Carboxylate-derived calixarenes with high selectivity for actinium-225

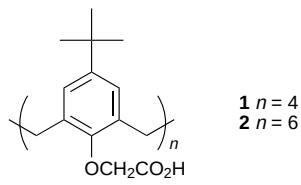
Xiaoyuan Chen,[a] Min Ji,[a] Darrell R. Fisher[b] and Chien M. Wai[a]

[a] *Department of Chemistry, University of Idaho, Moscow, Idaho 83844, USA*
[b] *Molecular Biosciences Department, PNNL, Richland, Washington 99352, USA*

The binding properties of two ligands, 5,11,17,23-tetra-*tert*-butyl-25,26,27,28-tetrakis(carboxymethoxy)calix[4]arene 1 and 5,11,17,23,29,35-hexa-*tert*-butyl-37,38,39,40,41,42-hexakiscarboxymethoxy)calix[6]arene 2, which show high selectivity for $^{225}Ac^{3+}$ (an α-emitter with $t_{1/2}$ = 10 days) over Na^+, K^+, Mg^{2+}, Ca^{2+} and Zn^{2+} are described.

In recent years there has been an increased interest in the development of monoclonal antibodies that may be linked with a radioisotope as targeting agents in radioimmunodiagnosis and radioimmunotherapy of cancer tumors.[1] The success of such approaches depends on the development of bifunctional complexing agents that can bind a specific radioisotope tightly and selectively and can be linked to antibodies. Chelators that can hold the desired radioisotope with high stability under physiological conditions are essential to deliver the radioisotope to the antigen binding site on tumor cells.[2] Linkage of β-emitters such as ^{90}Y and ^{67}Cu to the moab part of monoclonal antibodies *via* bifunctional aza- and peraza-crown ether macrocycles has shown good results for the treatment of a number of cancer patients.[3] For radioimmunotherapy, α-emitters are much better cytotoxic agents than β-emitters since they dissipate a large amount of energy along straight particle tracks of 40–70 μm (*ca*. 8 cell diameters).[4] Among the α-emitters having an appropriate physical half-life for such an application is actinium-225 ($t_{1/2}$ = 10.0 d).[5] ^{225}Ac decays through a chain of daughter products to stable ^{209}Bi with the emission of a total of four α and two β particles, releasing about 28 MeV of radiation energy to the absorbing medium.[6]

Solvent extraction experiments performed recently in our laboratory indicate that 5,11,17,23-tetra-*tert*-butyl-25,26,27,28-tetrakis(carboxymethoxy)calix[4]arene **1** and 5,11,17,23,29,35-hexa-*tert*-butyl-37,38,39,40,41,42-hexakis-(carboxymethoxy)calix[6]arene **2** (Fig. 1) exhibit high Ac^{3+} selectivity over alkali, alkaline earth, and zinc metal ions under neutral and weak acidic conditions. These calixarene derivatives were synthesized in our laboratory according to the known procedures in the literature.[7,8] The two-phase solvent extraction experiments were carried out between water (1.5 ml, [^{225}Ac] = 10^{-3} mM) and chloroform (1.5 ml, [ionophore] = 2 mM). The pH of the aqueous phase was adjusted with HCl for pH 1–3, succinic acid–NH_4OH for pH 4–6, and Tris–HCl for pH 7–8. The mixture was shaken at 25 °C for 30 min, which was long enough to reach equilibrium based on our time variation studies. The distribution ratio *D* ([Ac^{3+}] in the organic phase/[Ac^{3+}] in the aqueous phase) was determined by measuring the ^{225}Ac activity in each phase using a Ge(Li) detector. Percentage extraction of Ac^{3+} (Ex%) was calculated by $D/(1 + D)$. Fig. 2 shows Ex% of Ac^{3+} with ligands **1** and **2** plotted *vs*. pH of the aqueous phase. For **1**, Ex% becomes appreciable at pH 2.0 and reaches nearly 100% around pH 4.0. Ex% decreases rapidly at pH > 7.3 reaching only 40% at pH 8.0. Ex% for **2** shows a similar pH dependence. The Ex% increases from pH 1.5, reaching saturation at pH around 3.0 and decreasing sharply above pH 7.5.



1 *n* = 4
2 *n* = 6

Fig. 1 Structures of the carboxylate-derived calixarenes which show high selectivity for Ac^{3+}

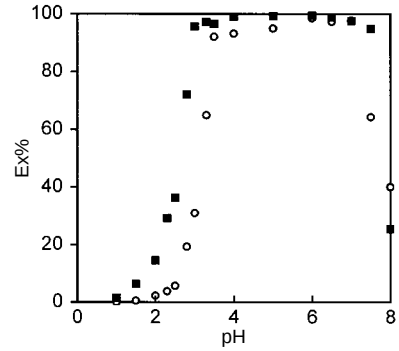


Fig. 2 pH dependence of extraction of actinium-225 with ligands **1** and **2**. The pH of the aqueous phase was adjusted with HCl for pH 1–3, succinic acid–NH_4OH for pH 4–6 and Tris–HCl for pH 7–8; (○) **1** (*n* = 4), (■) **2** (*n* = 6).

Fig. 3 shows plots of log *D vs*. −log[L] for the extraction of Ac^{3+} by ligands **1** and **2** at pH = 6.0, where [L] is the concentration of the ionophore in the organic phase. A linear relationship between log *D* and −log[L] is observed with the slope of both lines roughly equal to −1 suggesting that both **1** and **2** form a 1 : 1 complex with Ac^{3+}.

Owing to its short half-life and high radioactivity, it was not possible for us to obtain the stability constants of the ^{225}Ac complexes using common spectroscopic or potentiometric titration methods. We used a competition extraction method to obtain the relative extraction constants of Ac^{3+} by ligands **1** and **2** with respect to EDTA. To obtain the relative stability constants of the Ac complexes, the following competition experiments were performed. The Ac^{3+} in water at pH 7 was

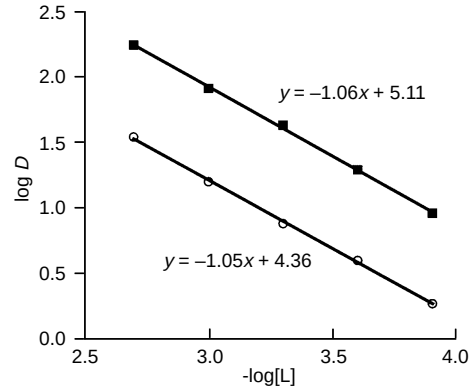


Fig. 3 Plot of log *D vs.* −log[L] for the extraction of Ac^{3+} by ligands **1** and **2**. The slopes were 1.05 and 1.06, respectively; (○) **1**, (■) **2**.

first extracted into the chloroform phase containing **1** or **2**. The organic phase was then back-extracted with an aqueous phase containing EDTA at pH = 7.0. No data for Ac^{3+} hydrolysis is available. We estimated the hydrolysis of Ac^{3+} based on the known values of Am^{3+} ($\log \beta_1 = -6.4 \pm 0.7$, $\log \beta_2 = -14.1 \pm 0.6$, $\log \beta_3 = -25.7 \pm 0.5$ at $I = 0$ and 25 °C), which fall in the range of the hydrolysis constants reported for the trivalent lanthanides.[9] According to these values, the distribution of Ac^{3+}, $Ac(OH)^{2+}$ and $Ac(OH)_2^{+}$ should be 71, 28 and 1%, respectively, at pH 6 and 17, 68 and 15%, respectively, at pH 7. Thus, ^{225}Ac could also be extracted as the Ac(OH)–calixarene complexes in near neutral solutions.

The following equilibrium relations were obtained based on the assumptions that neither $EDTA^{3-}$ nor $Ac(OH)(EDTA)^{-}$ was soluble in chloroform and the solubility of the H_2L^{2-} and $Ac(OH)(H_2L)$ in the aqueous phase was negligible. The new distribution ratio D' was taken as $[Ac(OH)(H_2L)]_{org}/[Ac(OH)(EDTA)^{-}]_{aq}$, where $Ac(OH)(H_2L)$ represented the Ac–calixarene complex.

$$EDTA^{3-} + Ac(OH)^{2+} = Ac(OH)(EDTA)^{-}$$

$$K_1 = [Ac(OH)(EDTA)^{-}]/[EDTA^{3-}][Ac(OH)^{2+}]$$

$$H_2L^{2-} + Ac(OH)^{2+} = Ac(OH)(H_2L)$$

$$K_2 = [Ac(OH)(H_2L)]/[H_2L^{2-}][Ac(OH)^{2+}]$$

$$K_2/K_1 = [Ac(OH)(H_2L)][EDTA^{3-}]/[Ac(OH)(EDTA)^{-}][H_2L^{2-}]$$

$$\log(K_2/K_1) = \log D' + \log[EDTA^{3-}]/[H_2L^{2-}]$$

A linear relationship is observed between log D' and log[EDTA]/[L] for both ligands (Fig. 4) with slope close to unity suggesting that the assumptions are reasonable. From the intercept, we obtain the extraction constants of the Ac–calixarene complexes relative to that of EDTA. Ligand **1** has $K_2 = 1.11\,K_1$ and **2** has $K_2 = 5.75\,K_1$, where K_1 is the extraction constant of Ac with H_4EDTA at pH 7.

We also investigated whether the actinium complexes could tolerate high concentrations of alkali, alkaline earth and zinc metal ions. We took aliquots of the organic phase containing the ^{225}Ac complexes and back-extracted with an aqueous solution

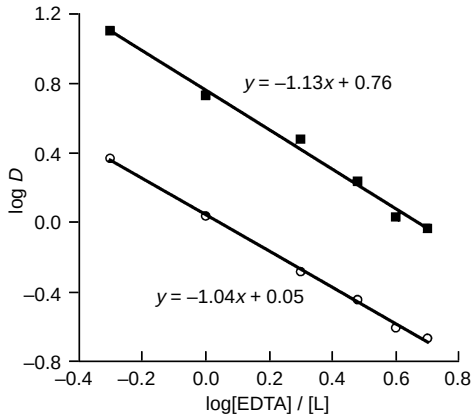


Fig. 4 Plot of log D *vs.* log[EDTA]/[L] at pH = 7.0 shows a straight line for both ligands; (○) **1**, (■) **2**

containing a mixture of 10 mM each of Ca^{2+}, Mg^{2+}, Na^{+}, K^{+} and Zn^{2+} at pH 7.0. After shaking for 5 h, ligand **1** showed no measurable loss of Ac^{3+} from the organic phase to the aqueous phase. For ligand **2**, about 5% of the Ac originally present in the organic phase was extracted to the aqueous phase. The high selectivity of the calixarene carboxylate ligands for Ac^{3+} may be related to the high charge density of the Ac^{3+} ion favoring electrostatic interactions with the anionic ligands.[10] Ligand **2** shows a slightly lower selectivity for Ac^{3+} than **1** which may be due to the structural difference between the two ligands. Because **2** has a larger and more flexible cavity, it probably can accommodate the competing cations better than **1**. Also **2** is more acidic than **1**, thus it can coordinate with the alkaline earth metal ions at lower pH values.

In conclusion, this study shows that both **1** and **2** have an ionophore cavity capable for selective complexation with Ac^{3+} in weak acid and neutral solutions. They appear to be suitable candidates as ^{225}Ac carriers. The next step in our research is to selectively functionalize the upper rim of the calixarene ring with amine, bromine, aldehyde or acid chloride forming bifunctional ligands that may be attached to the desired monoclonal antibodies.

This work was supported by the Pacific Northwest National Laboratory (PNNL).

Notes and References

* E-mail: cwai@uidaho.edu

1 S. J. DeNardo, J. S. Peng, G. L. DeNardo, S. L. Mills and A. L. Epstein, *Nucl. Med. Biol.*, 1986, **13**, 303; C. F. Meares and T. G. Wensel, *Acc. Chem. Res.*, 1984, **17**, 202; L. Yuanfang and W. Chuanchu, *Pure Appl. Chem.*, 1991, **63**, 427; D. Parker, *Chem. Soc. Rev.*, 1990, **19**, 271.

2 J. L. Klein, P. K. Leichner, K. M. Callahan, K. A. Kopher and S. E. Order, *Antibody, Immunoconjugates, Radiopharm.*, 1988, **1**, 55; S. V. Desphande, S. J. DeNardo, C. F. Meares, M. J. McCall, G. P. Adams, M. K. Moi and G. L. DeNardo, *J. Nucl. Med.*, 1988, **29**, 217; J. P. L. Cox, J. R. Morphy, K. J. Jankowski and D. Parker, *Pure Appl. Chem.*, 1989, **61**, 1637.

3 M. K. Moi and C. F. Meares, *J. Am. Chem. Soc.*, 1988, **110**, 6266; J. P. L. Cox, K. J. Jankowski, R. Kataky, D. Parker, M. A. W. Eaton, N. R. A. Beeley, A. T. M. Millican, A. Harrison and C. Walker, *J. Chem. Soc., Chem. Commun.*, 1989, 797; M. K. Moi, C. F. Meares, M. J. McCall, W. C. Cole and S. J. DeNardo, *Anal. Biochem.*, 1984, **148**, 249; J. R. Morphy, D. Parker, R. Alexander, A. Bains, M. A. W. Eaton, A. Harrison, A. Millican, R. Timas and D. Weatherby, *J. Chem. Soc., Chem. Commun.*, 1988, 156; C. J. Anderson, J. M. Connett, S. W. Schwarz, P. A. Rocque, L. W. Guo, G. W. Phillott, K. R. Zinn, C. F. Meares and M. J. Welch, *J. Nucl. Med.*, 1992, **33**, 2006.

4 D. S. Wilbur, *Antibody, Immunoconjugates, Radiopharm.*, 1991, **4**, 85.

5 M. W. Geerlings and R. Van der Hout, *Nucl. Med. Commun.*, 1993, **14**, 121.

6 M. W. Geelings, *Int. J. Biol. Markers*, 1993, **8**, 180; M. W. Geelings, F. M. Kaspersen, C. Apostolidis and R. Van der Hout, *Nucl. Med. Commun.*, 1993, **14**, 121.

7 S. Shinkai, H. Horeishi, K. Ueda and O. Manabe, *J. Chem. Soc., Chem. Commun.*, 1986, 733.

8 S. Shinkai, H. Horeishi, K. Ueda, T. Arimura and O. Manabe, *J. Am. Chem. Soc.*, 1987, **109**, 6371.

9 R. J. Silva, G. Bidoglio, M. H. Rand, P. B. Robouch, H. Wanner and I. Puigdomenech, in *Chemical Thermodynamics, Vol. 2: Chemical Thermodynamics of Americium*, Elsevier, 1995; C. F. Baes and R. E. Mesmer, in *The Hydrolysis of Cations*, Wiley, New York, 1976.

10 F. Arnaud-Neu, S. Cremin, S. Harris, M. A. McKervey, M.-J. Schwing-Weill, P. Schwinté and A. Walker, *J. Chem. Soc., Dalton Trans.*, 1997, 329.

Received in Cambridge, UK, 18th September 1997; 7/06776C