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"Choose-a-Size" Approach in Dynamic Combinatorial Chemistry: A Single Substrate Dynamic Combinatorial Library of Oligomacrocycles That Adapts to the Size and Shape of Carboxylates

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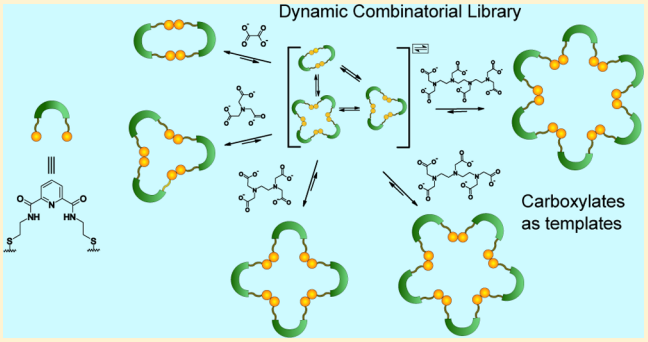
Supporting Information

ABSTRACT: A neutral anion binding receptor based on dipicolinic acid diamide was equipped with thiol groups in the amidic side arms. After the thiol was oxidized to disulfide groups with I_2, a mixture of cyclic oligomers (a library) was obtained. The distribution of macrocycles can be controlled kinetically during the oxidation process or thermodynamically at basic conditions via disulfide bond exchange. The library proved to be very sensitive to templation with various carboxylates in DMSO. The amplification pattern reflects the structural features of the anionic template and is sensitive to changes in the template's geometry. The application of carboxylates with multiple functional groups resulted in very strong amplification of the large penta- and hexameric macrocycles. The thermodynamic parameters of some templation effects were rationalized using a simple model and confirmed using competitive NMR titration.



1. INTRODUCTION

Selective recognition of organic molecules is a common yet crucial phenomenon in nature. It is responsible for many functions, such as communication within multicellular organisms (hormones, neurotransmitters), communication between organisms (pheromones), sensing the environment (smell, taste), and immunological response (antigen−antibody).

Compared to biological systems, artificial receptors are still far less selective and form weaker bonds. Studies in this field are driven by the need to fully understand the recognition processes taking place in nature and by the need for selective receptors in many practical applications.[1−3]

Organic molecules may adopt one of virtually unlimited variations in size, shape, and rigidity, combined with functional group distribution. Therefore, in principle, the molecules differ significantly, and a selective receptor can be designed rationally. However, any receptor design will always require experimental confirmation, and this is the most laborious step in the search for novel hosts. Dynamic combinatorial chemistry[4−11] (DCC) is a very useful tool in this regard, as it combines synthesis of multiple species with their evaluation in a single experiment that yields unambiguous results. This methodology was initially successfully applied for inorganic cations[12−25] and anions,[26−34] yet recently it also covered many types of organic molecules including ammonium cations,[35−45] barbituranes,[46,47] peptides,[48,49] nucleotides,[50,51] or even fullerenes.[52]

Organic anions remain relatively unexplored in DCC studies.[53−56] Anionic groups of oligocarboxylates act as well-defined anchoring points separated by various linkers, which makes these guests useful in model studies of molecular recognition. The total number of carboxylic groups and the size, shape, and flexibility of the linker are simple variables describing a family of guests. By analyzing the response of the dynamic combinatorial library (DCL) to the introduction of such anionic templates, the privileged structures of the highly selective and strongly binding receptors can be observed.

In this paper, we describe a DCL consisting of neutral macrocyclic anion receptors of various sizes, made from a single building block. The composition of the library is strongly affected by the anionic templates, and the changes reflect the structural parameters of the guests.

2. RESULTS AND DISCUSSION

2.1. Synthesis. The library substrate **1** is built on a dipicolinic acid bisamide motif, which has been found to be efficient in anion binding in pioneering work by Crabtree.[57] Macrocycles of various sizes employing this motif have been explored intensively in our group.[58−63] In this study, we equipped the receptor with arms containing a thiol group to enable reversible formation of oligomeric species. The receptor

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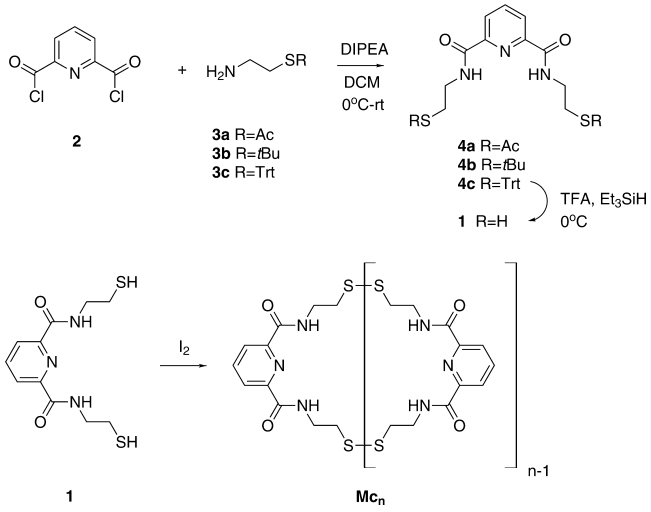
1 was synthesized from dipicolinic acid dichloride **2** and S-protected cysteamine (**3**). Acetyl protection of the thiol group (**3a**) was found to be too labile and bisamide **4a** could not be satisfactory purified; *tert*-butyl protection (**3b**), on the contrary, was too stable, and we could not deprotect **4b**. Finally, trityl protection proved to be very useful, with an efficient, easy multigram synthesis[64] of **3c** and mild, quantitative removal of the protecting groups, yielding **1**.

2.2. Library Formation. Kinetic Control. Upon oxidation of the thiol groups, disulfide can be obtained, which is stable under neutral and acidic conditions but may undergo exchange under basic conditions. Typically, oxidation is performed by exposing the aqueus solution of thiols to atmospheric oxygen.[65] A similar process occurring in water−DMSO mixtures or 100% DMSO as a solvent was recently described.[66] In our case, no reaction of **1** was observed even when pure oxygen was passed through the DMSO solution for several hours. Instead, the oxidation proceeded smoothly and quantitatively by reacting with I_2 (Scheme 1).

Scheme 1. Synthesis of Building Block 1 and Its Oxidation, Resulting in a Mixture of Oligomeric Macrocycles Mc_n



The products of this oxidation were analyzed by RP-HPLC, and it was determined that they are composed solely of macrocyclic oligomers Mc_n (Figure 1). No cyclic monomers (Mc_1) were found in the mixture, which indicates that such a 13-membered ring would be too strained to form. Cyclic oligomers with n = 2−9 were resolved and identified by LC-HRMS.

Under acidic conditions (hydrogen iodide evolved in the reaction), the library is kinetically stable (frozen) and its composition is the result of kinetic control. Because the oxidation reaction proceeds immediately, we could modify the Mc_2−Mc_9 distribution by changing the mode of adding the reagents. In the first mode, adding I_2 to a solution of **1**, the reaction proceeds for a high concentration of **1**, which facilitates the formation of larger oligomers. The second mode, adding **1** to I_2 solution, with a lower **1** concentration, results in the relative amplification of smaller macrocycles. Finally, a high dilution technique where both reagents were added via syringe pump to a large volume of solvent resulted in nearly quantitative formation of Mc_2, as depicted in Figure 2. We were able to isolate the pure compounds Mc_2−Mc_5 by using preparative RP-HPLC.

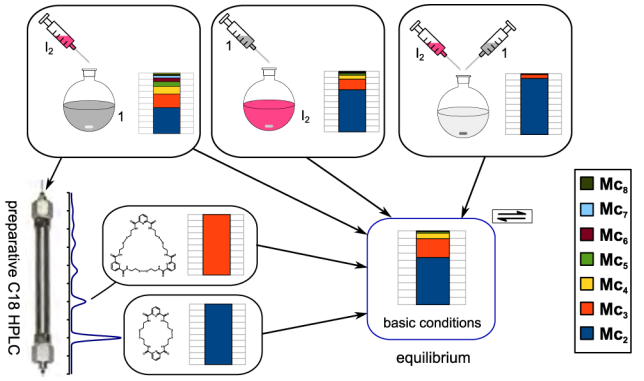


Figure 2. Kinetically controlled libraries and isolated single macrocycles equilibrate upon addition of base into a mixture of common composition. The bars represent the mass% compositions of the samples.

2.3. Equilibration. Thermodynamic Control. The mixtures obtained by the three different modes of oxidation reaction described above (after removing HI), as well as solutions of pure Mc_2 or Mc_3, were treated with 5 mol % of tetrabutylammonium hydroxide (TBAOH) to initiate library equilibration via disulfide bond exchange. After the mixture was stirred overnight, the same composition was found in all five cases, which shows that the distribution of the macrocycles is controlled purely thermodynamically.

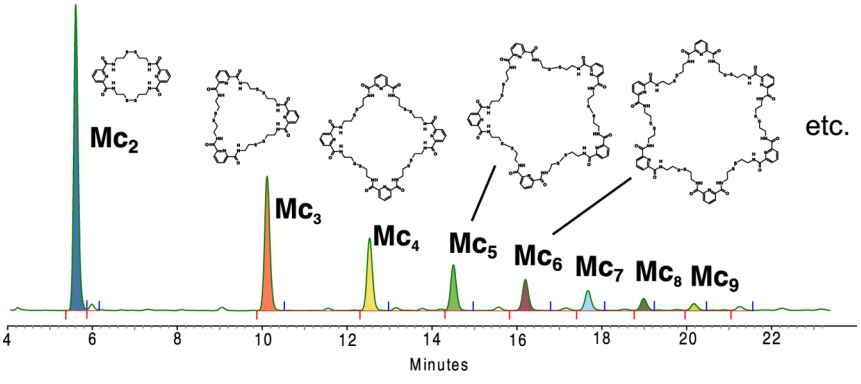


Figure 1. HPLC trace of the mixture obtained upon oxidation of **1** with iodine.

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A detailed kinetic study of equilibration was performed with a pure $\mathbf{Mc}_2$ component, which was equilibrated by adding TBAOH; higher oligomers were formed at the cost of $\mathbf{Mc}_2$, whose concentration decreased. As indicated by the graph in Figure 3, the reaction is fast and the concentration plateau of all

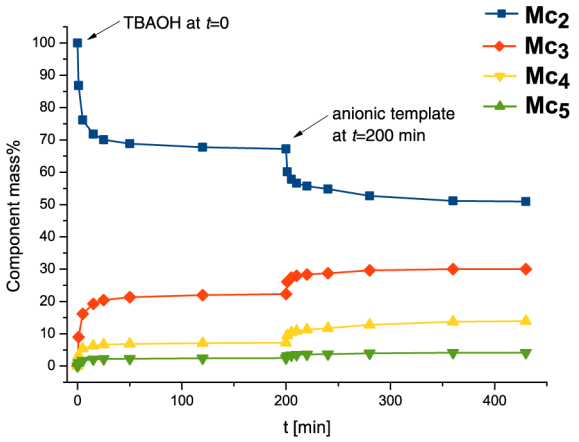

Figure 3. Kinetics of DCL equilibration. A solution of $\mathbf{Mc}_2$ was treated with 5 mol % of TBAOH at $t = 0$. Once equilibrium was reached, an anionic template (TBA salt of timesic acid) was introduced at $t = 200$ min.

species is reached within ca. 2 h. A similar time is required by the system to fully respond to stimulus via a template addition (see further in text).

The library composition under equilibrium conditions was measured for various $[\mathbf{Mc}_n]_0$ total concentrations, and the data were matched using DCLfit software tool[67] (see Supporting Information for details). The results show that the ratio of concentrations of the following macrocycles $([\mathrm{Mc}_n])/([\mathrm{Mc}_{n+1}])$ is constant (for a given $[\mathbf{Mc}_n]_0$) for the whole series apart from the first ratio, $([\mathrm{Mc}_2])/([\mathrm{Mc}_3])$, which is higher. This indicates that the dimer is slightly more stable than other oligomers, whose distribution can be described with a single aggregation constant.[68]

2.4. Templation Studies. In the next step, a set of 18 organic anions was used for DCL templation (Chart 1). All guests but one influenced the distribution of $\mathbf{Mc}_2$–$\mathbf{Mc}_9$; only in the case of malonate did we observe the disappearance of all HPLC peaks corresponding to macrocycles. The malonate with its nucleophilic carbon atom reacted with the disulfide bonds to form thiols and thioesters (see Supporting Information for details). We therefore employed unreactive 2,2-dimethylmalonate as a template of similar shape and size. Our set of organic templates consists of TBA salts of carboxylates with one to six carboxylic groups and squaric acid. For each template, we ran DCC experiments with several concentrations of guests (see Supporting Information). In some cases, the trend of amplification was quite regular (Figure 4a,b), while other templates induced changes with an irregular course (Figure 4c,d).

We analyzed the templated DCLs in terms of changes in library composition (Figure 5a) and normalized amplification factors[45] AF_n (Figure 5b):

$$\mathrm{AF}_n(\mathrm{A}, \mathrm{T}) = \frac{[\mathrm{A}]_\mathrm{T} - [\mathrm{A}]_0}{[\mathrm{A}]_{\max} - [\mathrm{A}]_0} \quad (1)$$

where A denotes library member, $[\mathrm{A}]_0$, $[\mathrm{A}]_\mathrm{T}$ are concentrations of A in untemplated and templated libraries, and $[\mathrm{A}]_{\max}$ denotes the maximum possible concentration of A (limited by the amounts of substrates). These results are presented in Figure 5.[69]

We managed to rationalize all the observed templation effects in this relatively simple single substrate system. Before we present the detailed analysis of the guests, some general remarks are required. In principle, each carboxylate group can be bound by one dipicolinic acid diamide moiety found in the monomer. We would expect that for a given number (n) of carboxylic groups in the template, $\mathbf{Mc}_n$ is the fittest host. If $\mathbf{Mc}_n$ cannot accommodate the guest for geometrical reasons, a larger ($\mathbf{Mc}_{n+1}$) should turn out to be the optimal one. As well as the fittest host, the templates can also be bound by smaller oligomers, leaving anionic group(s) uncomplexed, and by larger ones, leaving the host's hydrogen bond donor-free. Another important factor is highlighted by an analysis of the influence of the simplest guest–benzoate (**A**). This templation experiment was successfully fitted using DCLfit, and the association constants for complexes with all macrocycles were found, as expected, to be at the same level ($\log(K_a) \approx 2.5$). Although there is no preference for the host, the smallest one is gradually amplified, at the expense of all other macrocycles, with an increasing guest concentration (see Figure 4a). Shifting the equilibrium toward smaller components results in the formation of a higher number of receptor molecules, which is desirable for the system especially when the template is used in excess.[70] This trend must be taken into account in any analysis of the remaining templates and indicates that the amplification of higher oligomers is usually suppressed.

A subset of templates **B**–**F** consists of dianions with systematically increasing distances between the functional groups. Squarate (**B**) seems to fit well in the relatively small cavity of $\mathbf{Mc}_2$ and amplifies it to 90% by mass ($\mathrm{AF}_n = 0.69$). As the chain of the dianionic template elongates, the dimeric host gradually becomes less favored and the abundances of higher oligomers are systematically increased.

Although the distance between anchoring points in acetylenedicarboxylate (**G**) is between the distances in **E** and **F**, its behavior is quite different due to its high rigidity. In contrast to aliphatic templates, **G** has very restricted conformational freedom and $\mathbf{Mc}_3$ was found to match the geometry of this anion. Another rigid template–fumarate (**H**) has very little impact on the DCL, similarly to succinate (**E**) of analogous length. This seems to be a consequence of a special coincidence of association constants which results in zero net effect, although both guests do bind to the receptors.

Among the series of three phthalates, only isophthalate (**J**) significantly amplified the trimer, which can match both size and shape of this carboxylate. *o*-Phthalate (**I**) has nearly no effect probably due to the close proximity of the carboxylic groups and their unfavorable arrangement. In the case of terephthalate (**K**), the distance between binding points is about 5.7 Å, and $\mathbf{Mc}_4$ is the smallest host to bind both carboxylate groups; however, the formation of hydrogen bonds by just two pairs of amide groups does not provide an association constant large enough to amplify the tetrameric host.

Because the isophthalate turned out to fit to $\mathbf{Mc}_3$, we expected that trimesic acid trianion **L**, as an expansion of isophthalate with all carboxylate groups in relative meta positions, should amplify $\mathbf{Mc}_3$ even more strongly. The experimental results indicate, however, that $\mathbf{Mc}_4$ is the best

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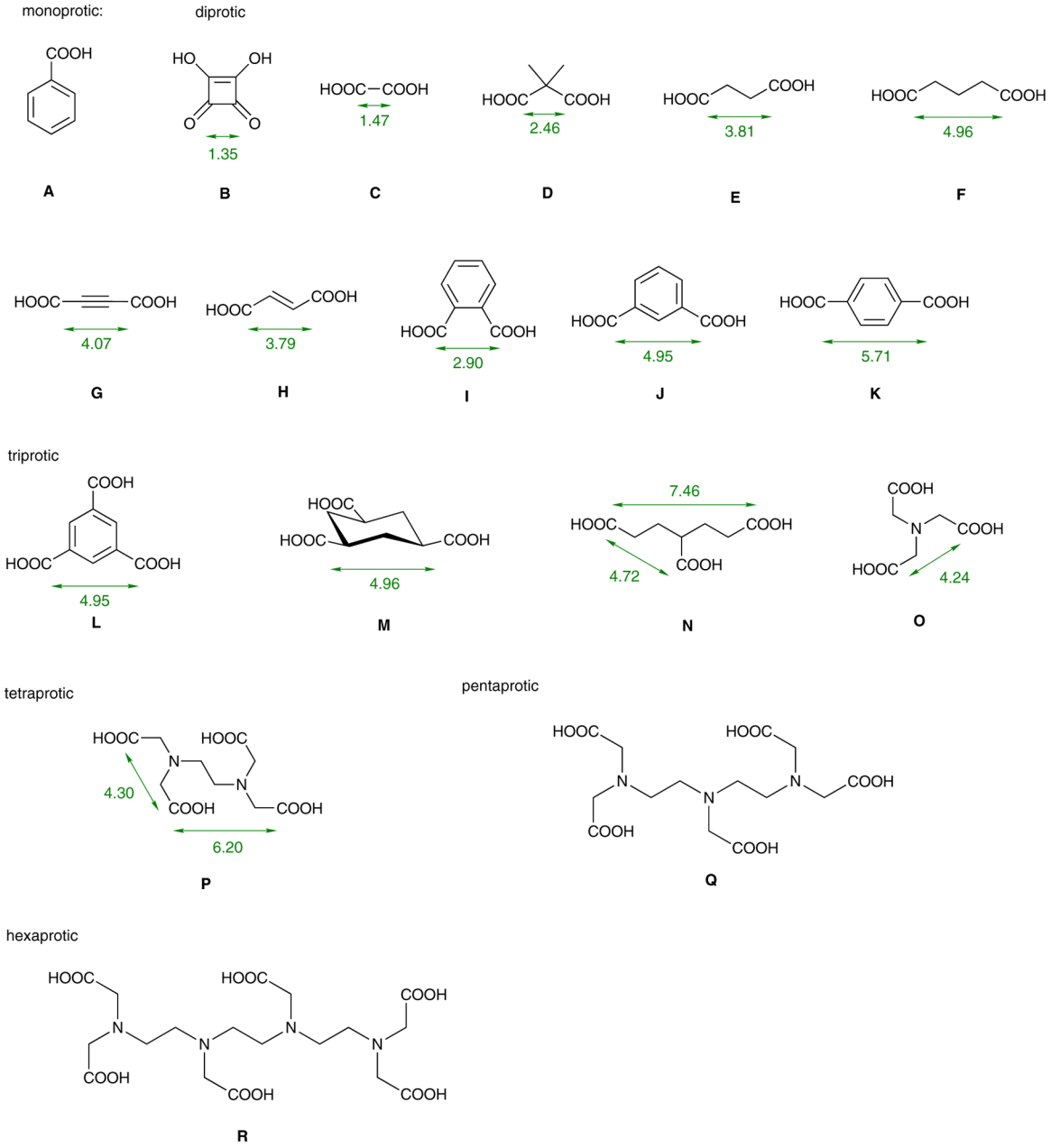
Chart 1. Organic Acids Used in Templation Experiments as the Respective TBA Salts[a]



[a]Approximate distance in angstroms between carbon atoms of carboxylic groups is given for selected acids (molecular mechanics, lowest energy conformer).

suited host and binds **L** stronger than $\mathbf{Mc}_3$. According to simple modeling, a trimesic acid anion can fit in the cavity of $\mathbf{Mc}_3$, but the binding must induce some strain in the macrocycle and limits its conformational freedom (loss of entropy). $\mathbf{Mc}_4$ can easily adapt to the size of the guest and is significantly amplified, although one dipicolinic moiety is left free. Quite interestingly, a template with very similar geometrical demands, 1,3,5-cyclohexyltricarboxylate (**M**), gives a different amplification profile, with $\mathbf{Mc}_3$ being the most amplified oligomer (AF_n = 0.21). This observation indicates that a slight change in the size and flexibility of the guest can strongly affect the distribution of library members. We can also conclude that in the formation of supermolecules, both host and guest adapt their geometries to maximize binding interactions.

Another two of the tricarboxylates tested lack rings and are therefore even more flexible. Template **N** amplified the trimeric host slightly higher than that for **M** (AF_n = 0.27), while nitrilotriacetic acid salt (NTA, **O**) was found to be perfectly suited to match the cavity of $\mathbf{Mc}_3$. The addition of 0.5 equiv of this template resulted in the formation of a trimer at 67% (by mass) and AF_n = 0.58. This excellent match is most probably due to the appropriate size for the guest, its flexibility, and C_3 symmetry.

Encouraged by the former result, we decided to employ other members of the aminopoly(carboxylic acid)s: EDTA (**P**), DTPA (**Q**), and TTHA (**R**) as templates. We expected these guests to selectively amplify the oligomeric host consistent with the number of carboxylic groups in the template. This assumption turned out to be true for tetra-, penta-, and

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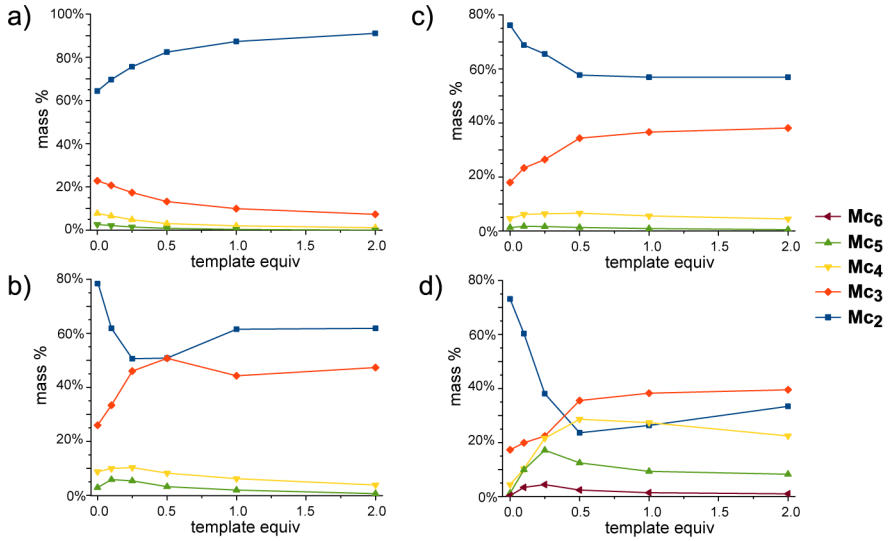

Figure 4. Various amplification trends induced by anions (a) **A**, (b) **G**, (c) **J**, (d) **Q**.

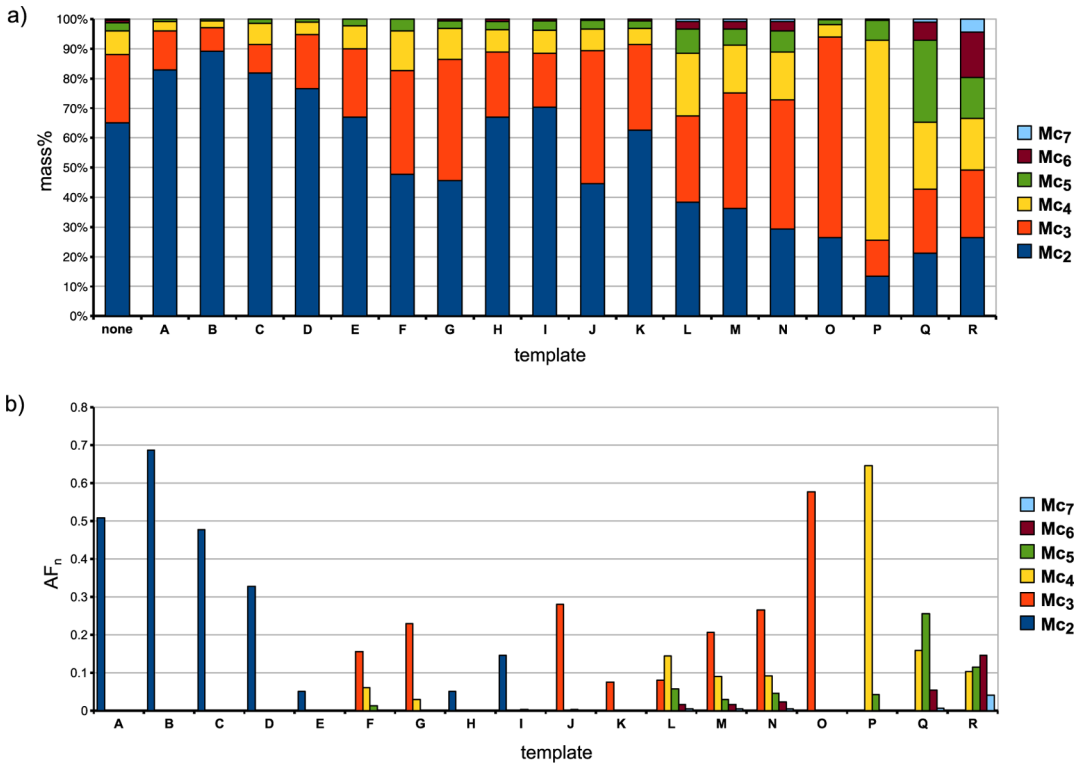

Figure 5. (a) Distributions of macrocycles in the untemplated and templated libraries (mass%). (b) Normalized amplification factors (AF_n) of macrocycles upon templation (only positive values are presented). In all DCLs: $[Mc_n]_0$ = 10 mM, template: 5 mM (0.5 equiv); only for **R** template: 1.5 mM (0.15 equiv).

hexaanions. The highest abundances and AF_n values were observed for $\mathbf{Mc}_4$, $\mathbf{Mc}_5$, and $\mathbf{Mc}_6$, respectively. Upon addition of EDTA, the tetrameric host was very strongly and selectively amplified (AF_n = 0.65) to constitute the vast majority of the library, 67% by mass. In the case of the two remaining larger guests, the amplification was lower and less selective. The higher the oligomer, the harder it is to amplify, because this requires overcoming the tendency of the system to favor smaller components. Moreover, templates **Q** and **R** are linear, which is an undesirable geometry for cyclic hosts. Templation of our DCL with pentaanionic DTPA resulted in a 10-fold increase in the concentration of $\mathbf{Mc}_5$, which corresponds to AF_n = 0.26; the pentamer is the major component of the templated library (27.5% by mass). Our largest tested anion, TTHA, afforded a 16-fold amplification of the hexamer and an 11.5-fold amplification of the heptamer. In terms of normalized amplification factors, the influence of this template is less spectacular: $AF_n(\mathbf{Mc}_6)$ = 0.14 and $AF_n(\mathbf{Mc}_7)$ = 0.04; the $AF_n(\mathbf{Mc}_6)$ value is just above the values for tetramer and pentamer, which is a reflection of lower selectivity.

Although the latter two templation effects seem quite weak compared to NTA and EDTA, they are among the highest

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amplifications for such large DCL members thus far described. With proper templation, it is possible to obtain a pentamer (a 65-membered ring in 27.5% yield), a hexamer (a 78-membered ring in 15.3% yield), and a heptamer (a 91-membered ring in 5% yield).[71]

2.5. Thermodynamics of Binding. To gain a better insight into the selectivity of anion binding for aminopoly(carboxylic acid)s (**O**-**R**), we performed multiple templation experiments (15 data points with various concentrations of guest) and succeeded in fitting the data with DCLfit (see Supporting Information for details). However, because of the high complexity of the system, the values obtained must be treated as approximate.[72] For the first two anions of this subset (NTA and EDTA), the K_a values increase quite regularly with the increasing size of the macrocycle until the number of binding sites in the receptor matches the number of carboxylate groups, and then K_a remain constant for higher oligomers (Figure 6). This distribution of association constants over the hosts can be rationalized by a model where the formation of each carboxylate−dipicolinic diamide bond adds a constant value of about 6.7 kJ/mol to the ΔG^0 of complex formation. Macrocycles larger than the fittest ones cannot form more hydrogen bonds with the guest, and no rise in association constant is observed. The other two templates (DTPA and TTHA) exhibit a more complicated behavior. For pentaanionic DTPA, the first two K_a, with $\mathbf{Mc}_2$ and $\mathbf{Mc}_3$, are consistent with the aforementioned model, but the following two association constants with higher oligomers are lower than expected. The expected maximum value for K_a (according to 5 × 6.7 kJ/mol) is not reached until $\mathbf{Mc}_6$. These observations indicate that because of the linear shape of the anion, hosts $\mathbf{Mc}_4$ and $\mathbf{Mc}_5$ cannot fully bind four or five carboxylate groups, respectively. Satisfactory complexation of all five anchoring points of DTPA is achieved by the hexamer. For the largest anion TTHA (**R**), the determined association constants are quite different compared to the other anions in this subset; the K_a values seem to have been overestimated, which we attribute to an error relating to the very complex numerical model and difficulties in the fitting procedure.

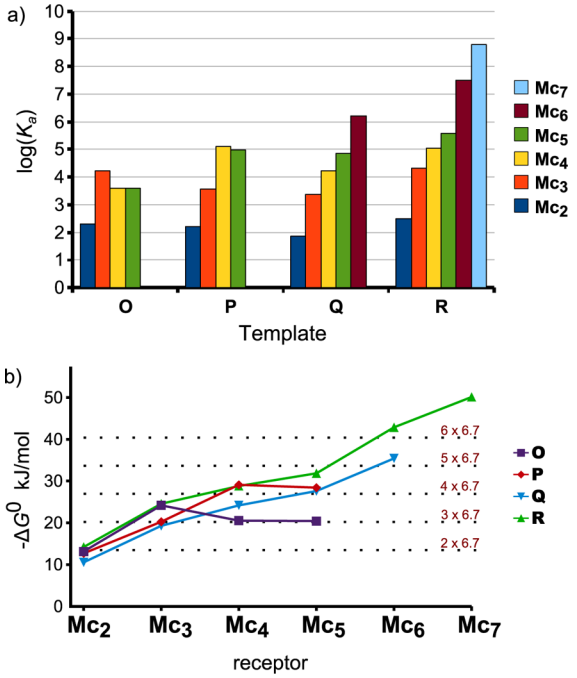

Figure 6. (a) Association constants of complexes of anions **O**-**R** with oligomeric receptors. (b) Enthalpy of formation of complexes vs macrocyle size. Dashed horizontal lines denote the multiplications of 6.7 kJ/mol (average value per carboxylate group bound).

We decided to use competitive ^{1}H NMR titration[73] to confirm the aforementioned distributions of association constants. Using the isolated macrocycles $\mathbf{Mc}_2$−$\mathbf{Mc}_5$, we prepared an equimolar mixture of these receptors which was then titrated in DMSO-d_6 with a TBA salt of either NTA, EDTA, or DTPA. The signals of amidic protons were followed, as they undergo the strongest changes upon addition of anionic guests. The titration profiles we obtained indicated that the kinetics of formation of the supramolecules are relatively complex. The equilibria of formation of some of the complexes are fast on the NMR time scale, which is reflected in the gradual shift of the signal. Other equilibria are slow—the signal of the host gradually decreases while the signal of the complex emerges. Finally, some complexes form at a rate comparable to the NMR time scale, resulting in the broadening and disappearance of the respective signals. Because of the signal broadening and overlapping, we were unable to analyze the titrations with NTA and DTPA, while that with EDTA could easily be rationalized (Figure 7). The first signals to undergo changes upon guest addition are those of $\mathbf{Mc}_4$ and $\mathbf{Mc}_5$, which follow the slow exchange equilibria. The signals of the two complexes increase at similar rates, indicating very close K_a values for these receptors. Once $\mathbf{Mc}_4$ and $\mathbf{Mc}_5$ have been saturated with the EDTA anion, the $\mathbf{Mc}_3$ signals begin to shift (fast exchange) until a plateau is reached. At that time, the $\mathbf{Mc}_2$ signals begin to shift slowly (low K_a, fast exchange). The order

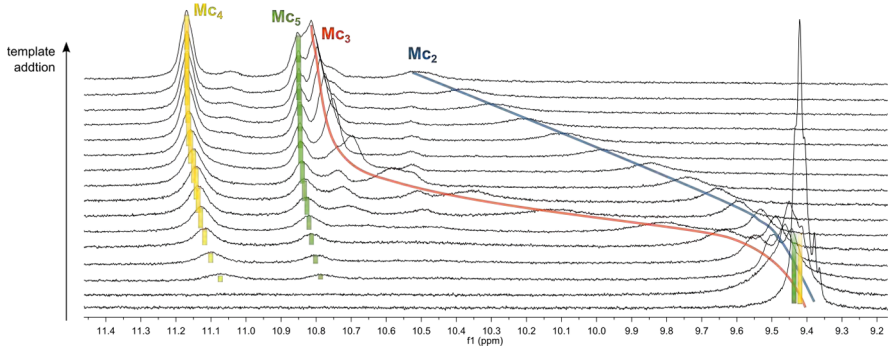

Figure 7. Competitive titration of macrocycles $\mathbf{Mc}_2$−$\mathbf{Mc}_5$ with the EDTA anion. Slow and fast kinetics of supermolecule formation are indicated by bars and solid curves, respectively.

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of association constants determined in this experiment: $K_a^{M_5} \approx K_a^{M_4} \gg K_a^{M_3} \gg K_a^{M_2}$ is in good agreement with results from the DCLfit analysis of templation effects.

3. CONCLUSIONS

We prepared a neutral anion receptor **1** capable of forming oligomacrocycles upon oxidation. The distribution of oligomers can be controlled kinetically, by adjusting the concentrations of reagents. Under basic conditions, the library equilibrates in 2 h with disulfide bond exchange to reach its minimum ΔG^0 for the given conditions. The DCL composition can be modified by adding various carboxylates. Monofunctional benzoate amplifies the smallest receptor $\mathbf{Mc}_2$; difunctional carboxylates are bound by a dimeric or trimeric host in a relatively regular manner depending on the template size and rigidity. Among the tricarboxylates, we found that flexible aliphatic tricarboxylates bind strongly to $\mathbf{Mc}_3$, while a large and rigid aromatic trimesic acid anion (**L**) amplifies $\mathbf{Mc}_4$. Four aminopoly(carboxylic acid)s were found to be exceptionally selective templates; each primarily amplifies the oligomeric host consistent with the number of carboxylate groups. This is the first time where high and selective amplifications of penta- and hexameric hosts upon templation have been obtained. The association constants with the latter templates can be rationalized by an analysis of the number of effective hydrogen bonds formed. In one case, the EDTA anion (**P**), the order of binding affinities was confirmed by competitive NMR titration. Although our system responds nicely to anionic templates, even better fitted hosts could be obtained by increasing the diversity of the DCLs by introducing substrates with various side arms as well as linker molecules. Such systems are currently under investigation.

4. EXPERIMENTAL SECTION

4.1. Synthesis. *General Remarks.* All reagents and solvents were of puriss p.a. quality. Dichloromethane was distilled over CaH_2. Column chromatography was performed with silica gel 60 (60−230 mesh). Ion exchange resin (Amberlite 400-IRA) in OH form was prepared from Cl form by washing the resin with 5% aqueous NaOH until an aliquot of the eluate added to $AgNO_3$ in diluted HNO_3 resulted in nearly no cloudiness. Next, the resin was washed with distilled water until pH < 9. After subsequent washing with methanol, the resin was dried on a rotary evaporator. HRMS were recorded with ESI ionization and TOF analyzer. NMR spectra were calibrated on residual solvent signal (2.50 ppm (^{1}H) and 39.52 ppm (^{13}C) for DMSO; 7.26 (^{1}H) and 77.00 (^{13}C) for $CDCl_3$).

Compound ***3c*** *N,N′-Bis[2-((triphenylmethyl)sulfanyl)ethyl]-pyridine-2,6-dicarboxamide.* A solution of *S*-trityl cysteamine (**3c**) (3.98 g, 12.5 mmol), DIPEA (2.55 mL, 15 mmol), and DMAP (20 mg) in dry dichloromethane (50 mL) was cooled to 0 °C. Dipicolinic acid dichloride (**2**) (1.02 g, 5 mmol) was added in five portions, the cooling bath was removed, and the reaction was stirred for 2 h. The solution was concentrated to about 15 mL and was subjected to column chromatography. The product was eluted with 2−5% MeOH in DCM. White foam, yield: 2.42 g (63%) mp 98−99; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.34 (2 H, t, *J* 6.0), 8.35−7.99 (3 H, m), 7.69−6.78 (30 H, m), 3.39 (4 H, dd, *J* 13.3, 6.7), 2.38 (4 H, t, *J* 7.0). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.0, 148.4, 144.3, 139.4, 129.0, 128.0, 126.7, 124.3, 65.9, 37.7, 31.4. HRMS: calcd for $C_{49}H_{43}N_3O_2NaS_2$ ([M + Na]): 792.2694, found: 792.2682.

Compound ***1*** *N,N′-Bis(2-sulfanylethyl)pyridine-2,6-dicarboxamide.* A solution of **4c** (2 g, 2.6 mmol) in dichloromethane (10 mL) was cooled to 0 °C, TFA (5 mL) was added, and the solution changed to intense yellow (trityl cation). Triethylsilane (Et_3SiH) (0.91 mL, 5.72 mmol) was added in one portion, and the solution gradually became colorless (trityl cation reduction). After about 10 min, the solution was evaporated to dryness on a rotary evaporator. The solid residue was dissolved in dichloromethane and chromatographed with 2−10% MeOH in DCM. Fractions containing the product were evaporated, the residue was dissolved in a minimal volume of dichloromethane:methanol (5:1), and a pure crystalline product was precipitated with hexane. White crystals, yield: 0.682 g (92%) mp 188−189. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.36 (1 H, d, *J* 7.8), 8.05 (1 H, t, *J* 7.8), 3.71 (2 H, q, *J* 6.3), 2.84 (2 H, dd, *J* 14.8, 6.4), 1.47 (1 H, t, *J* 8.5). ^{13}C NMR (100 MHz, $CDCl_3$) δ:163.4, 148.7, 139.2, 125.2, 42.2, 24.8. HRMS: calcd for $C_{11}H_{15}N_3O_2NaS_2$ ([M + Na]): 308.0503, found: 308.0504.

4.2. Library Formation. *4.2.1. Kinetically Controlled Libraries. Mode 1.* To a solution of **1** in DCM:MeOH (3:1) (0.05 M) was added an equal volume of I_2 in DCM (0.05 M) over 1 h via a syringe pump. The solution of macrocylces was analyzed by HPLC.

Mode 2. To a solution of I_2 in DCM (0.05 M) was added an equal volume of **1** in DCM:MeOH (3:1) (0.05 M) over 5 h via a syringe pump. The solution of macrocylces was analyzed by HPLC.

Mode 3. Solutions of I_2 in DCM and **1** in DCM:MeOH (3:1) (0.05 M, 5 mL each) were added simultaneously via a syringe pump over 5 h into 300 mL of DCM:MeOH (3:1). The solution was concentrated to ∼10 mL and analyzed by HPLC.

4.2.2. Thermodynamically Controlled Libraries. Stock Solution of M_n. Dithiol **1** (285 mg, 1 mmol) was dissolved in DCM:MeOH (3:1) (10 mL). Iodine (232 mg, 1.8 mmol, 1.8 equiv) was added. When all crystals of iodine were dissolved and the solution became colorless, a solution of I_2 in DCM (1 M) was added dropwise until a pale yellow color remained. Amberlite IRA-400 OH form (1.8 mmol/g, 3 g) was added, and the solution was stirred mechanically for 10 min. The resin was filtered off and washed twice with DCM:MeOH (3:1) (2 × 10 mL). The collected filtrates were evaporated to dryness. The solid residue was dissolved in DMSO+0.5% H_2O (40 mL) to obtain 0.025 M (calculated for monomer **1**) solution. A solution of tetrabutylammonium hydroxide (TBAOH, 1.0 M in MeOH, 50 μL, 5 mol %) was added to initiate equilibration.

Stock Solution of Anionic Template. To a solution of organic acid (0.15 mmol) in methanol (5 mL) was added TBAOH solution (1 M, 1.05 equiv per carboxylic group). The resulting solution was evaporated on a rotary evaporator (40 °C). The syrup obtained was dissolved in DMSO+0.5% H_2O (3 mL) to obtain 0.05 M solution.

Typical Templation Experiment. Appropriate volumes of stock solutions were mixed and diluted with DMSO+0.5% H_2O to obtain the desired concentrations. In all templation experiments the concentration of M_n was 0.01 M. The solutions were stirred for at least 3 h, and the equilibrium was frozen by addition of 1 M TFA in DMSO (1/3 volume of the library).

4.3. Kinetic Study of Equilibration. A solution of $\mathbf{Mc}_2$ (isolated as described above) in DMSO+0.5% H_2O (2 mL, 0.01 M) was treated with TBAOH solution (1 μL, 1.0 M in MeOH) and stirred at 20 °C. At the given time, an aliquot of this solution (20 μL) was added to a solution of TFA in DMSO (40 μL of 0.5 M solution) and was analyzed by HPLC. After an equilibrium was reached (200 min), template **P** was introduced (185 μL, 0.5 equiv) and stirring and sampling were continued.

4.4. HPLC Analyses. The libraries were analyzed by HPLC on an ACE-C18 column (100 Å, 5 μm, I.D. 4.6 mm, *L* 250 mm) at 25 °C, flow 1 mL/min with gradient elution: from 40% MeCN 60% H_2O to 70% MeCN 30% H_2O in 25 min. Both solvents contained 0.01% TFA. The chromatograms were analyzed at λ = 220 nm.

In all cases, with various compositions for the libraries, the total sums of integrals were the same, indicating that the molar absorptions coefficients of $\mathbf{Mc}_n$ (ϵ_n) are multiplications of the coefficient of the monomer ($\epsilon_n = n \times \epsilon_1$). Quantitative analyses of libraries compositions were based on this relation.

■ ASSOCIATED CONTENT

Supporting Information

LC-MS data, DCLfit models and data, titration procedures and data, and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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