ORGANIC LETTERS

2004
Vol. 6, No. 8
1225−1228

Facile Purification of Rare Cucurbiturils by Affinity Chromatography

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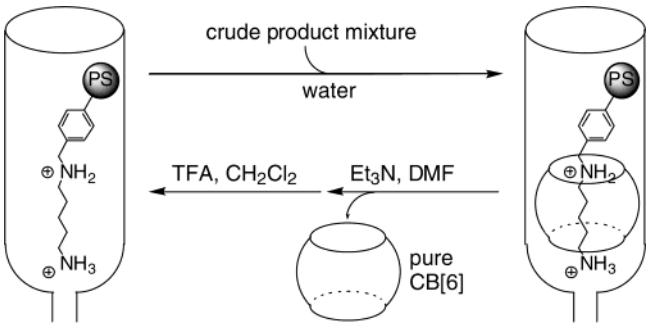
Received January 2, 2004

ABSTRACT



A practical method for the separation and purification of cucurbituril (CB) hexamers was developed on the basis of affinity chromatography using aminopentylaminomethylated polystyrene beads. This recyclable resin, which can be used repeatedly, facilitates the general preparation of cucurbituril derivatives and compensates for the usually moderate yields and mixed products that characterize the acid-catalyzed synthesis of CB derivatives. This technique allows convenient, rapid isolation of rare substituted cucurbiturils, including hexacyclohexanocucurbit[6]uril and dodecamethylcucurbit[6]uril.

Cucurbituril, **1**, is a macrocyclic cavitand that is made of six glycoluril molecules, **2**, and 12 formaldehyde units.[1] Although it has been known since 1905,[2] it was first characterized by Mock and co-workers in 1981.[3] A few substituted cucurbiturils and homologues, CB[*n*], $n = 5-8$, comprising 5, 7, and 8 glycoluril units, have also been prepared and characterized,[4] all having a hydrophobic cavity that is accessible through two identical carbonyl-fringed portals. The rigid structure and the combination of a hydrophobic cavity with polar portals allow these cavitands to host various molecules and cations, rendering the CBs attractive synthetic receptors and useful building blocks of various supramolecular structures. For example, **1** forms stable host−guest complexes with doubly protonated diaminoalkanes, such as 1,4-diaminobutane, 1,5-diaminopentane, and 1,6-diaminohexane ($K_d = 1.5 \times 10^{-6}$, 2.4×10^{-7}, 2.7×10^{-7} M, respectively).[5] This property has been extensively used by Kim[6] and others[7] to construct many supramolecular assemblies, including catenanes, rotaxanes, and pseudopolyrotaxanes.

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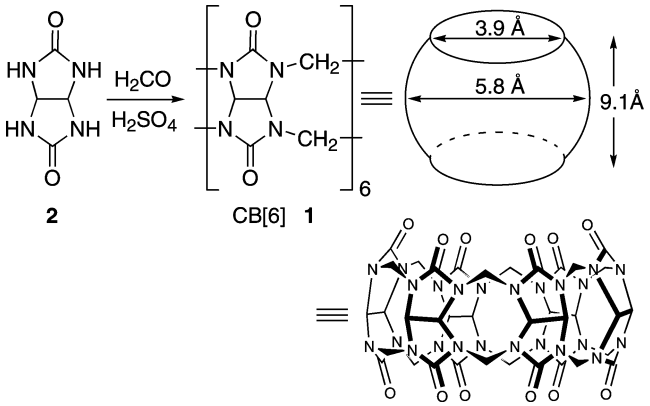
10.1021/ol0499755 CCC: $27.50
Published on Web 03/12/2004

As has been the case with the well-studied families of other host molecules, including crown ethers, cryptands,[8] cyclodextrins,[9] and calixarenes,[10] one might expect that the extensive utilization of CBs in various fields would be highly dependent on their accessibility, namely, their facile synthesis, separation, and purification methods. Although these macropolycycles are easily assembled via an acid-catalyzed condensation of the appropriate glycolurils with formaldehyde (Scheme 1),[1] they are usually obtained in the form of

Scheme 1



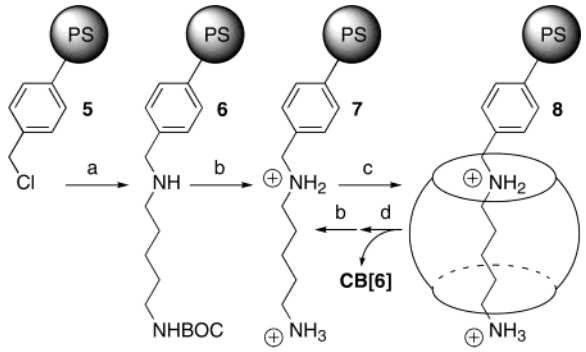
complex mixtures that contain many cyclic and acyclic oligomers and polymers, including insoluble polymers. Thus, the isolation of pure CBs has become the major impediment to their availability, particularly when large-scale synthesis is required.

Here, we report on an efficient, fast, and general purification method that is applicable to a broad range of substituted cucurbiturils.

The current methods of isolating CBs from their reaction mixtures are based mainly on differential solubility in various solvents and on fractional crystallization. These methods, however, are not general and may not be suitable for substituted CBs. In most cases, separation by column chromatography is difficult due to the high polarity and limited solubility of these compounds. It is somewhat easier to separate CB[6] from its product mixture by creating an inclusion complex with a diaminoalkane. However, we found that although the formed complexes could be separated from the mixture by precipitation, it is difficult to obtain pure, diamine-free CBs. One way to circumvent this problem is to employ a weaker amine binder, such as 4-aminopyridine, which can be easily removed from the complex by treatment with K_2CO_3 in DMSO at room temperature. This approach, however, cannot discriminate CB[6] from the other CB[*n*] homologues.

We envisioned that the strong binding interactions between CBs and protonated amines or diamines could be harnessed for the design of an affinity chromatography purification strategy.[11] This could be achieved by the employment of polymer-bound polyamines (Scheme 2), taking advantage of the fact that the binding affinities are solvent- and pH-dependent.

Scheme 2[a]



[a] Key: (a) $H_2N(CH_2)_5NHBOC$, DMF, Py; (b) TFA, CH_2Cl_2; (c) crude product mixture from Scheme 3; (d) Et_3N, DMF.

We decided to use chloromethylated polystyrene beads (Merrifield resins) for this purpose. Although such resins are commercially available with a loading of 0.5−1.6 mmol/g (NovaBiochem), we preferred to work with higher density functional groups. To that end, we started with cross-linked polystyrene beads (Rohm & Haas, Amberlite XE-305, 2% divinylbenzene, 20−50 mesh, average pore size 1400 Å, surface area 48 m^2/g). The resin (8 g) was chloromethylated by mixing it with chloromethylmethyl ether (75 mL), dropwise addition of tetrachlorostannane (2.4 mL),[12] and refluxing for 3 h. The mixture was then cooled to room temperature, poured into methanol (200 mL), and filtered.

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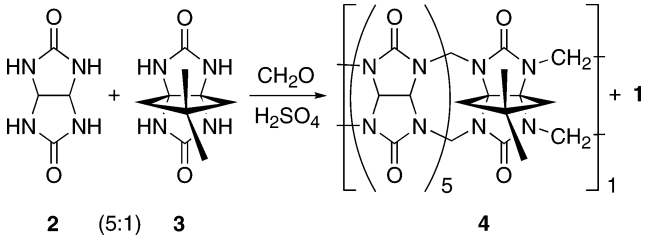
The beads were washed thoroughly with methanol and then dried under reduced pressure to give the chloromethylated resin **5**. Elemental analysis (C, 71.38; H, 5.99; Cl, 19.73) indicated loading of 2.7 mmol/g (85% ring substitution). IR: 1611, 1510, 1442, 1420 cm^{-1}.

Amination of resin **5** (Scheme 2)[13] was achieved by mixing it (7 g) with DMF (50 mL) followed by dropwise addition of a solution of the tosylate salt of mono-*tert*-butoxycarbonyl-1,5-diaminopentane (Novabiochem, 5.2 g, 14 mmol) in pyridine−DMF (2:1, 30 mL). The mixture was agitated at 50 °C for 24 h, filtered, washed with DMF, CH_2Cl_2, and MeOH, and dried in vacuo to give the Boc derivative **6**. Elemental analysis (C, 64.06; H, 6.61; N, 4.65) indicated loading of 1.67 mmol/g. IR: 3381, 1631, 1485, 1450, 1425 cm^{-1}.

Removal of the Boc group in **6** was achieved by packing it (3 g) in a 1 × 12 cm column and washing it with trifluoroacetic acid (10% v/v in CH_2Cl_2, 50 mL) at a flow rate of 1 mL/min. The column was then washed with CH_2Cl_2 (50 mL) and finally with DMF (50 mL) at a flow rate of 2 mL/min, producing the fully protonated resin, **7**, as could be concluded from its IR spectrum (see the Supporting Information).

We first demonstrated the affinity purification approach by the separation of all hexameric CB products from a complex reaction mixture that was obtained by heating a 5:1 mixture of **2** and dimethylcyclopentanoglycoluril, **3**, with formaldehyde in concentrated sulfuric acid, a mixture that contained mainly dimethylcyclopentano-CB, **4**, and **1** (Scheme 3).[14,15] The water−soluble fraction (740 mg) of the crude,

Scheme 3



heterogeneous mixture (1.3 g) was dissolved in neutral water (50 mL) and passed through the column at a flow rate of 0.5 mL/min.[16] The column was then washed with water, methanol, CH_2Cl_2, and again with methanol (a small sample of the resin was dried under vacuum overnight for FTIR analysis; see the Supporting Information). Removal of the solvent from the combined eluent afforded a solid residue (510 mg). This residue was used for collecting a second harvest of CB[6] using the same column (vide infra).

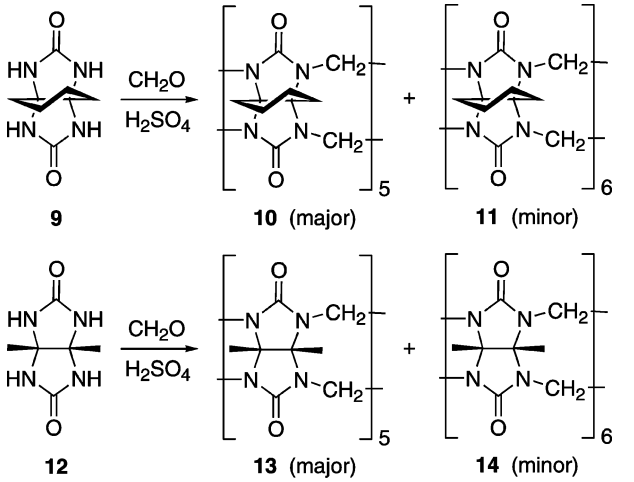
The resin-bound CB was released from the column by elution with a 1:2 mixture of triethylamine−DMF (100 mL) at a flow rate of 2 mL/min. The column was then washed with water (200 mL), the combined eluent was concentrated under reduced pressure, methanol was added, and the precipitate was collected and dried. Analysis of the resultant white powder (195 mg) by ^1H NMR and ESI-MS (see the Supporting Information) indicated that it was mainly a mixture of **4** and **1** with a small amount of a di(dimethylcyclopentano)-CB[6].

The column was regenerated by washing with 10% (v/v) trifluoroacetic acid in CH_2Cl_2 and used again to harvest additional amounts of CB[6] from the CB-depleted remnants from the first harvest (510 mg). That residue was dissolved in neutral water and loaded on the column as described above. Unloading of the column with triethylamine−DMF yielded a second crop of pure CB[6] (165 mg).

To check the performance of this column over multiple cycles of affinity chromatography, we loaded and unloaded a sample of purified CB[6] (150 mg) four times. The sample was trapped and released quantitatively in all eight operations with no apparent loss of the column capacity. It may thus be concluded that the resin can be used repeatedly over many cycles for the separation and purification of CB derivatives.

An appropriate testing ground for the above-described affinity chromatography technique would be the isolation of rare CB derivatives. It has been shown by calculations that CB[6] and CB[7] are the most stable homologues among the unsubstituted CB[n]. However, for highly substituted CBs, the smaller homologues are preferred, on the basis of both thermodynamic and kinetic considerations.[17] For example, the reaction of cyclohexanoglycoluril, **9**, with formaldehyde under strong acidic conditions (Scheme 4) produces

Scheme 4



pentacyclohexano-CB[5], **10**, and hexacyclohexano-CB[6], **11**, in 16% and 2% yield, respectively.[18] These products were previously separated after a series of dissolution and

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(16) Since **4** is partially soluble in neutral water while **1** is essentially not, these two compounds can be separated by dissolution of the mixture in water.
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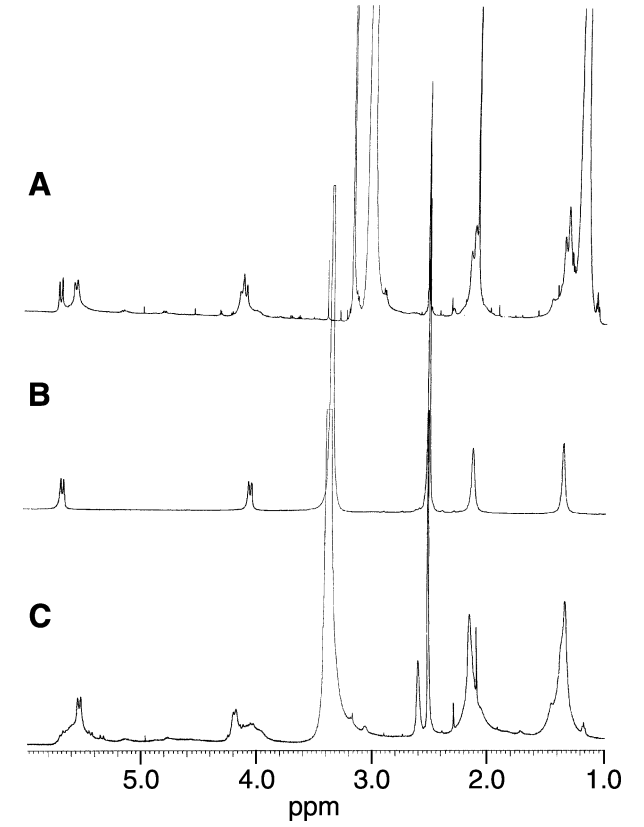

Figure 1. ^{1}H NMR spectra of components obtained in the reaction of **9** with formaldehyde: (A) crude product mixture containing mainly **10** and **11**; (B) compound **11** that was separated from the mixture by one cycle of affinity chromatography; (C) the residue obtained after removal of **11** from the mixture by one cycle of affinity chromatography.

fractional crystallizations.[18] Even higher selectivity for CB-[5] was reported for the reaction of dimethylglycoluril, **12**, under similar conditions, in which only one product, decamethylcucurbit[5]uril, **13**, could be isolated from the reaction mixture.[19]

Using our affinity chromatography approach, we rapidly separated the minor CB[6] product, **11**, from the crude reaction mixture described by Kim.[18] The NMR spectra of the mixture before and after the separation (Figure 1) indicates that pure **11** was quantitatively recovered from the product mixture. Moreover, when the reaction mixture that was described by Stoddart[19] was subjected to this separation technique, the very rare dodecamethycucurbit[6]uril, **14**, was obtained in 0.2% yield. Clearly, it would be extremely difficult to isolate this compound by any alternative approach. The purity of both **11** and **14** was evident from their ^{1}H, ^{13}C NMR and MS data (see the Supporting Information).

In conclusion, a very practical method for the separation and purification of CB[6] derivatives was developed. The approach is based on affinity chromatography using amino-pentylaminomethylated cross-linked polystyrene beads. This recyclable resin, which can be used in multiple operations, facilitates the general preparation of cucurbituril derivatives and compensates for the usually moderate yields and mixed products that characterize the acid-catalyzed CB syntheses. The method was demonstrated by the separation of rare CB-[6] analogues, such as **11** and **14**. Analogous affinity chromatography resins that are specific for higher CB homologues, including CB[7] and CB[8], are currently being developed in our laboratories.

Acknowledgment. We thank the US−Israel Binational Science Foundation and the German−Israeli Project Cooperation (DIP). E.K. is a member of the Lise Meitner-Minerva Center for Computational Quantum Chemistry. S.S. acknowledges the Skaggs Institute for Chemical Biology for fellowships.

Supporting Information Available: ^{1}H NMR (300 MHz) and ESI-MS of a mixture of **4** and **1**; ^{1}H NMR (600 MHz), ^{13}C NMR, ESI-MS, and HRMS spectra of **11** and **14**; HMBC spectrum of **14**; FTIR spectra of resin XE-305, resins **5**−**8**, and the neutral version of **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0499755

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