Discovery of Powerful Uranyl Ligands from Efficient Synthesis and Screening

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Abstract: New tripodal *gem*-(bis-phosphonates) uranophiles were discovered by a screening method that allowed for the selection of ligands with strong uranyl-binding properties in a convenient microtiter-plate format. The method is based on competitive uranium binding by using Sulfochlorophenol S as chromogenic chelate. This dye compound was found to present high uranyl complexation properties and allowed to highlight ligands presenting association constants for UO_2^{2+} up to 10^{18} at pH 7.4 and 10^{20} at pH 9. A collection of 40 known ligands including polycarboxylate, hydroxamate, catecholate, hydroxypyridonate and hydroxyquinoline derivatives was tested. Also screened was a combinatorial library prepared from seven amine scaffolds and eight acrylates bearing diverse chelating moieties. Among these 96 tested candidates, a tripod derivative bearing *gem*-bis-phosphonates moieties was found to present the highest complexation properties over a wide range of pH and was further studied.

Keywords: actinides · chelates · Michael addition · tripodal ligands · uranium

Introduction

The development of powerful chelating agents for actinides represents a challenging area of research which found multiple applications such as selective separation, nuclear waste management and in vivo chelation therapy.[1] Among the actinides, a renewed interest is found in uranium and in the means to chelate it because handling of uranium in the nuclear industry as well as for military purposes is increasing.[2] The uranyl ion UO_2^{2+}, the most stable uranium form in biological media, is a hard Lewis acid adopting unusual pseudo-planar penta- or hexa-coordinated complexes structures. This particular coordination mode stimulated the design and synthesis of macrocyclic ligands hopefully useful for the selective detection[3] or extraction of uranium from sea water or nuclear wastes.[4] Stimulated by the lack of effectiveness of existing treatments,[5] several specific ligands for UO_2^{2+} (i.e., uranophiles) were also prepared for in vivo uranium removal. After contamination, UO_2^{2+} is rapidly transferred from the blood stream to its target organs, essentially kidney and bone. Uranyl develops a chemical toxicity in the kidney[6] whereas long-term accumulation in the bone might induce cancer.[7] Much of the current research concerns the synthesis of well designed uranyl ligands which are then directly evaluated in vivo on mice or rats. The most significant advances in this area were obtained by K. N. Raymond, P. W. Durbin and co-workers. Several multidentate catecholate and hydroxy-pyridonate ligands were synthetized and their in vivo efficiencies evaluated by this group.[8] This research highlighted several chelates displaying significant uranyl removal efficiencies.[9] However, these efficiencies were often observed when the ligands are administrated immediately (5 to 30 min) after contamination, which shows that the development of new ligands is still of interest.

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Current combinatorial or parallel synthetic methodologies allow for the preparation of high number of new molecules. This approach combined with a screening method has provided a powerful tool for the discovery of new metal complexes.[10]

The aim of this study was to develop a simple and fast in vitro test in order to screen the ligands with strong uranyl binding properties in a library of compounds. With the help of the assay presented herein, screening of candidate ligands in a parallel manner offers a rapid readout and allows the focus on interesting candidates which can then be analyzed more carefully in view of physico-chemical characterizations and/or in vivo experiments. Herein are described new powerful uranophiles discovered by this approach.

Results and Discussion

Screening method development: One of the most typical methods to determine stability constants of uranophiles is displacement of carbonate from the $[UO_2(CO_3)_3]^{4-}$ complex after addition of the ligand investigated.[11] The apparition of the new complex is usually followed spectrophotometrically. Unfortunately, such a method usually requires basic pH, suffers of a large range of already described $[UO_2(CO_3)_3]^{4-}$ association constants (from $10^{18.3}$ to 10^{23})[12] and is not easily applicable to a screening strategy since λ_{max} absorption might vary from a complex to another.

However, the displacement method might be used for fast ligands screening by following the disappearance of a preformed chromogenic uranyl complex.[13] Addition of another complexing agent should induce a significant decrease in absorbance related to its chelating properties. This implies that a well-characterized reference chelate can by used, which displays a good affinity to uranium and generates a stable complex with strong UV/Vis absorption ($\lambda_{max} > 500$ nm). We found that sulfochlorophenol S (SCP, Figure 1), a well-known indicator grade for rare earth elements,[14] matches with these criteria.

In aqueous solution, this compound displays a violet colour whereas the solution of uranyl complex is blue. The colorimetric properties of the **SCP**/UO_2^{2+} complex allowed to perform the screening assay at acidic, neutral or basic pH. This point is of importance because pH varies between the different biological compartments, thus the development of metal chelators displaying high complexation properties in a large range of pH could be of great interest for medical applications.[15] We focused our interest on biological relevant pH values of 5.5, 7.4 and 9 using MES, HEPES and CHES buffers, respectively. Control experiments proved that these buffers do not interfere with the **SCP**/UO_2^{2+} complex.[16] At these pH values, a 1:1 complex is formed instantaneously, which was confirmed by mass spectroscopy[16] and the Job method of continuous variation (Figure 1).[17] The complex presents strong colorimetric features (Figure 1; $\varepsilon_{690} = 21\,580$, 23 390 and 20 490 $M^{-1}cm^{-1}$ for pH 5.5, 7.4 and 9, respectively) and good stability properties (absorbance remains stable after several weeks).

Further spectrophotometric experiments carried out at various pH values confirmed the high complexation properties of **SCP** for UO_2^{2+}. The thermodynamic complexation constants (Figure 2) were found particularly high therefore allowing the screening of powerful uranyl ligands. For example, the method should revealed ligands with conditional constants $K_{cond} > 10^{13}$ at pH 5.5, $K_{cond} > 10^{18}$ at pH 7.4 and $K_{cond} > 10^{20}$ at pH 9 (if they formed a 1:1 complex). The effect of the addition of a competitive ligand is easily visualized by eye or quantified by UV/Vis spectroscopy by staining at 690 nm using a 96-well absorbance reader; this allows for a fast and inexpensive screening without interference due to the absorbance of most of organic uranophiles.

Synthesis and screening of the ligand library: This screening assay was then applied to a chelate library obtained by collecting commercially available compounds and published ligands,[20,21] (Figure 3) or by parallel synthesis (Figure 4).

Recently, we described a modular approach for the parallel synthesis of multidentate ligands libraries.[22] The synthesis uses Michael addition of acrylates, which bear chelating moieties on the amine scaffolds as a key step. This work was extended by using four new acrylates (**B5**, **B6**, **B7** and **B8**). Combination of seven amines (six dipodals and one tripo-

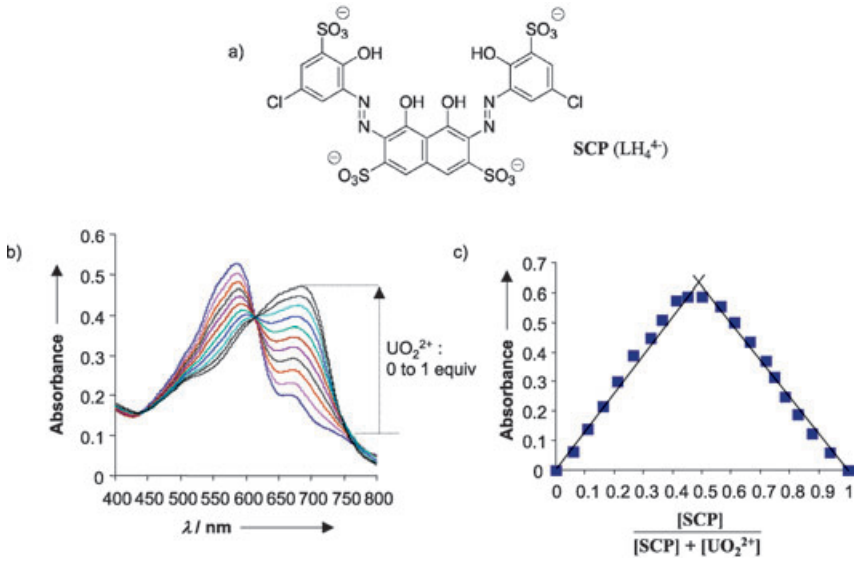

Figure 1. a) Sulfochlorophenol S (**SCP**) structure; b) UV/Vis spectra and c) Job plot of **SCP** and **SCP**/UO_2^{2+} mixtures at pH 7.4 (absorbance measured at 640 nm).

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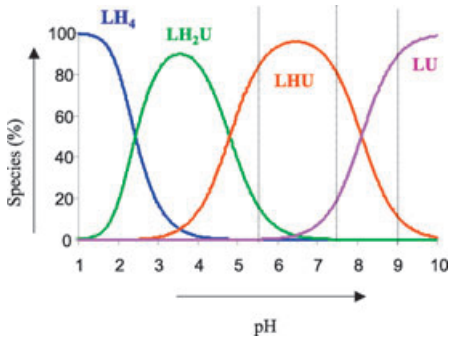

Figure 2. Thermodynamic constants and speciation diagram of **SCP**/UO_2^{2+} complexes calculated by using pHab[18] and HYSS[19] programs, respectively (see Experimental Section). Values in parentheses give the estimated in the least significant figure based on the variation between replicate experiments. **LH_2U**: $\log\beta_{112} = 37.8(1)$, **LHU**: $\log\beta_{111} = 33.0(1)$, **LU**: $\log\beta_{110} = 24.9(3)$.

dal) and eight acrylates conducted to the formation of 56 new chelates with high structural diversity (Figure 4).

The 96 ligands were then screened in a parallel manner on microtiter plates for their uranium binding properties at pH 5.5, 7.4 and 9 by using **SCP** as a reference chelate. The results are summarized in Figure 5.

Remarkably, the compounds bearing bis-phosphonate chelating moieties were found to be the most potent among the library containing polycarboxylate, hydroxamate, catecholate, hydroxypyridonate and hydroxyquinoline derivatives. This is highlighted by the comparison of the results obtained at pH 5.5 and 7.4 with a family of tripods **3A**–**H** and **4A** constructed with the same tris(2-aminoethyl)amine group anchoring arms. At pH 7.4, 3,4,3-LIHOPO **2G** was found the only compound without a bis-phosphonate chelating function, which was able to displace around 60% of the **SCP**/UO_2 complex. This ligand has already been tested for in vivo uranyl removal with some success.[23] Comparison of the results obtained with dipodal bis-phosphonates **A1**–**5B6** (Figure 5, pH 7.4) show the influence of the spacer separating the two chelating moieties, the maximum of uranyl-binding was observed with ligand **A4B6** (12 atoms separating the chelating moieties). The uranyl binding properties of bis-phosphonates overall remains superior regardless of the pH value. More interesting is the fact that, among the bis-phosphonate family, tripods **4B** and **A7B6** presented the highest K_{cond} values at pH 9 comparable to catechol based-ligand TRENCAMS (**3B**) which exhibit high K_{cond} enhancement in basic conditions in accordance with previous findings.[24]

Next we focused our interest on ligand **A7B6** which induced the highest displacement value of the **SCP**/UO_2 complex at all the tested pH.

Studies on compound A7B6: Electrospray mass spectrometry (ES-MS) was first used to confirm the 1:1 stoichiometry of the **A7B6**/UO_2^{2+} complex. In the negative mode, ES-MS showed one major peak at m/z 367 corresponding to $[(UO_2)(H_7L)]^{3-}$ (**A7B6** = $H_{12}L$).[16] No polymetallic complex were detected even in the presence of an excess of UO_2^{2+}.

The **A7B6**/UO_2 complex presents remarkable fluorescence properties which were investigated by using time-resolved laser-induced fluorescence (TRLIF, Figure 6). The fluorescence spectrum of the aqueous uranyl ion reflects the symmetrical vibration of the U−O bond. The observed emission bands correspond to the electronic transition $S_{11}\rightarrow S_{00}$ (473 nm) and $S_{10}\rightarrow S_{0\nu}$ with $\nu = 0$–4 (488, 510, 535, 560, and 587 nm).[25] In the absence of the ligand, two species are present at pH 5.5: UO_2^{2+} ($\tau = 2\,\mu s$) and UO_2OH^+ ($\tau = 80\,\mu s$).[26] Progressive addition of ligand **A7B6** on a solution of UO_2^{2+} induced a hyperchromic and bathochromic shift (λ_{em}: 499, 520, 546 and 572 nm) of the uranyl emission. Increase of fluorescence reached until saturation corresponding to 1 equiv of **A7B6** therefore confirming the 1:1 stoichiometry of the complex. Nonlinear regression fit showed a tight slope demonstrating the high complexation properties of **A7B6** and making calculation of K_{cond} not possible in this experiment. The strong enhancement of the fluorescence intensity and lifetime ($\tau = 176 \pm 8\,\mu s$) allowed us to detect less than 10^{-11} M of uranyl ion envisioning analytical applications of this compound.

We then determined the stability constant of **A7B6** at pH 9 using first the competitive displacement method of the **SCP**/UO_2 complex.[27] When **A7B6** was added to a solution of **SCP**/UO_2 complex (80 μM in CHES buffer), competitive displacement takes place and reaches equilibrium after 24 h according to the spectral changes. We varied the concentration of **A7B6** from 0 to 10 equiv[16] and estimated the K_{cond} values at each concentration. On the basis of these measurements accurate association constant was found to be $\log K_{cond} = 19.2 \pm 0.4$. In a parallel manner, we took advantage of the fluorescence properties of the **A7B6**/UO_2 complex to carry out a competitive displacement of the nonfluorescent uranyl carbonate complex $[UO_2(CO_3)_3]^{4-}$. For this purpose, uranyl (40 μM) was dissolved in 30 mM carbonate buffer (pH 9) and the compound **A7B6** was added (4 to 400 μM). Displacement of carbonate by **A7B6** takes place and the formation of **A7B6**/UO_2 complex was followed by fluorescence staining.[16] Association constants, determined at each **A7B6** concentration, were found to be $\log K_{cond} = 19.1 \pm 0.1$. Both relative constants independently determined are in good agreement, affording a reliable value.

The selectivity of the complex was then investigated using TRLIF experiments which consist in adding a competing metal cation (M^{n+}) in the presence of the **A7B6**/UO_2 complex. A decrease of the fluorescence signal will be observed if those metals form a new complex with **A7B6**. Competing metal cations, such as K^+, Ca^{2+}, Mg^{2+}, Zn^{2+} and Fe^{3+} have been added to the solution of the 1:1 **A7B6**/UO_2 complex at pH 5.5. The **A7B6**/UO_2 complex lifetime has been examined in order to verify that no interference due to the addition of complexing cations occurred (variation of the lifetime would indicate dynamic quenching and not complexation). Representative results are displayed on Figure 7.

The data revealed that the **A7B6**/UO_2 complex presents a good stability even in the presence of excess of other metals

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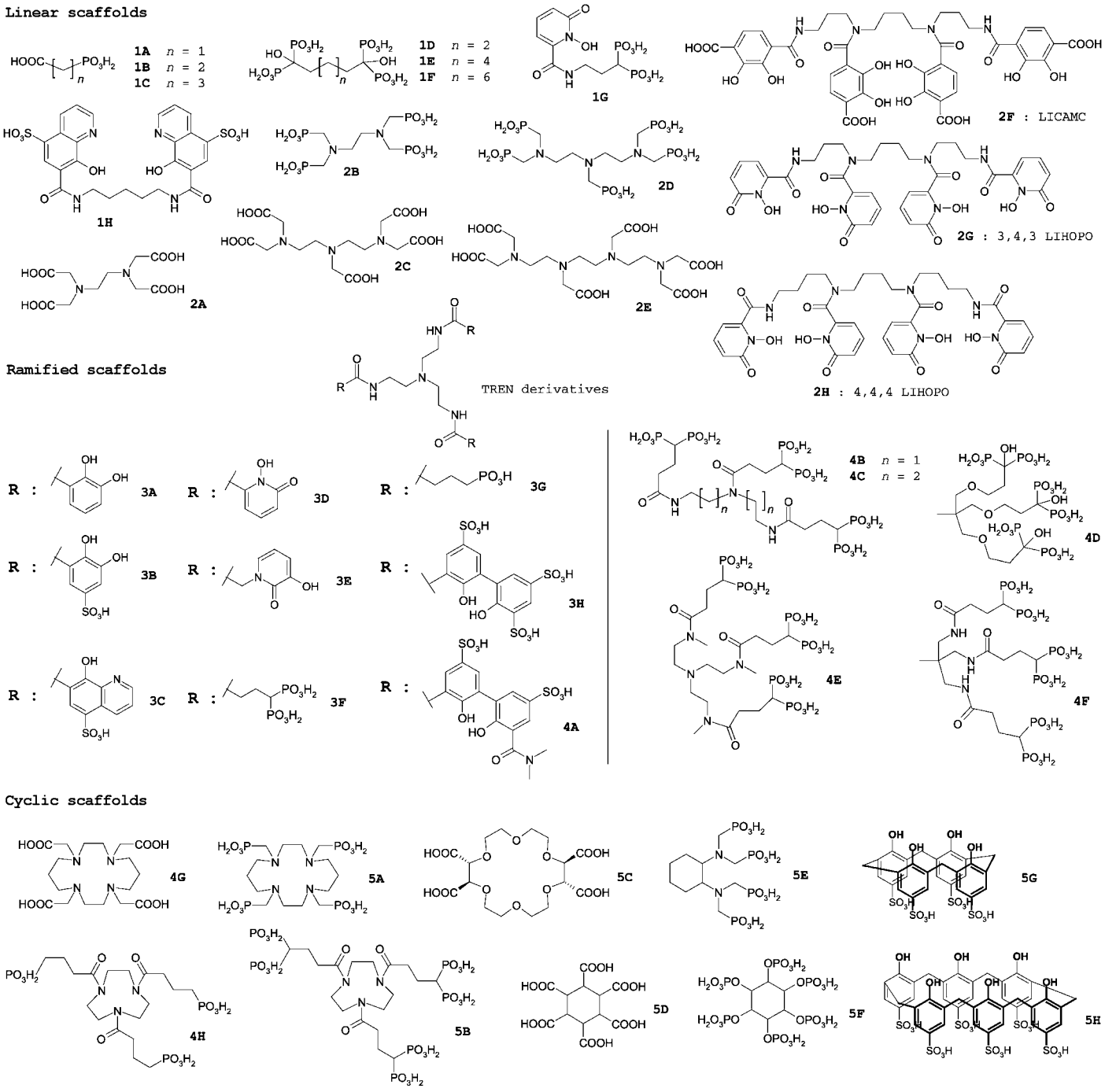

Figure 3. Ligand library.

(100 equiv) except for Fe^{3+} which dramatically affect **A7B6**/UO_2 complex.

In conclusion, **SCP** was found highly suitable for the rapid screening of putative uranium ligand library. The described procedure allowed us to compare the binding properties of 96 highly diverse ligands. The main chemical information is that ligands bearing bis-phosphonate moieties display by far the strongest binding features for the uranyl ion. Tris(bis-phosphonates) ligands, especially compounds **4B** and **A7B6**, were found the most powerful uranyl ligands among our library. These ligands display large association constants at acidic, neutral and basic pH envisioning analytical and medical possible applications. The complexing properties of phosphonates are well known,[28] but to the best of our knowledge, this is the first time that such a comparative study of highly diverse ligands was accomplished with uranium.

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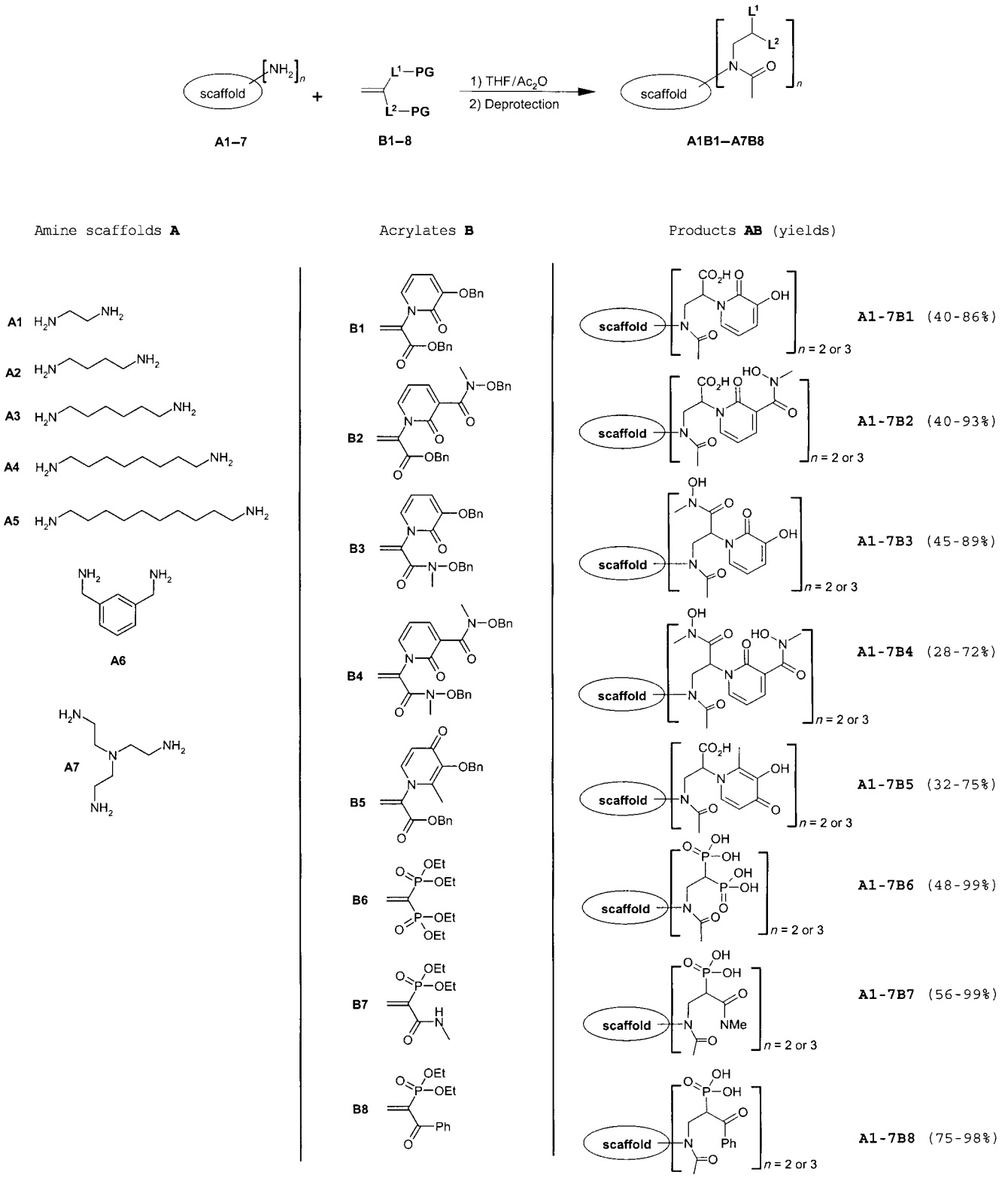

Figure 4. Parallel synthesis of ligands **A1B1** to **A7B8**. Michael additions were run with an excess of acrylates **B** in THF or DMF and then quenched by an excess of Ac_2O. Crude mixtures were then concentrated and purified through filtration on silica gel. Removal of the protective groups (**PG**) of the chelating moieties ($\mathbf{L^1}$ and $\mathbf{L^2}$) were carried out by Pd hydrogenation or by TMSBr to afford **A1–7B1–5** and **A1–7B6–8**, respectively (see Experimental Section). LC-MS controls showed more than 85% purities for each products. All compounds have been fully characterized by using ^1H, ^{31}P NMR and mass spectroscopy.

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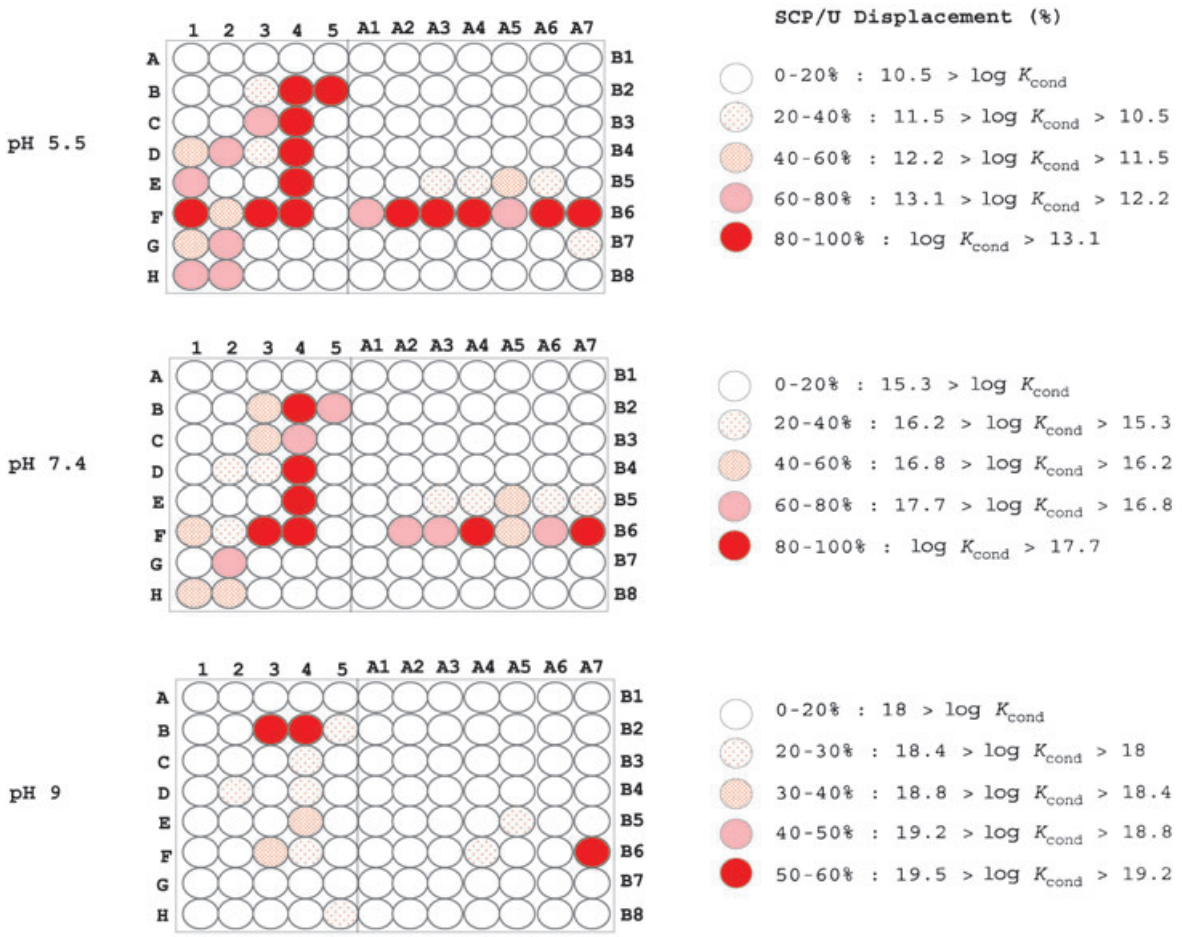

Figure 5. Screening results: Displacements (%) of the **SCP**/UO_2^{2+} complex are presented in a color-coded format for clarity reason. $\log K_{cond}$ (estimates) were calculated by using the HYSS program supposing that the complex competitive ligand/UO_2^{2+} is 1:1. For details on the procedure, see the Experimental Section.

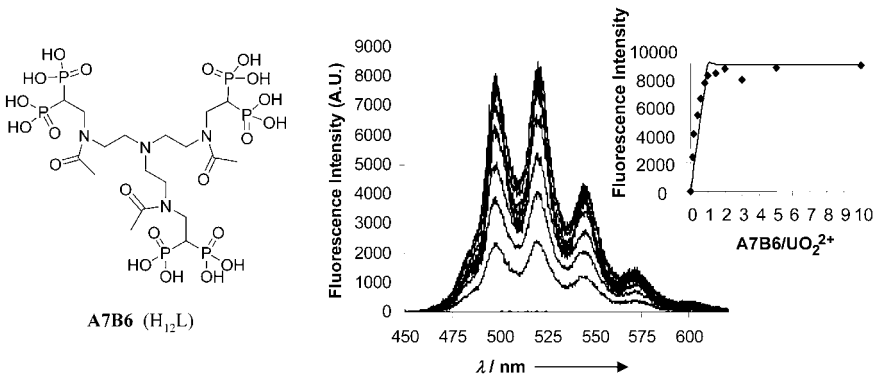

Figure 6. Structure of ligand **A7B6**. Spectrofluorescence titration of a solution of UO_2^{2+} (4.2 μM) by **A7B6** in 0.1 M $NaClO_4$, pH 5.5. The inset shows the experimental and theoretical uranyl fluorescence intensity as a function of **A7B6**/UO_2^{2+} ratio at λ_{exc} 266 nm, λ_{em} 519 nm.

Experimental Section

General methods: Unless otherwise stated, starting materials were obtained from commercial suppliers and used without purification. THF was distilled from sodium/benzophenone before use. Analytical thin layer chromatography (TLC) was performed by using 0.25 mm silica gel coated MERCK 60 F_{254} plates. Visualization of the chromatogram was carried out by UV absorbance, ethanolic solution of phosphomolybdic acid and iodine. Flash chromatography was performed using compressed air with the indicated solvent system and silica gel 60 (Merck, 230–400 mesh). Purifications through silica cartridges were performed by using Bond Elut Jr. 18C (1 g, Varian) as reverse phase.

UV measurements were carried out on a VARIAN CARRY 50 Scan UV/Vis spectrophotometer in a 1 cm quartz Suprasil cells purchased from HELLMA operating with Cay Win UV software. The pH of the solutions was measured with an automatic titrator system DMS 716 titrino with a combined glass electrode (Methrom filled with saturated KCL). NMR spectra were recorded on Bruker instrument (AC300). MS chromatograms were carried out on ESI/TOF Mariner spectrometer. Analytical LC-MS separations were carried out on Waters HPLC 2525 with a C_{18} column (Xterra, 5 μm, 4.6×50 mm), elution was done with H_2O/CH_3CN/

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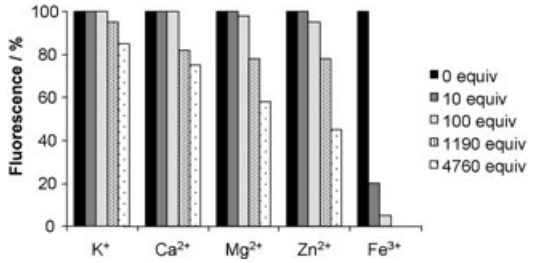

Figure 7. Selectivity of the **A7B6**/UO_2 complex in the presence of metal cations. [**A7B6**/UO_2] = 4.2 μM; [$NaClO_4$] = 0.1 M; [competitive metal] = 0 to 20 mM; pH 5.5

HCOOH (gradient from 95:05:0.1 to 0:100:0.1) with a 1.0 mL min^{-1} flow rate; detection were carried out by UV detector (254 nm) and DEDL PL-ELS 1000 (Polymer laboratories); mass spectra were taken on Waters Micromass ZQ by positive electrospray method (ES+). Screening was performed in the 96 well microtiter polystyrene plates purchased from NUNC. The plates were read using an absorbance plate reader SPECTRA max PLUS (Molecular Devices).

Time resolved laser-induced fluorescence (TRLIF) measurements were carried out by using a Nd/YAG laser (model Minilite, Continuum, Santa Clara, USA) operating at 266 nm (quadrupled) or 355 nm (tripled) and delivering an energy of 2 mJ in a 4 ns pulse duration at a repetition rate of 15 Hz was used as the excitation source. The laser output energy was monitored by a laser powermeter (Scientech, Boulder, USA). The excitation laser beam was focused on the cell of the spectrofluorometer "Fluo 2001" (Dilor, France). The light emitted from the cell was focused onto the entrance slit of the polychromator. Taking into account dispersion of the holographic grating used in the polychromator, measurement range extended to approximately 200 nm into the visible spectrum with a resolution of 1 nm. The detection was performed by an intensified array of photodiodes (1024 diodes) cooled by Peltier effect (−35 °C) and positioned at the polychromator exit. Recording of spectra was performed by integration of the pulsed light signal given by the intensifier. The integration time was adjustable from 1 to 99 s and allowed for variation in detection sensitivity. Logic circuits, synchronized with the laser shot, allowed the intensifier to be active with a defined time delay and during a selected aperture time. The whole system was controlled by a microcomputer. TRLIF experiments were performed by using a gate delay of 300 μs, a gate length of 100 μs and integration time of 0.5 s. The emission wavelength was 520 nm (excitation at 266 nm) and the temperature was 20 °C.

Electrospray ionization mass spectrometric detection of negative ions was performed by using a Quattro (Micromass, Manchester, UK). Samples were introduced into the source with a syringe pump (Harvard Apparatus, Cambridge, MA, USA). Nitrogen was employed as both the drying and spraying gas with a source temperature of 80 °C. The cone voltage was set to 22 and 30 V, the voltage applied on the capillary is 3500 kV, and the sample solution flow rate was 10 μL min^{-1}. Spectra were recorded by averaging 40 scans from 100 to 1000 m/z at a scan rate of 6 s per scan.

HRMS were performed in TOF MS ES$^+$ mode at the "Institut de Chimie des Substances Naturelles" (Gif sur Yvette, France).

Synthesis: The preparation of compounds **A1–7B1–4** has been published elsewhere.[22] The synthesis of acrylates **B5–8** is described in the Supporting Information.

General procedure for the synthesis of ligands A1–7B5: Acrylate **B5** (94 mg, 0.25 mmol, 4 equiv for amines **A1–6** or 188 mg, 0.5 mmol, 8 equiv for amine **A7**) was added to a solution of amine **A1–7** (0.063 mmol, 1 equiv) in dry THF (2 mL). After 2 h of reaction at room temperature, acetic anhydride (100 μL, 1.07 mmol, 17 equiv for amines **A1–6** or 150 μL, 1.6 mmol, 25 equiv for amine **A7**) was added. The solution was stirred for 15 min at room temperature, evaporated and then directly filtered through a silica gel (5 g, Et_2O/MeOH 9:1 to remove the excess of **B5**, then CH_2Cl_2/MeOH 8:2) to yield protected chelate. After removal of the solvents, the residues were then dissolved in a solution of 0.37 M ammonium formate in 9:1 DMF/H_2O (10 equiv per benzyl group) and stirred overnight in the presence of Pd/C (10% by weight). The solutions were filtered, acidified with 10% formic acid to pH 4, then evaporated. Sublimation at 50 °C under vacuum eliminated excess of formate salts. After filtration through a reverse phase silica cartridge (water/methanol 3:7) and evaporation, the target chelates **A1–7B5** were dissolved in MeOH and precipitated by addition of Et_2O (32–75%).

Analytical data for selected compounds (data for compounds **A1–7B5** are available in Supporting Information):

For A6B5: Yield 49%; ^{1}H NMR (300 MHz, DMSO): δ = 1.83, 1.93 (2s, 6H), 2.16 (s, 6H), 3.65–4.00 (m, 4H), 4.05–4.70 (m, 4H), 4.82 (m, 2H), 6.13 (m, 2H), 6.87 (m, 1H), 7.00 (m, 2H), 7.28 (m, 1H), 7.51 (m, 2H); ^{13}C NMR (75.47 Hz, DMSO): δ = 13.14, 13.50, 22.11, 22.88, 35.43, 48.41, 53.11, 111.84, 126.30, 126.56, 127.63, 130.35, 131.05, 139.28, 146.29, 170.18, 170.89, 171.54; IR (NaCl, cm^{-1}): 3392, 3078, 1905, 1727, 1633, 1504, 1421, 1248, 1174, 1026, 991, 829; HRMS: calc. for [M+H]$^+$: 611.2353; found: 611.2339.

For A7B5: Yield 75%; ^{1}H NMR (300 MHz, DMSO): δ = 1.80, 1.84, 1.95 (3s, 9H), 2.32 (s, 9H), 2.95–3.80 (m, 12H), 3.85–4.30 (m, 6H), 5.58 (m, 3H), 6.77 (m, 3H), 7.19 (m, 3H); ^{13}C NMR (75.47 Hz, DMSO): δ = 14.18, 22.62, 22.72, 35.40, 47.50, 51.30, 112.10, 127.61, 134.16, 145.36, 169.78, 172.79, 173.58; IR (NaCl): $\tilde{\nu}$ = 3147, 3045, 2808, 2007, 1739, 1634, 1403, 1260, 1025, 995, 828, 659 cm^{-1}; HRMS: m/z: calcd for: 858.3521; found: 858.3549 [M+H]$^+$.

General procedure for the synthesis of ligands A1–7B6–8: Acrylate **B6** (60 mg, 0.2 mmol, 8 equiv for amines **A1–6** or 135 mg, 0.45 mmol, 18 equiv for amine **A7**) or acrylate **B7** (22 mg, 0.1 mmol, 4 equiv for amines **A1–6** or 44 mg, 0.2 mmol, 8 equiv for amine **A7**) or acrylate **B8** (27 mg, 0.1 mmol, 4 equiv for amines **A1–6** or 54 mg, 0.2 mmol, 8 equiv for amine **A7**) was added to a solution of amine **A1–7** (0.025 mmol, 1 equiv) in dry DMF (2 mL). After reaction at room temperature (48 h for acrylate **B6**, 24 h for acrylate **B7** and 4 h for acrylate **B8**), acetic anhydride (40 μL, 0.43 mmol, 17 equiv for amines **A1–6** or 60 μL, 0.63 mmol, 25 equiv for amine **A7**) was added. The solution was stirred for 15 min at room temperature, evaporated and then directly filtered through a silica gel (5 g, hexane/acetone 1:1 to remove the excess of acrylate, then acetone/MeOH 7:3) to yield protected chelate. After removal of the solvents, protected chelates were then dissolved in dry CH_3CN and TMSBr (3 equiv per functional group) was added under argon. The mixture was stirred under reflux for 3 h and then quenched with water (3 equiv per functional group). After refluxing for additional 15 min, the solvents were evaporated. The residue co-evaporated with MeOH and subsequently dissolved in a minimum of MeOH and precipitated with diethyl ether. The resulting slurry was decanted, washed with diethyl ether and dried under high vacuum in the presence of P_2O_5 to afford the target chelates **A1–7B6–8** (48–99%).

Analytical data for selected compound (data for compounds **A1–7B6–8** are available in Supporting Information):

For A6B6: Yield 90%; ^{1}H NMR (300 MHz, D_2O): δ = 2.18, 2.23, 2.32, 2.52 (4s, 6H), 2.83–3.32 (m, 2H), 4.06 (m, 4H), 4.84, 4.95 (2m, 4H), 7.25 (m, 1H), 7.40(m, 2H), 7.60 (m, 1H); ^{31}P NMR (121.5 MHz, D_2O): δ = 18.73, 19.34; ^{13}C NMR (75.47 Hz, D_2O): δ = 21.63; 37.12 (t, ^1J(C,P) = 124.5 Hz), 44.37 (t, ^1J(C,P) = 73.1 Hz), 53.59, 126.54, 126.96, 129.84, 137.54, 175.19, 175.68; IR (NaCl): $\tilde{\nu}$ = 3112, 2150, 1610, 1442, 1204, 1176, 990, 915, 821, 760, 699 cm^{-1}; HRMS: m/z: calcd for: 595.0413; found: 595.0408 [M−H]$^+$.

For A7B6: Yield 48%; ^{1}H NMR (300 MHz, D_2O): δ = 2.23, 2.41, 2.47 (4s, 9H), 2.77–3.02 (m, 3H), 3.70 (m, 6H), 3.98 (m, 6H), 4.17 (m, 6H); ^{31}P NMR (121.5 MHz, D_2O): δ = 18.43; ^{13}C NMR (75.47 Hz, D_2O): δ = 21.46, 38.09 (t, ^1J(C,P) = 123.1 Hz), 42.13 (t, ^1J(C,P) = 72.8 Hz), 47.12, 52.78, 176.61; IR (NaCl): $\tilde{\nu}$ = 3331, 2920, 2360, 1615, 1428, 1148, 998, 914, 702 cm^{-1}; HRMS: calcd for: 837.0809; found: 837.0846 [M+H]$^+$.

Screening: Uranyl stock solution (20 mM) was prepared by dissolving $UO_2(OAc)_2$·2H_2O in 0.1 M perchloric acid. Sulfochlorophenol S (**SCP**, purchased from Fluka) stock solution (4 mM) was prepared by dissolving

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SCP in Millipore quality water. All experiments were carried out at 25 °C in buffers with a fixed ionic strength of 0.1 M prepared as follows: MES (for pH 5.5), HEPES (for pH 7.4) or CHES (for pH 9) (acid forms) (12.5 mmol) and nBu$_4$N$^+$Cl$^-$ (112.5 mmol) were dissolved in Millipore quality water (1 L). Adjustment of the pH was done using nBu$_4$N$^+$OH·30 H$_2$O. A 100 μM SCP/UO_2^{2+} complex solution was prepared by mixing: **SCP** stock solution (6.25 mL) diluted in the appropriate buffer (12.5 mM, 242.5 mL) with uranyl stock solution (1.25 mL) for 1 h at room temperature. Candidates ligands were dissolved in water to get 400 μM solutions.

General procedure for the screening of the ligands library: In each well of a microtiter plate 100 μM SCP/UO_2^{2+} solution (200 μL) and 400 μM ligand solution (50 μL) were mixed at 25 °C for 36 h. Controls experiments (without ligand and without UO_2^{2+}) were made in each plates. All experiments were done in duplicate. Control experiments (**SCP** alone, **SCP**/UO_2 and **SCP**/competitive ligand mixtures) were carried out on each plate and used to calculate the percentage of **SCP**/UO_2 displacement or to control that the competitive ligand did not interfere with the UV/Vis properties of **SCP**. Absorbance measurements were carried out on an absorbance plate reader by staining at 690 nm.

Using the speciation program Hyss, it was possible to determine the value of the conditional constants, defined in Equation (1), of the competitive ligands L′ in case of the formation of UL′ 1:1 complex.

$$K_{cond} = [UL']/([U]([L'] + [HL'] + [H_2L'] + \ldots + [H_nL'])) \quad (1)$$

Determination of the SCP/UO_2 complexation constants: The complexation constants were determined by measuring the absorbance spectra of an equimolar solution of **SCP**/UO_2 at pH ranging from 1 to 10. An aqueous stock solution (250 mL) containing nBu$_4$N$^+$Cl$^-$ (0.1 M), **SCP** (20 μM) and $UO_2(OAc)_2$·2 H_2O (20 μM) was prepared and the pH adjusted to 1 with an aqueous solution of HCl. A series of 44 samples were prepared in separate sealed containers at various pH using nBu$_4$N$^+$OH$^-$·30 H_2O. After the samples had been equilibrated in the dark at 25 °C for at least 48 h, a potentiometric measurement of pH and an absorbance spectrum were recorded for each solution. Data analysis was performed with the pHab spectral componentization and least-square program. All equilibrium constants were defined as cumulative formation constants according to Equation (2); **SCP** is designated as L.

$$mU + nL + zH \rightleftharpoons [U_mL_nH_z];\ \beta_{mlh} = [U_mL_nH_z]/([U]^m[L]^n[H]^z) \quad (2)$$

A typical analysis of an experiment included approximately 15 equilibrium constants (K_a and UO_2^{2+} constants for **SCP**, UO_2^{2+} hydrolysis constants and K_w). Despite this complexity the refinements were stable since only three constants were refined (formation constants β_{110}, β_{111}, and β_{112} are given in Figure 2). The theoretical UV spectra are in good agreement with the experimental UV spectra (see Supporting Information).

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