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Molecular Design of Calix[4]arene-Based Extractants Which Show High Ca^{2+} Selectivity

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It has been established that calix[4]aryl esters and amides show very high Na^+ selectivity because of the size-fit ionophoric cavity and the conformationally rigid calix[4]arene platform supporting the cavity.[1–6] By using these calix[4]arene-based ionophores, one can design Na^+-selective electrodes,[7–9] and in some cases the Na^+/K^+ selectivity exceeds a factor of 10^3.[9] The purpose of the present study is to apply the above-mentioned advantages in calix[4]arene-based ionophores to the design of Ca^{2+}-selective extractants. We considered this idea to be feasible because the ion radius of Ca^{2+} (0.99 Å) is very similar to that of Na^+ (0.96 Å). In addition to the similarity in the ion size, we took several factors into account which are indispensable to achieve high Ca^{2+} selectivity in two-phase solvent extraction. First, the extractant should be dianionic to keep the complex neutral and to facilitate the extraction into the organic phase. Second, Ca^{2+}-selective ionophores (e.g., those designed by Simon et al.[10]) mostly include amide groups which can strongly interact with alkali and alkaline earth metal ions. The advantage of the amide group has also been corroborated in calix[4]arenes: calix[4]aryl amides generally show much higher metal affinity than calix[4]aryl esters.[5,11,12] Third, the ionophoric cavity composed of neutral ligands and the two anionic functional groups neutralizing the metal charge must act cooperatively upon the binding of metal ions. We noticed that 5,11,17,23-tetra-*tert*-butyl-25,27-bis(carboxymethoxy)-26,28-bis[(diethylaminocarbonyl)methoxy]-calix[4]arene (**1**), which can be synthesized from 5,11,17,23-tetra-*tert*-butylcalix[4]arene-25,26,27,28-tetrol via three steps, fully satisfies these prerequisites. Two-phase solvent extraction studies have established that, as expected, **1** shows "perfect" Ca^{2+} selectivity among alkaline earth metal ions at an appropriate pH region.

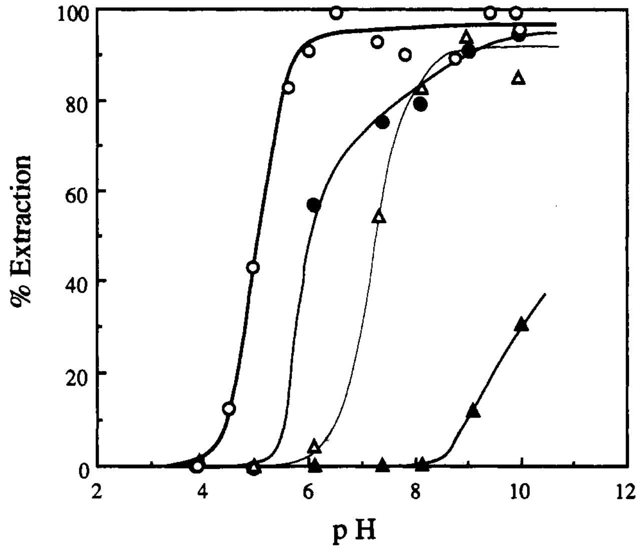
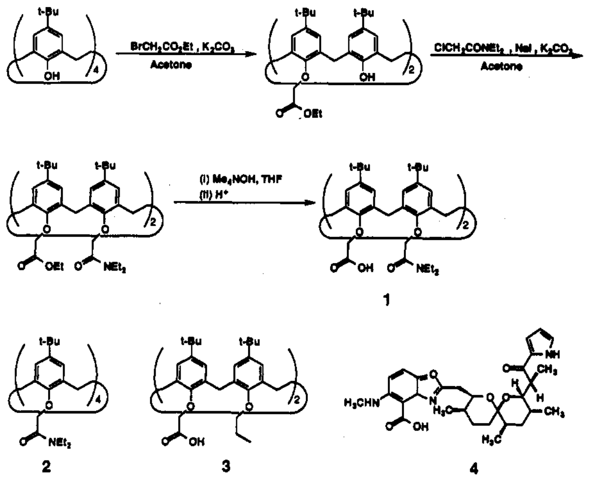


Figure 1. Two-phase solvent extraction of alkaline earth metal ions with **1**. The extraction conditions are recorded in the text. The pH of the aqueous phase was adjusted with succinic acid–NH_4OH for pH 4–6, Tris-HCl for pH 7–9, and 3-(cyclohexylamino)propanesulfonic acid (CAPS)–NH_4OH for pH 10. Ca^{2+} (○), Sr^{2+} (●), Ba^{2+} (△), and Mg^{2+} (▲).

Scheme 1



Compound **1** with a cone conformation was synthesized according to the reaction route shown in Scheme 1. The product was identified by IR and ^1H NMR spectral evidence and elemental analysis.[13] Compound **2** with four amide groups[11,12] and compound **3** with two carboxylic acid groups,[14] both having a cone conformation, were used as reference compounds. Compound **4** is a naturally occurring Ca^{2+} ionophore (A23187). Two-phase solvent extraction was carried out between water (4 mL, $[M(NO_3)_2]$ = 0.10 mM) and chloroform (4 mL, [ionophore] =

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(13) Mp 258–259 °C; IR (Nujol) $\nu_{C=O}$ 1655 and 1755 cm^{-1}, ν_{OH} 3050–3100 cm^{-1}; ^1H NMR ($CDCl_3$, 25 °C, 250 MHz) δ 0.85 and 1.31 (*t*-Bu, s each, 18H each), 1.17 (CH_3 in Et, t, 12H), 3.28 and 4.61 ($ArCH_2Ar$, d each (J = 13.0Hz), 4H each), 3.3–3.4 (NCH_2, m, 8H), 4.58 and 4.77 (OCH_2, s each, 4H each), 6.60 and 7.13 (ArH, s each, 4H each). Anal. Calcd for $C_{60}H_{82}N_2O_{10}$: C, 72.69; H, 8.34; N, 2.83. Found: C, 72.48; H, 8.47; N, 2.91.

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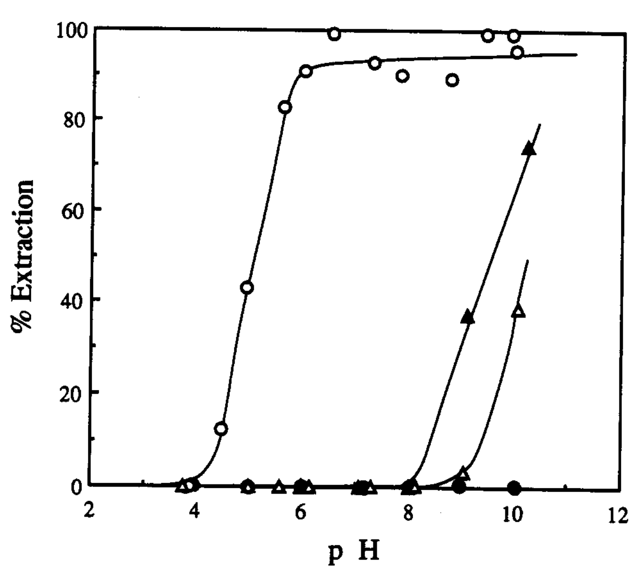

Figure 2. Two-phase solvent-extraction of Ca^{2+} with **1**–**4**. The extraction conditions are similar to those employed in Figure 1. Extraction of Ca^{2+} with **1** (○), **2** (●), **3** (△), and **4** (▲).

Table 1. Percent Extraction of Alkaline Earth Metal Ions from Water to $CHCl_3$ at pH 5.3 and 25 °C[a]

	extractability/%			
ionophore	Mg^{2+}	Ca^{2+}	Sr^{2+}	Ba^{2+}
1	0	59.3	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0

[a] The extractability "0" means Ex% < 0.1%.

0.050 mM). The two-phase mixture was shaken for 30 min at 25 °C. We confirmed that this period is enough to attain the distribution equilibrium. The organic phase was reextracted with water (5 mL, 1.0 M aqueous HCl) and the extractability (Ex%) was determined by ICP analysis of the aqueous solution.

Figure 1 shows Ex% for alkaline earth metal ions with **1** plotted against pH in the aqueous phase. Ex% for Ca^{2+} increases from pH 4.0 and is saturated at around pH 6, giving 100% extractability. In contrast, Ex% for Sr^{2+}, Ba^{2+}, and Mg^{2+} increases from pH 5.2, 6.0, and 8.2, respectively. Thus, the extraction of alkaline earth metal ions occurs from acidic pH region in the order of $Ca^{2+} > Sr^{2+} > Ba^{2+} > Mg^{2+}$. Figure 1 tells us that the highest Ca^{2+} selectivity is attained at around pH 5. Figure 2 shows extraction of Ca^{2+} with **1**–**4** plotted against pH in the aqueous phase. Compound **2**, a neutral ionophore, could not extract a perceptible amount of Ca^{2+} at whole pH range. Compounds **3** and **4** showed significant extractability at above pH 8. The difference clearly supports the contribution of the two amide groups to the Ca^{2+} binding. This view was further corroborated by the ^1H NMR study of the **1**–Ca^{2+} complex. A D_2O solution (4 mL) containing $Ca(NO_3)_2$ (4.0×10^{-3} M; pH 9.0 with 2.5×10^{-2} M Tris-HCl) and a $CDCl_3$ solution (4 mL) containing **1** (2.0×10^{-3} M) was shaken at 25 °C for 30 min. The 100% extractability was achieved under the extraction conditions. The ^1H NMR spectrum of the $CDCl_3$ solution showed that the chemical shifts for the OCH_2CON and NCH_2 methylene protons move to higher magnetic field by 0.03 ppm[15] and lower magnetic field by 0.22 ppm, respectively. The $\Delta\delta$ for the AB pattern of the $ArCH_2Ar$ methylene protons decreased from 1.33 to 0.92 ppm, indicating that the phenol groups are flattened upon the metal binding. The foregoing findings consistently reveal that **1** has high affinity as well as high specificity toward Ca^{2+}.

Table 2. Competitive Extraction of Ca^{2+} from a Mixture of Four Alkaline Earth Metal Ions

		extractability/%			
pH	ionophore	Mg^{2+}	Ca^{2+}	Sr^{2+}	Ba^{2+}
5.3	**1**	0	61.4	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
6.0	**1**	0	73.4	3.3	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
7.0	**1**	0	78.7	3.3	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
8.0	**1**	0	83.7	2.7	0
	2	0	0	0	0
	3	0	0	0	0
	4	41.8	0	0	0
9.0	**1**	0	79.2	3.7	0
	2	0	0	0	0
	3	0	0	0	0
	4	58.2	0	0	0
10.0	**1**	0	82.9	4.6	0
	2	0	0	0	0
	3	4.8	25.5	4.5	2.6
	4	69.4	0	0	0

Ex% values determined at pH 5.3 are summarized in Table 1. It is clearly seen from Table 1 that at this pH only compound **1** shows significant extractability and only Ca^{2+} ion is extracted with **1**. The results establish that ***1** acts as an excellent extractant for Ca^{2+}*.

Finally, we investigated whether **1** can selectively extract Ca^{2+} from a mixture of four alkaline earth metal ions and whether Ca^{2+} selectivity of **1** is superior to that of naturally occurring Ca^{2+} ionophore **4**. The results are summarized in Table 2. At pH 5.3, **1** can selectively extract Ca^{2+} even from a mixture of four metal ions (0.10 mM each). At pH 6.0–7.0, the Ex% for Ca^{2+} is enhanced, but a small amount of Sr^{2+} is extracted as well. At this pH region, **4** does not show significant extractability. At basic pH region (pH 8.0–10.0), Ca^{2+} is predominantly extracted by **1** although the selectivity is no not as high. In contrast, **3** shows significant affinity for the first time at pH 10.0, but the extraction occurs rather nonselectively. The results imply that the pK_a of the carboxylic acid groups in **1** is lowered owing to the effective binding of Ca^{2+} to the ionophoric cavity.

In conclusion, the present study demonstrated that **1** has an ionophoric cavity which shows excellent Ca^{2+} selectivity at appropriate pH region. In the molecular design of this molecule, hole-size selectivity, rigidity of the ionophoric cavity, charge neutralization of the complex, cooperativity of the metal ligation groups, etc. are all taken into account. Particularly, nearly "perfect" Ca^{2+} selectivity is realized owing to the rigid, size-fitting molecular skeleton of a calix[4]arene. We believe that compound **1** can be successfully applied to many chemical and biological systems where severe discrimination of Ca^{2+} is required or Ca^{2+} acts as a motive force for biological events.

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(15) It is known that in calix[4]aryl esters and amides the metal binding induces the upfield shift of the OCH_2CO methylene protons in spite of the electron-withdrawing nature of the bound metal: Yamada, A.; Murase, T.; Kikukawa, K.; Arimura, T.; Shinkai, S. *J. Chem. Soc., Perkin Trans. 2* **1991**, 793. This is attributed to a metal-induced conformational change which forces the OCH_2CO methylene protons to be located on the benzene ring.