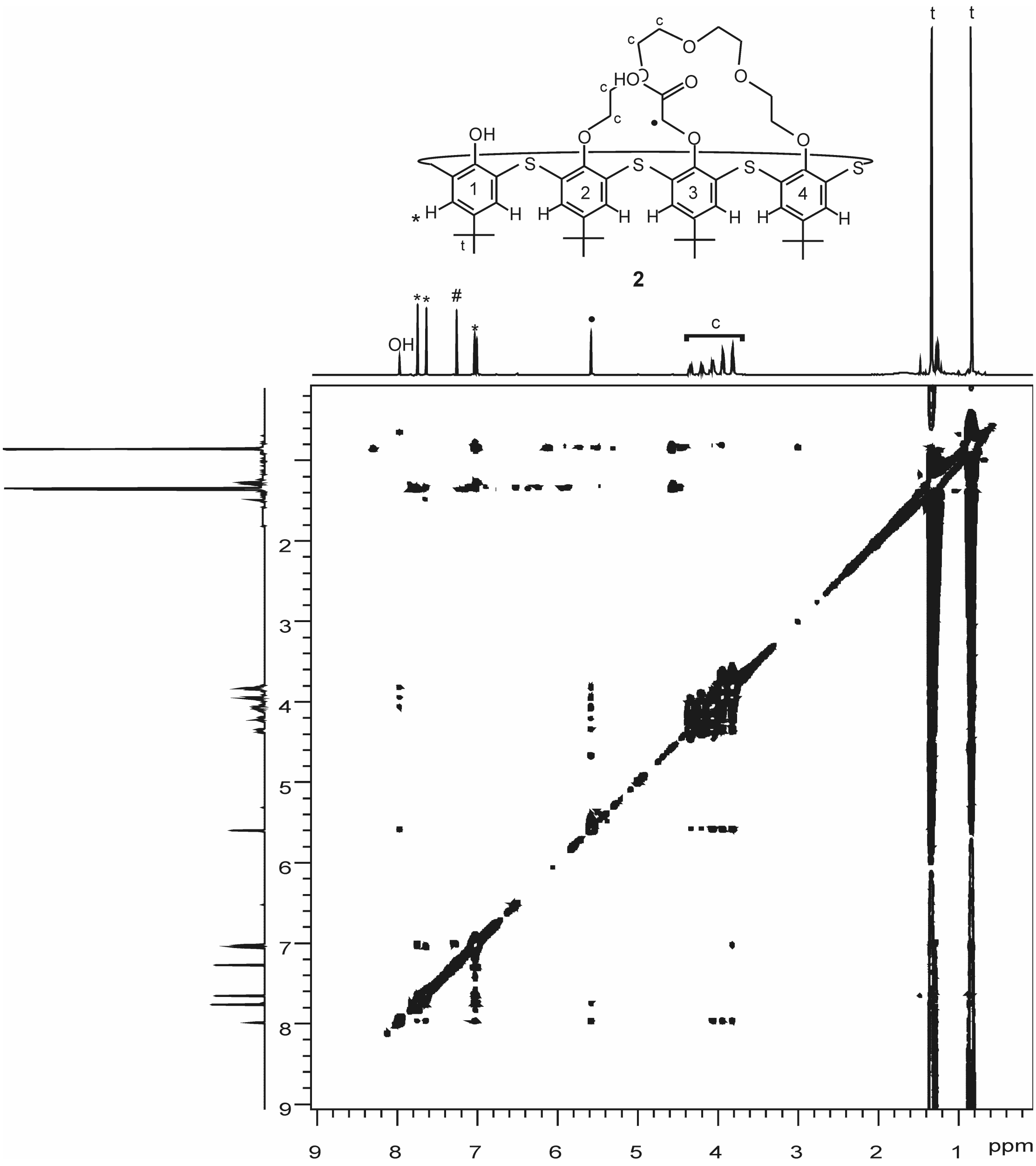


Figure S1. ROESY spectrum of the thiacalix[4]crown monocarboxylic acid (**2**) (cone) in $CDCl_3$ at 25 °C. The $CHCl_3$ peak is indicated with $^{\#}$.

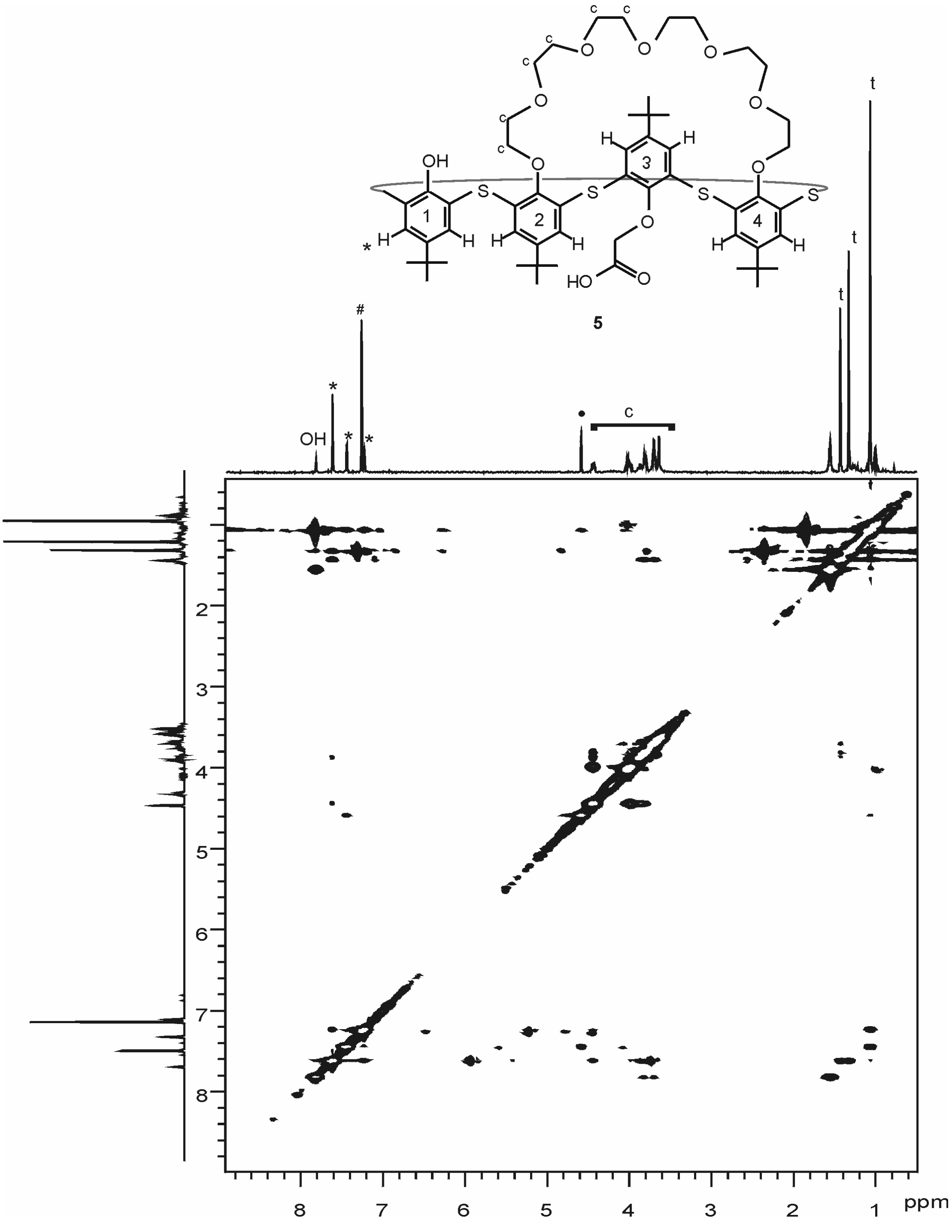


Figure S2. NOESY spectrum of the thiacalix[4]crown monocarboxylic acid (**7**) (partial cone) in CDCl$_3$ at 25 °C. The CHCl$_3$ peak is indicated with $^{\#}$.

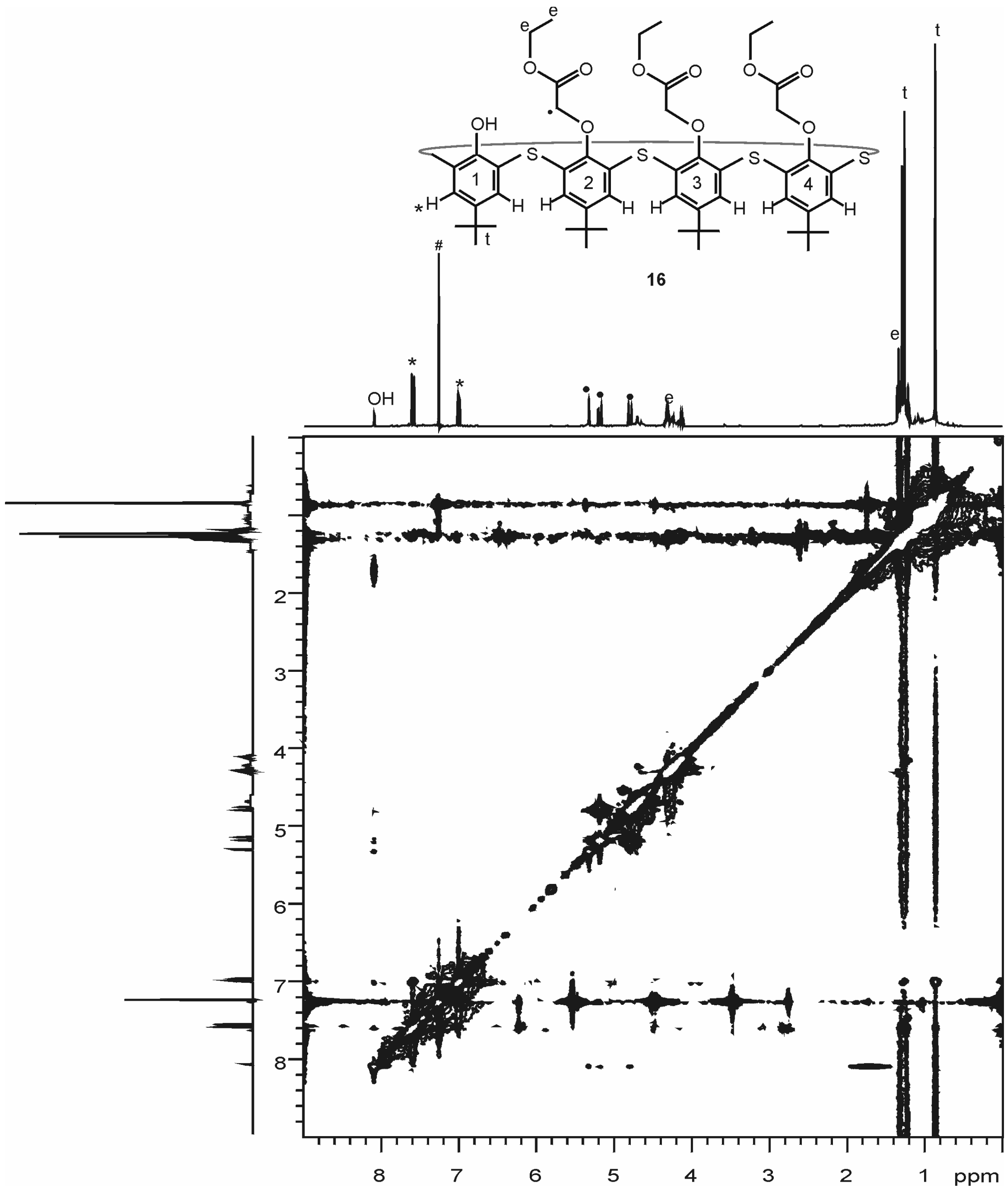


Figure S3. NOESY spectrum of the thiacalix[4]arene-crown tricarboxylic ethyl ester (**16**; cone) in $CDCl_3$ at 25 °C. The $CHCl_3$ peak is indicated with $^{\#}$.

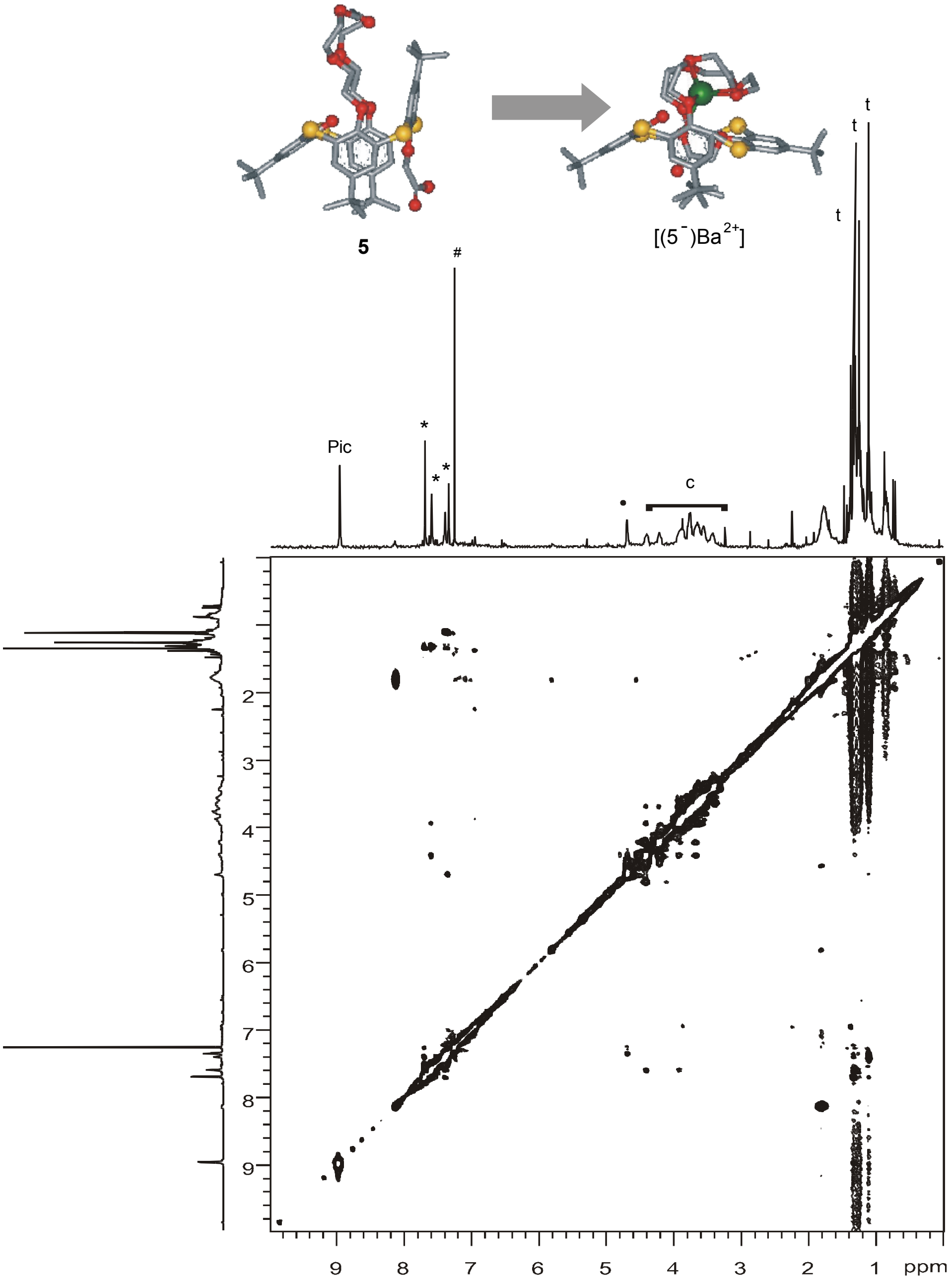


Figure S4. NOESY spectrum of the thiacalix[4]crown monocarboxylic acids (**5**) (partial cone) Ba^{2+} complex in $CDCl_3$ at 25 °C. Indicated are the resonances of: the $–OCH_2CO_2$- group ($^{\bullet}$), the crown-ether bridge (^{c}), the ArH groups (*), the *t*-butyl groups (^{t}), and $CHCl_3$ ($^{\#}$).The expected conformational change of **5** (Figure S2) after complexation of Ba^{2+} is depicted as molecular model (top Figure S4).

Extraction equilibria and stoichiometries used to obtain extraction constants

Comparison of the extraction constants of the competing cations (Ca^{2+}, Sr^{2+}, and Ba^{2+}) and of $^{226}Ra^{2+}$, allows the quantification of the differences between the various extractants used (**2**, **3a,b**, **4a,b**, **5**, **6**, **7**, and **8**). In order to obtain these extraction constant ratios, several equilibria have to be considered.

Since the p*K*a of tris is 8.1[1] and all the competition experiments were performed at pH 8.9 ($[\text{tris}]_{tot} = 5.0 \times 10^{-2}$ M), $[\text{Htris}^+]_{aq} = 6.8 \times 10^{-3}$ M. Consequently, because of the basic nature of the aqueous phase, the uncomplexed ligands ($[L]_{tot} = 10^{-4}$ M) in the organic phase are assumed to exist as neutral (Htris)L, $(\text{Htris})_2L$, $(\text{Htris})_3L$, or $(\text{Htris})_4L$ salts, depending on the charge of the ligand.

*Singly Charged Extractants, 1: 1 Stoichiometry (**2** and **5**)*

Extraction of M^{2+} cations by the singly charged thiacalix[4]crown monocarboxylic acids (**2** and **5**) is shown in Figure S.5. The best fits were obtained assuming a 1:1 complex stoichiometry, and therefore the equilibrium for the extraction of the divalent cations can be written as equation 1, with equation 2 describing the corresponding extraction constant.

$$[M^{2+}]_{aq} + [X^-]_{aq} + [\text{HtrisL}]_{org} \rightleftharpoons [\text{MLX}]_{org} + [\text{Htris}^+]_{aq} \qquad (1)$$

$$K^M_{ex} = [\text{MLX}]_{org}[\text{Htris}^+]_{aq}/[M^{2+}]_{aq}[\text{HtrisL}]_{org}[X^-]_{aq} \qquad (2)$$

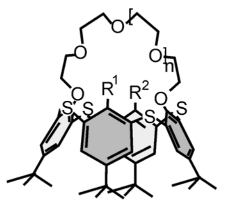
Because of the equal volumes used, a decrease of $[M^{2+}]_{aq}$ in the aqueous phase leads to an equal increase of $[\text{MLX}]_{org}$ in the organic phase, allowing for direct comparison of the concentrations of the two phases. This results in the equations 3 and 4 for the mass balances of M^{2+} and L, respectively.

$$[M^{2+}]_{tot} = [M^{2+}]_{aq} + [MLX]_{org} \qquad (3)$$

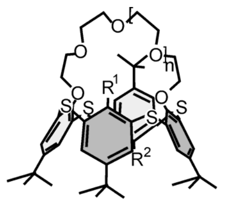
$$[L]_{tot} = [(Htris)_2L]_{org} + [MLX]_{org} \qquad (4)$$

If the extraction percentages obtained for the competing cations (see Figure S.5) are incorporated as $p_M = [MLX]_{org}/[M^{2+}]_{tot}$, $[MLX]_{org}$ is equal to $p_M[M^{2+}]_{tot}$, and $[M^{2+}]_{aq} = (1-p_M)[M^{2+}]_{tot}$.

The extraction percentages of the competing M^{2+} cations in a concentration range of (0.2-3.0) x 10^{-4} M are provided in Figure S.5. From these extraction curves, K^{M}_{ex} can be obtained by fitting calculated p_M values to the experimentally determined values (Table 2), using a non-linear least squares fitting procedure.



2 $R^1 = OH$, $R^2 = OCH_2CO_2H$ (n= 1)



5 $R^1 = OH$, $R^2 = OCH_2CO_2H$ (n= 2)

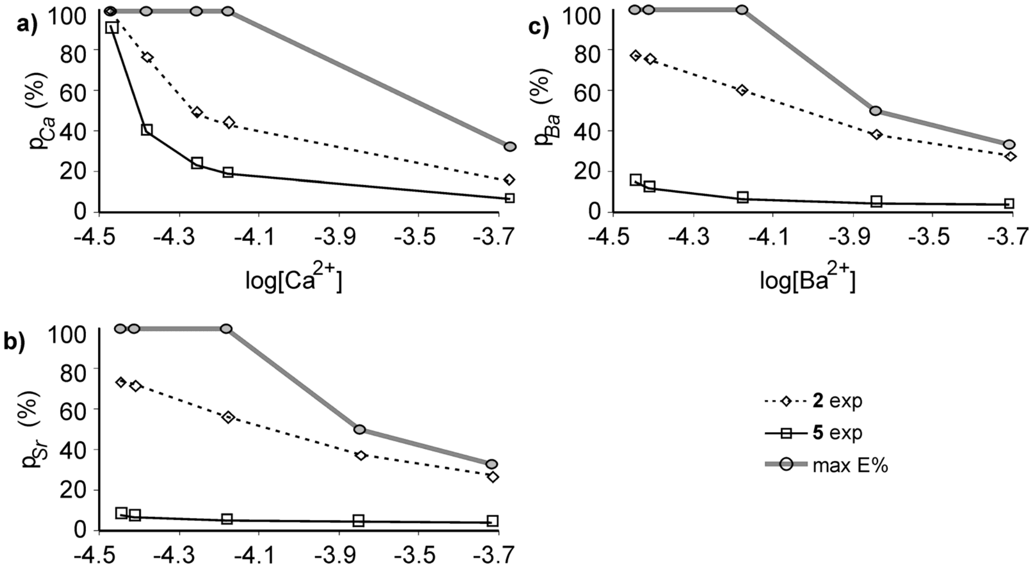


Figure S.5. M^{2+} extraction percentages ($p_M = [M^{2+}]_{org}/[M^{2+}]_{tot}(\%)$) for extractants **2** and **5** (10^{-4} M; 1 ml of CH_2Cl_2) as a function of $\log[M^{2+}]$ ($M^{2+} = Ca^{2+}$(a), Sr^{2+}(b), and Ba^{2+}(c); 1 ml of water pH 8.9 tris-HCl buffer). The line through the circles gives the extraction percentages calculated for the complex formation of a 1:1 [MLX] complex.

When the $^{226}Ra^{2+}$ experiments, in competition with the divalent cations Ca^{2+}, Sr^{2+}, and Ba^{2+} (Figure 3), are modelled, additional equilibria and mass balances have to be considered. The extraction equilibrium for $^{226}Ra^{2+}$ and its extraction constant are given in equations 5 and 6, respectively. The $^{226}Ra^{2+}$ mass balance is given in equation 7, and the ligand mass balance (equation 4) is now expanded to equation 8.

$$[^{226}Ra^{2+}]_{aq} + [X^-]_{aq} + [HtrisL]_{org} \rightleftharpoons [^{226}RaLX]_{org} + [Htris^+]_{aq} \tag{5}$$

$$K^{Ra}_{ex} = [^{226}RaLX]_{org}[Htris^+]_{aq}/[^{226}Ra^{2+}]_{aq}[HtrisL]_{org}[X^-]_{aq} \tag{6}$$

$$[^{226}Ra^{2+}]_{tot} = [^{226}Ra^{2+}]_{aq} + [^{226}RaLX]_{org} \tag{7}$$

$$[L]_{tot} = [HtrisL]_{org} + [MLX]_{org} + [^{226}RaLX]_{org} \tag{8}$$

However, because of the small amount of $[^{226}Ra^{2+}]_{tot}$ (2.9 x 10^{-8} M), $[^{226}RaLX]_{org}$ can be neglected in equation 8. Similar to p_M for the competing cations, p_{Ra} is defined as $[^{226}RaLX]_{org}/[^{226}Ra^{2+}]_{tot}$, resulting in $[^{226}RaLX]_{org} = p_{Ra}[^{226}Ra^{2+}]_{tot}$, and $[^{226}Ra^{2+}]_{aq} = (1-p_{Ra})[^{226}Ra^{2+}]_{tot}$. Thus the K^{Ra}_{ex}/K^{M}_{ex} ratio (equation 9) can be written as equation 10.

$$K^{Ra}_{ex}/K^{M}_{ex} = ([^{226}RaLX]_{org}/[^{226}Ra^{2+}]_{aq}) * ([M^{2+}]_{aq}/[MLX]_{org}) \tag{9}$$

$$K^{Ra}_{ex}/K^{M}_{ex} = p_{Ra}(1-p_M)/(1-p_{Ra})p_M \tag{10}$$

Since the K^{M}_{ex} values of the different extractants have different dimensions, only K^{Ra}_{M}/K^{M}_{ex} ratios are given. These ratios are obtained by fitting calculated p_{Ra} values to the experimentally determined values (Figure 3), using a non-linear least squares fitting procedure.

*Di- or Trivalently Charged Extractants, 1: 1 Stoichiometry (**3a**,**b**, **4a**,**b**, **6**, and 7)*

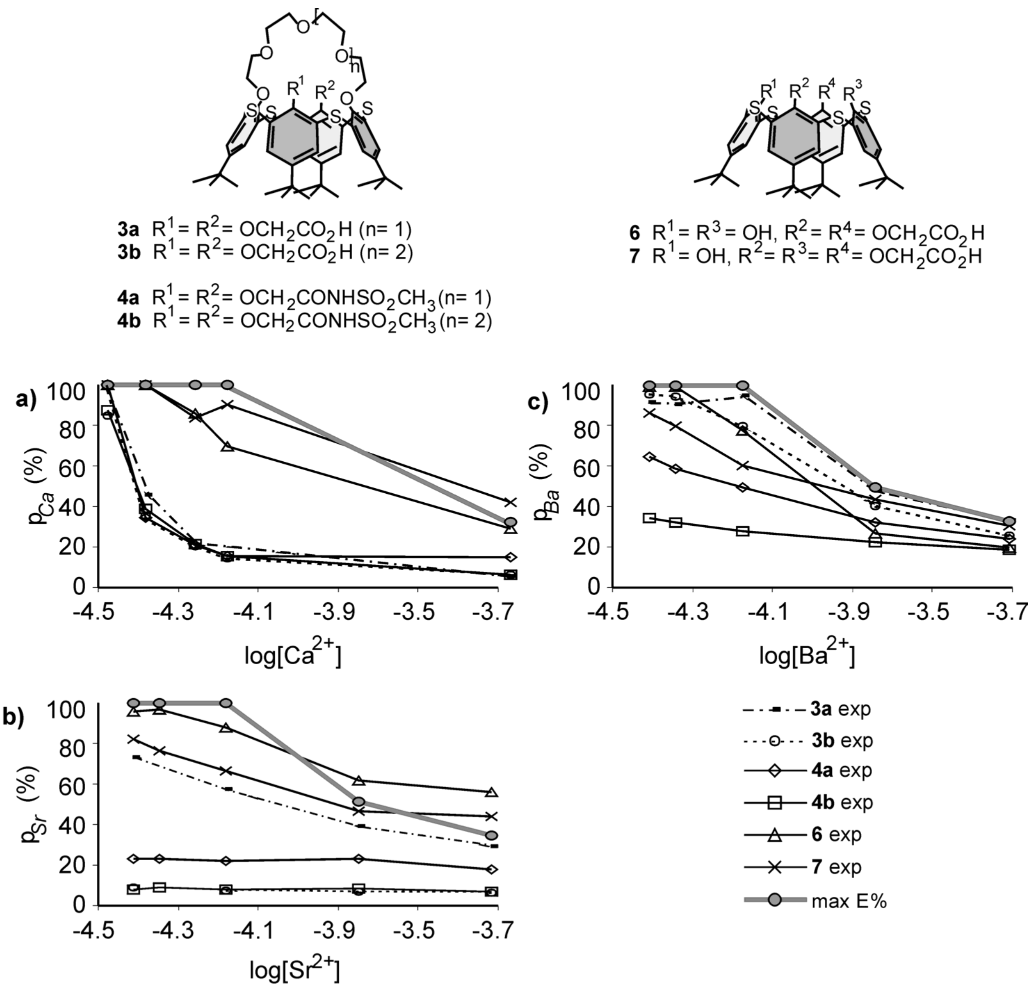


Figure S.6. M^{2+} extraction percentages ($p_{M} = [M^{2+}]_{org}/[M^{2+}]_{tot}$ (%)) for extractants **3a**,**b**, **4a**,**b**, **6**, and **7** (10^{-4} M; 1 ml of CH_2Cl_2) as a function of $\log[M^{2+}]$ ($M^{2+} = Ca^{2+}$(a), Sr^{2+}(b), and Ba^{2+}(c); 1 ml of water pH 8.9 tris-HCl buffer). The line through the circles gives the extraction percentages calculated for a 1:1 [ML] complex.

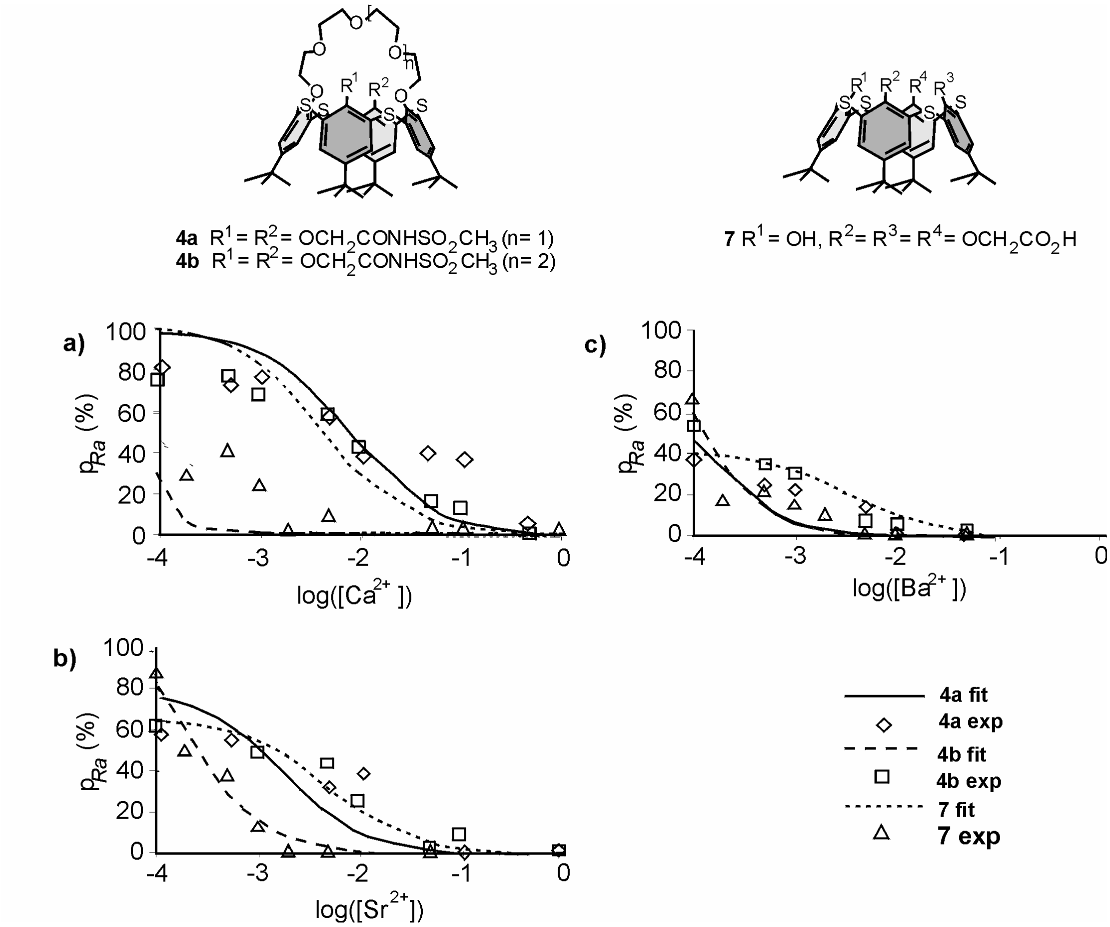
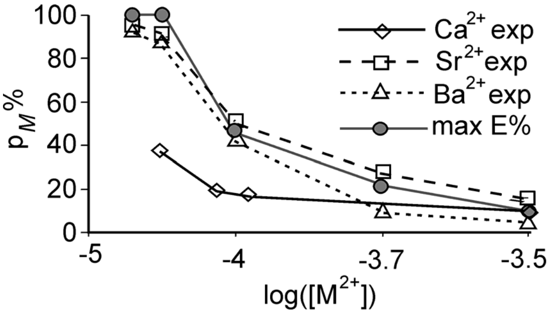


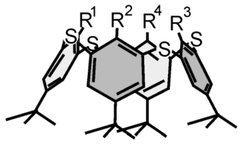
Figure S.7. $^{226}Ra^{2+}$ extraction percentages (p_{Ra} = $[^{226}Ra^{2+}]_{org}/[^{226}Ra^{2+}]_{tot}$ (%)) for extractants **4a** (a)**, 4b** (b), and **7** (c) (10^{-4} M; 1 ml of CH_2Cl_2), as a function of the $M(NO_3)$ (M^{2+} = Ca^{2+}, Sr^{2+}, or Ba^{2+}; 1 ml of water pH 8.9 tris-HCl buffer) concentration, with 2.9 x 10^{-8} M $^{226}Ra^{2+}$.

The equations used to determine the extraction constants and selectivity coefficient of the divalently charged thiacalix[4]crown dicarboxylic acids (**3a,b**) have previously been described.[2] For thiacalix[4]crown bis(methylsulfonyl) carboxamides

(**4a**,**b**), thiacalix[4]arene dicarboxylic acid (**6**), and thiacalix[4]arene tricarboxylic acid (**7**) the same equations can be used, which results in equation 10 for the $^{226}Ra^{2+}$ selectivity coefficient. Here only the extraction behavior of **3a**,**b**, **4a**,**b**, **6**, and **7** towards Ca^{2+}, Sr^{2+}, and Ba^{2+} are depicted in Figure S.6, as well as the $^{226}Ra^{2+}$ extraction curves of **4a,b** and **7** (Figure S.7).

*Tetravalent Charged Extractant, 1: 2 Stoichiometry (**8**)*





8 $R^1 = R^2 = R^3 = R^4 = OCH_2CO_2H$

Figure S.8. M^{2+} extraction percentages (p_M = $[M^{2+}]_{org}/[M^{2+}]_{tot}$ (%)) recorded with **8** at different extractant to $M(NO_3)_2$ ratios (M^{2+} = Ca^{2+}, Sr^{2+}, and Ba^{2+}; 1 ml of pH 8.9 tris-HCl buffer), using a fixed extractant (10^{-4} M; 1 ml of CH_2Cl_2). The line through the circles gives the extraction percentages calculated for a 1:2 $[M(Htris)_3L)_2]$ complex stoichiometry .

Surprisingly, extraction data obtained for the thiacalix[4]arene tetracarboxylic acid (**8**), with the competing cations Ca^{2+}, Sr^{2+}, and Ba^{2+} (Figure S.8) suggest a 1:2 (ML_2) complex stoichiometry. Consequently, models based on a 1:2 stoichiometry could be used to fit these curves, while models based on more plausible 1:1 and 2:1 stoichiometries or a combination, did not give proper fits. As a result thereof, the equilibrium for the extraction of the divalent cations can be written as equation 11, with equation 12 describing the corresponding extraction constant.

$$[M^{2+}]_{aq} + 2[(Htris)_4L]_{org} \rightleftharpoons [M((Htris)_3L)_2]_{org} + 2[Htris^+]_{aq} \tag{11}$$

$$K^M_{ex} = [M((Htris)_3L)_2]_{org}[Htris^+]_{aq}^2/[M^{2+}]_{aq}[(Htris)_4L]_{org}^2 \tag{12}$$

Because of the equal volumes used, a decrease of $[M^{2+}]_{aq}$ in the aqueous phase leads to an equal increase of $[M((Htris)_3L)_2]_{org}$ in the organic phase, allowing for direct comparison of the concentrations of the two phases. This results in the equations 13 and 14 for the mass balances of M^{2+} and L, respectively.

$$[M^{2+}]_{tot} = [M^{2+}]_{aq} + [M((Htris)_3L)_2]_{org} \tag{13}$$

$$[L]_{tot} = [(Htris)_4L]_{org} + 2[M((Htris)_3L)_2]_{org} \tag{14}$$

The extraction percentages obtained for the competing cations (see Table 1) are incorporated in equations 12-14 as $p_M = [M((Htris)_3L)_2]_{org}/[M^{2+}]_{tot}$, $[M((Htris)_3L)_2]_{org} = p_M[M^{2+}]_{tot}$, and $[M^{2+}]_{aq} = (1-p_M)[M^{2+}]_{tot}$.

The extraction percentages of the competing M^{2+} cations in a concentration range of (0.2-3.0) x 10^{-4} M are provided in Figure S.8. From these extraction curves, K^M_{ex} can be obtained by fitting calculated p_M values to the experimentally determined values (Table 2), using a non-linear least squares fitting procedure.

When the $^{226}Ra^{2+}$ experiments, in competition with the divalent cations Ca^{2+}, Sr^{2+}, and Ba^{2+} (Figure S.9), are modelled, additional equilibria and mass balances have to be considered. The extraction equilibrium and its extraction constant are given in equations 15 and 16, respectively. The $^{226}Ra^{2+}$ mass balance is given in equation 17, and the ligand mass balance (equation 4) is now expanded to equation 18.

$$[^{226}Ra^{2+}]_{aq} + 2[(Htris)_4L]_{org} \rightleftarrows [^{226}Ra((Htris)_3L)_2]_{org} + 2[Htris^{+}]_{aq} \qquad (15)$$

$$K^{Ra}_{ex} = [^{226}Ra((Htris)_3L)_2]_{org}[Htris^{+}]_{aq}^{2}/[^{226}Ra^{2+}]_{aq}[(Htris)_4L]_{org}^{2} \qquad (16)$$

$$[^{226}Ra^{2+}]_{tot} = [^{226}Ra^{2+}]_{aq} + [^{226}Ra((Htris)_3L)_2]_{org} \qquad (17)$$

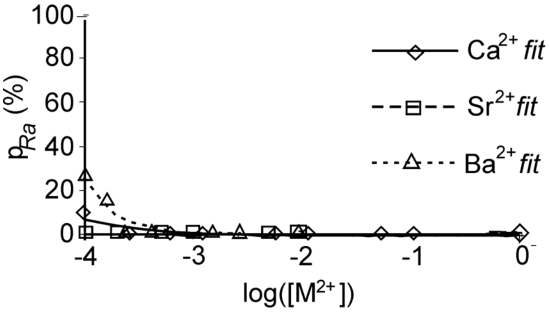
$$[L]_{tot} = [(Htris)_4L]_{org} + 2[M((Htris)_3L)_2]_{org} + 2[^{226}Ra((Htris)_3L)_2]_{org} \qquad (18)$$

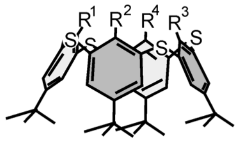
However, because of the small amount of $[^{226}Ra^{2+}]_{tot}$ (2.9 x 10^{-8} M), $[^{226}Ra((Htris)_3L)_2]_{org}$ can be neglected in equation 18. Similar to p_M for the competing cations, p_{Ra} is defined as $[^{226}Ra((Htris)_3L)_2]_{org}/[^{226}Ra^{2+}]_{tot}$, resulting in $[^{226}Ra((Htris)_3L)_2]_{org} = p_{Ra}[^{226}Ra^{2+}]_{tot}$, and $[^{226}Ra^{2+}]_{aq} = (1-p_{Ra})[^{226}Ra^{2+}]_{tot}$. Thus the K^{Ra}_{ex}/K^{M}_{ex} ratio (equation 19) can be written as equation 20.

$$K^{Ra}_{ex}/K^{M}_{ex} = ([^{226}Ra((Htris)_3L)_2]_{org}/[^{226}Ra^{2+}]_{aq}) * ([M^{2+}]_{aq}/[M((Htris)_3L)_2]_{org}) \qquad (19)$$

$$K^{Ra}_{ex}/K^{M}_{ex} = p_{Ra}(1-p_{M})/(1-p_{Ra})p_{M} \qquad (20)$$

Since the K^{M}_{ex} values of the different extractants have different dimensions, only K^{Ra}_{M}/K^{M}_{ex} ratios are given. These ratios are obtained by fitting calculated p_{Ra} values to the experimentally determined values (Figure S.9), using a non-linear least squares fitting procedure.





8 $R^1 = R^2 = R^3 = R^4 = OCH_2CO_2H$

Figure S.9. ^{226}Ra^{2+} extraction percentages (p_{Ra} = $[^{226}Ra^{2+}]_{org}/[^{226}Ra^{2+}]_{tot}$ (%)) for extractant **8** (10^{-4} M; 1 ml of CH_2Cl_2), as a function of the $M(NO_3)$ (M = Ca^{2+}, Sr^{2+}, or Ba^{2+}; 1 ml of water pH8.9 tris-HCl buffer) concentration, with 2.9 x 10^{-8} M ^{226}Ra^{2+}. The fitted curves are depicted.

References

1. Kearns, A.; Cole, L.; Haws, C. R.; Evans, D. E. *Plant Physiol. Biochem*. 1**998**, *36*, 879-887.
2. Van Leeuwen, F. W. B.; Beijleveld, H.; Miermans, C. J. H.; Huskens, J.; Verboom, W.; Reinhoudt, D. N. *submitted for publication.*