Supramolecular Protection with a Recyclable Molecular Container: an Efficient Strategy for the One-pot Selective Functionalization of Polyfunctional Substrates

Simon Lambert,[&,£] *Kristin Bartik,*[&*] *and Ivan Jabin*[£*]

[&] Université libre de Bruxelles (ULB), Ecole polytechnique de Bruxelles, Engineering of Molecular NanoSystems, Avenue F.D. Roosevelt 50, CP165/64, B-1050 Brussels, Belgium.

[£] Université libre de Bruxelles (ULB), Laboratoire de Chimie Organique, Avenue F.D. Roosevelt 50, CP160/06, B-1050 Brussels, Belgium.

Kristin.Bartik@ulb.be, Ivan.Jabin@ulb.be

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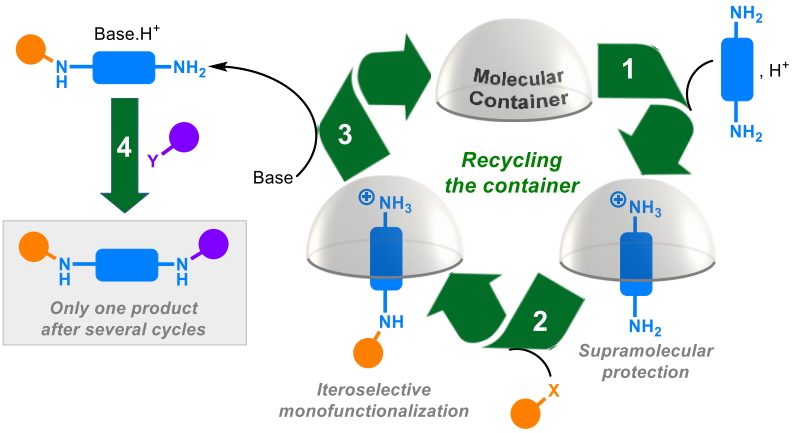


Abstract

The control of the selective transformation of a functional group on a substrate bearing multiple identical reactive functions is a major synthetic challenge. The use of macrocyclic molecular containers as supramolecular protecting units is in this context a promising strategy. We report here on a hexahomotrioxacalix[3]arene molecular container whose exceptional binding properties opens new directions for the supramolecular protection strategy. This macrocyclic receptor binds primary ammonium ions with high selectivity and affinity, allowing for the monofunctionalization of diamines. The reaction was readily achieved with various diamines and different types of reagents. The acid-base control of the protection/deprotection of the substrate furthermore allowed for the recovery and recycling of the molecular container and the subsequent *in-situ* accumulation of non-symmetric difunctionalized products, with remarkably

no loss of selectivity over the reaction cycles. This recycling strategy considerably increases the synthetic viability of the supramolecular approach with only a catalytic amount of molecular container needed. The synthesis of products, such as non-symmetric diamino-diureas, which are difficult to obtain via classical synthetic routes, was also achieved through the one-pot reaction of mixtures of difunctional substrates monoprotected by the molecular container. The supramolecular protection strategy was finally extended to the selective functionalization of more complex polyamines such as a triamine and a lysine derivative. These results show that the use of a supramolecular protecting container for the functionalization of polyfunctional substrates presents many synthetic advantages over strategies using traditional protecting groups: i) it enables high chemo-, regio- and iteroselectivities through a one-pot protection/functionalization/deprotection sequence, ii) it avoids the introduction and removal of covalently linked protecting groups thus leading to high yield , iii) it allows the formation of unstable intermediates and the control of their reactivity, iv) it gives access to complex polyfunctional products whose synthesis remains challenging through classical synthetic methods.

Keywords: Supramolecular protection – Homooxacalixarenes – Polyamines – Host-guest systems – Monofunctionalization – Iteroselectivity

Introduction

The development of reactions and strategies enabling the chemo-, regio- and/or stereoselective modification of polyfunctional compounds is an important field of research in organic chemistry.[1] Chemo- and regio-selectivity issues are most often overcome by using a protection/deprotection sequence for one or more of the substrate's functional groups. However, this strategy is not adapted to symmetrical substrates bearing identical remote groups as the transformation of one group has little or no influence on the reactivity of the others. With such substrates, the control of the iteroselectivity,[2] which is the selectivity that governs the number of repeating chemical transformations on a substrate bearing multiple identical reactive functions, remains a challenge.[3] For example, attempts to monofunctionalize (with one equiv. of a reagent) symmetrical substrates displaying two identical and independent reactive groups classically lead to a statistical mixture of three products (iteromers) and isolating the desired monofunctionalized product can furthermore be complicated (Figure 1a).[4] To overcome this problem, a classical trick consists in employing a large excess of the difunctional starting material to limit the formation of the difunctionalized product.[5] This method can however only

be applied to cheap difunctional substrates that can be easily separated from the monofunctionalized product.

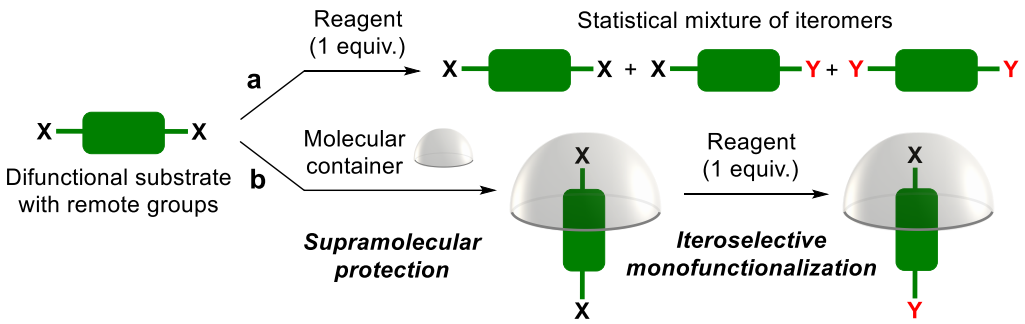


Figure 1. Reaction of a symmetrical substrate displaying two remote functional groups: a) nonselective functionalization leading to a statistical mixture of iteromers; b) iteroselective monofunctionalization through the supramolecular protection approach.

Recently, miscellaneous strategies based on supramolecular approaches have been reported for the iteroselective functionalization of polyfunctional substrates. For example, metal oxide particles,[6] shadow masks[7] and coordination chemistry[8] have been used to protect one or more functional groups of polyfunctional substrates. In this context, the use of macrocyclic molecular containers as supramolecular protecting units emerges as a promising strategy.[9] The general concept relies on the protection of a functional group of a polyfunctional substrate through its binding in the cavity of a molecular receptor and the subsequent selective transformation of an accessible functional group (Figure 1b). To the best of our knowledge, the first example of such a host-mediated site-selective reaction was reported by the group of Reinaud in 2009.[10] In this seminal work, a calix[6]arene-based zinc complex was used for the regio- and iteroselective mono-carbamoylation of *N*-(2-aminoethyl)-1,3-propanediamine with a 63% yield. More recently, the group of Rebek exploited the binding properties of resorcin[4]arene-based cavitands in water for the monofunctionalization of various symmetrical difunctional molecules.[11] In all cases, the supramolecular protection ensured by the resorcinarene-based cavitands was driven by the hydrophobic effect. These systems were notably applied to the iteroselective radical reduction of alkyl dihalides (>95% yield),[12] monohydrolysis of alkyl dibromides (up to 93% yield),[13] monoepoxidation of dienes (up to 84% yield),[14] selective reduction of diazides (>95% yield),[15] and monohydrolysis of diesters (>90% yield).[4] In 2021, Ballester and coworkers showed that super aryl-extended calix[4]pyrroles could be used as supramolecular protecting molecular containers for the selective monohydrolysis of bis-isonitriles in water.[16]

Very recently, we described the unique binding properties of a hexahomotrioxacalix[3]arene[17]-based molecular container (**Ox3**).[18,19] We showed that this host is able to accommodate primary ammonium ions (RNH_3^+) in its polyaromatic cavity with high binding constants (log K_a > 4 even in a protic environment) (Figure 2). Secondary, tertiary and quaternary ammoniums are not recognized and the selective recognition process for primary ammoniums relies on a three-point H-bonding complementarity between the ethereal macrocycle of **Ox3** and the ammonium head of the guest. The free amine form (RNH_2) is furthermore not recognized, enabling a control of the reversibility of the host-guest process through the addition of acids and bases. This molecular container, which can reversibly bind primary ammonium ions with high affinity and selectivity, offers a wonderful playground to explore new directions for the supramolecular protection strategy and widen its synthetic applications.

Herein we report on key advances in the field of supramolecular protection using this macrocycle. The first new concept that we explored was the possibility of regenerating the molecular container *in situ* after a supramolecular protection/monofunctionalization sequence. This would considerably increase the synthetic viability of the supramolecular approach as a functionalized product could then be accumulated through a *one-pot* cyclic process involving a small amount of molecular container. Another aim was to evaluate the synthesis of products, which are difficult to obtain via classical synthetic routes, through the one-pot reaction of mixtures of difunctional substrates monoprotected by a molecular container. Finally, we also explored the supramolecular approach with complex polyfunctional substrates for the development of regio- and/or iteroselective reactions.

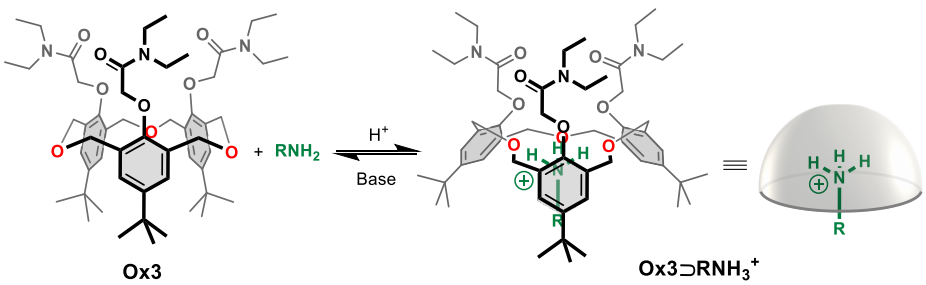


Figure 2. Host-guest properties of molecular container **Ox3** toward primary ammonium ions RNH_3^+.

Results

NMR study of the binding between host Ox3 and protonated symmetrical diamines. The binding properties of **Ox3** towards the monocationic form of linear symmetrical diamines H_2N-$(CH_2)_n$-NH_2 (with n ranging from 3 to 8) were first evaluated in $CDCl_3/CD_3CN$ (9:1). The

addition of aliquots of diamine and picric acid (PicH) (1:1 ratio) to **Ox3** led, in all cases, to the intra-cavity complexation of the monoammonium ion, as evidenced by the presence of high-field signals (i.e. below 1 ppm) in the ^{1}H NMR spectra (see Figure 3a-b for cadaverine $CadNH_2$, n = 5). With all guests, host-guest exchange was slow on the NMR chemical shift timescale and the signals of the host-guest complexes were assigned through COSY experiments.[20] The shifts of the host Ar*H* and ArCH_2 signals following complexation are characteristic of the more open conformation adopted by **Ox3** when an ammonium ion is bound in the cavity (see the structure displayed in Figure 2).[18] For the shorter diamines (n ≤ 6), integration of the ^{1}H signals showed the exclusive formation of 1:1 complexes over the course of the titration. The complexes were obtained quantitatively after the addition of a slight excess of the ammonium salt, confirming a high binding affinity (log K_a = 4.2 ± 0.2, 4.1 ± 0.2, 4.7 ± 0.2 and > 5 for diamines with n = 3, 4, 5, and 6 respectively). With the longer diamines (n = 7 and 8), NMR signals corresponding to the 2:1 host-guest complex (<10%) were also observed.[21] Similar results, albeit with slightly smaller affinity constants, were obtained when the binding studies were conducted in $CDCl_3/CD_3OD$ (4:1): log K_a = 3.7 ± 0.2, 3.7 ± 0.2, 4.0 ± 0.2 and 4.3 ± 0.3 for diamines with n = 3, 4, 5, and 6 respectively.[20]

Addition of an excess of PicH to the host-guest complexes led to the protonation of the free amino group dangling outside of the host cavity. For n = 3 and 4, the resulting diammonium ions were not soluble in $CDCl_3/CD_3CN$ (9:1). For n = 5 and 6, the binding properties of **Ox3** were only weakly affected by the protonation of the second amino group and similar binding affinities to those obtained with monoammonium ions were determined (log K_a = 4.8 ± 0.2 and >5 with n = 5 and 6 respectively).[20] With n = 7 and 8, 2:1 host-guest complexes were also detected over the course of the titration (identified through COSY experiments and integration of appropriate signals).[20]

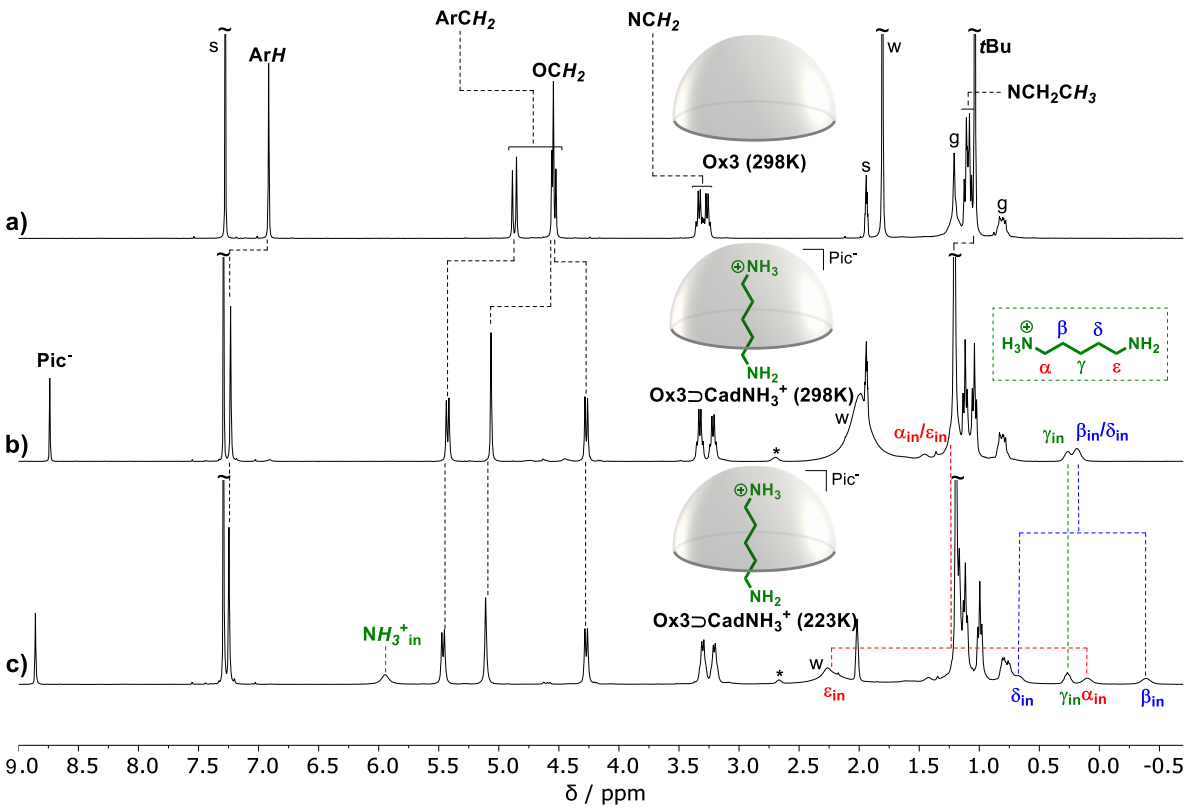


Figure 3. ^{1}H NMR spectra (400 MHz, $CDCl_3/CD_3CN$ 9:1) of **Ox3** (3 mM) at 298 K (a); **Ox3** + 1.1 equiv. of cadaverine + 1.1 equiv. PicH at 298 K (b) and at 223 K (c). *: free cadaverine; s: residual solvents; w: residual water; g: grease; "in" stands for "included".

In silico studies by molecular mechanics conformational analysis were performed for the 1:1 complex **Ox3⊃CadNH$_3^+$** (Figure 4a) and the 2:1 complex **(Ox3)$_2$⊃$^+$H$_3$N-(CH$_2$)$_8$-NH$_3^+$** (Figure 4b). In both cases, the host displays an open-cone conformation with the oxygen of the ether bridges oriented toward the center of the cavity. The bound ammonium groups are deeply inserted in the cavity of the receptor, establishing three H-bonds with the ether bridges. These results are consistent with the data obtained by NMR spectroscopy. Moreover, the minimized structure of **Ox3⊃CadNH$_3^+$** shows that a five methylene spacer (n = 5) is long enough for the free amine group of the guest to protrude from the cavity and thus be accessible to reactants in the solution.

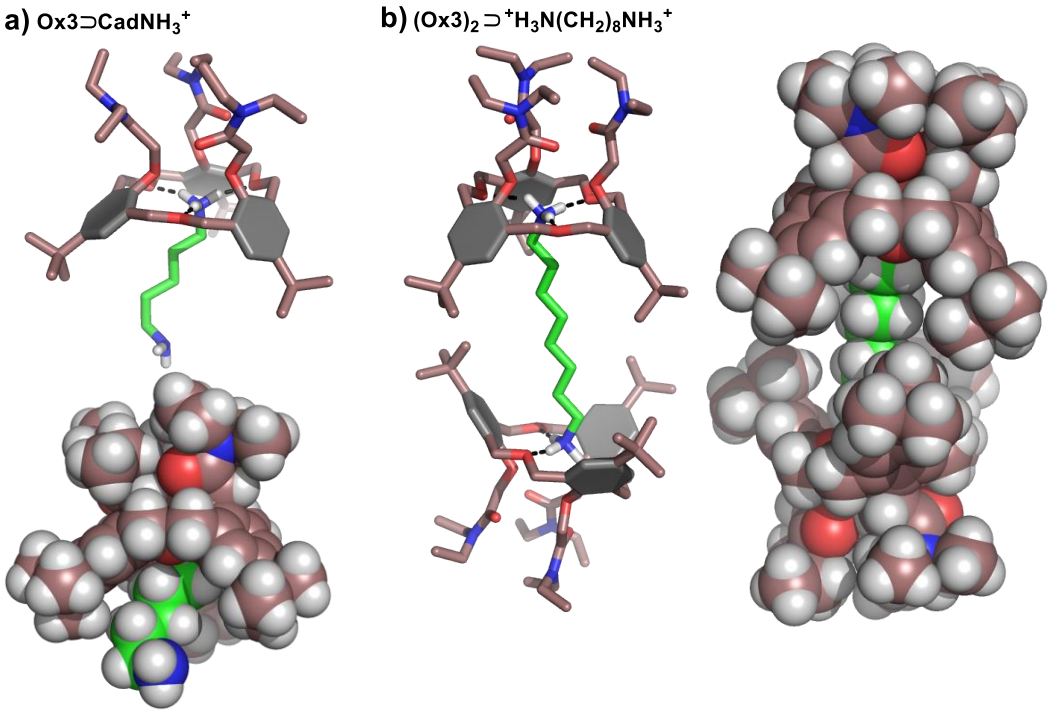


Figure 4. Energy-minimized structures (stick and space filling representations) of the 1:1 complex **Ox3⊃CadNH$_3^+$** (a) and the 2:1 complex **(Ox3)$_2$⊃$^+$H$_3$N-(CH$_2$)$_8$-NH$_3^+$** (b). In the case of the stick representations, with the exception of the polar H, all hydrogen atoms are omitted for clarity. Hydrogen bonds are indicated by dashed lines. Average O(host)–N(guest) distances (Å): 2.85, 2.88 and 3.13 for **Ox3⊃CadNH$_3^+$** and 2.90, 2.91, 2.92, 2.93, 3.01 and 3.05 for **(Ox3)$_2$⊃$^+$H$_3$N-(CH$_2$)$_8$-NH$_3^+$**.

Host-guest exchange dynamics. Interestingly, for the diamines with n ≤ 6, the number of ^{1}H signals for the complexed monoammonium guest was less than expected in the spectra recorded at 298 K.[20] In the case of cadaverine (n = 5), only three high-fielded signals instead of five were observed for the methylene groups (Figure 3b). The five expected signals were however clearly observed in the spectrum recorded at 223 K (Figure 3c) and their chemical shifts confirm a fast exchange process at 298 K between the CH$_2$α and CH$_2$ε protons and between the CH$_2$β and CH$_2$δ protons (see inset Figure 3b for the assignment of the methylene protons). At 298 K, the exchange process was slow on the NMR timescale for the longer guests (n = 7 and 8), as distinct high field signals were observed for all the guest methylene groups.[20] Two possible degenerate processes can be envisaged to explain the reduced number of signals for the shorter guests at 298 K: an associative process (Figure 5, path a) consisting in the folding of the guest carbon chain in the cavity followed by an internal proton transfer from one amino group to the other (guest tumbling) or a dissociative process with reversal of the guest upon re-complexation (Fig 5, path b). The dissociative mechanism can be ruled out as slow *in-out* host-guest exchange is observed in all cases. It is noteworthy that the tumbling process was not observed with a rigid

diamine (i.e. 1,4-phenylenediamine = 1,4-Ph(NH_2)$_2$) unable to fold in the cavity, even at 323 K, or when the diamines were fully protonated.[20] In the latter case, the associative process is probably prevented because of the electrostatic repulsion that would result from the folding of the dicationic guest in the cavity.

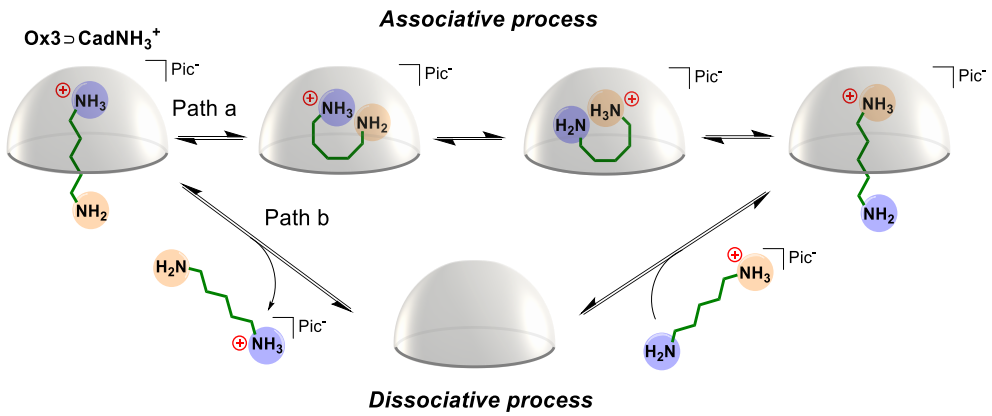
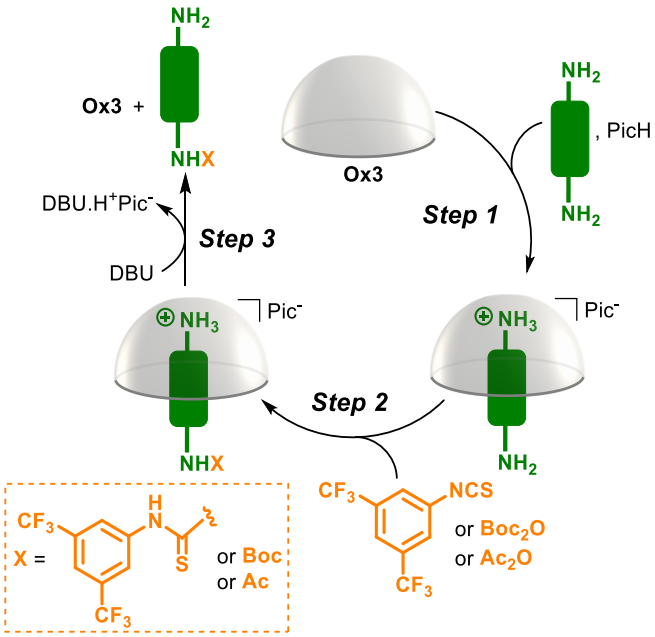


Figure 5. Associative (top) or dissociative (bottom) degenerate processes for the reversal of $CadNH_3^+$ in the cavity of **Ox3**.

Monofunctionalization of symmetrical diamines via supramolecular protection by Ox3. The monofunctionalization of symmetrical linear diamines of different lengths (H_2N-(CH_2)$_n$-NH_2 with n = 4, 5, 6 and 8) and of 1,4-phenylene diamine was investigated in $CDCl_3$/CD_3CN (9:1) with three different reagents (i.e. 3,5-bis(trifluoromethyl)phenyl isothiocyanate, di-*tert*-butyl dicarbonate and acetic anhydride). Control experiments in the absence of **Ox3** were first performed with 1 equiv. of 3,5-bis(trifluoromethyl)phenyl isothiocyanate added to cadaverine either in absence or in presence of 1 equiv. of PicH.[22] In both cases, a statistical ca. 1:2:1 mixture of unreacted starting material, monothiourea and dithiourea was obtained, showing that both amine groups react independently.[20]

The functionalization of the various diamines was then investigated in the presence of molecular container **Ox3** (Scheme 1) and, as a representative example, Figure 6 displays the ^{1}H spectra recorded during the process for cadaverine with the isothiocyanate. The host-guest complex with a reactive amine group protruding from the cavity (Step 1, Scheme 1 and Figure 6a-b) was first obtained through the addition of the diamine (0.9 equiv.) to host **Ox3** in the presence of PicH (0.9 equiv.). A slight excess of the host was used to ensure that no free monoammonium ion remains in the bulk solution. The subsequent addition of the reagent (0.9 equiv.) led to the formation of the complexed monofunctionalized product (Step 2, Scheme 1 and Figure 6c).[23] Finally, addition of the base 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (1 to

2 equiv.)[24] triggered the release of the monofunctionalized product through deprotonation of its ammonium group (Step 3, Scheme 1 and Figure 6d). The quantitative regeneration of free molecular container **Ox3** was observed in all cases, opening the way to its reuse for an additional cyclic three-step monofunctionalization of the diamine (*vide infra*).



Scheme 1. Iteroselective monofunctionnalization of diamines through a three-step sequence involving their supramolecular protection by **Ox3**.

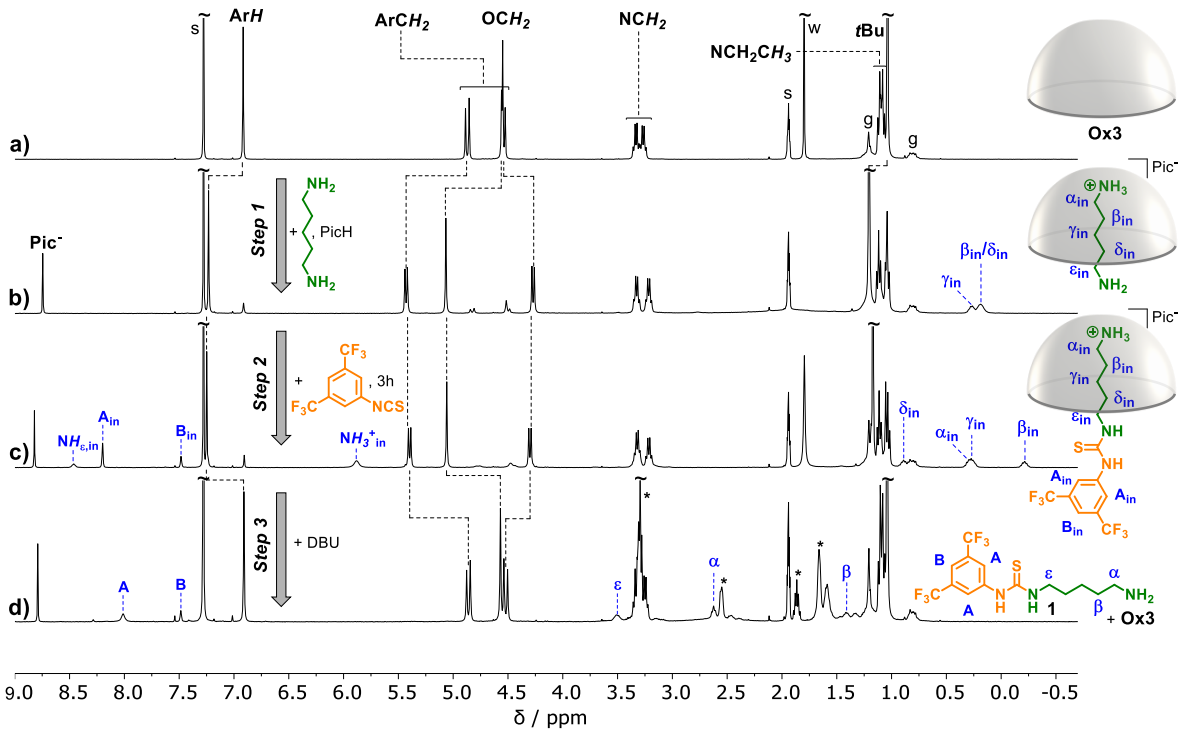


Figure 6. ^{1}H NMR spectra (298 K, 400 MHz, $CDCl_3/CD_3CN$ 9:1) of **Ox3** (3 mM) (a); after the successive addition of 0.9 equiv. of cadaverine and 0.9 equiv. of PicH (b); 0.9 equiv. of 3,5-bis(trifluoromethyl)phenyl isothiocyanate (after 3 hours) (c) and 1.3 equiv. of DBU (d). S: residual solvents; w: residual water; g: grease; *: DBU/DBU.H$^+$.

With all tested guests and reagents, a high iteroselectivity in favor of the monofunctionalized product was observed after the three-step sequence (Table 1). Remarkably, in the case of cadaverine and 1,4-Ph$(NH_2)_2$ (Table 1, entries 2 and 5), only the monofunctionalized products were observed with all three reagents (NMR yields > 93%) (see the Experimental Part). With the other diamines, very small signals resulting from the presence of the difunctionalized product were detected in the ^{1}H NMR spectra.[20] The reduced iteroselectivity observed with the smallest diamine (putrescine, n = 4) is certainly due to the smaller affinity constant for this guest combined with a decrease in the reactivity of the free amine group stemming from a steric clash between the reagent and the host (*vide supra*). For the longest diamine (n = 8), traces of the 2:1 host-guest complex between **Ox3** and the diammonium ion were observed at the protection step (Step 1), resulting in the presence of traces of free diamine in the sample.

Table 1. Monofunctionalization of diamines through supramolecular protection by host **Ox3**.

Entry	Substrate	NMR yield[a] of the monofunctionalized product (ratio[a] of mono- and difunctionalized products) using: isothiocyanate[b]	Boc_2O	Ac_2O
1	**$H_2N(CH_2)_4NH_2$** (putrescine)	85% (nd[c])	81% (8.7:1.3)	82% (nd[c])
2	**$H_2N(CH_2)_5NH_2$** (cadaverine)	> 94% (10:0)	> 93% (10:0)	> 93% (10:0)
3	**$H_2N(CH_2)_6NH_2$**	88% (nd[c])	83% (9:1)	84% (9:1)
4	**$H_2N(CH_2)_8NH_2$**	78% (nd[c])	81% (8.7:1.3)	80% (8.5:1.5)
5	**1,4-Ph$(NH_2)_2$**	> 95% (10:0)	-	-

[a] determined through integration of appropriate signals (see the experimental part).

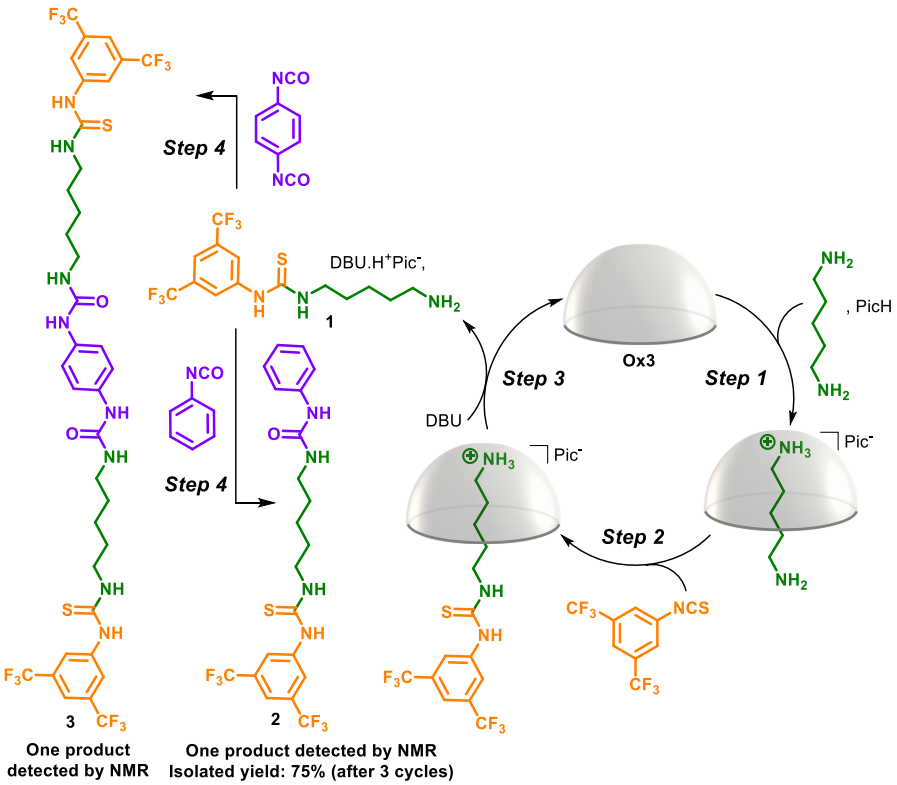
[b] i.e. 3,5-bis(trifluoromethyl)phenyl isothiocyanate.

[c] not determined because the very small ^{1}H signals of the difunctionalized product were superimposed to other signals, preventing their accurate integration.

Accumulation of the functionalized product through a *one-pot* cyclic process. The recycling of the molecular container in a one-pot cyclic process was investigated with cadaverine, the diamine with which the best results in terms of iteroselectivity were observed. Monothiourea **1** was obtained as the unique product following the three-step sequence described above (Scheme 2, steps 1-3). Phenyl isocyanate (0.9 equiv.) or 1,4-phenylene diisocyanate (0.45 equiv.) was then added and reacted *in situ* with the remaining free amino group of **1** (Scheme

2, Step 4). In both cases, no by-products were observed by ^{1}H NMR, indicating the exclusive formation of thiourea-urea **2** or dithiourea-diurea **3**. As host **Ox3** was regenerated, the cyclic four-step sequence could be repeated under similar conditions (see the Experimental Part). No differences were observed between the second and first cycles, products **1** and then **2** or **3** being quantitatively obtained without any trace of by-products.[20] This remarkable result validates the strategy that consists in accumulating selectively functionalized products through the *one-pot* recycling of the molecular container.

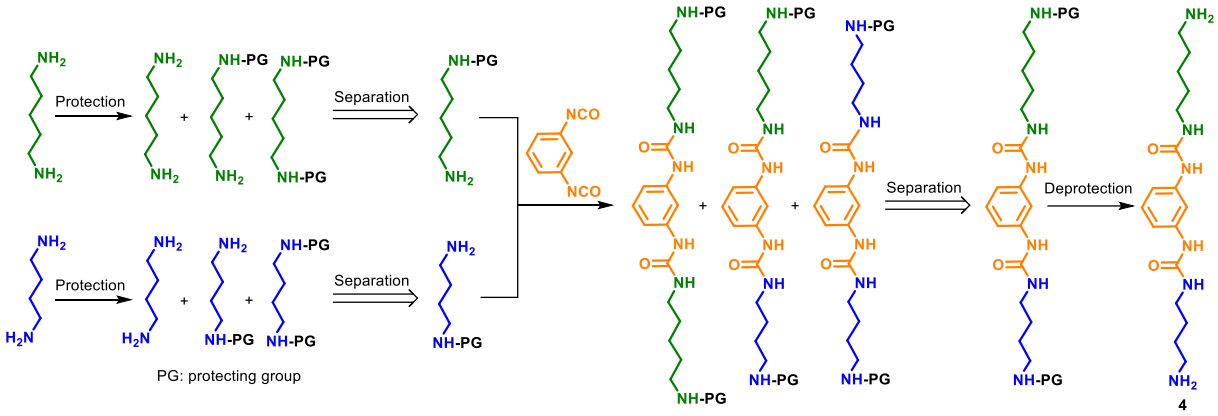
To illustrate the synthetic validity of the approach, the synthesis of thiourea-urea **2** through the four-step cyclic sequence was performed on a larger scale (1.2 10^{-2} mmol of **Ox3** instead of 1.8 10^{-3} mmol) in $CHCl_3/CH_3CN$ (9:1). After 3 cycles, only the presence of the desired thiourea-urea **2** was detected by ^{1}H NMR analysis of the crude mixture (> 90% NMR yield). After acidic washings to remove the DBU, thiourea-urea **2** was isolated through purification by flash chromatography (FC) in 75% isolated yield.[20] As one could have expected with a thiourea-urea compound, purification of **2** by FC was quite complicated, explaining in part the small discrepancy between the NMR and isolated yields.



Scheme 2. Four-step selective *one-pot* functionalization of cadaverine enabling the reuse of host **Ox3**.

Extension of the strategy to reactions between different monoprotected substrates. The next challenge was to see if the supramolecular protection approach could be applied to

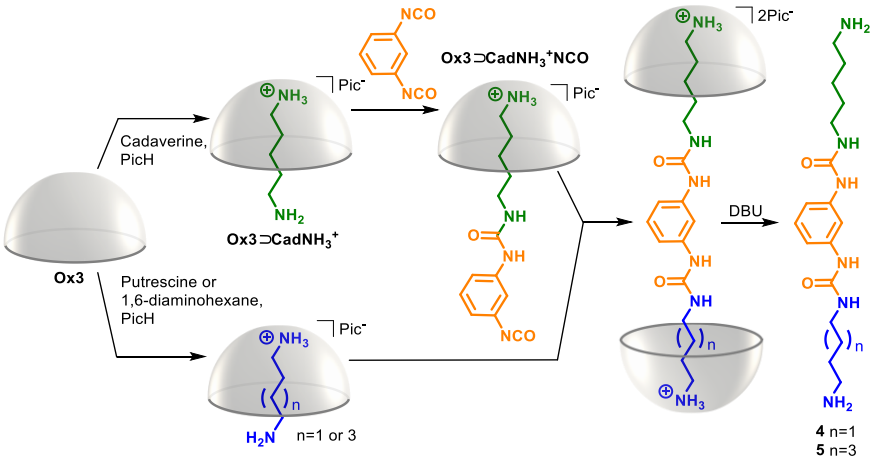
reactions between different monoprotected diamines. Such an extension of the strategy would enable the straightforward one-pot synthesis of products difficult to access through classical synthetic methods. For example, to avoid the formation of polymers, the classical preparation of the simple diamino-diurea **4** from symmetrical diamines would require a very demanding multistep synthesis involving non-selective protection/tricky separation/deprotection steps (Scheme 3). Only a very low global yield could be expected from such an approach.



Scheme 3. Synthesis of diamino-diurea **4** through a classical approach.

The one-pot syntheses of diamino-diurea derivatives **4** and **5** were investigated in $CDCl_3/CD_3CN$ (9:1) with the supramolecular protection approach using **Ox3** (Scheme 4). The host-guest complex **Ox3⊃CadNH$_3^+$** was first prepared through addition of cadaverine (0.9 equiv.) and PicH (0.9 equiv.) to **Ox3** (3 mM). The free amine group of the protected complexed guest was then left to react for 5 min with 0.9 equiv. of 1,3-phenylene diisocyanate. NMR analysis indicated the presence of the desired host-guest complex (i.e. **Ox3⊃CadNH$_3^+$NCO**) and of a trace of the 2:1 host-guest complex with as guest the symmetrical diurea-diammonium ion resulting from the reaction of the diisocyanate with two equiv. of cadaverine (Figure 7a).[20] The formation of this minor by-product is probably due to the reaction between the two host-guest complexes **Ox3⊃CadNH$_3^+$** and **Ox3⊃CadNH$_3^+$NCO** upon the addition of the diisocyanate. Note that attempts to totally prevent the formation of this by-product (either through slow addition of complex **Ox3⊃CadNH$_3^+$** to the diisocyanate or fast addition of the diisocyanate to the complex **Ox3⊃CadNH$_3^+$**) failed.[20] The ^{1}H NMR spectrum of **Ox3⊃CadNH$_3^+$NCO** remained unchanged after 20h, indicating the remarkable stability of the included guest.[20] The preparation and stabilization of such an ammonium-isocyanate derivative illustrates the power of the supramolecular protection approach. Further reaction of **Ox3⊃CadNH$_3^+$NCO** with 0.9 equiv. of another diamine monoprotected by **Ox3** (either

putrescine or 1,6-diaminohexane) led to the clean formation of the doubly protected diammonium-diurea in ca. 80% NMR yield for **4** and **5** (Scheme 4 and Figure 7b-c). No new by-products were formed during this step. Full assignment of the ^{1}H NMR signals of the 2:1 host-guest complexes was achieved with COSY and TOCSY NMR spectra.[20] Finally, addition of DBU (2 equiv.) led to formation of the free deprotected products **4** and **5** which were then isolated pure through solid-liquid extraction in CH_2Cl_2.[20]



Scheme 4. One-pot selective synthesis of diamino-diurea derivatives **4** and **5** through supramolecular protection by **Ox3**.

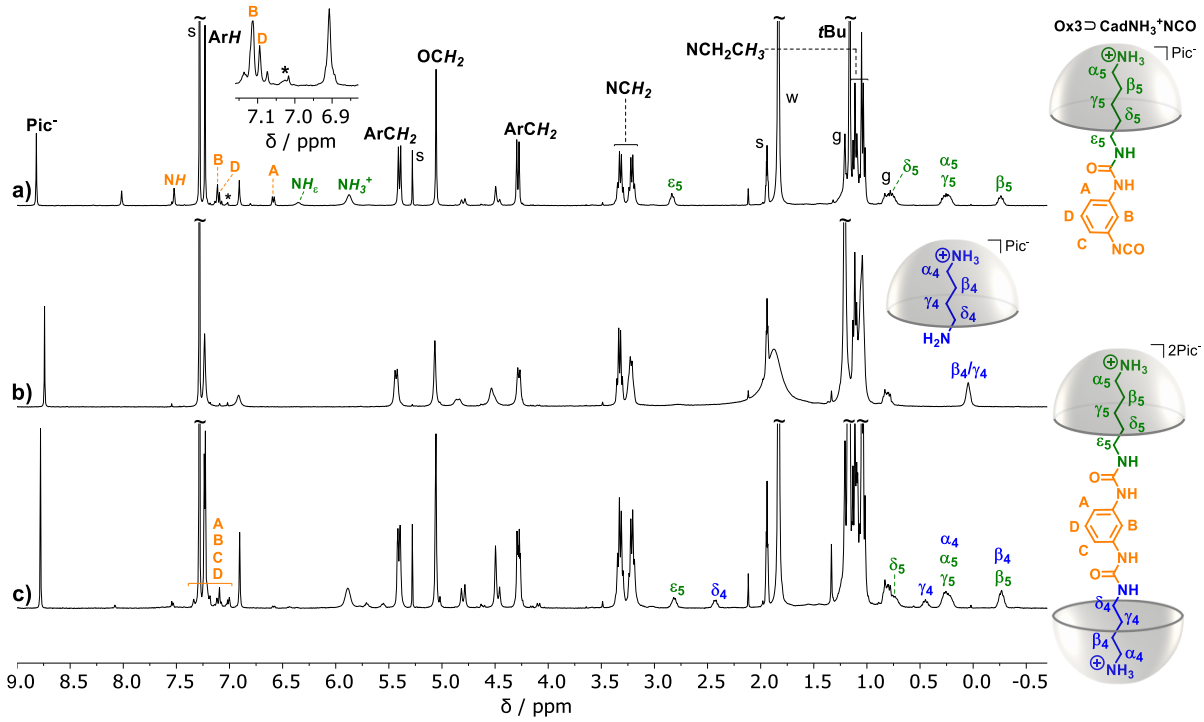
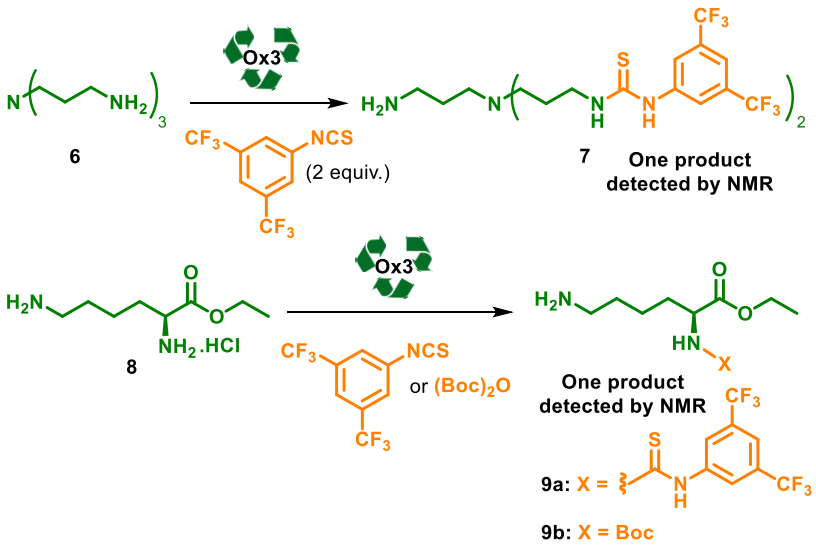


Figure 7. ^{1}H NMR spectra (298 K, 400 MHz, $CDCl_3/CD_3CN$ 9:1) of **Ox3** (3 mM) + 0.9 equiv. cadaverine + 0.9 equiv. PicH + 0.9 equiv. 1,3-phenylene diisocyanate (a); **Ox3** (3 mM) + 0.9 equiv. putrescine + 0.9 equiv. PicH

(b) and protected diamino-diurea derivative **4.2H**$^+$ (c). S: residual solvents; w: residual water; g: grease; *: symmetrical diurea-diammonium by-product.

Selective functionalization of complex polyamines through their supramolecular protection by Ox3. In a further series of experiments, we evaluated the potential of the supramolecular protection approach for the selective functionalization of more complex polyamines such as lysine derivatives and a triamine. The substrates were reacted with various reagents in the presence of molecular container **Ox3** (Scheme 5). First, the functionalization of tris(3-aminopropyl)amine **6** with 3,5-bis(trifluoromethyl)phenyl isothiocyanate was monitored in the presence of PicH (in $CDCl_3/CD_3CN$, 9:1). Difunctionalized product **7** was obtained as the only observable species (91% NMR yield) when using 2 equiv. of the isothiocyanate.[20] This result highlights the efficient protection provided by **Ox3** for one of the three amino groups of **6** and opens the door to the iteroselective functionalization of all but one amine groups of polyamines. Even more remarkably, L-lysine ethyl ester.HCl **8** was regio- and iteroselectively monofunctionalized (in $CDCl_3/CD_3OD$, 4:1) at the level of its less reactive *N*-terminal group, leading to products **9a** (90% NMR yield) or **9b** (95% NMR yield) as the only observable species (Scheme 5).[20] The regioselectivity originates from the selective supramolecular protection of the amine group of the side chain for steric reasons. The fact that molecular containers can provide a size and shape cavity-based selectivity[25] represents another advantage of their use as protection units.



Scheme 5. Selective functionalization of polyamines **6** and **8** thanks to the supramolecular protection by **Ox3**.

Conclusions

We have demonstrated that **Ox3** is able to bind the monocationic form of a family of diamines, with the ammonium group deeply inserted in the cavity of the receptor, and the second amino group protruding from the host cavity and thus accessible for a chemical transformation. These host-guest properties have been exploited in a supramolecular protection strategy for the monofunctionalization of various diamines. Selective monofunctionalization was readily obtained with different types of reagents and only one product was detected by NMR in most cases. A high host-guest affinity and a control of the host-guest stoichiometry were shown to be key parameters to achieve high iteroselectivity.

We have also shown that the use of **Ox3** presents many synthetic advantages over strategies using traditional protecting groups:

i) The supramolecular protection step is specific to primary amines and more specifically to those deeply included in the cavity of the molecular container, enabling high chemo-, regio- and iteroselectivities;

ii) The protection/functionalization/deprotection sequence is a one-pot process and the supramolecular character of the protection/deprotection steps allows to reach high yields for the whole sequence;

iii) The acid-base control of the host-guest properties enables the recycling of the receptor and, as a consequence, the accumulation of the functionalized product without any loss of selectivity over several cycles;

iv) Unstable intermediates bearing two non-compatible reactive groups, such as a primary ammonium bearing an isocyanate group, whose preparation would be highly challenging through classical methods can be stabilized and their reactivity can be controlled through the supramolecular protection of one of their reactive groups;

v) Products that are very difficult to access through classical synthetic methods, such as non-symmetric diamino-diureas, can be readily synthesized in high yield.

These results open the way to interesting perspectives in the field of organic synthesis. Given the recyclability of the receptor, different successive reactions, such as peptide coupling reactions with different amino acids, can be envisaged in a one-pot process using a difunctional substrate. With a molecular container displaying a chiral cavity, high stereoselectivity should also be possible, in addition to the chemo-, regio- and iteroselectivities. Automation of the process could lead to large scale syntheses, and the grafting of the receptor on a surface would enable an easier separation of the product and recycling of the host. Current research in our group is focused on the extension of the methodology to other types of host-guest systems and other types of stimuli for the control of the capture/release of the guest. The use of receptors

with an extended cavity, in order to have a better control over the host-guest stoichiometry and thus the iteroselectivity, is also under investigation.

Experimental Section

General Information. All solvents were reagent grade. Silica gel (230−400 mesh) was used for flash chromatography purifications. ^{1}H NMR spectra were recorded at 400 or 600 MHz and ^{13}C NMR spectra were recorded at 100 MHz. NMR parameters (acquisition time, recycling times and signal accumulation) were chosen to ensure that quantitative data could be obtained from signal integration in the ^{1}H NMR spectra. The NMR spectra were recorded at 298 K unless otherwise stated. Chemical shifts are expressed in ppm. Traces of residual solvents were used as internal standards. $CDCl_3$ was filtered through a short basic alumina column to remove traces of DCl. The ^{1}H NMR signals were assigned on the basis of 1D and 2D NMR analyses (COSY). NMR signals multiplicity are described according to the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), m (multiplet) and br s (broad signal). **Ox3** was prepared as previously described.[26]

NMR Titration Experiments. All experiments were prepared following a similar protocol. A known volume (~600 μL) of a solution of known concentration of the host (~3 mM) was placed in an NMR tube, and the ^{1}H NMR spectrum recorded at 400 MHz. Aliquots of a stock solution of the guest (and PicH) were successively added, and the ^{1}H NMR spectrum recorded after each addition. In general, aliquots were added until no changes in the host signals were observed. In all cases, two (or more when 2:1 complexes are observed) sets of signals were observed over the course of the titration, indicating slow host–guest exchanges on the ^{1}H NMR chemical shift timescale. Association constants (log K_a) were determined via integration of the signals of the different species (host, guest and complex) when possible. The association constants were determined as the mean value of the constants calculated based on different spectra and with the integration of different signals. The error was then estimated as the difference between this mean value with the smallest and largest association constants determined. In the cases where free host and free guest could not be observed at the same time over the course of the titration, the association constant is too high to be determined precisely (log $K_a > 5$).

Monofunctionalization of the diamines. A solution (600 μL) of **Ox3** (3 mM) was prepared in $CDCl_3/CD_3CN$ (9:1). During step 1, 0.9 equiv. of the diamine and 0.9 equiv. of PicH were added (supramolecular protection). At step 2, 0.9 equiv. of reagent was added (monofunctionalization). Once the reaction is complete, DBU (1-2 equiv.) was added for step 3 (deprotection). In some cases, a second reagent (0.9 or 0.45 equiv.) is added for a step 4

(second functionalization). To determine the amount of the different species added or formed, **Ox3** was used as an internal standard. Integration of the Ar*H*, ArCH_2 and OCH_2 (free + complex) signals were used and compared to the integration of the signals of the species added or formed.

Determination of the NMR yields of the monofunctionalized products. Integration of the signals attributed to the protected monofunctionalized product were compared to integration of the signals of the receptor (complexed + free) during step 2 of the monofunctionalization of the diamines. From the integration of these signals, an estimated number of moles for the monofunctionalized product was determined and compared to the amount of diamine added to determine the NMR yields.

One-pot synthesis of thiourea-urea 2 through three subsequent cycles. To a solution (600 µL) of **Ox3** 20 mM (11.0 mg, 1.2 10^{-2} mmol) in $CDCl_3/CD_3CN$ (9:1), cadaverine (54 µL solution at 0.2 M, 0.9 equiv.) and picric acid (PicH) (54 µL solution at 0.2 M, 0.9 equiv.) were added forming the complex **Ox3⊃CadNH$_3^+$**. 3,5-bis(trifluoromethyl)phenyl isothiocyanate (54 µL solution at 0.2 M, 0.9 equiv.) was then added to give the protected monothiourea product **1** after 3h. Subsequent addition of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (90 µL solution at 0.2 M, 1.5 equiv.) gave the deprotected amino-thiourea product that upon addition of phenyl isocyanate (54 µL solution at 0.2 M, 0.9 equiv.) afforded compound **2**. A second cycle was performed with the recovered molecular container **Ox3**. Accumulation of product was performed without prior purification or separation starting with additions of cadaverine (54 µL solution at 0.2 M, 0.9 equiv.) and PicH (90 µL solution at 0.2 M, 1.5 equiv.), followed by subsequent additions of 3,5-bis(trifluoromethyl)phenyl isothiocyanate (54 µL solution at 0.2 M, 0.9 equiv.), then after 3h DBU (90 µL solution at 0.2 M, 1.5 equiv.) and finally phenyl isocyanate (54 µL solution at 0.2 M, 0.9 equiv.). A third cycle was then performed in the same conditions as the second cycle with additions of cadaverine (54 µL solution at 0.2 M, 0.9 equiv.), PicH (90 µL solution at 0.2 M, 1.5 equiv.), 3,5-bis(trifluoromethyl)phenyl isothiocyanate (54 µL solution at 0.2 M, 0.9 equiv.), then after 3h DBU (90 µL solution at 0.2 M, 1.5 equiv.) and phenyl isocyanate (54 µL solution at 0.2 M, 0.9 equiv.). The mixture was then washed with HCl 1 M (2 x 1 mL). The organic phase was evaporated under reduced pressure. Purification through flash chromatography (CH_2Cl_2/EtOAc 80:20) then afforded compound **2** as a white solid (12.0 mg, 75% yield). Mp = 66°C. IR (cm^{-1}): υ = 1659, 1549, 1276, 1131. ^{1}H NMR (298 K, $CDCl_3$, 400 MHz): δ (ppm) 8.81 (s, 1H, N*H*), 7.89 (s, 2H, Ar*H*), 7.58 (s, 1H, N*H*), 7.55 (s, 1H, Ar*H*), 7.24-7.15 (m, 4H, Ar*H*), 7.00 (quint, 1H, *J* = 4.4 Hz, Ar*H*), 6.94 (s, 1H, N*H*), 5.32 (t, 1H, *J* = 6.0 Hz, N*H*CH_2), 3.56 (br s, 2H, NHCH_2), 3.26 (q, 2H, *J* =

6.4 Hz, NHC*H*$_2$), 1.63 (quint, 2H, *J* = 6.8 Hz, NHCH$_2$C*H*$_2$), 1.52 (quint, 2H, *J* = 6.6 Hz, NHCH$_2$C*H*$_2$), 1.40 (quint, 2H, *J* = 7.1 Hz, NHCH$_2$CH$_2$C*H*$_2$). ^{13}C NMR (298 K, $CDCl_3$, 100 MHz): δ (ppm) 181.4, 157.3, 140.7, 137.8, 131.7, 129.5, 124.7, 123.9, 121.9, 121.5, 118.1, 44.7, 39.1, 29.5, 27.1, 23.4. ESI-MS (+) *m/z* 493.25 ([M + H]$^+$, 100%).

One-pot synthesis of diamino-diurea derivative 4. To a solution (600 µL) of **Ox3** 3 mM in $CDCl_3$/CD_3CN (9:1), cadaverine (54 µL solution at 30 mM, 0.9 equiv.) and picric acid (PicH) (54 µL solution at 30 mM, 0.9 equiv.) were added forming the complex **Ox3⊃CadNH$_3^+$**. 1,3-phenylene diisocyanate (54 µL solution at 30 mM, 0.9 equiv.) were then added to give the protected ammonium-urea product after 5 min. Addition of a $CDCl_3$/CD_3CN (9:1) solution (540 µL) of **Ox3** 3 mM, putrescine 2.7 mM and picric acid (PicH) 2.7 mM afforded protected compound **4**. 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (200 µL solution at 30 mM) was then added to deprotect compound **4**. The mixture of solvents ($CDCl_3$/CD_3CN (9:1)) was evaporated under reduced pressure. Purification through solid-liquid extraction in CH_2Cl_2 then afforded compound **4** as a white solid. As the reaction was achieved on a very small scale (amount of **4** expected with a 100 % yield: 0.6 mg), an amount of less than 1 mg of **4** was isolated and the yield cannot be thus calculated with accuracy. ^{1}H NMR (298 K, D_2O, 400 MHz): δ (ppm) 7.31 (t, 1H, *J* = 8.2 Hz, Ar*H*), 7.31 (s, 1H, Ar*H*), 7.02 (d, 2H, *J* = 8.2 Hz, Ar*H*), 3.23 (t, 2H, *J* = 6.9 Hz, NHC*H*$_2$), 3.20 (t, 2H, *J* = 7.0 Hz, NHC*H*$_2$), 3.03 (t, 2H, *J* = 8.6 Hz, NH$_2$C*H*$_2$), 3.01 (t, 2H, *J* = 8.4 Hz, NH$_2$C*H*$_2$), 1.77-1.65 (m, 4H, NH$_2$CH$_2$C*H*$_2$), 1.65-1.52 (m, 4H, NHCH$_2$C*H*$_2$), 1.42 (quint, 2H, *J* = 8.4 Hz, NH$_2$CH$_2$CH$_2$C*H*$_2$). ESI-MS (-) *m/z* 385.33 ([M + Cl]$^-$, 100%).

One-pot synthesis of diamino-diurea derivative 5. To a solution (600 µL) of **Ox3** 3 mM in $CDCl_3$/CD_3CN (9:1), cadaverine (54 µL solution at 30 mM, 0.9 equiv.) and picric acid (PicH) (54 µL solution at 30 mM, 0.9 equiv.) were added forming the complex **Ox3⊃CadNH$_3^+$**. 1,3-phenylene diisocyanate (54 µL solution at 30 mM, 0.9 equiv.) were then added to give the protected ammonium-urea product after 5 min. Addition of a $CDCl_3$/CD_3CN (9:1) solution (540 µL) of **Ox3** 3 mM, 1,6-diaminohexane 2.7 mM and picric acid (PicH) 2.7 mM afforded protected compound **5**. 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (200 µL solution at 30 mM) was then added to deprotect compound **5**. The mixture of solvents ($CDCl_3$/CD_3CN (9:1)) was evaporated under reduced pressure. Purification through solid-liquid extraction in CH_2Cl_2 then afforded compound **5** as a white solid. As the reaction was achieved on a very small scale (amount of **5** expected with a 100 % yield: 0.7 mg), an amount of less than 1 mg of **5** was isolated and the yield cannot be thus calculated with accuracy. ^{1}H NMR (298 K, D_2O, 400 MHz): δ (ppm) 7.30 (t, 1H, *J* = 8.0 Hz, Ar*H*), 7.29 (s, 1H, Ar*H*), 7.01 (d, 2H, *J* = 8.0 Hz, Ar*H*),

3.20 (t, 2H, J = 6.7 Hz, NHCH_2), 3.18 (t, 2H, J = 6.4 Hz, NHCH_2), 3.00 (br s, 4H, NH_2CH_2), 1.75-1.61 (m, 4H, $NH_2CH_2CH_2$), 1.61-1.49 (m, 4H, $NHCH_2CH_2$), 1.47-1.34 (m, 6H, J = 8.4 Hz, $NH_2CH_2CH_2CH_2$ and $NHCH_2CH_2CH_2$).

Molecular Modeling. Monte Carlo multiple minimum (MCMM)[27] conformational searches (100 steps per torsion angle, maximum 1000 steps in total) were performed in Schrödinger Release 2019-1, using the OPLS3 or the MMFF force field[28] with $CHCl_3$ as selected solvent in Maestro MacroModel (version 11.9.011).

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Supporting information: NMR spectra (^{1}H, COSY) relative to the binding studies and mono- or difunctionalization reactions (PDF). The Supporting Information is available free of charge at https://

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[20] See the Supporting Information.

[21] The detection of the 2:1 host-guest complex (<10%) suggests the presence of a small amount of free non-protonated diamine. However, the minor ^{1}H signals of the free diamines were not detected as they were superimposed to other signals.

[22] The control experiment in presence of 1 equiv. of PicH was conducted in $CDCl_3/CD_3OD$ (4:1) because of the low solubility of the doubly protonated cadaverine in $CDCl_3/CD_3CN$ (9:1).

[23] While the reaction with acetic anhydride was conducted in absence of a base, full conversion of the cadaverine was still observed in 24 h, indicating that the AcOH formed during the reaction is not acidic enough under these conditions to prevent the acetylation reaction.

[24] In the case of the monoprotection with Ac_2O, it was necessary to add 2 equiv. of DBU as 1 equiv. of AcOH was formed during the acetylation reaction.

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