High Yielding Flow Synthesis of a Macrocyclic Molecular Hinge

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High-Yielding Flow Synthesis of a Macrocyclic Molecular Hinge

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KEYWORDS Macrocycles, molecular hinge, continuous flow, supramolecular, host-guest chemistry

ABSTRACT: Many molecular machines are built from modular components with well-defined motile capabilities, such as axles and wheels. Hinges are particularly useful, as they provide the minimum flexibility needed for a simple and pronounced conformational change. Compounds with multiple stable conformers are common, but molecular hinges almost exclusively operate via dihedral rotations rather than truly hinge-like clamping mechanisms. An ideal molecular hinge would better reproduce the behavior of hinged devices, such as gates and tweezers, while remaining soluble, scalable and synthetically versatile. Herein, we describe two isomeric macrocycles with clamp-like open and closed geometries, which crystallize as separate polymorphs but interconvert freely in solution. An unusual one-pot addition cyclization reaction was used to produce the macrocycles on a multigram scale from inexpensive reagents, without supramolecular templating or high-dilution conditions. Using mechanistic information from NMR kinetic studies and at-line mass spectrometry, we developed a semi-continuous flow synthesis with maximum conversions of 85-93% and over 80% selectivity for a single isomer. The macrocycles feature voids that are sterically protected from guests, including reactive species such as fluoride ions, and could therefore serve as chemically inert hinges for adaptive supramolecular receptors and flexible porous materials.

INTRODUCTION

Biological processes depend strongly on the ability of molecules to undergo reliable and reversible changes in shape. Finely controlled conformational transitions play important roles, for example, in muscle contraction, transmembrane ion transport and ATP synthesis. 1-3 A key ambition of supramolecular chemists is to engineer molecular machines capable of performing useful work, such as catalysis, transport and host-guest binding, with comparable precision. 4, 5 To date, synthetic molecular machines have featured complex arrangements of moving parts, including rings that shuttle between stations on a linear or circular track, 6, 7 and crane-like arms that can transfer labile moieties between reactive docking sites. 8, 9

Molecular machines typically consist of modular components linked by covalent or mechanical bonds. Each component must deliver reversible conformational changes in a simple and predictable fashion. For example, a nanocar may be constructed by connecting pseudo-spherical adamantane or fullerene wheels to a central dipolar chassis via an alkyne axle. 10 Rotors 11 and shuttles 7 commonly incorporate a catenane or rotaxane, while hinges are built from moieties with interconvertible geometric isomers. Suitable structures include photoisomerizable double bonds, such as stilbenes, imines and azobenzenes, 12 and fused aliphatic rings with distinct chair, boat and skew conformations. 13, 14 Hinging provides a mechanism for controlling resonance energy transfer 15 and other physical phenomena dependent on the separation of interacting groups. Alternatively, hinged architectures may function as molecular clips or tweezers, varying the distance between their closing “jaws” to maximize binding with an encapsulated guest. 16, 17

Figure 1 (a) Schematic mechanism of a typical molecular hinge based on dihedral rotations around a rigid spacer, such as a double bond or disubstituted ring; (b) clamp-like mechanism of a macrocyclic molecular hinge (this work).

Despite their simple mechanical function, synthesizing molecular hinges with widespread utility remains a challenge. Hinges based on a flexible single bond, 18-20 double bond, 15, 21-23 or disubstituted ring system 24-27 operate through dihedral rotations, analogous to the twisting of a crank around a shaft. Since they cannot undergo a clamping motion, such hinges are sub-optimal scaffolds for pincer-like receptors (Figure 1a). In addition, isomerization of these structures is
often triggered by inputs such as heat, light or redox reactions.\textsuperscript{28} The need for a stimulus is disadvantageous in applications such as adaptive host-guest binding, where switching must occur rapidly and reversibly under ambient conditions.\textsuperscript{29}

A truly versatile molecular hinge must function in a range of chemical environments without disrupting target reactions and supramolecular motifs. Thus, hinges should be soluble, simple to synthesize, chemically inert and incapable of significant host-guest interactions. To ensure that hinging is predictable and entropically feasible, the process should involve well-defined open and closed structures with no alternative stable conformations. Unfortunately, synthetic scaffolds displaying suitable conformational isomerism\textsuperscript{30-32} are often insoluble, reactive or difficult to prepare, making them inconvenient building blocks for modular functional materials.\textsuperscript{33}

Macrocycles represent a promising starting point for the construction of more reliable clamp-like molecular hinges (Figure 1b). Even complex macrocycles may be highly rigid, exhibiting a precise arrangement of functional groups around a well-defined central cavity.\textsuperscript{34} Thus, conformational isomerism in macrocycles often involves a small number of structures with shapes that are easy to distinguish and usefully diverse.\textsuperscript{35-38} Conformers are described as atropisomers if they can be manipulated as separate compounds, interconverting only at elevated temperatures or through the breaking of a bond.\textsuperscript{39} By contrast, non-atropisomeric conformers may equilibrate readily in solution under ambient conditions. Atropisomerism is widely exploited in medicinal macrocycles, such as vancomycin, to fix the compounds in their most biologically active conformations.\textsuperscript{40} Conformationally flexible macrocycles, meanwhile, may favor different forms in response to physical or chemical stimuli.\textsuperscript{29} Since conformers typically display pronounced differences in their spectroscopic, host-guest binding and solid-state properties, flexible macrocycles may be integrated into remotely switchable molecular containers and other stimuli-responsive materials.\textsuperscript{41}

Another important advantage of macrocycles is that binding sites are confined to fixed locations within an easily modified intrinsic void.\textsuperscript{42} The shape of this void may be tuned to ensure tight complementarity with a target guest, for applications such as catalysis, drug delivery and molecular recognition.\textsuperscript{43} Likewise, undesirable guest uptake by a macrocyclic hinge may be avoided by the inclusion of competitive intramolecular motifs or bulky peripheral substituents, which present a steric barrier to incoming species.\textsuperscript{30} Introducing more flexibility into a macrocycle can lead to a stronger induced fit with guests,\textsuperscript{44-46} provided the increase in complementarity compensates for the greater loss of entropy on binding.\textsuperscript{47} However, it may also provide a gating mechanism for limiting the possibility of host-guest interactions. For example, a labile coordination site\textsuperscript{48} or isomerizable double bond\textsuperscript{49} may allow the structure to switch to a more closed conformation, restricting access to internal binding sites.

Designing and synthesizing a flexible macrocycle can be a challenging task. In the absence of preorganized intermediates, it is often necessary to use protecting groups, supramolecular templates and high-dilution conditions to promote macrocycle formation over oligomerization pathways.\textsuperscript{50} Syntheses may thus be slow and low-yielding or involve multiple protection and deprotection steps. Problems of this nature are increasingly resolved through the use of flow reaction platforms,\textsuperscript{51,52} in combination with in- or on-line reaction monitoring and automated optimization techniques,\textsuperscript{53} to identify the most efficient and selective reaction conditions. Reactants in flow may be mixed, heated, and cooled more uniformly, and the synthetic protocol can be adjusted continuously in response to real-time conversion and kinetic measurements. By enabling more controlled addition of reagents and higher reaction temperatures, flow technology has allowed macrocycles to be generated more rapidly, in higher yields, and with fewer side products than conventional batch methods.\textsuperscript{54}

In this investigation, two isomeric macrocycles, 1 and 2, (Figure 2) were prepared from readily available reagents via a one-pot addition-cyclization reaction. Remarkably, each macrocycle transitions between a pair of distinct conformers in solution, which can be isolated as separate concomitant single crystals for analysis by single-crystal X-ray diffraction (SCXRD) (Figure 3). Furthermore, the ratio of macrocycles in the product may be tuned by varying the sequence in which the starting materials are mixed. A semi-continuous synthetic method was used to maximize the rate of macrocycle formation and attain high selectivity for product 2. Synthesizing the macrocycles in flow allows the intermediates and products to be monitored over a range of temperatures, aiding optimization of the reaction conditions and kinetic analysis of key mechanistic steps.

Figure 2 Formulae and crystal structures of isomeric macrocycles 1 and 2 in their syn conformations. Gray, white, blue and red atoms in the crystal structures correspond to C, H, N and O, respectively. Oxazolidine rings are angled out of the macrocycle plane, producing clamp-like geometries.
As molecular hinges, macrocycles 1 and 2 display several useful properties. Firstly, syn-anti transitions offer access to well-defined open and closed geometries, which can be readily characterized in both solution and the solid state. Based on variable-temperature NMR (VT-NMR), crystallographic and computational studies, we propose that the hinging mechanism differs from that of similarly flexible macrocycles, such as calixpyrroles, in that it involves a simple rapid clamping motion with no alternative conformers. In addition, NMR and computational studies suggest that the macrocycles interact only weakly with guests, including protic solvents and highly basic species such as fluoride ions, due to shielding of their internal voids by bulky methyl groups and oxazolidine rings. Finally, both compounds are readily soluble and may be synthesized easily and inexpensively on a large scale. Given the simplicity and derivatizability of the amine and isocyanate reactants, these structures would serve as useful scaffolds for clamp-like receptors, hinged molecular machines and porous framework materials, enabling reliable changes in shape and adaptive recognition of target guests.

RESULTS AND DISCUSSION

Synthesis and characterization

A mixture of 1 and 2 is generated by reacting tetramethylxylylene diisocyanate with 2-chloroethylamine or 2-bromoethylamine in the presence of triethylamine. In our proposed mechanism (Figure 4), the reaction generates a mono-urea 3 or bis-urea 4, which slowly cyclize to form the nucleophilic 2-iminoxazolidinones 5 and 6. These intermediates likely exist as a mixture of tautomers and stereoisomers but react further to form macrocycles only in the Z configurations, which allow for a stabilizing intramolecular hydrogen bond in the final product. Macrocycle 1 is produced by the homocoupling of 5, while its isomer 2 results from the addition of 6 to a second equivalent of diisocyanate. The structures of the oxazolidine intermediates and their mechanism of formation is highly unusual. Although cyanate ions can undergo cyclization reactions with substituted alkylamines, comparable O-alkylation of an isocyanate is rarely reported. Indeed, to the best of our knowledge, this type of reaction has been described only once before and has never been exploited for macrocycle synthesis.

Macrocycles 1 and 2 can be separated by column chromatography and purified by recrystallization from methanol. The structures of the compounds were confirmed by SCXRD (Supporting Information, Figures S17-20 and Table S1) and are consistent with elemental analysis, mass spectrometry and NMR spectroscopy data (SI, Figures S1-12). Intriguingly, each compound adopts two stable conformations that crystallize separately as concomitant polymorphs. The conformers differ in the orientations of the oxazolidine rings, displaying either U-shaped syn or Z-shaped anti configurations (Figure 3). Isomers anti-1 and syn-2 are achiral, while the chiral compounds syn-1 and anti-2 give rise to intrinsically racemic crystal forms. The conformers are rigidified by intramolecular hydrogen bonds between the urea and imine groups, but otherwise exhibit no significant supramolecular motifs in the solid state. Consequently, the compounds can be dissolved readily in dichloromethane or chloro-
form for assessment of their stereodynamic and host-guest binding properties. The solution processability of the compounds could also be an advantage for their large-scale synthesis and functionalization, potentially leading to more optimal clamp-like receptors for practical applications.

**Molecular hinge behavior**

The conformational isomerism of compounds 1 and 2 is unusually well-defined. Each macrocycle exhibits distinct syn and anti forms, which can be readily distinguished by powder X-ray diffraction (PXRD). When 10 mM chloroform solutions of the compounds are slowly evaporated, the resulting precipitates consist mainly of the syn-1 (Figure 5a) and anti-2 (Figure 5b) polymorphs. However, recrystallization of the materials from methanol causes the anti-1 and syn-2 polymorphs to preferentially form. The 1H NMR spectrum of each macrocycle at room temperature contains one set of resonances that can be assigned to the thermal average of its syn and anti conformations, with no indication of separate atropisomeric species (SI, Figures S1 and S7). We conclude that interconversion of the conformers is not fully restricted by the bulky methyl groups of 1 and 2 and occurs rapidly in solution on the NMR timescale, as in the case of the similarly methylated calixpyrroles. This switching behavior allows the ratio of conformers to vary during crystallization, favoring different polymorphs depending on the crystal growth conditions.

Variable-temperature 1H NMR (VT-NMR) offers further insight into the mechanism of conformational switching (SI, Figures S22-24 and Table S3). Each macrocycle displays two triplet signals in the range 3.5-4.3 ppm that were attributed to the four methylene protons of the oxazolidine ring, matching the NMR assignments of literature analogues (Figure 6a). At room temperature, the protons of each CH₂ site are chemically and magnetically equivalent due to rapid interconversion of the syn and anti conformers. However, when dichloromethane-d₂ solutions of 1 (Figure 6b) and 2 (Figure 6c) are cooled below -20 and -40°C, respectively, the inequivalent protons α1 and α2 and β1 and β2 are clearly resolved as separate with matching integrals. We propose that protons α1 and β1 give rise to downfield signals due to interactions with the opposing oxazolidine rings and, in macrocycle 1, the attached urea carbonyl groups. Indeed, the crystal structure of syn-1 displays interatomic CH···OC distances of 2.6-2.9 Å (with a mean value of 2.73 Å), while CH···HC contacts in both syn-1 and syn-2 lie in the range 2.3-2.8 Å (with means of 2.49 Å and 2.57 Å for 1 and 2, respectively).

**Figure 5** Calculated PXRD patterns of (a) 1 and (b) 2 from the crystal structures of their syn and anti conformers, and the experimental patterns obtained after recrystallization of the compounds from chloroform and methanol.

**Figure 6** (a) Assignment of the four methylene proton environments in 1, with close CH···OC contacts illustrated in red. The methylene protons are enlarged and other protons omitted for clarity; (b) VT-NMR spectra of 1, showing splitting of the 1H methylene signals below 253 K; (c) VT-NMR spectra of 2, showing splitting of the 1H methylene signals below 233 K.

The coalescence temperature, Tᶜ, for each methylene group was estimated by extrapolating the chem-
ical shifts of the split signals to the point of convergence. This value was used to estimate the activation energy for switching, $\Delta G^\ddagger$, via the Eyring equation:

$$\Delta G^\ddagger = -RT \ln \left( \frac{h k_r}{k T_c} \right)$$

(1)

where $k$ is the Boltzmann constant, $h$ the Planck constant, $R$ the molar gas constant and $k_r$ the rate constant for the conformational change. The value of $k_r$ is determined from $\Delta \delta$, the maximum separation of the syn and anti signals in Hz:

$$k_r = \frac{\pi}{\sqrt{2}} \Delta \delta$$

(2)

For 1 and 2, this analysis produces $\Delta G^\ddagger$ values of 54 ± 1 and 47 ± 1 kJ mol$^{-1}$, respectively. Macrocycle 1 displays a slightly larger barrier for the syn-anti transition, suggesting that opening of the syn form is resisted by stronger interactions between the oxazolidine rings.

To rationalize their relative stabilities, the syn and anti conformers of 1 and 2 were modeled using the density functional theory (DFT) method B3LYP$^{64}$ in the basis sets 6-31++G$^{**}$,$^{65}$ def2-TZVP$^{66}$ and aug-cc-pVDZ (SI, Table S4).$^{67}$ After geometry optimization, syn-1 is approximately 13 kJ mol$^{-1}$ lower in energy than anti-1, while anti-2 is 6 kJ mol$^{-1}$ more stable than syn-2. The oxazolidine rings of syn-1 interact more strongly due to the antiparallel alignment of dipoles and close contacts between the methylene and carbonyl groups. In syn-2, where the oxazolidine rings exhibit a parallel configuration, no such CH···OC interactions are possible. Thus, syn-1 and anti-2 are expected to be the dominant conformers of the macrocycles in solution. This hypothesis is supported by our PXRD studies, which indicate a greater abundance of the predicted low-energy conformers when 1 and 2 are precipitated from chloroform (Figure 5). Preferential crystallization of the higher-energy anti-1 and syn-2 conformers from methanol could result from solvent-macrocycle hydrogen bonding or other stabilizing solvation effects, which are not accounted for in our DFT calculations.

Additional DFT modeling was undertaken to explore the mechanism of conformational switching (SI, Figures S25-29 and Table S5). For each conformer, one torsion angle was altered in increments of 0.2–5.0° until the alternative macrocycle structure was reached. The geometry was optimized for each fixed torsion angle in the 6-31+G* basis set and its energy compared with that of the starting conformation (Figure 7a). The calculations suggest that the two conformational changes are mechanistically similar, involving rotations of the phenyl groups out of the plane of the macrocycle (Figure 7b). The intramolecular hydrogen bonds are strongly preserved, forcing each urea-oxazolidine motif to move as a single rigid structure like the jaw of a clamp. Following refinement of the highest-energy geometries in a range of larger basis sets, we estimated an activation barrier of 37–41 kJ mol$^{-1}$ for conversion of anti-2 to the less stable syn form. Conversion of anti-1 to syn-1 is opposed by a similar energy barrier, but the reverse transition displays a much larger activation energy of 51–54 kJ mol$^{-1}$ due to the relatively high stability of the syn geometry.

Strong agreement between these results and those of our VT-NMR experiments suggests that the behavior of the macrocycles in solution has been accurately described. Interestingly, further refining the model with a D3BJ dispersion correction$^{68}$ does not significantly alter the mechanisms of the conformational transitions or the energy barrier for macrocycle 2 (SI, Figures S27-29 and Tables S4 and S5). However, the correction increases the stability of syn-1, and thus the barrier for the syn-anti transition, by 16–19 kJ mol$^{-1}$. This discrepancy could be due to an overestimation of dispersion forces between the oxazolidine rings or the omission of solvation effects from the modeled system.

![Figure 7](image_url)  
Figure 7 (a) DFT energies (B3LYP/6-31+G*) of 1 and 2 for varying values of the methyl-imine torsion angle (inset); (b) the highest-energy conformations of 1 (top) and 2 (bottom), in which the oxazolidine rings are approximately perpendicular and the phenyl groups twist out of the macrocycle plane.

Given the simplicity and rapidity of their conformational switching behavior, compounds 1 and 2 represent appealing scaffolds for the construction of larger clamp-like macrocycles. There is much scope for derivatizing the xylylene spacers or decorating the oxazolidine rings with functional substituents, like the rim substituents of calixarenes$^{31}$ and calixpyrroles,$^{32}$ to deliver stronger host-guest binding or stimulus-responsive properties. The ability to incorporate a reliable hinge into more complex materials, such as polymers and framework solids, could enable better control of useful properties such as porosity and gelation capacity. Exploring this modular synthetic approach will be a key objective of our future research.

**Batch synthesis**

Macrocycles 1 and 2 can be produced in different ratios by varying the sequence in which reagents are mixed (Figure 8a). If the amine and isocyanate react in an equimolar ratio over six hours (Method A), neither isomer is strongly favored. By contrast, adding two equivalents of the amine to the neat isocyanate fol-
allowed by a second equivalent of neat isocyanate after three hours (Method B) results in a high selectivity for compound 2. It is proposed that the ureas 3 and 4 form rapidly but only slowly cyclize to yield the oxazolidines 5 and 6. Thus, Method B allows for near-quantitative conversion of mono-urea 3 to bis-urea 4, preventing the formation of 1 via intermediate 5.

In both Methods A and B, higher temperatures and longer reaction times enable total isolated macrocycle yields of 60-90%. Furthermore, the selectivity of Method B for isomer 2 exceeds 90% even if both steps occur at 50°C with a total reaction time of 24 hours. Higher reaction rates are possible if the synthesis is performed as a semi-continuous process (vide infra). Nonetheless, as one-pot processes involving inexpensive and readily available starting materials, the batch reactions offer a viable route for the large-scale manufacture of macrocyclic hinges. We anticipate reactions of this type finding widespread use in the synthesis of more complex molecular architectures, generating flexible structures with unusual three-dimensional morphologies in a single reaction step. Efforts to derivatize macrocycles for this purpose are currently underway.

Mechanistic studies

The high selectivity of reactions involving 2-chloroethylamine suggests that conversion of the bis-urea 4a to oxazolidine 6a is a rate-determining step at room temperature. Indeed, by performing the first step of Method B at 0°C and evaporating the reaction mixture after one hour, the intermediate 4a was obtained in a 45% yield (SI, Figures S13-16). Like other bis-ureas derived from tetramethylenylene disiocyanate and an alkylamine, 4a can be recrystallized from polar solvents to yield single crystals suitable for analysis by SCXRD (Figure 9a). Molecules of the bis-urea adopt an extended conformation and interact via urea-urea tape motifs, crystallizing from acetonitrile as a three-dimensional hydrogen bonding network of hydrogen bonded tapes and from methanol as a lamellar structure (SI, Figure S21 and Table S2). Intriguingly, NMR analysis of the compound in DMSO-d$_6$ indicates that cyclization may be induced by heating, without risk of oligomerization (SI, Figure S33). Thus, it may be possible to generate oxazolidine 6 in a more controlled fashion for incorporation into asymmetric macrocyclic species.

To gain further insight into the rate-determining reaction step, formation of the urea and oxazolidine intermediates was monitored at room temperature by in situ NMR spectroscopy (SI, Figures S35-38 and Tables S8-10). Due to strong overlap of their NMR signals, urea intermediates such as 3 and 4 cannot be quantified separately. Nonetheless, monitoring the disappearance of urea signals in the range 5.5-6.5 ppm allows the average cyclization rate constant to be measured (Figure 9b). The two amine starting materials react with the isocyanate at similarly high rates, delivering maximum urea concentrations within 15 minutes. However, the intermediates produced from 2-chloroethylamine, 3a and 4a, cyclize 87 ± 3 times more slowly. The cyclization displays pseudo-first order kinetics, with a rate constant $k$ of $(7.0 ± 0.1) \times 10^{-4}$ s$^{-1}$ for 2-bromoethylamine and just $(8.0 ± 0.2) \times 10^{-6}$ s$^{-1}$ for 2-chloroethylamine at 21°C. The half-life of 3a and 4a is $24.0 ± 0.5$ hours, while 3b and 4b display a half-life of $16.5 ± 0.3$ minutes.
Select formation of macrocycle 2 is possible only if the initial amine-isocyanate addition reaction is considerably faster than the cyclohexane step. Once the unwanted intermediate 3a has been consumed, cyclohexane may be safely accelerated to optimise the rate of macrocycle formation. We assessed the thermal dependency of cyclization in Method B by performing NMR kinetic studies at several temperatures and estimating the activation energy \( E_a \) from an Arrhenius plot (Figure 9c). The results reveal an \( E_a \) value of 76.5 ± 1.4 kJ mol\(^{-1}\) and frequency factor \( A \) of \((4 ± 2) \times 10^{18} \) s\(^{-1}\), meaning that a reaction temperature of 70 ± 1°C is needed to match the room-temperature \( k \) value of 2-bromoethylamine. Since the boiling point of chloroform at standard pressure is only 61°C, heating a batch reaction mixture is unlikely to produce the optimum cyclization rate.

In the final stage of the reaction, macrocyclization is favored over oligomerization due to preorganization of the reaction intermediates. It is likely that 6 adopts a C-shaped conformation similar to the rigid geometries of the bis-oxazoline (BOX) and related "boxman" compounds, which are used as chelating ligands for asymmetric catalysis.\(^72\)\(^-\)\(^72\) The methyl groups of the xylylene spacer are highly important, as reactions using the non-methylated isocyanate as a starting material produce insoluble ureas with no significant macrocycle formation (SI, Figure 34). To rationalize this observation, DFT energies were calculated for varying conformations of 6 and its non-methylated analogue 7, spanning all possible xylylene-oxazolidine torsion angles \( \phi_1 \) and \( \phi_2 \) (Figure 10). Due to the symmetry of the molecules, the energies are recorded on triangular contour plots, and convergence may be assessed by comparing symmetry-equivalent combinations of \( \phi_1 \) and \( \phi_2 \) (SI, Figure S30 and Table S6). Optimizations were performed with a D3BJ correction for dispersion forces, but omitting this adjustment was found to have little effect on the final appearance of the contour plot (SI, Figure S31).

The energy landscapes of the intermediates reveal large differences in molecular flexibility. Compound 7 can adopt many conformations with a maximum difference in energy of just 9.5 kJ mol\(^{-1}\). By contrast, conformations of 6 differ by up to 25 kJ mol\(^{-1}\) and are most stable when the phenyl ring and C-N bonds are approximately co-planar, as in macrocycles 1 and 2. We conclude that methylation of the xylylene group lowers the entropic cost of macrocycle formation and increases the abundance of suitable precursor geometries.\(^73\) This conformational bias, which is comparable to the Thorpe-Ingold effect,\(^73\) usefully eliminates the need for templating or high-dilution conditions typically encountered in macrocycle syntheses.\(^50\)

**Figure 9** (a) Crystal structure of 4a in its orthorhombic polymorph, obtained by slow evaporation of an acetonitrile solution; (b) first-order kinetic plot comparing urea conversions over time \( t \) at different reaction temperatures \( T \), with a dashed line marking the maximum urea concentration; (c) Arrhenius plot for the urea cyclization reaction, from which the activation energy \( E_a \) and pre-exponential factor \( A \) may be calculated. Error bars represent the standard error in \( \ln(k) \) and the standard deviation in \( T \) for replicate experiments \( n = 4 \).

**Figure 10** DFT energies (B3LYP/6-31+G*) of bis-oxazolidine 6 and its theoretical analogue 7 for 5° increments of the torsion angles \( \phi_1 \) and \( \phi_2 \). The contour plots are calculated by averaging the results of replicate geometry optimizations \( n = 8 \) with different initial molecular conformations. Crystal-structure geometries of bis-urea 4a and macrocycle 2 are plotted for comparison.
**Flow synthesis**

Selective production of 2 allows the macrocycle to be isolated without wasteful separation steps. However, the reliability of the synthesis is limited by the need for stepwise addition of the isocyanate. Furthermore, the reaction is inconveniently slow and could be challenging to scale up, as effective mixing is needed to maintain a constant stoichiometric ratio of the starting materials. While increasing the temperature can improve the efficiency of the process, excess heating may lower reactivity by enabling early accumulation of intermediate 5. This problem could be minimized by completing each step of the synthesis at a different temperature. However, it is difficult to make rapid changes to the conditions of a batch reaction while ensuring uniformity of the reaction mixture, particularly if the process is conducted on a larger scale.

The yield, consistency and scalability of the reaction can be enhanced by transferring the batch process to a continuous flow platform (Figure 11). By performing the synthesis in a flow reactor with two heated coils, we were able to ensure a high mixing rate and automate reagent additions at fixed time points. Use of a dynamic backpressure regulator (BPR) set to 3.0 bar allowed the reaction temperature to be safely increased up to 100°C. Furthermore, a switching valve enabled automatic sampling of the reaction mixture for analysis by ultra-performance liquid chromatography mass spectrometry (UPLC-MS). These at-line measurements provided additional insight into the reaction mechanism by allowing intermediates and side products to be rapidly detected.

For each flow reaction, stock solutions of the starting materials were prepared using a 300 mM solution of triethylamine in chloroform and mixed after the first coil in a 1:1 volumetric ratio. A switching valve may be used to sample the reaction mixture before or after the second coil for at-line analysis by UPLC-MS. A methanol quench is included to allow reaction conversions to be accurately measured.

Figure 11 Flow reactor schematic for the semi-continuous synthesis of macrocycle 2. The solutions are prepared with 300 mM triethylamine in chloroform and mixed after the first coil in a 1:1 volumetric ratio. A switching valve may be used to sample the reaction mixture before or after the second coil for at-line analysis by UPLC-MS. A methanol quench is included to allow reaction conversions to be accurately measured.

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For each flow reaction, stock solutions of the starting materials were prepared using a 300 mM solution of triethylamine in chloroform and mixed after the first coil in a 1:1 volumetric ratio. A switching valve may be used to sample the reaction mixture before or after the second coil for at-line analysis by UPLC-MS. A methanol quench is included to allow reaction conversions to be accurately measured.

The solution of macrocycle was heated to temperature $T_1$ in the first coil to drive formation of the oxazolidine species. Finally, the reaction was completed by mixing the solution with another equivalent of isocyanate in a second coil at temperature $T_2$. The selectivity of the synthesis for product 2 depends on minimizing macrocycle formation in the first reaction step. Indeed, in less selective reactions, UPLC-MS measurements after the first coil (Figure 12) reveal positive ion signals for the protonated macrocycle ($m/z$ 575) and its sodium adduct ($m/z$ 597). A signal at $m/z$ 288 is also observed after both coils, corresponding to the unwanted intermediate 5 in the first step and a fragment ion of 1 in the final product mixture (SI, Figure S40).

Figure 12 At-line mass spectra for reaction mixtures sampled after the first reaction step at different temperatures ($T_1$). Molecular ions of the macrocycles, intermediates and a proposed adduct of 4a and 2-chloroethylamine are assigned. Mono-cyclization of 4a produces a further intermediate, for which the protonated molecular ion ($m/z$ 367) and sodium adduct ($m/z$ 389) are marked with asterisks. All peaks occur at the predicted $m/z$ values of the assigned species.

Products of the flow reactions were quenched immediately in methanol and quantified against an acetonitrile standard as in the batch reaction studies (SI, Figure S41 and Tables S11 and S13). Repeat experiments confirm the reliability of the protocol, demonstrating that replicate conversions and selectivities typically vary by less than two percentage points (SI, Tables S12 and S14). The impact of the first heated coil was assessed by varying $T_1$ and fixing $T_2$ at a low value of 50°C, to minimize oxazolidine formation in the second coil. Flow syntheses at $T_1 > 60^\circ$C are higher-yielding than the batch reactions of both 2-chloro and 2-bromoethylamine at room temperature, despite being performed over shorter times and with higher dilutions in the macrocyclization step. This result suggests that oxazolidine formation is not rate-limiting when $T_1$ is high, allowing changes in $T_2$ to strongly influence the macrocycle yield.
As $T_1$ is increased from 50 to 75°C, more macrocycle is generated due to greater formation of intermediate 6 (Figure 13a). However, a decrease in conversion above 90°C is evidence of competing reaction pathways, which inhibit the cyclization process. Likewise, the selectivity for product 2 remains above 90% when $T_1 < 75°C$ but falls sharply at higher temperatures (Figure 13b). UPLC-MS analysis after the first coil at $T_1 = 100°C$ reveals a strong signal matching the sodium adduct of 8 ($m/z$ 468), a bis-urea derived from the reaction of 4a with 2-chloroethylamine (Figure 12). It is concluded that excess heating in the first coil promotes off-target amine alkylation reactions, preventing the later formation of macrocyclic products.

The final reaction step was optimized by varying $T_2$ at a fixed value of $T_1 = 70°C$. Although increasing $T_2$ also lowers the selectivity for 2, more complete formation of intermediate 6 in the first coil limits the potential for alkyl adducts and other side products. Thus, selectivity varies more gradually with temperature than in the first coil, decreasing by just 3 percentage points per 10°C of heating. Conversion, however, rises steeply with $T_2$, reaching 85-93% between 90 and 100°C with a maximum 70-74% yield of 2. The maximum macrocycle production rate for our system is 0.25 g/h under steady-state conditions. All major NMR signals of the crude products at high $T_2$ can be assigned to 1, 2 and triethylamine, confirming that the reaction approaches completion while avoiding off-target reaction pathways (SI, Figure S42).

The optimized values of $T_1$ and $T_2$ exceed the boiling point of the chloroform solvent (61°C). Thus, the high-yielding synthesis of 2 is only possible due to pressurization of the system in flow. Indeed, batch and flow syntheses conducted at 60°C with equal reaction times produce similar total conversions of just 28 ± 1 and 27 ± 2%, respectively, with selectivities of 97.8 ± 0.5 and 95.5 ± 0.5% (SI, Figure S43 and Table S15). We conclude that the use of a flow reactor allows yields to be more than tripled by providing safe and reliable access to higher reaction temperatures.

**Host-guest binding**

In their syn conformations, macrocycles 1 and 2 are geometrically similar to molecular clips. These C-shaped molecules are designed to provide a rigid concave binding surface to encapsulate guests in a shape-selective fashion. Host-guest interactions could limit the usefulness of a molecular hinge, however, by impeding changes in conformation and competing with the binding sites of attached receptor moieties. To investigate this possibility, solutions of the macrocycles in CDCl$_3$ were titrated with 0-120 equivalents of various guests (Figure 14a and SI, Figures S44-46 and Table S16). Changes in the NMR chemical shift of the NH proton, $\Delta\delta$, were measured relative to a reference tetramethylsilane (TMS) signal and fitted to a suitable isotherm if significant binding ($\Delta\delta > 0.02$ ppm) was observed (Figure 14b).

Remarkably, neither macrocycle interacts strongly with most of the neutral guests tested. Indeed, only methanol was found to produce measurable values of $\Delta\delta$ for 1 as well as 2, interacting in a 1:1 stoichiometry with an association constant ($K_{11}$) of approximately 0.8 M$^{-1}$ in both cases (Figure 14c). Similarly low $K_{11}$ values have been recorded for hydrogen bonded complexes of esters, suggesting that methanol forms OH···OC hydrogen bonds but does not bind directly to the NH groups. Macrocycle 2 also displays small but significant $\Delta\delta$ values with hydrogen bond acceptors such as acetonitrile, acetone and dimethylformamide. Titration studies with acetonitrile reveal a $K_{11}$ value smaller than that of methanol, consistent with the formation of weaker CH···nitrile and CH···carbonyl interactions. We hypothesize that acetonitrile and other small polar guests can bind loosely to 2 by entering the narrow void of the macrocycle with only slight disruption of its stable conformation.

The mechanisms of binding were explored further by modeling the interactions of methanol, acetonitrile, acetone and chloroform with the syn and anti conformers of both macrocycles (SI, Figures S49-51 and Table S17). DFT optimizations were performed from a variety of starting configurations in the 6-31+G* basis set, then refined in the larger basis set 6-31+G**. Counterpoise corrections for basis set superposition error were omitted due to their negligible impact on energy values (SI, Table S18). The energy of each host-guest interaction ($E_{int}$) was calculated by subtracting the total energy of the free host and guest from the energy of the geometry-optimized complex. Finally, the favorability of the structures was estimated by comparing their $E_{int}$ values with those of the corresponding chloroform complexes. Although $E_{int}$ does not account for guest-guest interactions or changes in solvation, it nonetheless offers insight into the relative binding strengths of 1 and 2 and the structural differences between their host-guest assemblies.
Fig. 14 (a) $^1$H NMR signals of the NH groups of 1 (marked in orange) and 2 (blue), before and after the addition of different guests (50 eq.). Spectra were recorded using separate 9 mM solutions of 1 and 2 in CDCl$_3$ and concatenated to aid comparison; (b) $^1$H NMR chemical shifts of the NH groups in 1 and 2 relative to TMS, with fixed macrocycle concentrations (9 mM) and varying amounts of a neutral or ionic guest. Trend lines correspond to the best-fit binding isotherms for the Δδ values; (c) mean 1:1 association constants for the macrocycle-guest combinations, with error bars representing the standard error in $K_{11}$ for replicate experiments ($n = 3$).

Fig. 15 (a) Calculated (B3LYP/6-31++G**) interaction energies ($E_{int}$) for complexes of 1 and 2 with neutral guests, and the most stable binding complexes of acetonitrile with (b) 2 and (c) 1; (d) $E_{int}$ values for complexes of 1 and 2 with anionic guests, and the most stable (e) NH···F$^-$ and (f) CH···F$^-$ contacts in fluoride complexes of 2. All $E_{int}$ values are expressed relative to the corresponding chloroform complexes. Hydrogen bonds and major dipole-dipole interactions are marked in red and parts of the macrocycles are omitted for clarity.

As predicted, the DFT results suggest that all macrocycle conformers engage in methanol-carbonyl hydrogen bonding without undergoing significant deformation. However, only syn-2 can interact effectively with acetone and acetonitrile, establishing multiple interactions with the guests via the oxazolidine methylene and urea carbonyl groups (Figure 15a). Chloroform associates less strongly as it is unable to establish the same bifurcated dipole-dipole motifs. Likewise, 1 displays smaller $E_{int}$ values because guests cannot interact simultaneously with both carbonyl groups (Figure 15b). In the syn-1 conformer, binding is further weakened by the presence of CH···OC contacts, which compete with the formation of intermolecular hydrogen bonds and prevent separation of the oxazolidine rings.

Titration of the macrocycles with anionic species, in the form of tetrabutylammonium (TBA) salts, also
produces measurable Δδ values. For 1, these changes are too small for the association constants to be reliably quantified. Conversely, 2 interacts with fluoride and chloride to give Δδ values in the range 0.06–0.07 ppm. The smaller Δδ values of other salts indicate relatively weak binding to the TBA cation, while comparisons of hydrated and anhydrous TBACl suggest that interactions with water of crystallization are similarly minor (SI, Figure S47). In addition, the absence of a triplet peak in the region 15–17 ppm confirms that macrocycles are not deprotonated by fluoride to form bifluoride (HF₂⁻) ions (SI, Figure S48). Both halides conform to a 1:1 binding model and interact more strongly than the neutral guests. However, the K1 values of the anions are smaller than those of typical NH-halide complexes by 3–4 orders of magnitude. For 90% of the molecules of 2 to participate in 1:1 binding, at least 0.88 ± 0.09 M (98 ± 10 eq.) TBAF or 3.3 ± 0.9 M (370 ± 100 eq.) TBACl would be required. It is likely that halide-macrocycle interactions are destabilized by competing intramolecular interactions or heavily disfavored by the compact macrocycle geometry.

DFT modeling suggests that the binding of anions by 2 is controlled by steric crowding around the NH sites. In both the syn and anti conformers, chloride and nitrate ions are too large to fit within the macrocycle void so interact primarily with external CH groups. The fluoride ion exhibits a larger Eint value and can penetrate further between the methyl groups and oxazolidine rings, even engaging in NH···F hydrogen bonding (Figure 15c). However, these interactions are weakened by the resulting conformational strain, making them less stable than the alternative CH···F contacts (Figure 15d). As expected from the NMR data, compound 1 binds anions consistently less strongly in both of its conformers. Though NH···F hydrogen bonds are still possible, particularly in the anti geometry, these offer only a small enthalpic advantage over alternative binding modes so are unlikely to be strongly favored in solution.

Overall, our results illustrate that the macrocycles are resistant to interactions with a variety of species, including highly basic fluoride ions. Though the carbonyl groups act as weak hydrogen bond acceptors, the urea protons are shielded by intramolecular hydrogen bonds and bulky peripheral groups. Thus, the shape and flexibility of the macrocycles can be considered independent of their chemical environment. This predictability is crucial if the structures are to function as modular molecular hinges, providing synthetic scaffolds for a novel family of clamp-like receptors and other conformationally adaptive materials.

Conclusions

A pair of flexible clamp-like macrocycles has been produced from inexpensive starting materials via a multigram, one-pot addition-cyclization reaction. The mechanism of this process has been explored in detail, allowing key intermediates to be isolated and characterized and the ratio of isomeric products to be reliably controlled. Furthermore, by performing part of the process in continuous flow, we have achieved conversions of 85–93% with over 80% selectivity for a single isomer. Both macrocycles act as molecular hinges, undergoing simple clamp-like transitions between isolable syn and anti conformers. Thus, this work represents a valuable addition to the synthetic toolbox of supramolecular chemists, providing an efficient route to inert, versatile and scalable building blocks for the modular assembly of molecular machines.

ASSOCIATED CONTENT

Supporting Information. Experimental details, 1H and 13C NMR data, mass spectra, elemental analyses, crystallographic information, kinetic plots, reaction optimization data and DFT models. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions
The manuscript was written by CDJ and AGS. CDJ and LJKC were responsible for acquiring, processing and interpreting the XRD data, while KVL performed the VT-NMR experiments. DMG assisted in the set up of flow experiments and provided code for automation of the atline measurements. CDJ performed the remaining computational and experimental studies. JWS oversaw the preliminary research, while CDJ and AGS managed the overall research direction and project design. All authors have given approval to the final version of the manuscript.

Notes
The authors declare no competing financial interest.

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ABBREVIATIONS

NMR, nuclear magnetic resonance; VT-NMR, variable-temperature nuclear magnetic resonance; SCXRD, single-crystal X-ray diffraction; PXRD, powder X-ray diffraction;
DFT, density functional theory; BPR, backpressure regulator; UPLC, ultra-performance liquid chromatography mass spectrometry; TMS, tetramethylsilane; TBA, tetra- butylammonium.

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High-Yielding Flow Synthesis of a Macrocyclic Molecular Hinge

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Supplementary Data

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1 Experimental procedures

1.1 Materials and methods

All solvents, reagents and starting materials were obtained from commercial suppliers and used without further purification. Elemental (CHN) analysis was performed using a Thermo FlashSmart Elemental Analyzer. NMR spectra were recorded on Bruker Avance I and Avance III 400 MHz spectrometers, with typical sample concentrations of 30 mM, and processed using Bruker TopSpin 4.0 software. Electrospray ionization (ESI+) mass spectra were obtained from 0.001% (w/v) solutions in methanol doped with 0.1% (v/v) formic acid using a Waters Acquity UPLC-MS (H-class) instrument. Powder X-ray diffraction studies were performed on a PANalytical X'Pert PRO MPD, with a Cu X-ray source, used in high throughput transmission mode with Ka focusing mirror and PIXCEL 1D detector. Single-crystal X-ray structures for syn-1 and the monoclinic polymorph of 4a were obtained at 100 K using a Rigaku 007HF Mo rotating anode (λ = 0.70926 Å) with a Saturn 724+ CCD detector and Oxford Cryostream 700+. All other single-crystal X-ray diffraction experiments were performed at 150 K using a Bruker D8 Venture dual microfocus diffractometer with a Photon 100 CMOS detector, Oxford Cryostream 700 and Mo radiation source. Structures were solved in Olex2 with the ShelXT structure solution program using Intrinsic Phasing and refined with the ShelXL refinement package using least-squares minimisation.

1.2 Flow reactions

Reactor platform

Semi-continuous syntheses were performed using a Vapourtec R-Series Flow System fitted with peristaltic pumps, PFA coiled tube reactors and a Vapourtec SF-10 pump used as an active BPR (3.0 bar). A 5 mL reactor was used for the first step of the reaction and a 10 mL reactor for the second, and the two reagent solutions were mixed at a T-piece between the two reactors. Products were collected in the steady-state regime and sampled via a VICI Valco 4-port switching valve. At-line mass spectra were obtained with a Waters Acquity UPLC-MS fitted with a BEH C18 1.7 μm 2.1 x 50 mm column, using a flow rate of 0.4 ml (typical pressure 3500 psi) and 0.1% (v/v) formic acid in methanol as the carrier solvent.

Switching valve setup

Flow reaction sample queues were prepared in the Waters software MassLynx. The switching valve was connected to the flow path of the reactor platform and the solvent inlet (port 5) and column inlet (port 6) of the UPLC Sample Manager injection valve. Sampling was performed at 6 min intervals by switching the valve from Position A to Position B for 5 s. Switching was controlled via a custom Python script and used to trigger data acquisition via a hardware connection to the Inject Hold port of the Sample Manager.

Python script for valve control

#!/usr/bin/env python

""
Author: David Marquez-Gamez, Christopher Jones
University of Liverpool
Date: July 2019
Python Version: 2.7
```python
import serial
import time

# Serial communication parameters and control command list can be found
# on the Universal Electric Actuator Instruction Manual Models EUH, EUD, and EUT
# https://www.vici.com/support/manuals/universal-actuator.pdf

# Open serial port
ser = serial.Serial(
    port = 'COM4',  # Adjust to current PC setting
    baudrate = 9600,
    parity = serial.PARITY_NONE,
    stopbits = serial.STOPBITS_ONE,
    bytesize = serial.EIGHTBITS
)
ser.isOpen()

# Delay interval in milliseconds
set_delay_ = 5000  # 5 seconds
set_delay = str(set_delay_)

# Number of runs
set_runs = 30  # 15 samples if using 1 reactor, 30 if using 2 reactors

# Wait time in seconds
wait_time = 355  # Time from injection to next experiment; wait_time + set_delay = 360 s

# COMMANDS
current_mode = 'AM'  # Displays the current actuator mode
current_pos = 'CP'  # Displays the current position
position_AB = 'CC'  # Sends the actuator from position A to B
position_BA = 'CW'  # Sends the actuator from position B to A
delay_time = 'DT'  # Displays the current delay time. If "DTnnnnn", sets the
                  # delay time from 0 to 65535 milliseconds
actuator_go = 'GO'  # Sends the actuator to a specified position.
                    # E.g. "GOB" moves the actuator from position A to B
toggles_pos = 'TO'  # Toggles the actuator to the opposite position
toggles_wait_back = 'TT'  # Toggles the actuator to the opposite position, waits for
                         # the delay time then returns to the original position
actuator_help = '/?'  # Displays a list of valid commands

# Loop
for n in range(set_runs):
    print("Experiment number: ", n+1)
    print("Init position A, filling with sample")
    ser.write(actuator_go + 'A' + '\r')
    # Send command: move the actuator to Position A
    ser.write(delay_time + set_delay + '\r')
    # Send command: set the delay interval to 5 seconds
    print("Going to position B, experiment started")
    ser.write(toggles_wait_back + '\r')
    # Send command: timed toggle from A to B
    time.sleep(set_delay_/1000)  # Time in seconds
    print("Back to position A, waiting for experiment end")
    # wait for next injection
    time.sleep(wait_time)

# Close serial port
ser.close()
```
1.3 Synthesis

Batch synthesis of 1 and 2

A mixture of 2-bromoethylamine hydrobromide (2.25 g, 11.0 mmol) and triethylamine (0.300 M, 2.1 eq.) in chloroform (75 mL) was stirred at 50°C until a clear solution was obtained. The cooled solution was poured onto neat tetramethylyxylene diisocyanate (2.70 g, 11.0 mmol, 1.0 eq.), stirred at room temperature for 15 minutes, then at 50°C for 6 hours. The reaction was quenched with methanol (40 mL) and the resulting solution evaporated to dryness. The residue was sonicated with cold methanol (50 mL), filtered and washed with further cold methanol (2 × 50 mL) to obtain a mixture of 1 and 2 as a white crystalline solid (2.40 g, 4.19 mmol, 76% yield, 73% selectivity for 1). A sample of the mixture (1.00 g, 1.74 mmol) was separated by column chromatography (1:1 DCM/ethyl acetate) to obtain compounds 1 (0.35 g, 48% recovery, 36% net yield, \( R_I \) 0.27) and 2 (0.11 g, 42% recovery, 32% net yield, \( R_I \) 0.20). Single crystals suitable for SCXRD studies were obtained by recrystallizing the compounds from methanol (10 mL, 7.0 mM) with slow evaporation at room temperature. Whilst the anti-1 and syn-2 polymorphs accounted for the majority of the solid products, small quantities of the alternative polymorphs were observed.

![Chemical Structures](image)

Compound 1: \( m/z \) (ESI+) 575.3 [M + H] (theor. 575.3346), 597.3 [M + Na] (theor. 597.3165), 613.3 [M + K] (theor. 613.2905). Elem. Anal. Calc. (%) (C\(_{33}\)H\(_{42}\)N\(_{6}\)O\(_{5}\)) C 66.88, H 7.37, N 14.62; Found (%) C 66.64, H 7.31, N 14.62. \(^1\)H NMR (400 MHz, CDCl\(_3\)) 10.84 (s, 2H, e), 7.49 (t, \( J = 0.9 \) Hz, 2H, c) 7.28 (t, \( J = 7.6 \) Hz, 2H, a), 7.22 (m, 4H, b), 3.97 (t, \( J = 3.8 \) Hz, 4H, g), 3.63 (t, \( J = 3.8 \) Hz, 4H, f), 1.70 (s, 12H, d, d′) 1.59 (s, 12H, h, h′). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) 151.5, 150.3, 148.5, 147.8, 127.7, 122.0, 121.3, 77.2, 57.8, 55.0, 42.5, 31.5, 30.1.

Crystal data for syn-1: orthorhombic, space group Pca2\(_1\) (no. 29), colorless plate, \( a = 19.6490(4) \) Å, \( b = 10.9456(2) \) Å, \( c = 28.5401(5) \) Å, \( V = 6138.1(2) \) Å\(^3\), \( Z = 8\), \( T = 100.0 \) K, \( \mu(\text{MoK}) = 0.084 \) mm\(^{-1}\), \( D_{\text{calc}} = 1.244 \) g cm\(^{-3}\), 72161 reflections measured (4.146° ≤ 2θ ≤ 52.772°), 12513 unique (\( R_{\text{int}} = 0.0968\), \( R_{\text{sigma}} = 0.0844\)) which were used in all calculations. The final \( R_I \) was 0.0714 (\( I > 2\sigma(I)\)), \( wR_I \) was 0.1578 (all data) and GoF was 1.044.

Crystal data for anti-1: triclinic, space group P-1 (no. 2), colorless block, \( a = 10.0018(12) \) Å, \( b = 10.9370(13) \) Å, \( c = 15.4860(17) \) Å, \( \alpha = 85.948(4)^{\circ}\), \( \beta = 72.043(3)^{\circ}\), \( \gamma = 76.061(4)^{\circ}\), \( V = 1564.0(3) \) Å\(^3\), \( Z = 2\), \( T = 150.0 \) K, \( \mu(\text{MoK}) = 0.082 \) mm\(^{-1}\), \( D_{\text{calc}} = 1.220 \) g cm\(^{-3}\), 49375 reflections measured (5.128° ≤ 2θ ≤ 56.696°), 7759 unique (\( R_{\text{int}} = 0.0316\), \( R_{\text{sigma}} = 0.0211\)) which were used in all calculations. The final \( R_I \) was 0.0541 (\( I > 2\sigma(I)\)), \( wR_I \) was 0.1521 (all data) and GoF was 1.037.
Solution A: A mixture of 2-chloroethylamine hydrochloride (0.128 g, 1.10 mmol) and triethylamine (0.300 M, 2.1 eq.) in chloroform (75 mL) was stirred at 50°C until a clear solution was obtained. The cooled solution was poured onto neat tetramethyldiisocyanate (1.35 g, 5.53 mmol, 0.50 eq.), stirred at room temperature for 1 hour, then at 50°C for 3 hours. Additional isocyanate (1.36 g, 5.57 mmol, 0.50 eq.) in chloroform (10 mL) was added and the mixture stirred at 60°C for a further 20 hours. The reaction was quenched with methanol (40 mL) and the mixture evaporated to dryness. The residue was sonicated with cold methanol (50 mL), filtered and washed with further cold methanol (2 x 50 mL) to obtain a mixture of 1 and 2 as a white crystalline solid (2.21 g, 3.85 mmol, 69% yield, 91% selectivity for 2). A sample of the mixture (1.11 g, 1.94 mmol) was recrystallized twice from methanol to obtain pure compound 2 as a crystalline white solid (0.730 g, 1.27 mmol, 72% recovery, 45% net yield, 98% purity).

Solution B: A mixture of tetramethyldiisocyanate (0.135 g, 0.55 mmol, 0.50 eq.) and triethylamine (0.300 M) in chloroform (11.4 g).

Solution A (6.00 ml) was passed into Reactor 1 (5 mL, T₁ = 50-100°C) with a residence time of 30 min and flow rate of 0.167 mL min⁻¹. Solution B (5.14 ml) was added in a 1:1 steady-state volumetric ratio and the mixture transferred to Reactor 2 (10 mL, T₂ = 50-100°C) at the same flow rate. The resulting solution was collected in the steady-state regime (75-100 min, collection volume 7.98 ml), quenched immediately in methanol (20 mL) and evaporated to dryness. The composition of the crude product was determined by dissolving the residue in a weighed CDCl₃ solution of acetonitrile (typically 1 M, 3 g) with swirling and recording the ¹H NMR
spectrum of the resulting mixture. Each macrocycle was quantified by normalizing the integral of the corresponding NH signal to the CH₃ signal (δ = 2.0 ppm) of the acetonitrile standard.

**Batch synthesis of 4a**

A mixture of 2-chloroethylamine hydrochloride (1.29 g, 11.1 mmol) and triethylamine (0.300 M, 2.1 eq.) in chloroform (75 mL) was stirred at 50°C until a clear solution was obtained. The cooled solution was poured onto neat tetramethylyxylene diisocyanate (1.36 g, 5.57 mmol, 0.50 eq.) and the mixture stirred at 0°C for 1 hour. The reaction was quenched with methanol (40 mL) and the mixture evaporated to dryness. The resulting white solid was washed with cold chloroform (3 x 20 mL), filtered and dried in air at room temperature. Compound 4a was obtained as a white solid (1.04 g, 2.58 mmol, 46%). Single crystals of polymorphs I and II were obtained by recrystallizing the compound from methanol (2.0 mL, 38 mM) and acetonitrile (3.5 mL, 17 mM) solutions, respectively, with slow evaporation at room temperature.

![Chemical structure of 4a](image)

**Crystal data for 4a, Form I:** monoclinic, space group I2/a (no. 15), colorless needle, a = 15.9023(7) Å, b = 9.1526(3) Å, c = 30.4646(14) Å, β = 105.031(4)°, V = 4282.3(3) Å³, Z = 8, Z' = 1, T = 99.99(16) K, μ(MoKα) = 0.322 mm⁻¹, Dcalc = 1.251 g/cm³, 33084 reflections measured (3.294° ≤ 2θ ≤ 50.052°), 3776 unique (Rint = 0.0628, Rsigma = 0.0321) which were used in all calculations. The final R₁ was 0.0664 (I > 2σ(I)), wR₂ was 0.1686 (all data) and GoF was 1.108.

**Crystal data for 4a, Form II:** orthorhombic, space group P2₁2₁2₁ (no. 19), colorless block, a = 10.6959(6) Å, b = 10.9131(8) Å, c = 18.0408(11) Å, V = 2105.8(2) Å³, Z = 4, Z' = 1, T = 150.0 K, μ(MoKα) = 0.327 mm⁻¹, Dcalc = 1.272 g/cm³, 42165 reflections measured (4.516° ≤ 2θ ≤ 67.082°), 8232 unique (Rint = 0.0485, Rsigma = 0.0371) which were used in all calculations. The final R₁ was 0.0475 (I > 2σ(I)), wR₂ was 0.1358 (all data) and GoF was 1.039.

**1.4 Analytical studies**

**Rate constant measurements (room temperature)**

A mixture of 2-chloroethylamine hydrochloride (128 mg, 1.10 mmol) or 2-bromoethylamine hydrobromide (226 mg, 1.10 mmol) in a CDCl₃ solution of triethylamine (0.300 M, 2.1 eq., 11.4 g) was stirred at 50°C until a clear solution was obtained. The cooled solution was poured onto neat tetramethylyxylene diisocyanate (135 mg, 0.55 mmol, 0.50 eq.) and stirred. Kinetic data were obtained by taking 6-10 in-situ NMR measurements of an individual sample over a period of 5 hours for the reaction of 2-chloroethylamine and 30 minutes for the reaction of 2-bromoethylamine. Integrals of the macrocycle NH signals were normalized to the weighted average integrals of the triethylamine CH₂ (quartet, δ = 2.95 ppm) and CH₃ (triplet, δ = 1.30 ppm) signals. First-order rate...
constants were calculated by plotting the natural logarithms of the normalized and corrected NH integrals against the sampling time and measuring the gradient of the initial straight-line region.

Rate constant measurements (heated)

A mixture of 2-chloroethylamine hydrochloride (128 mg, 1.10 mmol) in a CDCl$_3$ solution of triethylamine (0.300 M, 2.1 eq., 11.4 g) was stirred at 50°C until a clear solution was obtained. The cooled solution was poured onto neat tetramethylyxylene diisocyanate (135 mg, 0.55 mmol, 0.50 eq.) and stirred continuously in a thermostatically controlled water bath set to 30, 40 or 50°C. Kinetic data for heated solutions were measured by taking aliquots at intervals of 15-60 minutes and recording their $^1$H NMR spectra. Integrals of the macrocycle NH signals were normalized to the weighted average integrals of the triethylamine CH$_2$ (quartet, $\delta = 2.95$ ppm) and CH$_3$ (triplet, $\delta = 1.30$ ppm) signals. For each measurement, the sampling and NMR acquisition times were both recorded, and the NH integrals corrected based on the measured rate laws of the room-temperature reactions. First-order rate constants were calculated from 5-10 data points as in the room-temperature kinetic studies. Fewer data points were used at higher temperatures due to the shorter timescale of the reaction and more rapid divergence from first-order kinetics.

Conversion and selectivity measurements

A mixture of 2-chloroethylamine hydrochloride (128 mg, 1.10 mmol) or 2-bromoethylamine hydrobromide (226 mg, 1.10 mmol) in a chloroform solution of triethylamine (0.300 M, 2.1 eq., 11.4 g) was stirred at 50°C until a clear solution was obtained. In Method A, the cooled solution was poured onto neat tetramethylyxylene diisocyanate (269 mg, 1.10 mmol, 1.0 eq.) and stirred continuously at room temperature for 6 hours. In Method B, the cooled solution was poured onto the neat isocyanate (135 mg, 0.55 mmol, 0.50 eq.), stirred continuously at room temperature for 3 hours, mixed with additional neat isocyanate (135 mg, 0.55 mmol, 0.50 eq.) then stirred at room temperature for a further 3 hours. The reaction was quenched by shaking with methanol (5 mL) and the resulting solution evaporated to dryness. The residue was dissolved in a weighed CDCl$_3$ solution of acetonitrile (typically 1 M, 3 g) with swirling and a sample taken for $^1$H NMR analysis. Each macrocycle was quantified by normalizing the integral of the corresponding NH signal to the CH$_3$ signal (singlet, $\delta = 2.0$ ppm) of the acetonitrile standard.

Flow and batch comparison at 60°C

A mixture of 2-chloroethylamine hydrochloride (97 mg, 84 mmol) in a chloroform solution of triethylamine (0.300 M, 2.1 eq., 8.64 g) was stirred at 50°C until a clear solution was obtained. The cooled solution was poured onto neat tetramethylyxylene diisocyanate (102 mg, 42 mmol, 0.50 eq.) and the mixture was left to stand at room temperature for 30 minutes, matching the average time for steady-state conditions to be established in flow. The mixture was stirred at 60°C for 30 minutes, mixed with a solution of tetramethylyxylene diisocyanate (102 mg, 42 mmol, 0.50 eq.) and triethylamine in chloroform (0.300 M, 8.64 g) and stirred at 60°C for a further 30 minutes. The reaction was quenched by shaking with methanol (5 mL) and the resulting solution evaporated to dryness. The residue was dissolved in a weighed CDCl$_3$ solution of acetonitrile (typically 1 M, 3 g) with swirling and a sample taken for $^1$H NMR analysis. Each macrocycle was quantified by normalizing the integral of the corresponding NH signal to the CH$_3$ signal ($\delta = 2.0$ ppm) of the acetonitrile standard. The results were compared with semi-continuous flow experiments performed at $T_1 = T_2 = 60°C$. 

6
Host-guest binding measurements

To quantify the binding of guests by 1 and 2, a range of species (100-200 eq.) were added to stock solutions of the macrocycles in CDCl₃ (9.0 mM) with tetramethylsilane (TMS, 0.05% w/v) as an internal reference (δ = 0.0 ppm). Ionic guests were added as tetrabutylammonium (TBA) salts. For each guest producing significant changes in the NMR spectrum (1 + methanol, 2 + methanol, acetonitrile, TBAF trihydrate and TBACl), weighed aliquots (15-200 mg) of the host-guest solution were added sequentially to a known mass of the macrocycle stock (typically 1.0 g) in a single NMR tube. After each addition, the sample was inverted multiple times to ensure complete mixing then analyzed by ¹H NMR spectroscopy at room temperature. A total of 10-14 aliquots were added, reaching total guest concentrations of 40-130 eq. (maximum concentrations of the TBA salts were lower due to their limited solubility). Binding isotherms were produced by measuring changes in the chemical shift of the macrocycle NH signal, Δδ, for increasing concentrations of the added guest. Data were fitted to 1:1 binding isotherms using a Nelder-Mead algorithm in the online software BindFit.⁴,⁵

1.5 Computational studies

Geometry optimizations

Atomic coordinates of the macrocycles were extracted from their single-crystal X-ray structures and optimized in Gaussian 16, Revision A.03,⁶ using the DFT method B3LYP⁷ with tight SCF convergence. Structures were modeled in the basis set 6-31+G* and refined in the larger basis sets 6-31++G**, def2-TZVP⁹ and aug-cc-PVDZ.¹⁰ Optimizations were performed both with and without the D3BJ dispersion correction,¹¹ and their accuracy evaluated by comparison with VT-NMR data.

Conformational transitions

Activation energies for conformational transitions were estimated by varying the C=N-C-C torsion angle between one oxazolidine ring and its anti methyl group and re-optimizing the structure after each scan step. Geometry optimizations were performed using the method B3LYP and basis set 6-31+G*. Torsions were incremented in steps of 0.2° near the energetic maximum and 1-5° elsewhere, depending on the smoothness of the geometric changes. The final activation energies were refined by fixing the scanned torsion angle of the highest-energy geometry and re-optimizing the structure in the larger basis set aug-cc-PVDZ. All calculations were performed both with and without the D3BJ dispersion correction, and their accuracy evaluated by comparison with VT-NMR data.

Conformational energy landscapes

The conformational energies of 6 and its theoretical analogue 7 were analyzed by fixing one methyl-imine (C=N-C-C) torsion angle, incrementing the other in steps of 5°, and re-optimizing the structure after each scan step. Geometry optimizations were performed using the method B3LYP and basis set 6-31+G* with the D3BJ dispersion correction. The calculations were repeated using eight different starting conformations, resulting in 8-16 replicate energy values for each combination of torsion angles. Conformational energies were obtained by calculating the mean energies of symmetry-unique geometries and subtracting the average energy of the most stable conformer. The results were analyzed graphically using triangular contour plots, while convergence of was assessed by comparing the mean energies of symmetry-equivalent torsion angle pairs.
**Binding energy calculations**

Model 1:1 host-guest complexes of 1 and 2 were based on low-energy, partially open macrocycle geometries, selected from the calculated pathways of the syn-anti and anti-syn conformational transitions. Guest molecules were added manually to produce a variety of configurations, spanning all possible relative orientations and hydrogen bond donor-acceptor pairs. Geometries were optimized using the method B3LYP and basis set 6-31+G*, then refined in the larger basis set 6-31++G**. Binding energies were calculated by subtracting the energy of the complex from the energies of the free host and guest in their optimized geometries. A sample of structures were re-optimized using a counterpoise correction for basis set superposition error,12 but this procedure was found to have a negligible impact on the calculation outcome while substantially increasing computation time.
2 Characterization

2.1 Compound 1

Fig. S1 $^1$H NMR spectrum of 1 in CDCl$_3$ with structural assignments (inset) and integrals shown. Accurate integration of the multiplet corresponding to the aryl proton $a$ is not possible due to overlap with the CHCl$_3$ signal.

Fig. S2 $^1$H,$^1$H-COSY NMR spectrum of 1 in CDCl$_3$. 
Fig. S3 $^{13}$C($^1$H) NMR spectrum of 1 in CDCl$_3$.

Fig. S4 $^1$H,$^{13}$C-HSQC NMR spectrum of 1 in CDCl$_3$. 
**Fig. S5** $^1$H,$^{13}$C-HMBC NMR spectrum of 1 in CDCl$_3$.

**Fig. S6** Mass spectrum (ESI+) of 1 in methanol doped with 0.1% (v/v) formic acid.
2.2 Compound 2

Fig. S7 $^1$H NMR spectrum of 2 in CDCl$_3$ with structural assignments (inset) and integrals shown. Accurate integration of the multiplet corresponding to aryl protons $a$, $b$, $j$ and $k$ is not possible due to overlap with the CHCl$_3$ signal.

Fig. S8 $^1$H,$^1$H-COSY NMR spectrum of 2 in CDCl$_3$. 
Fig. S9 $^{13}$C(H) NMR spectrum of 2 in CDCl$_3$.

Fig. S10 $^1$H,$^{13}$C-HSQC NMR spectrum of 2 in CDCl$_3$. 

13
Fig. S11 $^1$H,$^{13}$C-HMBC NMR spectrum of 2 in CDCl$_3$. 

Fig. S12 Mass spectrum (ESI+) of 2 in methanol doped with 0.1% (v/v) formic acid.
2.3 Compound 4a

Fig. S13 $^1$H NMR spectrum of 4a in DMSO-$d_6$ with structural assignments (inset) and integrals shown.

Fig. S14 $^1$H, $^{13}$C-COSY NMR spectrum of 4a in DMSO-$d_6$. 
Fig. S15 $^{13}\text{C}(^1\text{H})$ NMR spectrum of 4a in DMSO-\text{d$_6$}.

Fig. S16 Mass spectrum (ESI$^+$) of 4a in methanol doped with 0.1\% (v/v) formic acid.
3 Single-crystal X-ray diffraction

|                           | syn-1      | anti-1     | syn-2      | anti-2     |
|---------------------------|------------|------------|------------|------------|
| Formula weight            | 574.71     | 574.71     | 574.71     | 574.71     |
| T / K                     | 100.0      | 150.0      | 150.0      | 150.0      |
| Crystal system            | orthorhombic | triclinic  | monoclinic | triclinic  |
| Space group               | Pca2₁      | P-1        | P2₁/n      | P-1        |
| a / Å                     | 19.6490(4) | 10.0018(12)| 15.4684(14)| 10.8032(7) |
| b / Å                     | 10.9456(2) | 10.9370(13)| 10.8078(9) | 15.6010(11)|
| c / Å                     | 28.5401(5) | 15.4860(17)| 19.8946(19)| 20.3666(15)|
| α / °                     | 90         | 85.948(4)  | 90         | 68.586(3)  |
| β / °                     | 90         | 72.043(3)  | 111.816(3)| 80.258(2)  |
| γ / °                     | 90         | 76.061(4)  | 90         | 87.278(2)  |
| V / Å³                    | 6138.1(2)  | 1564.0(3)  | 3087.8(5)  | 3149.2(4)  |
| Z                         | 8          | 2          | 4          | 4          |
| Z'                        | 2          | 1          | 1          | 2          |
| ρcalc / g cm⁻³            | 1.244      | 1.220      | 1.236      | 1.212      |
| µ / mm⁻³                  | 0.084      | 0.082      | 0.083      | 0.082      |
| F(000)                    | 2464       | 616        | 1232       | 1232       |
| Crystal size / mm³        | 0.18 x 0.04 x 0.04 | 0.25 x 0.15 x 0.15 | 0.55 x 0.34 x 0.11 | 0.40 x 0.25 x 0.10 |
| Radiation                 | MoKα       | MoKα       | MoKα       | MoKα       |
| 2θ range / °              | 4.146 to 52.772 | 4.738 to 56.696 | 4.716 to 56.712 | 4.354 to 56.804 |
| Index ranges              | -24 ≤ h ≤ 24 | -13 ≤ h ≤ 13 | -20 ≤ h ≤ 20 | -14 ≤ h ≤ 14 |
|                           | -10 ≤ k ≤ 13 | -14 ≤ k ≤ 14 | -20 ≤ k ≤ 20 | -20 ≤ k ≤ 20 |
|                           | -35 ≤ l ≤ 35 | -26 ≤ l ≤ 26 | -26 ≤ l ≤ 27 | -26 ≤ l ≤ 27 |
| Reflections collected     | 72161      | 49375      | 58309      | 98172      |
| Independent reflections   | 12513      | 7759       | 7696       | 15672      |
| Rint                      | 0.0968     | 0.0316     | 0.0532     | 0.0373     |
| Rsigma                     | 0.0844     | 0.0211     | 0.0353     | 0.0278     |
| Data/restraints/parameters| 12513/1/773 | 7759/0/395 | 7696/0/395 | 15672/0/789 |
| Goodness-of-fit on F²     | 1.044      | 1.037      | 1.027      | 1.025      |
| Final R indexes [I ≥ 2σ(I)| R₁ = 0.0714  | R₁ = 0.0541 | R₁ = 0.0456 | R₁ = 0.0469 |
|                           | wR₂ = 0.1440 | wR₂ = 0.1460 | wR₂ = 0.1054 | wR₂ = 0.1157 |
| Final R indexes [all data]| R₁ = 0.1014  | R₁ = 0.0619 | R₁ = 0.0695 | R₁ = 0.0630 |
|                           | wR₂ = 0.1578 | wR₂ = 0.1521 | wR₂ = 0.1173 | wR₂ = 0.1245 |
| Largest peak/hole / e Å⁻³ | 0.72/-0.31  | 0.37/-0.45 | 0.36/-0.24 | 0.47/-0.44 |
| Flack parameter           | 0.5(9)     | -          | -          | -          |

Table S1 Crystal data for syn and anti polymorphs of 1 and 2. The structure of syn-1 unambiguously confirms the connectivity of the molecule but displays high R₁ and low I/o values due to the small size and weak diffraction of the sample crystal. Attempts to obtain larger, more strongly diffracting crystals were unsuccessful. The asymmetric units of the chiral isomers syn-1 and anti-2 are racemic, containing one molecule each of the two possible enantiomers. It should be noted that no chiral excess is possible due to the lack of chirality in the starting materials. The absolute structure of the non-centrosymmetric crystal of syn-1 is in an arbitrary configuration due to the low precision of the calculated Flack parameter.
**Fig. S17** SCXRD geometry of *syn*-1 viewed from the (a) top and (b) side and (c) along the phenyl-phenyl axis, and crystal packing viewed along (d) (010) and (e) (101).

**Fig. S18** SCXRD geometry of *anti*-1 viewed from the (a) top and (b) side and (c) along the phenyl-phenyl axis, and crystal packing viewed along (d) (010) and (e) (100).
Fig. S19 SCXRD geometry of *syn*-2 viewed from the (a) top and (b) side and (c) along the phenyl-phenyl axis, and crystal packing viewed along (d) (010) and (e) (100).

Fig. S20 SCXRD geometry of *anti*-2 viewed from the (a) top and (b) side and (c) along the phenyl-phenyl axis, and crystal packing viewed along (d) (010) and (e) (100).
|                         | 4a Form I | 4a Form II |
|-------------------------|-----------|------------|
| Formula weight          | 403.34    | 403.34     |
| T / K                   | 100.0     | 150.0      |
| Crystal system          | monoclinic| orthorhombic|
| Space group             | I2/a      | P2₁2₁2₁    |
| a / Å                   | 15.9023(7)| 10.6959(6) |
| b / Å                   | 9.1526(3) | 10.9131(8) |
| c / Å                   | 30.4646(14)| 18.0408(11)|
| α / °                   | 90        | 90         |
| β / °                   | 105.031(4)| 90         |
| γ / °                   | 90        | 90         |
| V / Å³                  | 4282.3(3) | 2105.8(2)  |
| Z                       | 8         | 4          |
| Z'                      | 1         | 1          |
| \(\rho_{\text{calc}}\) / g cm\(^{-3}\) | 1.251 | 1.272 |
| \(\mu\) / mm\(^{-1}\)  | 0.322     | 0.327      |
| \(F(000)\)              | 1712      | 856        |
| Crystal size / mm\(^3\) | 0.20 x 0.05 x 0.04 | 0.25 x 0.22 x 0.15 |
| Radiation               | MoKα      | MoKα       |
| 2θ range / °            | 3.294 to 50.052 | 4.516 to 67.082 |
| Index ranges            | -18 ≤ h ≤ 18 | -16 ≤ h ≤ 16 |
|                        | -10 ≤ k ≤ 10 | -13 ≤ k ≤ 16 |
|                        | -36 ≤ l ≤ 36 | -26 ≤ l ≤ 28 |
| Refractions collected   | 33084     | 42165      |
| Independent reflections | 3776      | 8232       |
| \(R_{\text{int}}\)      | 0.0628    | 0.0485     |
| \(R_{\sigma}\)         | 0.0321    | 0.0371     |
| Data/restraints/parameters | 3776/0/240 | 8232/0/273 |
| Goodness-of-fit on \(F^2\) | 1.108 | 1.039 |
| Final \(R\) indexes [\(I \geq 2\sigma(I)\)] | \(R_I = 0.0664\) | \(R_I = 0.0475\) |
|                        | \(wR_2 = 0.1634\) | \(wR_2 = 0.1303\) |
| Final \(R\) indexes [all data] | \(R_I = 0.0746\) | \(R_I = 0.0548\) |
|                        | \(wR_2 = 0.1686\) | \(wR_2 = 0.1358\) |
| Largest peak/hole / e Å\(^{-3}\) | 0.82/-0.49 | 0.57/-0.76 |
| Flack parameter         | -         | 0.14(2)    |

**Table S2** Crystal data for polymorphs of 4a. The absolute structure of the non-centrosymmetric crystal of Form II is in an arbitrary configuration due to the low precision of the calculated Flack parameter.
Fig. S21 (a) SCXRD geometry of 4a in crystals of Form I; (b) urea tape network in layers of Form I, which lie parallel to the a-b plane and comprise a “brick-wall” [AB] repeat unit; (c) layering of two-dimensional urea tape networks in Form I, viewed along (100); (d) SCXRD geometry of 4a in crystals of Form II, illustrating the disorder in one chloroethyl end-group (modeled over two sites with equal occupancies); (e) urea tape network in Form II, which exhibits a three-dimensional [ABCD] hydrogen bonding topology; (f) orientation of molecules around the urea tape axes of Form II, which lie parallel to (001).
4 Variable-temperature NMR spectroscopy

Fig. S22 $^1$H NMR spectra (a) 1, (b) a repeat sample of 1 and (c) 2 in dichloromethane-d$_2$ at varying temperatures, $T$. Spectra were recorded upon lowering the temperature, and peak positions calibrated to the reference dichloromethane-d$_2$ peak at $\delta = 5.355$ ppm.

Fig. S23 $^1$H NMR chemical shifts, $\delta$, of the (a) $\alpha$ and (b) $\beta$ oxazolidine CH$_2$ environments in 1 for a dichloromethane-d$_2$ solution of the compound at 190-290 K. Peak positions are calibrated to the reference dichloromethane-d$_2$ peak at $\delta = 5.355$ ppm. Data for the (c) $\alpha$ and (d) $\beta$ environments of a replicate sample are also shown, illustrating the reproducibility of the variable-temperature measurements. For each plot, the interpolated coalescence temperature, $T_C$, is labelled, in addition to the estimated activation energy for the conformational transition, $\Delta G^\ddagger$. 
Fig. S24 $^1$H NMR chemical shifts, $\delta$, of the (a) $\alpha$ and (b) $\beta$ oxazolidine CH$_2$ environments in 2 for a dichloromethane-d$_2$ solution of the compound at 190-290 K. Peak positions are calibrated to the reference dichloromethane-d$_2$ peak at $\delta = 5.355$ ppm. For each plot, the interpolated coalescence temperature, $T_C$, is labelled, in addition to the estimated activation energy for the conformational transition, $\Delta G^\ddagger$.

Table S3 Measured peak splitting parameters ($\Delta\delta$), coalescence temperatures ($T_C$) and transition rate constants ($k_r$) for dichloromethane-d$_2$ solutions of 1 and 2, and the resulting activation energy estimates for syn-anti interconversions ($\Delta G^\ddagger$). Peak positions ($\delta$) are calibrated to the reference dichloromethane-d$_2$ peak at $\delta = 5.355$ ppm. Values of $\Delta\delta$ are measured at the point of maximum splitting in the variable-temperature NMR plots, while $k_r$ is calculated using an NMR frequency of 400.13 MHz. Errors in $\Delta G^\ddagger$ are calculated assuming a conservative systematic uncertainty in $T_C$ of $\pm 5$ K for each experiment.
Conformational energy calculations

Fig. S25 Changes in the geometry of (a) syn-1 and (b) syn-2 as one methyl-imine torsion angle ($\phi$) is increased. Values of $\phi$ for each optimized geometry are shown in bold and hydrogen atoms are omitted for clarity. Geometries were optimized in the basis set 6-31+G* using the DFT method B3LYP with no dispersion correction. The modeling reveals that the syn $\rightarrow$ anti transitions for the two macrocycles are mechanistically similar.

Fig. S26 Changes in the geometry of (a) anti-1 and (b) anti-2 as one methyl-imine torsion angle ($\phi$) is increased. Values of $\phi$ for each optimized geometry are shown in bold and hydrogen atoms are omitted for clarity. Geometries were optimized in the basis set 6-31+G* using the DFT method B3LYP with no dispersion correction. The modeling reveals that the anti $\rightarrow$ syn transitions for the two macrocycles are mechanistically similar.
Fig. S27 Changes in the geometry of (a) syn-1 and (b) syn-2 as one methyl-imine torsion angle (φ) is increased. Values of φ for each optimized geometry are shown in bold and hydrogen atoms are omitted for clarity. Geometries were optimized in the basis set 6-31+G* using the DFT method B3LYP with the D3BJ dispersion correction. The modeling reveals that the \textit{syn} \rightarrow \textit{anti} transitions for the two macrocycles are mechanistically similar.

Fig. S28 Changes in the geometry of (a) anti-1 and (b) anti-2 as one methyl-imine torsion angle (φ) is increased. Values of φ for each optimized geometry are shown in bold and hydrogen atoms are omitted for clarity. Geometries were optimized in the basis set 6-31+G* using the DFT method B3LYP with the D3BJ dispersion correction. The modeling reveals that the \textit{anti} \rightarrow \textit{syn} transitions for the two macrocycles are mechanistically similar.
**Fig. S29** Changes in energy of macrocycles 1 and 2 for increasing methyl-imine torsion angles (φ), beginning with the DFT-optimized (a) syn and (b) anti conformations. The macrocycle geometries were optimized after each scan step (keeping φ fixed) using the DFT method B3LYP in the basis set 6-31+G* with no dispersion correction. Repeating the scans with the dispersion correction D3BJ produced the plots shown in (c) and (d). Only small changes are observed in the energy landscape of 2, whereas the barrier for the transition of syn-1 to anti-1 is greatly increased.

| Method       | Compound | Energy difference (syn-anti) / kJ mol⁻¹ |
|--------------|----------|---------------------------------------|
|              |          | 6-31++G**   | def2-TZVP | aug-cc-PVDZ |
| B3LYP        | 1        | -13.29      | -12.09     | -13.46       |
| B3LYP-D3BJ   | 1        | -31.39      | -29.14     | -30.73       |
| B3LYP        | 2        | 6.24        | 6.32       | 5.43         |
| B3LYP-D3BJ   | 2        | 6.53        | 1.83       | 0.77         |

**Table S4** Energy differences between the syn and anti conformers of 1 and 2, calculated in a range of basis sets via the DFT method B3LYP with and without the D3BJ dispersion correction. The geometry of each molecule was optimized in the selected basis set after an initial optimization in the smaller basis set 6-31+G*. Negative energy values correspond to systems in which the syn conformer is more stable.
| Method    | Compound | Basis set                     | Activation energy for syn-anti transition / kJ mol⁻¹ |
|-----------|----------|-------------------------------|---------------------------------------------------|
|           |          |                               | Initial structure syn | Initial structure anti | Mean |
| B3LYP     | 1        | 6-31++G** def2-TZVP aug-cc-PVDZ Mean | 48.02 | 53.86 | 54.79 | 57.0 ± 2.6 | 52.6 ± 3.0 | 51.4 ± 3.4 |
|           |          |                               | 54.81 | 47.68 | 55.25 | 52.6 ± 3.0 | 51.4 ± 2.6 | 50.8 ± 3.1 |
|           |          |                               | 54.81 | 47.68 | 55.25 | 52.6 ± 3.0 | 51.4 ± 2.6 | 55.0 ± 0.2 |
| B3LYP-D3BJ| 1        | 6-31++G** def2-TZVP aug-cc-PVDZ Mean | 71.22 | 68.02 | 69.81 | 69.7 ± 1.1 | 69.9 ± 0.2 | 69.8 ± 0.6 |
|           |          |                               | 70.28 | 69.86 | 69.64 | 69.9 ± 0.2 | 69.8 ± 0.6 | 69.7 ± 0.1 |
|           |          |                               | 70.28 | 69.86 | 69.64 | 69.9 ± 0.2 | 69.8 ± 0.6 | 69.7 ± 0.1 |
| B3LYP     | 2        | 6-31++G** def2-TZVP aug-cc-PVDZ Mean | 38.28 | 33.25 | 43.13 | 39.2 ± 3.5 | 40.6 ± 1.6 | 40.3 ± 2.5 |
|           |          |                               | 43.26 | 39.43 | 39.10 | 40.6 ± 1.6 | 40.3 ± 2.5 | 36.3 ± 3.1 |
|           |          |                               | 40.2 ± 2.0 | 41.1 ± 2.0 | 39.4 ± 1.9 | 40.2 ± 2.0 | 41.1 ± 2.0 |
| B3LYP-D3BJ| 2        | 6-31++G** def2-TZVP aug-cc-PVDZ Mean | 42.21 | 43.47 | 41.91 | 42.5 ± 0.6 | 38.3 ± 0.6 | 39.7 ± 2.2 |
|           |          |                               | 38.25 | 39.21 | 37.53 | 38.3 ± 0.6 | 39.7 ± 2.2 | 40.4 ± 0.6 |

Table S5 Energy barriers for interconversion of the syn and anti conformers of 1 and 2, calculated via the DFT method B3LYP with and without the dispersion correction. The conformational change was simulated by varying the torsion angle between one oxazolidine ring and its anti methyl group and re-optimizing the structure after each scan step. Torsions were incremented in steps of 0.2° near the energetic maximum and 1-5° elsewhere, depending on the smoothness of the geometric changes. Geometry optimizations were performed in the basis set 6-31+G*, and the highest-energy geometry refined in a range of larger basis sets while fixing the scanned torsion angle. Mean activation energies (most reliable in bold) correspond to the difference between the most stable macrocycle conformer (syn-1 or anti-2) and the maximum-energy geometry, which was calculated for both the syn → anti and anti → syn transitions. Errors in the mean values for each basis set are equal to half the difference between the two calculations. All other errors correspond to standard errors in the mean values.

| Compound | Conformational energy / kJ mol⁻¹ |
|----------|---------------------------------
|          | Maximum | Mean  | Standard deviation |
| 6        | 25.4    | 13.3  | 5.3 |
| 7        | 9.5     | 4.1   | 1.8 |

Table S6 Maximum and mean and standard deviation values extracted from the conformational energy landscapes of 6 and 7. Conformational energies were calculated by optimizing the geometries of the molecules for different combinations of torsion angles $\phi_1$ and $\phi_2$, spanning the full range of possibilities $0 \leq \phi_1 < 360^\circ$ and $0 \leq \phi_2 \leq 180^\circ$. The final conformational landscapes were constructed by performing eight replicate calculations with different initial molecular conformations and averaging the results.
Fig. S30 Convergence tests for the conformational energy landscapes of (a) 6 and (b) its theoretical non-methylated analogue 7. Mean energy values were calculated from eight replicate analyses with different initial combinations of the torsion angles $\phi_1$ and $\phi_2$. Illustrated in the contour plots are differences in energy for pairs of symmetry-equivalent combinations of $\phi_1$ and $\phi_2$. Small differences (<2 kJ mol$^{-1}$) are indicative of consistency in the DFT calculations and convergence of the mean results. The mean differences in (a) and (b) are 1.5 and 0.23 kJ mol$^{-1}$, respectively, suggesting that most areas of the energy landscapes have reached convergence.
Conformational energy landscapes of 6 calculated using the DFT method B3LYP/6-31+G* (a) with and (b) without the D3BJ correction for dispersion forces, and (c) the absolute differences between these alternative results. Calculations were performed once for each combination of torsion angles \( \phi_1 \) and \( \phi_2 \) using the same initial molecular conformations. Applying the correction slightly increases the separation of peaks and troughs and raises the energies of conformations near \( \phi_1 = \phi_2 = 180^\circ \), although the latter discrepancy is largely eliminated with further repeats (see Fig. 10). The qualitative appearance of the plot, the positions of the peaks and troughs and the majority of energies are only weakly affected. The maximum conformational energies in (a) and (b) are 25.8 and 24.8 kJ mol\(^{-1}\), respectively, while the mean energies are 11.6 and 10.0 kJ mol\(^{-1}\) and the standard deviations 5.4 and 5.7 kJ mol\(^{-1}\). The mean difference between equivalent combinations of \( \phi_1 \) and \( \phi_2 \) is 1.9 kJ mol\(^{-1}\), with a standard deviation of 1.4 kJ mol\(^{-1}\).
6 Batch synthesis

Fig. S32 Representative $^1$H NMR spectra of the crude products of batch syntheses performed with different methods and starting materials at room temperature. The intensities of the macrocycle NH signals (labelled in bold) are scaled by concentration via normalization against an acetonitrile internal standard. For ease of comparison, the NH signal of 2 is shifted to a fixed position of $\delta = 10.63$ ppm in all experiments. The use of 2-bromoethylamine greatly increases the total conversion but with lower selectivity for product 2. Selectivities are also higher when Method B is used.

| Method | Hal | Sample | Conversion (total) / % | Conversion (2) / % | Selectivity / % |
|--------|-----|--------|------------------------|--------------------|----------------|
| A Cl   | 1   | 1.99   | 1.10                   | 55.5               |
|        | 2   | 1.75   | 1.01                   | 57.3               |
|        | 3   | 1.68   | 1.00                   | 59.3               |
|        | 4   | 1.48   | 0.97                   | 65.6               |
|        | Mean| 1.7 ± 0.2 | 1.02 ± 0.06 | 59 ± 4             |
| B Cl   | 1   | 2.46   | 2.46                   | 100                |
|        | 2   | 2.05   | 2.05                   | 100                |
|        | 3   | 2.15   | 2.12                   | 98.3               |
|        | 4   | 2.71   | 2.65                   | 98.0               |
|        | Mean| 2.3 ± 0.3 | 2.3 ± 0.3 | 99 ± 1             |
| A Br   | 1   | 20.6   | 12.2                   | 59.1               |
|        | 2   | 19.1   | 11.9                   | 62.4               |
|        | 3   | 23.1   | 14.9                   | 64.7               |
|        | 4   | 23.1   | 13.5                   | 58.6               |
|        | Mean| 21.4 ± 2.0 | 13.1 ± 1.4 | 61 ± 3             |
| B Br   | 1   | 18.7   | 15.5                   | 82.7               |
|        | 2   | 14.2   | 12.3                   | 87.1               |
|        | 3   | 14.5   | 13.3                   | 91.6               |
|        | 4   | 14.3   | 12.5                   | 87.5               |
|        | Mean| 15.4 ± 2.2 | 13.4 ± 1.5 | 87 ± 4             |

Table S7 Conversions and selectivities of replicate macrocycle syntheses performed using Methods A and B. Values were obtained through $^1$H NMR analysis of the crude products after quenching with methanol, removal of the solvent in vacuo and dissolution of the residue in CDCl$_3$. Selectivities for 2 were calculated from the relative integrals of the macrocycle NH signals, while conversions were estimated by comparison with the CH$_3$ integral of an acetonitrile internal standard. Errors are equal to the standard deviations of the replicate results.
Fig. S33 (a) One possible scheme of oxazolidine formation from 4a in DMSO-d$_6$, deduced from tentative NMR assignments; (b) $^1$H NMR spectrum of 4a (60 mM) in wet DMSO-d$_6$ before heating; (c) $^1$H NMR spectrum of the same solution of 4a after heating and stirring at 75°C for 8 hours, with new signals marked by their proposed assignments; (d) comparison of the NH and OH signals of 4a (red line) and the proposed oxazolidine products (blue), with the relative integrals of unique product signals labelled. We hypothesize that the new signals correspond to the oxazolidine NH groups in the two geometric (cis/trans) isomers of the product (each potentially existing in two rapidly equilibrating tautomeric forms) and downfield alcohol groups of their hydrolysates. The positions of the OH signals may differ between geometric isomers due to intramolecular hydrogen bonding; (e) aryl CH and urea signals of the reactant and product; (f) alkyl CH signals of the reactant and product.

The relative integral of the new signal at $\delta = 1.65$ ppm is measured as 5.74, approximately matching the expected integrals of CH$_3$ protons (6.00).
Fig. S34 (a) Attempted macrocycle synthesis from 2-bromoethylamine hydrobromide and \textit{m}-xylylene diisocyanate, performed in a stirred chloroform solution of triethylamine (0.300 M, 10 mL) over 24 hours at room temperature. The solution of 2-bromoethylamine (0.145 M) was added to neat isocyanate (0.5 eq.) and further neat isocyanate (0.5 eq.) was added after 3 hours, resulting in a yellow solution. Rotary evaporation of the reaction mixture produced an off-white solid, which could not be redissolved in triethylamine/chloroform with heating; (b) \textsuperscript{1}H NMR spectrum of the crude product in DMSO-\textit{d}_6 after rotary evaporation; (c) \textsuperscript{1}H NMR spectrum of the solid product (318 mg) after washing with chloroform (10 mL); (d) downfield NH signals of the crude product before washing with chloroform; (e) downfield NH signals of the product after washing with chloroform. Note that the weak triplets in the region \(\delta = 9.5\text{--}10.5\) ppm were not affected by the chloroform wash, so are unlikely to correspond to the urea NH groups of the target macrocycles.
7 Kinetic studies

Fig. S35 $^1$H NMR spectra measured during the reaction of 2-bromoethylamine hydrobromide with tetramethylexyylene diisocyanate in triethylamine/chloroform at 21°C. Spectra are normalized against the integral of the triethylamine CH$_2$ (quartet, $\delta = 2.95$ ppm) and CH$_3$ (triplet, $\delta = 1.30$ ppm) signals. For clarity, each spectrum is shifted to coincide with the peak positions of the initial spectrum.

Fig. S36 $^1$H NMR spectra measured during the reaction of 2-chloroethylamine hydrochloride with tetramethylexyylene diisocyanate in triethylamine/chloroform at (a) 21°C, (b) 30°C, (c) 40°C and (d) 50°C. Spectra are normalized against the integral of the triethylamine CH$_2$ (quartet, $\delta = 2.95$ ppm) and CH$_3$ (triplet, $\delta = 1.30$ ppm) signals. For clarity, each spectrum is shifted to coincide with the peak positions of the initial spectrum.
Fig. S37 First-order kinetic plots for replicate reactions of 2-bromoethylamine hydrobromide with tetramethylxylylene diisocyanate in a 0.3 M solution of triethylamine in chloroform at 21°C. The reaction time t is measured from the start of the NMR experiment rather than the mixing of the reagents, as the variable effect of agitation on the initial value of [urea] prevents meaningful extrapolation of the plotted trend lines.

Fig. S38 First-order kinetic plots for replicate reactions of 2-chloroethylamine hydrochloride with tetramethylxylylene diisocyanate in a 0.3 M solution of triethylamine in chloroform at (a) 21°C, (b) 30°C, (c) 40°C and (d) 50°C. The reaction time t is measured from the point at which reagents were mixed.
of triethylamine used as normali...determining a lower value than a typical unimolecular process.

Table S8 Measured temperatures and rate constants for replicate reactions of 2-chloro and 2-bromoethylamine with tetramethylyxylene diisocyanate. Conversions were measured from changing integrals of urea signals in 1H NMR spectra of the reaction mixtures, with the CH2 and CH3 integrals of triethylamine used as normalization factors. Rate constants were calculated from the gradients of first-order kinetic plots, for which the correlation coefficients (R2) and number of fitted points (n) are shown. Values of ln(k) were used to construct Arrhenius plot, yielding an estimate for the activation energy (Ea) of the reaction with 2-chloroethylamine. Uncertainties in temperature correspond to the standard deviations for the aggregated experiments, while all other uncertainties correspond to standard errors in the mean values.

| T / °C | Hal | Measured mean T / °C | Sample | k / s⁻¹ | R² of first-order plot | Mean ln(k / s⁻¹) |
|-------|-----|----------------------|--------|---------|-----------------------|-----------------|
| 21    | Br  | 21.0 ± 0.5           | 1      | (6.80 ± 0.19) x 10⁻⁴ | 0.994 (n = 10)  | -7.27 ± 0.02 |
|       |     |                      | 2      | (6.91 ± 0.29) x 10⁻⁴ | 0.988 (n = 9)   |                |
|       |     |                      | 3      | (7.00 ± 0.27) x 10⁻⁴ | 0.990 (n = 9)   |                |
|       |     |                      | 4 Mean | (7.27 ± 0.21) x 10⁻⁴ | 0.994 (n = 9)   |                |
| 21    | Cl  | 21.0 ± 0.5           | 1      | (8.38 ± 0.20) x 10⁻⁶ | 0.998 (n = 6)   | -11.73 ± 0.02 |
|       |     |                      | 2      | (8.00 ± 0.21) x 10⁻⁶ | 0.997 (n = 6)   |                |
|       |     |                      | 3      | (7.67 ± 0.35) x 10⁻⁶ | 0.992 (n = 6)   |                |
|       |     |                      | 4 Mean | (8.02 ± 0.22) x 10⁻⁶ | 0.997 (n = 6)   |                |
| 30    | Cl  | 30.0 ± 0.9           | 1      | (1.97 ± 0.06) x 10⁻⁵ | 0.993 (n = 10)  | -10.74 ± 0.06 |
|       |     |                      | 2      | (2.02 ± 0.11) x 10⁻⁵ | 0.982 (n = 8)   |                |
|       |     |                      | 3      | (2.42 ± 0.08) x 10⁻⁵ | 0.996 (n = 6)   |                |
|       |     |                      | 4 Mean | (2.22 ± 0.12) x 10⁻⁵ | 0.989 (n = 6)   |                |
| 40    | Cl  | 40.1 ± 0.8           | 1      | (5.56 ± 0.09) x 10⁻⁵ | 0.999 (n = 6)   | -9.78 ± 0.03  |
|       |     |                      | 2      | (5.34 ± 0.21) x 10⁻⁵ | 0.994 (n = 6)   |                |
|       |     |                      | 3      | (6.03 ± 0.45) x 10⁻⁵ | 0.963 (n = 9)   |                |
|       |     |                      | 4 Mean | (5.80 ± 0.30) x 10⁻⁵ | 0.982 (n = 9)   |                |
| 50    | Cl  | 49.6 ± 0.5           | 1      | (1.18 ± 0.08) x 10⁻⁴ | 0.987 (n = 5)   | -8.96 ± 0.05  |
|       |     |                      | 2      | (1.23 ± 0.07) x 10⁻⁴ | 0.992 (n = 5)   |                |
|       |     |                      | 3      | (1.29 ± 0.17) x 10⁻⁴ | 0.937 (n = 6)   |                |
|       |     |                      | 4 Mean | (1.44 ± 0.12) x 10⁻⁴ | 0.972 (n = 6)   |                |

Table S9 Linear regression fit and derived Arrhenius parameters for the reaction of 2-chloroethylamine with tetramethylyxylene diisocyanate. It is noted that formation of the oxazolidinone must be accompanied by a deprotonation step. Thus, it is probable that triethylamine participates in the rate-determining step (i.e. k is dependent on [NEt₃]), causing the reaction to exhibit a lower A value than a typical unimolecular process15 (10¹⁰-10¹³ s⁻¹).

| Linear regression parameter | Value | Arrhenius parameter | Value |
|-----------------------------|-------|--------------------|-------|
| Slope / K                   | (-9.20 ± 0.17) x 10³ | Eₐ / kJ mol⁻¹ | 76.5 ± 1.4 |
| Intercept                   | 19.6 ± 0.6 | A / s⁻¹           | (3.6 ± 1.9) x 10⁸ |
| Parameter         | Mean     | Upper limit | Lower limit |
|-------------------|----------|-------------|-------------|
| $E_a / \text{kJ mol}^{-1}$ | 76.46    | 77.91       | 75.02       |
| $\ln(A / \text{s}^{-1})$     | 19.56    | 20.12       | 19.00       |
| $k_B / \text{s}^{-1}$         | $6.99 \times 10^{-4}$ | $7.11 \times 10^{-4}$ | $6.88 \times 10^{-4}$ |
| $T_{eq} / ^\circ\text{C}$    | 69.68    | 70.64       | 68.75       |

**Table S10** Estimation of the temperature ($T_{eq}$) needed for 2-chloroethylamine to equal the reaction rate of 2-bromoethylamine at room-temperature. The value of $T_{eq}$ is estimated by rearranging the Arrhenius relation $k_B = A \exp(-E_a/R T_{eq})$ to obtain $T_{eq} = -E_a/(R(\ln k_B - \ln A))$, where $E_a$ and $A$ are the activation energy and pre-exponential constant of the 2-chloroethylamine reaction. To obtain conservative estimates for the uncertainty in $T_{eq}$, the calculation was repeated for the upper and lower bounds of the input parameters. In all cases, these bounds were estimated by raising or lowering the mean value by the corresponding standard error.
8 Semi-continuous flow synthesis

Fig. S39 $^1$H NMR spectra of (a) 2-chloroethylamine hydrochloride (0.145 M) and (b) tetramethylxylylene diisocyanate (0.145 M) in a 0.300 M solution of triethylamine in CDCl$_3$, measured at multiple intervals over 24 hours. Comparison of the spectra reveals little alteration over the duration of the experiment.
Fig. S40 At-line mass spectra for reaction mixtures sampled after (a) the first reaction step at different values of $T_1$ and (b) the second reaction step at different values of $T_1$ and $T_2$. Spectra were recorded in methanol doped with 0.1% (v/v) formic acid.
Fig. S41 ¹H NMR spectra of the crude products from semi-continuous flow syntheses performed at varying values of (a) $T_1$ with $T_2 = 50^\circ C$ and (b) $T_2$ with $T_1 = 70^\circ C$. The intensities of the macrocycle NH signals (labelled in bold) are scaled by concentration via normalization against an acetonitrile internal standard. For ease of comparison, the NH signal of 2 is shifted to a fixed position of $\delta = 10.63$ ppm in all experiments. Increasing $T_1$ leads to a moderate rise in conversion with substantial loss of selectivity for 2. Increasing $T_2$, however, produces a sharp increase in conversion with a smaller decrease in selectivity. At the highest values of $T_2$, the intermediate signals around $\delta = 10.4$ ppm are almost absent, indicating complete conversion to the target products.

Fig. S42 ¹H NMR spectra of 1 and 2 and the crude product of a semi-continuous flow synthesis performed at $T_1 = 70^\circ C$ and $T_2 = 100^\circ C$. All spectra were recorded in CDCl₃. The product of the flow reaction contains triethylamine and triethylammonium chloride and an acetonitrile standard for conversion measurements. However, all other major peaks can be assigned to 1 and 2, suggesting near-complete formation of the target compounds. A total macrocycle conversion of 93% and selectivity of 80% were calculated for this reaction trial.
Table S11 Conversions and selectivities of semi-continuous flow syntheses performed at $T_2 = 50^\circ$C.

Values were obtained through $^1$H NMR analysis of the crude products after quenching with methanol, removal of the solvent in vacuo and dissolution of the residue in CDCl$_3$. Selectivities for 2 were calculated from the relative integrals of the macrocycle NH signals, while conversions were estimated by comparison with the CH$_3$ integral of an acetonitrile internal standard. Where replicate experiments were performed, mean values are shown (see Table S12).

| $T_1$ / $^\circ$C | Conversion (total) / % | Conversion (2) / % | Selectivity / % |
|------------------|------------------------|-------------------|------------------|
| 50               | 7.5                    | 7.2               | 94.8             |
| 55               | 12.4                   | 11.7              | 94.3             |
| 60               | 15.4                   | 14.4              | 93.6             |
| 65               | 21.2                   | 19.7              | 93.1             |
| 70               | 24.5                   | 22.7              | 92.7             |
| 75               | 24.6                   | 22.8              | 92.7             |
| 80               | 23.8                   | 19.5              | 81.8             |
| 85               | 24.3                   | 18.2              | 75.0             |
| 90               | 25.4                   | 17.2              | 67.8             |
| 95               | 23.2                   | 14.8              | 64.0             |
| 100              | 19.7                   | 12.2              | 62.1             |

Table S12 Replicate conversion and selectivity measurements for semi-continuous flow syntheses performed at $T_2 = 50^\circ$C. Errors correspond to the separation of each pair of measurements from the mean. The replicates are reasonably concordant, exhibiting deviations from the mean of less than two percentage points.
Table S13 Conversions and selectivities of semi-continuous flow syntheses performed at $T_1 = 70^\circ$C. Values were obtained through $^1$H NMR analysis of the crude products after quenching with methanol, removal of the solvent in vacuo and dissolution of the residue in CDCl$_3$. Selectivities for 2 were calculated from the relative integrals of the macrocycle NH signals, while conversions were estimated by comparison with the CH$_3$ integral of an acetonitrile internal standard. Where replicate experiments were performed, mean values are shown (see Table S14).

| $T_2$ / °C | Conversion (total) / % | Conversion (2) / % | Selectivity / % |
|------------|------------------------|-------------------|----------------|
| 50         | 23.7                   | 22.4              | 94.7           |
| 55         | 27.0                   | 25.5              | 94.4           |
| 60         | 31.1                   | 28.6              | 92.0           |
| 65         | 44.4                   | 40.5              | 91.2           |
| 70         | 61.1                   | 54.2              | 88.7           |
| 75         | 72.2                   | 62.9              | 87.2           |
| 80         | 80.8                   | 69.3              | 85.7           |
| 85         | 82.8                   | 68.7              | 83.0           |
| 90         | 84.9                   | 69.8              | 82.2           |
| 95         | 88.9                   | 72.8              | 81.9           |
| 100        | 92.7                   | 74.2              | 80.0           |

Table S14 Replicate conversion and selectivity measurements for semi-continuous flow syntheses performed at $T_1 = 70^\circ$C. Errors correspond to the separation of each pair of measurements from the mean. The replicates are reasonably concordant, exhibiting deviations from the mean of less than two percentage points.

| $T_2$ / °C | Sample | Conversion (total) / % | Conversion (2) / % | Selectivity / % |
|------------|--------|------------------------|-------------------|----------------|
| 55         | 1      | 25.49                  | 24.13             | 94.68          |
|            | 2      | 28.42                  | 26.76             | 94.17          |
|            | Mean   | 27.0 ± 1.5             | 25.5 ± 1.3        | 94.4 ± 0.3     |
| 65         | 1      | 44.06                  | 40.33             | 91.53          |
|            | 2      | 44.71                  | 40.64             | 90.91          |
|            | Mean   | 44.4 ± 0.3             | 40.5 ± 0.2        | 91.2 ± 0.3     |
| 75         | 1      | 74.00                  | 64.07             | 86.58          |
|            | 2      | 70.32                  | 61.78             | 87.84          |
|            | Mean   | 72.2 ± 1.8             | 62.9 ± 1.1        | 87.2 ± 0.6     |
| 80         | 1      | 82.69                  | 70.56             | 85.33          |
|            | 2      | 78.97                  | 67.96             | 86.05          |
|            | Mean   | 80.8 ± 1.9             | 69.3 ± 1.3        | 85.7 ± 0.4     |
| 90         | 1      | 84.73                  | 69.88             | 82.48          |
|            | 2      | 85.14                  | 69.80             | 81.98          |
|            | Mean   | 84.9 ± 0.2             | 69.8 ± 0.1        | 82.2 ± 0.2     |
| 95         | 1      | 89.97                  | 73.57             | 81.78          |
|            | 2      | 87.82                  | 72.02             | 82.02          |
|            | Mean   | 88.9 ± 1.1             | 72.8 ± 0.8        | 81.9 ± 0.1     |
Table S15 Replicate conversion and selectivity measurements for batch and semi-continuous flow syntheses performed for equal durations at $T_1 = T_2 = 60^\circ$C. To account for the time taken to reach steady-state conditions in flow, batch mixtures were left to stand at room temperature ($21^\circ$C) for 30 minutes before being stirred at $60^\circ$C. Errors correspond to the standard deviations of the replicate experiments.

| Test | Batch | Flow |
|------|-------|------|
|      | Conversion (total) / % | Conversion (2) / % | Selectivity / % | Conversion (total) / % | Conversion (2) / % | Selectivity / % |
| 1    | 28.72 | 28.07 | 97.71    | 26.12 | 24.93 | 95.42 |
| 2    | 29.19 | 28.46 | 97.51    | 25.76 | 24.59 | 95.44 |
| 3    | 26.73 | 26.33 | 98.52    | 29.21 | 27.71 | 94.87 |
| 4    | 28.54 | 27.84 | 97.58    | 27.39 | 26.36 | 96.21 |
| Mean | 28.3 ± 1.1 | 27.7 ± 0.9 | 97.8 ± 0.5 | 27.1 ± 1.6 | 25.9 ± 1.4 | 95.5 ± 0.5 |

Fig. S43 $^1$H NMR spectra of the crude products from replicate (a) batch and (b) semi-continuous flow syntheses at $60^\circ$C. The intensities of the macrocycle NH signals (labelled in bold) are scaled by concentration via normalization against an acetonitrile internal standard. For ease of comparison, the NH signal of 2 is shifted to a fixed position of $\delta = 10.63$ ppm in all experiments. The two synthetic methods produce similar NH signals, indicating that there is little variation in their conversions or selectivities. Differences between the non-macrocycle NH signals around $\delta = 10.4$ ppm suggest the use of a flow platform affects the formation of other products and intermediates, perhaps due mixing of reagents in flow before the second reaction step. These effects will be investigated further in future work.
9 Host-guest binding studies

Fig. S44 $^1$H NMR spectra showing the macrocycle NH signals in solutions of (a) 1 and methanol, (b) 2 and methanol, (c) 2 and acetonitrile, (d) 2 and TBAF trihydrate and (e) 2 and anhydrous TBACl. All spectra were recorded in CDCl$_3$ with TMS (0.05% w/v) as an internal reference ($\delta = 0.0$ ppm).
Fig. S45 ¹H chemical shifts of macrocycle NH peaks in CDCl₃ solutions of (a) 1 and methanol, (b) 2 and methanol, (c) 2 and acetonitrile, (d) 2 and TBAF trihydrate and (e) 2 and anhydrous TBACl. The titration data were obtained by varying the guest concentration at a fixed macrocycle concentration of 9.0 mM, with TMS (0.05% w/v) as an internal reference (δ = 0.0 ppm). Trend lines illustrate 1:1 binding isotherms fitted to the data via a Nelder-Mead algorithm in the online software BindFit. For some host-guest combinations, different guest concentration ranges were used to assess the effect of the experimental design on the consistency of the fitted binding models. Divergence of replicate plots at high guest concentrations is likely attributable to small differences in sample preparation and has little effect on the fitted $K_{11}$ values (Table S16).

| Host | Guest | $K_{11}$ / M⁻¹ | Mean / M⁻¹ | Host bound by 50 eq. guest / % |
|------|-------|----------------|-------------|-----------------------------|
|      |       | Trial 1        | Trial 2     | Trial 3                    |
| 1    | MeOH  | 0.841 ± 0.035  | 0.929 ± 0.026 | 0.585 ± 0.016 | 0.78 ± 0.13 | 26 ± 3 |
|      | MeCN  | 0.498 ± 0.013  | 0.584 ± 0.013 | 0.434 ± 0.014 | 0.84 ± 0.09 | 27 ± 2 |
|      | TBAF  | 11.9 ± 2.0     | 10.5 ± 1.2   | 8.9 ± 0.8      | 10.4 ± 1.0 | 90 ± 1 |
|      | TBACl | 3.99 ± 0.16    | 2.97 ± 0.05  | 1.79 ± 0.11    | 2.9 ± 0.8  | 56 ± 7 |

Table S16 Binding constants for host-guest complexes of 1 and 2, measured from ¹H NMR spectra of 9.0 mM solutions of the macrocycles in CDCl₃ with varying concentrations of the added guest. TMS (0.05% w/v) was included as an internal reference (δ = 0.0 ppm). Data were fitted to 1:1 binding isotherms using a Nelder-Mead algorithm in the online software BindFit. Errors in the individual $K_{11}$ measurements correspond to uncertainty in the fits, while standard errors are shown for the mean values.
**Fig. S46** $^1$H NMR spectra illustrating changes in the NH signals of (a) 1 with neutral guests, (b) 2 with neutral guests, (c) 1 with ionic guests and (d) 2 with ionic guests. In all experiments, 50 eq. of guest were added to a 9.0 mM macrocycle solution in CDCl$_3$. Ionic guests were added as TBA salts and TMS (0.05% w/v) was used as an internal reference ($\delta = 0.0$ ppm).
Fig. S47 1H NMR spectra illustrating changes in the NH signals of 1 and 2 (9.0 mM) in CDCl₃ with varying concentrations of hydrated and anhydrous TBACl. Equivalents of TBACl hydrate were estimated assuming a water-chloride ratio of 3:1, and TMS (0.05% w/v) was used as an internal reference (δ = 0.0 ppm). The hydrated and anhydrous salts produce similar changes in the NH chemical shifts, suggesting that water of crystallization has little effect on host-guest binding. The weak influence of water relative to other hydrogen bond donors such as methanol may be attributable to the immiscibility of the guest with CDCl₃.

Fig. S48 1H NMR spectra of (a) 1 and (b) 2 with different concentrations of TBAF trihydrate in CDCl₃. To increase the signal-to-noise ratio of potential product signals, elevated macrocycle concentrations of 17 mM were used in all experiments. No signals are visible in the region 15-17 ppm, suggesting that the macrocycles are not deprotonated by fluoride to form the bifluoride (HF₂⁻) ion.
10 Binding energy calculations

**Fig. S49** Possible orientations of guests relative to macrocycle 1 in the (a) *syn* and (b) *anti* conformations. Host-guest interaction energy ($E_{\text{int}}$) values were calculated using manually constructed model systems of 1 and 2 with all of the guest orientations shown. In some cases, multiple models with similar guest orientations were used, in order to assess the relative stabilities of different packing modes, dipole-dipole interactions and hydrogen bond donor-acceptor motifs. These orientations were not always preserved during the DFT geometry optimizations.

| Guest     | $E_{\text{int}}$ / kJ mol$^{-1}$ |
|-----------|----------------------------------|
|           | syn-1 Orientation                | anti-1 Orientation | syn-2 Orientation | anti-2 Orientation |
|           | A      | B    | C    | A      | B    | C    | A      | B    | C    | A      | B    | C    |
| Chloroform| -52.7  | -34.9 | -38.9| -23.7  | -23.6 | -21.0| -24.2  | -6.8  | -23.9| -23.9 | -10.8|
|           | -52.7  |      |      |        |       |      |        |       |      |       |      |
| Acetone   | -48.3  | -33.4 | -35.6| -19.7  | -11.9 | -17.7| -28.4  | -28.3 | -17.7| -20.4  | -11.6|
|           | -35.6  | -48.3 |      | -18.4  |       |      | -44.6  |       |      | -10.8  |      |
| Acetonitrile| -50.7  | -32.4 | -35.2| -20.9  | -2.1  | -16.6| -33.7  | -33.7 | -16.6| -21.6  | -21.3|
|           | -42.7  |      |      | -12.5  |       |      |        |       |      | -12.4  |      |
| Methanol  | -66.7  | -9.8  | -41.8| -32.6  | -13.8 | -17.4| -38.9  | -10.3 | -17.4| -32.8  | -32.7|
|           | -66.7  | -11.7 |      | -26.9  | -13.9 |      | -54.9  | -12.6 |      | -32.8  | -14.0|
|           | -66.6  | -11.7 |      | -32.6  | -9.8  |      | -38.8  | -12.6 |      | -32.8  | -14.0|
| Fluoride  | -173.3 | -134.7| -187.3| -149.9 | -128.0| -166.9| -181.3 | -138.8| -166.9| -158.9 | -128.4|
|           | -170.9 |      |      | -128.0 |       |      | -181.3 |       |      | -158.9 |      |
|           | -187.3 |      |      |        |       |      | -132.3 |       |      |        |      |
|           | -147.5 |      |      |        |       |      | -166.9 |       |      |        |      |
| Chloride  | -110.4 | -62.0 | -94.2| -69.3  | -48.1 | -78.1| -111.8 | -54.8 | -78.1| -69.1  | -49.9|
|           | -100.0 |      |      | -69.3  |      |      | -98.2  |       |      | -69.1  | -69.1|
| Nitrate   | -95.6  | -76.7 | -68.2| -67.1  | -59.4 | -69.1| -92.1  | -77.6 | -69.1| -67.5  | -59.5|
|           | -106.2 |      |      | -67.1  |      |      | -93.8  |      |      | -67.5  |      |

Table S17 Calculated interaction energy ($E_{\text{int}}$) values for complexes of 1 and 2 with a variety of guests. Each complex was optimized from a range of estimated initial configurations with different relative orientations of the host and guest (see Fig. S49), using the DFT method B3LYP in the basis set 6-31+G*. The output structures were further optimized in the larger basis set 6-31++G**, and the $E_{\text{int}}$ values calculated by subtracting the energies of the separate macrocycles and guest species. For ease of comparison, relative $E_{\text{int}}$ values were determined (Figs. S50 and S51 and Fig. 15, main article) from the absolute $E_{\text{int}}$ values shown in this table by subtracting the energy of the most stable chloroform complex from the most negative $E_{\text{int}}$ value of each complex (highlighted in bold).
| Guest     | BSSE / kJ mol⁻¹ |
|-----------|----------------|
| Chloroform| 0.093          |
|           | 0.079          |
| Acetone   | 0.070          |
|           | 0.062          |
| Acetonitrile| 0.052        |
|           | 0.024          |
| Methanol  | 0.206          |
|           | 0.116          |
|           | 0.221          |
| Fluoride  | 0.034          |
|           | 0.035          |
|           | 0.014          |
|           | 0.010          |
| Chloride  | 0.006          |
|           | 0.063          |
| Nitrate   | 0.130          |
|           | 0.095          |

**Table S18** Basis set superposition error (BSSE) values for DFT optimizations of syn-2 in complexes with various guests. All guests were bound between the oxazolidine rings in the starting structures (orientation A) and optimized with the B3LYP method in the basis set 6-31+G*, followed by refinement in the larger basis set 6-31++G**. BSSE values were calculated by performing the final optimization step with and without counterpoise corrections and calculating the difference between the converged energy values. Energies calculated without counterpoise corrections were more negative in all cases, indicating that BSSE increases the apparent stabilities of the host-guest complexes. However, BSSE accounts for a negligible proportion (<0.6%) of the $E_{int}$ values.
**Fig. S50** Most stable calculated host-guest complexes of chloroform, acetone, acetonitrile and methanol with (a) syn-1, (b) anti-1, (c) syn-2 and (d) anti-2. Geometry optimizations were performed using the DFT method B3LYP in the basis set 6-31+G*, followed by refinement in the larger basis set 6-31++G**. Close contacts (<2.7 Å) between hydrogen bond donor and acceptor sites are marked with red dashed lines, and parts of the macrocycles are omitted for clarity. In all cases, the most stable complexes are formed by guests in orientation A (Fig. S49). $E_{int}$ values are expressed relative to the corresponding chloroform complexes.
Fig. S51 Most stable calculated host-guest complexes of fluoride, chloride and nitrate with (a) syn-1, (b) anti-1, (c) syn-2 and (d) anti-2. Where NH···F and CH···F motifs are similarly stable, both complexes are shown. Geometry optimizations were performed using the DFT method B3LYP in the basis set 6-31+G*, followed by refinement in the larger basis set 6-31++G**. Close contacts (<2.7 Å) between ions and hydrogen atoms are marked with red dashed lines, and parts of the macrocycles are omitted for clarity. In all cases, the most stable complexes are formed by ions in orientation A (Fig. S49). $E_{int}$ values are expressed relative to the corresponding chloroform complexes.
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