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**Synthesis of phosphorylated calix[4]arene derivatives
for the design of solid phases immobilizing uranyl cations.**

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Keywords:	: Phosphorous-Calix[4]arenes, Uranyl immobilization, Solid supports, Anchoring arms

With the aim of developing supports for uranyl cation immobilization, new 1,3-alternate bearing both phosphonic acid functions as chelating site and N-succinimide-4-oxabutyrates as anchoring arm were synthesized. These compounds were proved to be coupled on a hydrazide gel and to complex uranium successfully.

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Synthesis of new phosphorylated calix[4]arene derivatives for the conception of novel supports immobilizing uranyl.

Synthesis of phosphorylated calix[4]arene derivatives
for the design of solid phases immobilizing uranyl cations.

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Abstract

With the aim of developing supports for uranyl cations immobilization, new 1,3-alternate calix[4]arenes bearing both phosphonic acid functions as chelating sites and N-succinimide-4-oxabutyrate as the anchoring arm were synthesized in good yields. The coupling of such calixarenes to a gel was performed and a successful immobilization of uranyl cations was obtained.

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Keywords: Phosphorous-Calix[4]arenes; Uranyl cations immobilization; Solid supports; Anchoring arms

Introduction

Uranium, especially under its uranyl ion form (UO_2^{2+}), is widespread in the environment, naturally occurring in various minerals but also resulting from both nuclear civil and military uses. Regarding to the complexity of biological matrices, studying its speciation *in vivo* still requires innovative tools. Thus, the design and synthesis of macrocyclic ligands which could preorganize uranyl chelating functions is of primary importance in the development of such tools.

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Among them, calixarenes represent an interesting class of macrocycles. On the one hand, they might adopt pseudoplanar configuration. However, in contrast to homooxacalix[4]arenes and tetrathiacalix[4]arenes [1-3], the crystal structure of the complex between the simple calix[4]arene and UO_2^{2+} evidenced that the 1,3-oxygen atom distance is far from the ideal distance required for an internal complex [3]. On the other hand, calix[4]arenes can adopt an 1,3-alternate conformation where the uranyl cation binding sites can be remote from the anchoring arm designed for a covalent bond to a polymer support.

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Calix[4]arenes functionalized by various complexing groups were then used as extractants of uranyl ion, i.e. carboxylic acid [4-8], carboxylic acid-amide [9,10], ester [11], hydroxamate [12] or semicarbazone functions [13]. Phosphoryl groups have also been proved to be suitable for

uranyl [cation](#) complexation. The solvent extraction of uranium(VI) was widely studied by neutral or acid organo-phosphorus extractants [14-21]. A recent work of Taran *et al.* reported that bis-phosphonates were powerful uranyl ligands [22]. Several papers described the synthesis of phosphonatocalix[4]arenes [23-31]. Some of them were also functionalized with an anchoring arm to be immobilized on solid supports [7,8,13]. However, none of these phosphorus-containing calixarenes were used to extract the uranyl ion.

Consequently, the purpose of the present work was also to provide bifunctional ligands which could simultaneously bind uranium and be immobilized on solid supports.

In this work, the synthesis of two phosphorus-containing calix[4]arenes in the 1,3 alternate conformation bearing both phosphonic acid and N-hydroxysuccinimide ester entities is reported. Their incorporation into a macromolecular matrix is also demonstrated.

Results and discussion

1. Conception of uranyl [cation](#) immobilizing agents: Synthesis of several (phosphonic acid)calix[4]arenes

Phosphorus containing calixarenes [modified to](#) provide an N-hydroxysuccinimide moiety able to react with NH_2 groups of a solid support [were synthesized](#). As it is established that the 1,3-alternate conformation of calixarenes seems to be the most favorable for metal complexation [32-34], the synthesis was [designed to produce](#) the new calixarenes [with this preferred](#) conformation.

Calixarenes **6** and **9** were obtained via a five-step synthesis. The synthetic pathway is illustrated in Fig.4.

[insert figure 1 about here]

Calix[4]arene **1** was first O-alkylated in the presence of K_2CO_3 with 1 equivalent of methoxyethoxy-p-toluenesulfonate to obtain the monoalkoxycalix[4]arene **2** in a 68 % yield. The cone conformation, stabilized by hydrogen bonds, [was revealed by the presence of two AB](#) systems [at 4.49 and 3.48 ppm \(\$J = 13.0\$ Hz\) and at 4.31 and 3.46 ppm \(\$J = 13.0\$ Hz\), in the \$^1\text{H}\$ -](#)

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[NMR spectrum](#), attributed to the methylenic protons ArCH_2Ar . The calixarene derivative **2** was then functionalized by reacting 1 equivalent of ethyl-4-bromobutyrate to obtain compound **3** in a 56 % yield. The cone conformation was proved to be maintained by the ^1H NMR spectrum of compound **3** which revealed two AB systems at 4.42 and 3.38 ppm ($J = 13.0$ Hz) and at 4.28 and 3.38 ppm ($J = 13.0$ Hz), attributed to the methylenic protons ArCH_2Ar .

The introduction of the phosphorus-containing functions was performed by O-alkylation with 2.1 equivalents of diethylphosphonoyl-methoxy-*p*-toluenesulfonate (**a**) or diethylphosphonoyl-propoxy-*p*-toluenesulfonate (**b**) in the presence of K_2CO_3 to yield calixarene **4** and a mixture of compounds **7** and **7'** respectively. The 1,3-alternate conformation of compound **4** was confirmed by the presence in the ^1H NMR spectrum of a singlet for the methylenic protons ArCH_2Ar at 3.49 ppm and by the ^{31}P NMR spectrum which showed a singlet at 21.8 ppm. Compounds **7** and **7'** were further separated by gel chromatography and were shown to be the expected diethylphosphonate calix[4]arene in two different conformations. Compound **7** was proved to be in the 1,3-alternate conformation, confirmed by the absence of any AB system in the ^1H NMR spectrum for the methylenic protons ArCH_2Ar and the presence of a singlet at 33.5 ppm in the ^{31}P NMR spectrum indicating the presence of two equivalent phosphorous atoms. Compound **7'** was shown to be in the partial cone conformation. The ^1H NMR spectrum revealed an AB system at 4.05 ppm and at 3.09 ppm ($J = 13.0$ Hz), corresponding to 4 methylenic protons ArCH_2Ar . The singlet corresponding to the other 4 methylenic protons ArCH_2Ar was located in the multiplet at 3.92-3.57 ppm. The ^{31}P NMR spectrum confirmed this conformation, revealing two singlets at 34.1 ppm and 33.1 ppm for the two different phosphorous atoms.

Calixarenes **5** and **8** were obtained by transesterification using trimethylbromosilane and subsequent hydrolysis of the trimethylsilylestere in a nearly quantitative yield. Finally, the activation of the carboxylic groups was performed using 2 equivalents of NHS and 2 equivalents of EDC to obtain calixarenes **6** and **9** with a 64 % and a 45% yield respectively. For all these compounds, the absence of any AB system in the ^1H NMR spectra confirmed that the 1,3-alternate conformation was maintained.

2. Immobilization of calixarenes **6** and **9** on a gel column

Immobilization was performed by incubating the Ultralink hydrazide gel (15 μmol NH_2 functions per g) with calixarenes quantities corresponding to 20% of the total amount of NH_2 groups.

Since the calixarenes synthesized in this paper are based on a succinimide ester that is commonly used for reactive amine crosslinking, the ester hydrolysis is a limiting factor. Two buffers were tested for the coupling: a HEPES buffer (pH 8.0) and a borate buffer (pH 9.2). A higher pH was shown to favor the nucleophilic attack to the detriment of the ester hydrolysis, and pH 9.2 was thus used in further experiments.

The repeatability of the coupling was then evaluated on 2 different gels using compound **6**. An average value of $(32 \pm 2)\%$ (% mol) of coupled calixarene **6** was found.

The same experiment conducted with calixarene **9** led to 28.5% (% mol), which seems to indicate that the alkyl chain length has no influence on the coupling.

Improvement of the coupling extent was investigated by performing successive calixarene **6** additions and incubations. Results are reported in Table 1 and show that a maximum of 2 μmol calixarene **6** / g gel is obtained.

3. Immobilization of uranyl ions on a gel column

Assessment of the complexation efficiency on a modified gel was performed. 1 g of the Ultralink hydrazide gel coupled with 2 μmol calixarene **6** / g was incubated overnight in the presence of 5

equivalents of uranyl acetate. A similar experiment was also conducted with the same quantity of a non-coupled gel. The uranyl cation content in the eluates was quantified and found to be 7.5 μmol for the eluates of the gel bound with calixarene **6** and 9.9 μmol for the blank.

Consequently, (2.4 ± 0.1) μmol uranyl cation / g were immobilized on the modified gel, which corresponds to a 100% immobilization (referring to the calixarene). For the non-coupled gel, the immobilized concentration was found to be as low as (0.1 ± 0.1) μmol uranyl cation / g. It seems

then that the immobilization of the uranyl cation occurs through the formation of a 1:1 (uranyl:calixarene) complex. The total release of the uranyl cation from the blank gel shows the absence of non specific interactions and reinforces this assumption.

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Conclusion

The synthesis of new phosphorous-containing calix[4]arenes bearing phosphonic acid functions on one side and NHS activated carboxylic functions on the other side, was achieved. With the aim of providing solid supports, the coupling of these compounds to a solid support was performed. A coupling extent of 2 μmol calixarene **6** / g gel was obtained. Under the used conditions, a quantitative immobilization of uranyl cations was shown to occur through the formation of an 1:1 UO_2^{2+} :calixarene **6** complex.

Further studies will be performed to improve the coupling extent of calixarene **6** on such support. However, the use of these heterofunctional macrocycles for many other usages into supramolecular devices could be further envisioned.

Experimental

1. Synthesis

All reagents and solvents were commercial and were used without further purification. Reagents for the synthesis were all Sigma-Aldrich and Prolabo products. Calix[4]arene was prepared according to the literature [35]. Chromatography used SiO_2 columns with Kieselgel Merck (art. 11567). The melting points were taken on a Büchi 535 apparatus in capillaries sealed under nitrogen. ^1H NMR and ^{31}P NMR spectra were respectively recorded at 300 MHz and 400 MHz on a Bruker Avance spectrometer. For ^1H NMR spectra, CHCl_3 ($\delta = 7.26$ ppm) was used as an internal standard in CDCl_3 and CHD_2OD ($\delta = 3.31$ ppm) was used as an internal standard in CD_3OD . For ^{31}P NMR spectra, 85 % H_3PO_4 was used as an external reference. MALDI-TOF mass spectra were obtained with a Bruker Autoflex II equipped with a N_2 laser ($\lambda = 337\text{nm}$) using α -cyano-4-hydroxycinnamic acid as matrix. Elemental analyses were performed at the Service de Microanalyse of the Institut de Chimie de Strasbourg.

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1.1 Synthesis of 1,3-[di-(oxamethyl-phosphonic acid)], 2-(N-succinimide-4-oxabutyrate), 4-(methoxyethoxy)-calix[4]-arene (6).

1.11. Mono-methoxyethoxy-calix[4]arene (2). A suspension of calix[4]arene **1** (12.73 g, 30.0 mmol) and K_2CO_3 (2.16 g, 15.6 mmol) in acetonitrile (500 mL) was stirred for 30 min at room temperature under a nitrogen atmosphere. Methoxyethyl *p*-toluenesulfonate (6.91 g, 12.0 mmol) was then added and the mixture was stirred and refluxed for 4 days. After removal of the solvent, 400 mL of CH_2Cl_2 and 400 mL of water were added and the mixture was stirred and acidified with HCl 1M. The organic layer was recovered and dried over anhydrous Na_2SO_4 . After removal of the solvent, compound **2** was purified by column chromatography (SiO_2 , eluent: CH_2Cl_2) and obtained as a white powder.

Yield: 9.91 g (68%) mp 224-225 °C. 1H -NMR (300MHz, $CDCl_3$) δ (ppm): 9.86 (s, 1H, ArOH), 9.24 (s, 2H, ArOH), 7.12-7.00 (m, 8H, ArH_{meta}), 6.89 (t, 1H, $J = 7.5$ Hz, ArH_{para}), 6.72-6.65 (m, 3H, ArH_{para}), 4.49 (d, 2H, $J = 13.0$ Hz, $ArCH_2Ar$), 4.35-4.32 (m, 2H, $CH_2CH_2OCH_3$), 4.31 (d, 2H, $J = 13.0$ Hz, $ArCH_2Ar$), 4.04-4.01 (m, 2H, $CH_2CH_2OCH_3$), 3.62 (s, 3H, OCH_3), 3.48 (d, 2H, $J = 13.0$ Hz, $ArCH_2Ar$), 3.46 (d, 2H, $J = 13.0$ Hz, $ArCH_2Ar$). Anal. Calcd. for $C_{31}H_{30}O_5$ (%): C, 77.16; H, 6.27. Found C, 77.43; H, 6.00

1.12. 1-(Ethyl-4-oxabutyrate), 3-(methoxyethoxy)-calix[4]arene (3). Mono-methoxyethoxy-calix[4]arene **2** (4.83 g, 10.0 mmol) and K_2CO_3 (0.72 g, 5.2 mmol) were suspended in acetonitrile (200 mL) and stirred for 1 h at room temperature under a nitrogen atmosphere. $Br(CH_2)_3C(O)OCH_2CH_3$ (2.34 g, 12.0 mmol) was then added and the resulting solution was refluxed for 4 days. After evaporation of the solvent in vacuo, the residue was taken up in CH_2Cl_2 (300 mL) and in water (300 mL) and the resulting mixture was acidified then separated in order to recover the organic layer which was dried over anhydrous Na_2SO_4 . After evaporation of the solvent, compound **3** was purified by column chromatography (SiO_2 , eluent: CH_2Cl_2 /acetone 98/2 v/v) and obtained as a white powder.

Yield: 3.36 g (56%), mp 165-166 °C. 1H -NMR (300MHz, $CDCl_3$) δ (ppm): 7.93 (s, 2H, ArOH), 7.06 (d, 4H, $J = 7.5$ Hz, ArH_{meta}), 6.90 (d, 4H, $J = 7.5$ Hz, ArH_{meta}), 6.76-6.71 (m, 2H, ArH_{para}),

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6.65 (t, 2H, $J = 7.5$ Hz, ArH_{para}), 4.42 (d, 2H, $J = 13.0$ Hz, $ArCH_2Ar$), 4.28 (d, 2H, $J = 13.0$ Hz, $ArCH_2Ar$), 4.23-4.16 (m, 4H, $COOCH_2CH_3 + CH_2CH_2OCH_3$), 4.07 (t, 2H, $J = 6.1$ Hz, $CH_2CH_2CH_2COOEt$), 3.95-3.92 (m, 2H, $CH_2CH_2OCH_3$), 3.56 (s, 3H, CH_2OCH_3), 3.38 (d, 4H, $J = 13.0$ Hz, $ArCH_2Ar$), 2.90 (t, 2H, $J = 7.4$ Hz, CH_2COOEt), 2.41-2.32 (m, 2H, $CH_2CH_2CH_2COOEt$), 1.29 (t, 3H, $J = 7.2$ Hz, $COOCH_2CH_3$). Anal. Calcd. for $C_{37}H_{40}O_7$ (%): C, 74.47; H, 6.76. Found C, 74.68; H, 6.52

1.13. Diethylphosphonoylmethoxy-*p*-toluenesulfonate (a). A solution of triethylamine (10.12 g, 100.0 mmol) in CH_2Cl_2 (50 mL) was added dropwise to a stirred mixture of diethyl(hydroxymethyl)phosphonate (8.41 g, 50.0 mmol) and *p*-toluenesulfonyl chloride (10.01 g, 52.5 mmol) in CH_2Cl_2 (450 mL) at ca. 0 °C. The resulting mixture was cooled to room temperature and stirred for 15 h. It was then extracted with 300 mL of acidified aqueous solution and dried over anhydrous Na_2SO_4 . After removal of the solvent, the residue was purified by column chromatography on silica gel. CH_2Cl_2 was used as the first mobile phase then CH_2Cl_2 /acetone (98/2, v/v). Compound **a** was recovered as a viscous liquid.

Yield: 12.93 g (80%). 1H -NMR (300 MHz, $CDCl_3$) δ (ppm): 7.79 (d, 2H, $J = 8.1$ Hz, ArH), 7.36 (d, 2H, $J = 8.1$ Hz, ArH), 4.19-4.09 (m, 6H, $SO_3CH_2P + POOCH_2CH_3$), 2.45 (s, 3H, CH_3Ar), 1.31 (t, 6H, $J = 7.1$ Hz, $POOCH_2CH_3$).

1.14. 1,3-[Di-(oxamethyl-diethylphosphonate)], 2-(ethyl-4-oxabutyrate), 4-(methoxyethoxy)-calix[4]arene (4). A suspension of 1-(ethyl-4-oxabutyrate), 3-(methoxyethoxy)-calix[4]arene **3** (1.79 g, 3.0 mmol) and K_2CO_3 (4.15 g, 30.0 mmol) in acetonitrile (100 mL) was stirred for 2 h at room temperature under nitrogen atmosphere. Diethylphosphonoylmethoxy-*p*-toluenesulfonate **a** (2.03 g, 6.3 mmol) was then added and the mixture was refluxed for 10 days. After filtration of the mixture, the solvent was removed under vacuo and the residue was taken up in CH_2Cl_2 (200 mL). 200 mL of water were added and the mixture was acidified with 1 M HCl. The organic layer was separated, dried over anhydrous Na_2SO_4 , filtered and concentrated. The resulting residue was purified by column

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chromatography (SiO₂, eluent: CH₂Cl₂/acetone 90/10 v/v). Compound **4** was obtained as a pure yellow viscous liquid.

Yield: 1.69 g (63%). ¹H-NMR (300MHz, CDCl₃) δ (ppm): 7.25-7.21 (m, 4H, ArH_{meta}), 7.11-7.08 (m, 2H, ArH_{meta}), 6.99-6.96 (m, 2H, ArH_{meta}), 6.68-6.57 (m, 4H, ArH_{para}), 4.29-4.17 (m, 6H, ArOCH₂ + COOCH₂CH₃), 4.07 (d, 4H, J = 9.2 Hz, ArOCH₂P), 3.91-3.88 (m, 2H, CH₂OCH₃), 3.77-3.64 (m, 8H, POOCH₂), 3.59 (s, 3H, CH₂OCH₃), 3.49 (s, 8H, ArCH₂Ar), 2.53 (t, 2H, J = 7.2 Hz, CH₂COOEt), 2.20 (q, 2H, J = 7.2 Hz, CH₂CH₂CH₂COOEt), 1.42 (t, 12H, J = 7.1 Hz, POOCH₂CH₃), 1.24 (t, 3H, J = 7.1 Hz, COOCH₂CH₃). ³¹P NMR (400 MHz, CDCl₃) δ (ppm): 21.8. Anal. Calcd. for C₄₇H₆₂O₁₃P₂ (%): C, 62.94; H, 6.97. Found C, 62.76; H, 7.14.

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1.15. 1,3-[Di-(oxamethyl-phosphonic acid)], 2-(4-oxabutyric acid), 4-(methoxyethoxy)-calix[4]arene (5). Bromotrimethylsilane (5.64 g, 36.83 mmol) was added to a solution of compound **4** (1.10 g, 1.23 mmol) in 20 mL acetonitrile. The reaction mixture was stirred for 24 h at room temperature under nitrogen atmosphere. It was then evaporated under reduced pressure and a mixture of 10 mL of methanol/water (50/50, v/v) was added to the residue. The resulting solution was stirred at room temperature overnight. After removal of methanol and water, the residue was evaporated twice with 10 mL of dry toluene and filtered to obtain compound **5** as a pink solid.

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Yield: 0.91 g (98 %), mp 158-159 °C. ¹H-NMR (300MHz, CD₃OD) δ (ppm): 7.17 (d, 4H, J = 7.5 Hz, ArH_{meta}), 7.05-7.02 (m, 2H, ArH_{meta}), 6.95-6.92 (m, 2H, ArH_{meta}), 6.62-6.53 (m, 4H, ArH_{para}), 3.76-3.50 (m, 18H, ArOCH₂CH₂ + ArCH₂Ar + ArOCH₂P + CH₂OCH₃), 3.22 (s, 3H, CH₂OCH₃), 2.47-2.39 (m, 2H, CH₂COOH), 2.03-1.92 (m, 2H, CH₂CH₂COOH). ³¹P NMR (400 MHz, CD₃OD) δ (ppm): 19.9. Anal. Calcd. for C₃₇H₄₂O₁₃P₂ (%): C, 58.73; H, 5.59. Found C, 58.47; H, 5.44. Mass spectrum (MALDI-TOF): m/z = 755.2 [M-H]⁺.

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1.16. 1,3-[Di-(oxamethyl-phosphonic acid)], 2-(N-succinimide-4-oxabutyrate), 4-(methoxyethoxy)-calix[4]arene (6). Compound **5** (1.14 g, 1.5 mmol), EDC [1-Ethyl-3-(3-dimethyl-aminopropyl)-carbodiimide] (0.58 g, 3.0 mmol) and NHS (N-hydroxysuccinimide) (0.35 g, 3.0 mmol) were dissolved in DMF (90 mL) and the mixture was stirred overnight at room

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temperature and then for 3 h at 55 °C. After removal of the solvent, the residue was treated with acetonitrile to obtain compound **6** as white solid.

Yield: 0.82 g (64 %), mp 109-111 °C. ¹H-NMR (300MHz, DMSO-d₆) δ (ppm): 7.24 (d, 4H, *J* = 7.4 Hz, *ArH*_{meta}), 7.02-6.99 (m, 2H, *ArH*_{meta}), 6.93-6.90 (m, 2H, *ArH*_{meta}), 6.57-6.52 (m, 4H, *ArH*_{para}), 3.75-3.55 (m, 12H, ArOCH₂CH₂ + ArCH₂Ar), 3.37 (s, 3H, CH₂OCH₃), 3.03-2.89 (m, 6H, ArOCH₂P + CH₂OCH₃), 2.71 (s, 4H, NHS), 1.99-1.95 (m, 2H, CH₂COONHS), 1.76-1.71 (m, 2H, CH₂CH₂COONHS). ³¹P NMR (400 MHz, DMSO-d₆) δ (ppm): 16.4. Anal. Calcd. for C₄₁H₄₅NO₁₅P₂ (%): C, 57.68; H, 5.31; N, 1.64. Found C, 57.39; H, 5.45; N, 1.81. Mass spectrum (MALDI-TOF): *m/z* = 855.1 [MH⁺].

1.2. Synthesis of 1,3-[di-(oxapropyl-phosphonic acid)], 2-(N-succinimide-4-oxabutyrate), 4-(methoxyethoxy)-calix[4]arene (9).

1.21. Diethylphosphonoylpropoxy-*p*-toluenesulfonate (b). Diethyl 3-bromopropylphosphonate (5.18 g, 20.0 mmol) and silver *p*-toluenesulfonate (11.16 g, 40.0 mmol) were dissolved in 200 mL acetonitrile and stirred at room temperature for 3 days. The solution was then filtered and the solvent was evaporated to dryness under reduced pressure. The residue was then taken up in CH₂Cl₂ (400 mL) and water (400 mL). The organic layer was recovered and dried over anhydrous Na₂SO₄. After removal of the solvent, the residue was purified by column (SiO₂, eluent: CH₂Cl₂/acetone 80/20 v/v). Compound **b** was recovered as a viscous liquid.

Yield: 1.75 g (25 %). ¹H-NMR (300MHz, CDCl₃) δ (ppm): 7.78 (d, 2H, *J* = 8.2 Hz, *ArH*), 7.34 (d, 2H, *J* = 8.2 Hz, *ArH*), 4.12-3.99 (m, 6H, CH₃ArSO₃CH₂ + POOCH₂CH₃), 2.44 (s, 3H, CH₃Ar), 2.01-1.87 (m, 2H, SO₃CH₂CH₂CH₂P), 1.81-1.69 (m, 2H, SO₃CH₂CH₂CH₂P), 1.29 (t, 6H, *J* = 7.1 Hz, POOCH₂CH₃).

1.22. 1,3-[Di-(oxapropyl-diethylphosphonate)], 2-(ethyl-4-oxabutyrate), 4-(methoxyethoxy)-calix[4]arene (7 and 7'). The synthesis was performed as for compound **4**.

Calix[4]arene derivative **3**: 1.43 g, 2.4 mmol; K₂CO₃: 3.32 g, 24.0 mmol; acetonitrile: 100 mL; diethylphosphonoylpropoxy-*p*-toluenesulfonate **b**: 1.77 g, 5.0 mmol; CH₂Cl₂: 200 mL; H₂O: 200

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mL. Column chromatography (SiO₂, eluent: CH₂Cl₂/acetone 80/20 v/v). Compounds **7** and **7'** were obtained as yellow viscous liquids.

Compound **7** (1,3-alternate), yield: 0.53 g (23 %). ¹H-NMR (300MHz, CDCl₃) δ (ppm): 7.09-7.06 (m, 2H, ArH_{meta}), 7.02-7.00 (m, 6H, ArH_{meta}), 6.75-6.70 (m, 4H, ArH_{para}), 4.21-4.09 (m, 10H, ArOCH₂ + COOCH₂CH₃), 3.75-3.55 (m, 16H, POOCH₂CH₃ + ArCH₂Ar), 3.37-3.35 (m, 2H, CH₂CH₂OCH₃), 3.34 (s, 3H, CH₂CH₂OCH₃), 2.26 (t, 2H, *J* = 7.5 Hz, CH₂CH₂CH₂COOEt), 1.92-1.66 (m, 10H, CH₂CH₂CH₂POOEt + CH₂CH₂CH₂COOEt), 1.37 (t, 12H, *J* = 7.1 Hz, POOCH₂CH₃), 1.30 (t, 3H, *J* = 7.1 Hz, COOCH₂CH₃). ³¹P NMR (400 MHz, CDCl₃) δ (ppm): 33.5 ppm. Anal. Calcd. for C₅₁H₇₀O₁₃P₂ (%): C, 64.27; H, 7.40. Found: C, 64.17; H, 7.29. Mass spectrum (MALDI-TOF): *m/z* = 975.4 [MNa⁺].

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Compound **7'** (partial cone), yield: 0.43 g (19 %). ¹H-NMR (300MHz, CDCl₃) δ (ppm): 7.35-7.33 (m, 1H, ArH_{meta}), 7.25-7.22 (m, 1H, ArH_{meta}), 7.10-7.07 (m, 2H, ArH_{meta}), 7.01-6.88 (m, 4H, ArH_{meta}), 6.49-6.42 (m, 2H, ArH_{para}), 6.32-6.26 (m, 2H, ArH_{para}), 4.21-4.10 (m, 10H, ArOCH₂ + COOCH₂CH₃), 4.05 (d, 2H, *J* = 13.0 Hz, ArCH₂Ar), 3.92-3.57 (m, 12H, POOCH₂CH₃ + ArCH₂Ar), 3.45 (s, 3H, CH₂CH₂OCH₃), 3.09 (d, 2H, *J* = 13.0 Hz, ArCH₂Ar), 2.61-2.43 (m, 2H, CH₂CH₂OCH₃), 2.23-2.14 (m, 4H, CH₂POOEt + CH₂COOEt), 1.96-1.84 (m, 2H, CH₂POOEt), 1.76-1.53 (m, 6H, CH₂CH₂CH₂POOEt + CH₂CH₂CH₂COOEt), 1.43-1.34 (m, 12H, POOCH₂CH₃), 1.26 (t, 3H, *J* = 7.1 Hz, COOCH₂CH₃). ³¹P NMR (400 MHz, CDCl₃) δ (ppm): 34.1 and 33.1 ppm. Anal. Calcd. for C₅₁H₇₀O₁₃P₂ (%): C, 64.27; H, 7.40. Found C, 64.17; H, 7.29

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1.23. 1,3-[Di-(oxapropyl-phosphonic acid)], 2-(4-oxabutyric acid), 4-(methoxyethoxy)-calix[4]arene (8**)**. The synthesis was performed as for compound **5**.

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Calix[4]arene derivative **7**: 0.29 g, 0.3 mmol; bromotrimethylsilane: 1.38 g, 9.0 mmol; acetonitrile: 10 mL; methanol/water (50/50, v/v): 5 mL. Pink solid, yield: 0.24 g (98%); mp > 290 °C. ¹H-NMR (300MHz, CD₃OD) δ (ppm): 7.15-7.03 (m, 8H, ArH_{meta}), 6.92-6.77 (m, 4H, ArH_{para}), 3.84-3.49 (m, 18H, ArOCH₂ + ArCH₂Ar + CH₂CH₂OCH₃), 3.31 (s, 3H, CH₂OCH₃), 2.12-1.98 (m, 2H, CH₂COOH), 1.72-1.45 (m, 10H, CH₂CH₂CH₂POOH + CH₂CH₂CH₂COOH). ³¹P NMR (400 MHz, CD₃OD) δ (ppm): 33.5 ppm. Anal. Calcd. for C₄₁H₅₀O₁₃P₂ (%): C, 60.59; H, 6.20. Found C, 60.46; H, 6.13.

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1.24. 1,3-[Dj-(oxapropyl-phosphonic acid)], 2-(N-succinimide-4-oxabuturate), 4-(methoxyethoxy)-calix[4]arene (9). The synthesis was performed as for compound 6.

Calix[4]arene derivative 8: 0.22 g, 0.27 mmol; EDC: 0.10 g, 0.54 mmol; NHS: 0.06 g, 0.54 mmol; DMF: 16 mL. Brown solid, yield: 0.11 g (45%); mp > 290 °C. ¹H-NMR (300MHz, DMSO-d₆) δ (ppm): 7.13-6.94 (m, 8H, ArH_{meta}), 6.79-6.69 (m, 4H, ArH_{para}), 3.66-3.46 (m, 18H, ArOCH₂ + ArCH₂Ar + ArOCH₂CH₂OCH₃), 3.36 (s, 3H, CH₂OCH₃), 2.71 (s, 4H, NHS), 1.99-1.88 (m, 2H, CH₂COONHS), 1.57-1.21 (m, 10H, CH₂CH₂CH₂POOH + CH₂CH₂CH₂COONHS). ³¹P NMR (400 MHz, DMSO-d₆) δ (ppm): 32.1. Anal. Calcd. for C₄₅H₅₃NO₁₅P₂ (%): C, 59.40; H, 5.87; N, 1.54. Found C, 59.18; H, 5.60; N, 1.60. Mass spectrum not available due to the poor solubility in solvents compatible with mass spectrometry.

2. Coupling on the solid support

A column was packed with 1 g Ultralink hydrazide gel according to the protocol described by the manufacturer and conditioned with a 50 mM sodium borate buffer at pH 9. 40 mg/mL solutions of compound 6 and compound 9 were prepared in DMSO. Five successive additions of these solutions were performed in order to obtain a final concentration of 3 μmol/g calixarene and the coupling was performed during 2.5 h under agitation.

The column was then washed with 2 x 2 mL coupling buffer and 2 mL coupling buffer containing 1M NaCl and the eluates were analyzed using a fluorimeter CARY-Eclipse (Varian) at λ_{exc} = 268 nm and λ_{em} = 310 nm. λ_{exc} and λ_{em} were previously determined from a calixarene solution and the calixarene amount in the eluates was quantified from a calibration curve (0; 1.86; 3.70; and 7.36 x 10⁻⁴ M).

The calixarene content was determined from the mass-balance, by difference between the initial number of moles of calixarene and that found in the eluate fractions.

3. Uranyl cation binding

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3 Aliquots of gels (1 g) coupled with 2 μmol calixarene **6** / g were washed with 4 x 2 mL 50 mM
4 sodium acetate buffer pH 4.0. 5 x 20 μL of a 0.1 M uranyl acetate solution (i.e 10 μM) were
5 then added to the column and contacted overnight under agitation at room temperature. The
6 supernatant was recovered and the column was then rinsed using 3 x 2 mL 50 mM acetate
7 buffer pH 4 and 3 x 2 mL 50 mM HEPES buffer pH 7.4
8
9 UV spectra of uranyl solutions, recorded on a CARY 50 (Varian), showed a maximum peak
10 between 222 and 234 nm, correlated to the uranyl concentrations in the solutions. A calibration
11 curve (0; 1.5; 3; and 6 x 10⁻⁴ M) was used to quantify the uranyl amount in both supernatant
12 and eluates. The amount of gel sorbed uranyl cation was determined by difference between its
13 initial quantity and that measured in the liquid fractions.
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Captions:

Figure 1 : Synthetic pathway for phosphonic acid)calix[4]arenes possessing an anchoring arm.

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Table 1 : Successive couplings of calixarene 6 on the same gel

Experiment number	Addition of calixarene 6 (μmol)	Calixarene 6 found in the eluate (μmol)	% calixarene 6 coupled to the gel
1	2.99	1.99	33.5
2	2.99	1.97	34.1
3	6.00	6.15	-