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Synthesis of a Functionalizable Water-Soluble Cryptophane-111

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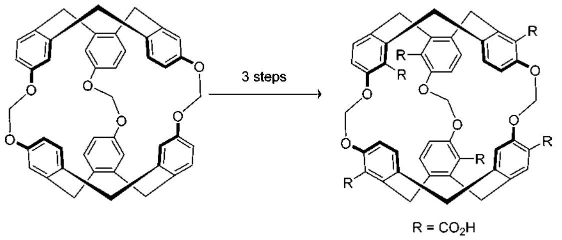
ABSTRACT



The development of optimized xenon host systems is of crucial importance for the success of molecular imaging using hyperpolarized ^{129}Xe MRI. Cryptophane-111 is a promising candidate because of its encapsulation properties. The synthesis of cryptophane-111-based biosensors requires both water-solubilizing and chemically activatable groups. An expeditious synthesis of a water-soluble and functionalizable cryptophane-111 is described.

Magnetic resonance imaging (MRI) is a powerful method for scanning deep tissues or opaque biological samples, but its low sensitivity precludes its use for imaging molecular targets. In this context, laser-polarized ^{129}Xe NMR spectroscopy is a highly attractive tool for *in vitro* and *in vivo* MRI. Xenon is a nontoxic gas, soluble in biological fluids, and readily delivered by inhalation. Moreover, thanks to an NMR signal enhanced by 4 or 5 orders of magnitude through optical pumping,[1] small amounts of gas dissolved in biological tissues can be easily detected with an excellent signal-to-noise ratio. Development of xenon biosensors, which capture xenon atoms in molecular cages suitably functionalized to bind the desired target, have been the subject of particular attention over the past decade.[2] Cryptophanes are molecular cages made by the connection of two cyclotriveratrylene units through covalent bridging (Figure 1). They exhibit outstanding properties for xenon encapsulation. The cavity size, which directly depends on the length of the molecular bridge, dramatically affects both the affinity for xenon and the rate of in and out exchange of xenon. Today, biosensors are only constructed from cryptophane A.[3] Although cryptophane-111 displays the highest affinity for xenon in both organic solvents[4] and water,[5] it has never been used for the synthesis of biosensors. Indeed, no congener that is simultaneously water-soluble and functionalizable has been described. In addition, the challenge of current chemical developments is rendering the cryptophane moiety more hydrophilic in order to avoid the biosensor's uptake by biological membranes[3f] or formation of self-assemblies.[3d]

Only two water-soluble cryptophanes-111 were described independently by Travis Holman and our group. The former, functionalized with six cationic electron-withdrawing $[(\eta^5\text{-}C_5Me_5)Ru^{II}]^+$ ([Cp*Ru]) moieties, is soluble at neutral pH ($> 30 \times 10^{-3}$ mol·dm^{-3}, 293 K) and exhibits a high xenon binding constant in water (2.9×10^4 mol^{-1}·dm^3) (Figure 2).[6] However, the possible instability of the organometallic moieties in biological fluids might be a drawback to the use of such derivatives. Moreover, the

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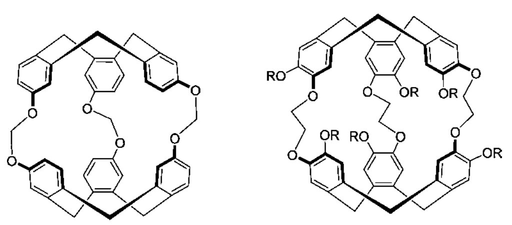


Cryptophane-111, 1

Cryptophane A (R = CH_3)

Figure 1. Structure of cryptophane-111 and cryptophane A.

vectorization of a biosensor built from this cage to a biological target seems difficult from a chemical point of view. In this context, we described the synthesis of a metal-free water-soluble cryptophane-111 (Figure 2).[7] This was achieved by grafting an original trisulfonated linker onto the cryptophane core. However, several functionalization experiments were unsucessful in our laboratory. As a result, we refocused on a completely different strategy and we describe here a straightforward synthesis of a water-soluble and functionalizable cryptophane-111.

A cryptophane-222 bearing six carboxylic acid groups has been successfully monofunctionalized and led to a Zn^{2+} biosensor.[31] Analogously utilizing this approach, a cryptophane-111 bearing six carboxylate groups would be a perfect precursor for the design of new biosensors (Figure 3).

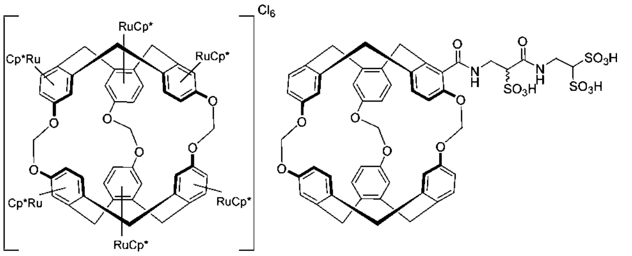
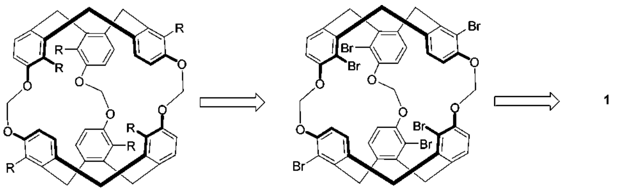


Figure 2. Structures of water-soluble cryptophanes-111: (left) metalated cryptophane-111[5]; (right) cryptophane-111 substituted by a trisulfonated group.[6]

Such a cryptophane-111, **3**, could be obtained from the corresponding hexabrominated compound **2**. The latter could be synthesized from cryptophane-111 **1** available at the multigram scale (Figure 3).[8]



3 (R = CO_2H)

2

Figure 3. Retrosynthetic pathway.

We started our investigation by studying the bromination step. As reported in a previous communication, cryptophane-111 **1** can be readily monobrominated using *N*-bromosuccinimide NBS in chloroform.[9] Using an excess of this reagent and longer times of reaction, we observed the formation of a mixture of polybrominated compounds (Table 1, entry 1). After 7 days at room temperature, the hexabrominated compound **2** was isolated in 35% yield (Table1, entry 2). Heating to reflux did not improve this result (Table 1, entry 3). The use of a large excess (120 equiv) of NBS accelerated the formation of compound **2** but complicated its purification due to the formation of di-, tri-, and tetrabrominated compounds (Table 1, entry 4). After 13 days of reaction at room temperature, the hexabrominated cryptophane-111 **2** was isolated in 60% yield (Table 1, entry 5). This product precipitated during the reaction and was isolated by simple filtration.

We were pleased to observe that the hexabromination takes place in a perfectly regioselective manner. Indeed, the ^{1}H NMR spectrum of compound **2** shows only two doublets in the aromatic area at 6.92 and 8.06 ppm (J = 6.0 Hz).[10] This demonstrates that compound **2** was regioselectively

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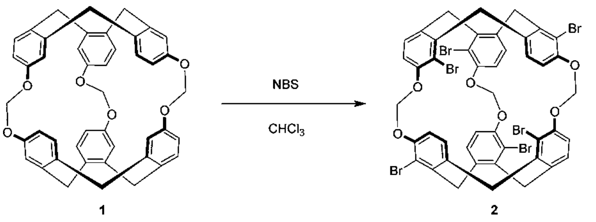
(10) See Supporting Information

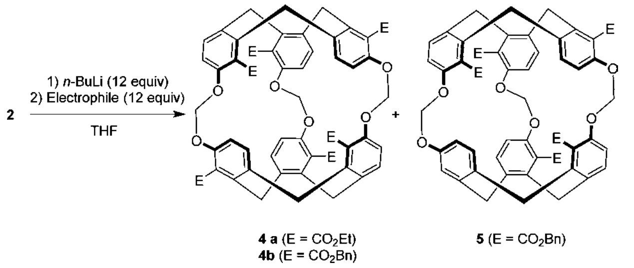
Table 1. Bromination of Cryptophane-111 **1**



entry	NBS (equiv)	reaction time	temp (°C)	compounds (yield, %)[a]
1	9	6 days	rt	mixture of polybrominated compounds
2	12	7 days	rt	**2** (35%)
3	12	4 days	reflux	hexabromocryptophane **2** (50%) and pentabromocryptophane (50%)[b]
4	120	4 days	rt	**2** (22%)
5	12	13 days	rt	**2** (60%)

[a] Isolated yield. [b] Monitoring by LC/MS.

Table 2. Optimization of Lithiation of Cryptophane



entry	electrophile	temp (°C)	result[a]
1	CO_2	−78	–[b]
2	CO_2	−15	**1**[b]
3	$ClCO_2Et$	−15	**1** and **4a**
4	$ClCO_2Et$	0	**1** and **4a**
5	$ClCO_2Bn$	−15	**4b** and **5**

[a] Monitoring by LC/MS. [b] Traces of mono- and dicarboxylic acid compounds.

brominated on the six *ortho* positions of the phenoxy rings, as shown in Figure 3.

We then turned our attention to the lithiation of compound **2** in order to obtain cryptophane-111 hexacarboxylic acid **3**. We started our investigations by using *n*-BuLi in THF and quenching lithiated species with CO_2. The reactions were monitored by LC/MS. After performing the reaction at −78 °C, the starting material was recovered along with small amounts of mono- and dicarboxylic acid compounds (Table 2, entry 1). This result demonstrated the possibility of performing the bromine/lithium exchange and of trapping the lithiated intermediate. In contrast, at −15 °C, all of the brominated precursor disappeared. However, cryptophane-111 **1** was obtained as the major product with small amounts of mono- and dicarboxylic acid compounds (Table 2, entry 2). This proved that the lithium/bromine exchange occurred, but the lithiated species were not efficiently trapped by CO_2. More reactive ethyl chloroformate, used as an electrophile, gave a mixture of cryptophane-111 hexaethyl ester **4a** and cryptophane-111 **1** in the same proportions (Table 2, entry 3). Similar results were obtained at 0 °C, demonstrating the stability of lithiated intermediates (Table 2, entry 4). Finally, using benzyl chloroformate as an electrophile led to hexa- and pentabenzyl ester compounds **4b** and **5** in the same proportions (Table 2, entry 5). In order to skip laborious purification steps, complete debenzylation by hydrogenolysis over Pd/C in tetrahydrofuran/methanol 1/1 was carried out on the crude mixture of products, leading to cryptophane-111 hexacarboxylic acid **3** in 18% isolated overall yield. The pentacarboxylic acid analog **6** was obtained in 23% overall yield as the main side product.

As expected, cryptophane-111 hexacarboxylate **3** and pentacarboxylate **6** are soluble in water at a concentration of 2.0 $mg \cdot mL^{-1}$. These compounds reversibly encapsulate xenon in D_2O at pH 6 and display a specific ^{129}Xe NMR signal for the caged noble gas of 87 and 84 ppm at 293 K, respectively.

In summary, we propose a strategy for the easy synthesis of a functionalizable and water-soluble cryptophane-111. Compound **3** can be readily obtained in three steps from cryptophane-111 **1** in moderate yields along with the corresponding pentacarboxylic acid **6**. As expected, cryptophane-111 hexacarboxylate **3** encapsulates xenon and therefore allows us to envision the development of new cryptophane-based bioprobes for ^{129}Xe MRI. Our ongoing research project aims to graft compound **3** onto various biomolecules through amide bond formation according to our previous work.[6]

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Supporting Information Available. Detailed experimental procedures, full characterization, and copies of ^1H and ^{13}C NMR spectra of all new compounds are available. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.