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Thiosemicarbazone Dynamic Combinatorial Chemistry: Equilibrator-Induced Dynamic State, Formation of Complex Libraries, and a Supramolecular On/Off Switch

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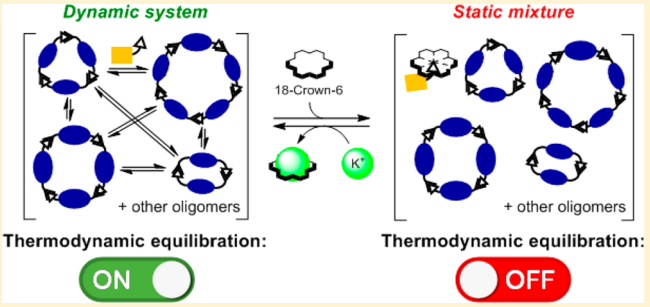
Supporting Information

ABSTRACT: Dynamic combinatorial libraries that equilibrate under thermodynamic control and can be trapped kinetically when desired are key to creating complex systems that can mimic dynamic biological systems, such as the biochemical system of life. A much-sought-after feature is the ability to turn off the dynamic exchange of the system, in order to investigate a transient state away from thermodynamic equilibrium, and then turn on the dynamic exchange again. We describe here the first use of thiosemicarbazone exchange to form dynamic combinatorial libraries. The libraries were found to require a nucleophilic catalyst, or equilibrator, in order to reach thermodynamic equilibrium. This equilibrator approach adds a supramolecular level of control over the dynamic system and allows the dynamic exchange to be turned off by addition of 18-crown-6, which binds the equilibrator in a nonnucleophilic complex. The dynamic exchange can be restarted by addition of potassium ions that competitively bind 18-crown-6, thus liberating the equilibrator. The highly complex thiosemicarbazone-based macrocyclic libraries contain both [2]catenanes and sequence isomers, which can be distinguished by HPLC-MS/MS.



1. INTRODUCTION

Abiogenesis,[1] one theory on the origin of life,[2] describes how life on Earth could have developed from inanimate matter through a wealth of interconnected equilibria by building up more and more complex dynamic systems, which eventually resulted in the formation of living cells.[3] In recent decades, the fields of dynamic combinatorial chemistry and systems chemistry have investigated how interconnected equilibria can lead to highly complex dynamic systems from mixtures of simple molecular building blocks,[4,5] thus providing a bottom-up approach toward understanding how complex systems, such as life itself, could have evolved from relatively simple small molecules.[6]

Dynamic combinatorial chemistry provides an appealing entry point to study systems chemistry.[5] In dynamic combinatorial chemistry, reversible chemical reactions mediate the formation of dynamic combinatorial libraries (DCLs) that equilibrate under thermodynamic control.[4a] The addition of templates to a DCL enables the selection of strong receptors for added templates from the DCLs.[4b]

"Ideal" reversible chemical reactions must live up to a number of requirements to create functioning DCLs; these requirements may differ depending on the individual application of the DCL. Universally, it is essential that the reversible chemical reaction proceeds to the thermodynamic equilibrium, and it is necessary to confirm this each time a new DCL is studied. Other requirements, which are often more difficult to achieve experimentally, are the ability to turn on and off the equilibrating reaction, and that the molecules produced in the DCL experiment are stable enough to be isolated and studied under the conditions of the equilibrating mixture. For example, the use of acylhydrazones as the reversible reaction in DCLs has received significant attention.[4a,7] The reaction between an aldehyde and a hydrazide produces acylhydrazones mediated by acidic conditions, typically the addition of 2−5% trifluoroacetic acid (TFA). This generally gives DCLs that equilibrate under thermodynamic control: the template-amplified species from the DCL can be isolated, and the interactions of the DCLs with the template can be studied. However, the amplification process occurs in the presence of TFA, and the molecular recognition event cannot be studied (e.g., by NMR spectroscopy) under the same conditions as the DCL, as it would cause the DCL to start equilibrating again.

Here we establish thiosemicarbazone exchange by the reaction between an aldehyde and a thiosemicarbazide as a new reversible reaction for dynamic combinatorial chemistry (Figure 1). We also demonstrate the use of a nucleophilic hydrazide catalyst, termed an equilibrator, to make the system reach a thermodynamic equilibrium. By exploiting the requirement for an equilibrator, we show how the dynamic system can be turned on and off via a supramolecular capture/release mechanism. It was found that the thermodynamic equilibration can be turned off by capturing the equilibrator as its ammonium

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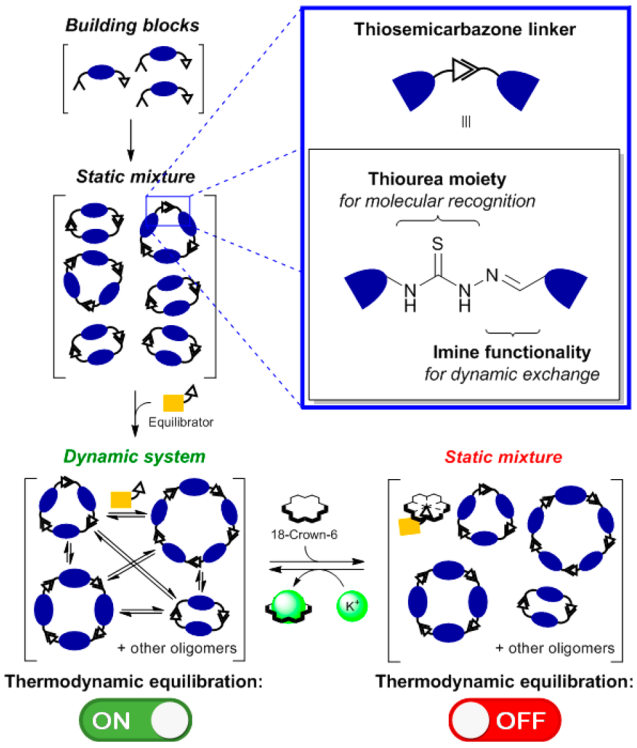


Figure 1. Principle of thiosemicarbazone-based dynamic systems presented in this study. Use of a nucleophilic catalyst, or equilibrator, allows for turning on or off the dynamic state of the system. (Inset) Structure of thiosemicarbazone motif, with thiourea and imine moieties highlighted.

ion complex with 18-crown-6, and by exploiting 18-crown-6's stronger affinity for potassium over ammonium ions, it was also possible to revert the static mixture to its dynamic state. We describe two different types of equilibrators: one that interferes with the DCL (addition of excess thiosemicarbazide) and one that mediates the equilibration but does not incorporate into the DCL (addition of excess hydrazide).

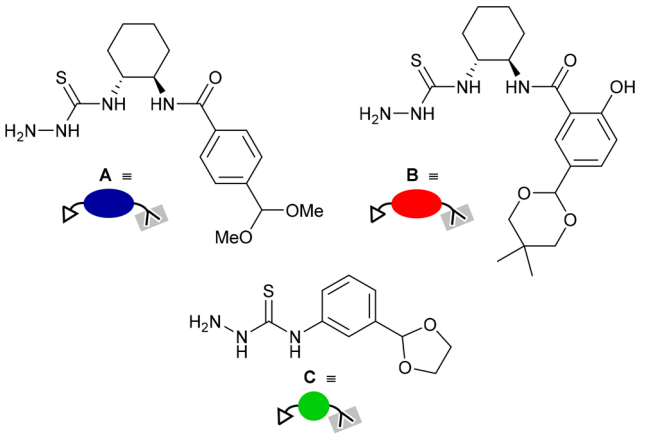
2. RESULTS AND DISCUSSION

Thiosemicarbazones were traditionally used to precipitate aldehydes from solution, and more recently they have been applied in anion recognition.[8] Furthermore, the last couple of decades has seen a vastly expanded use of thiourea-based receptors, for example, for organocatalysis,[9] and the inherent thiourea moiety of thiosemicarbazones is a desirable recognition motif to incorporate into DCLs.

In comparison to acylhydrazones, thiosemicarbazones are expected to be more resilient to hydrolysis (and thus exchange) because of attenuation of the electron-withdrawing effect from the thiocarbamoyl in thiosemicarbazones in comparison to the acyl in hydrazones. Therefore, use of an equilibrator is expected to be required for efficient thiosemicarbazone exchange, as has also been found necessary for some electron-deficient acylhydrazone-based DCLs.[10] This makes thiosemicarbazone exchange ideal for the formation of DCLs whose exchange reaction can be stopped by supramolecular means.

2.1. Building Block Design. Three building blocks, comprising a central scaffold onto which was placed a thiosemicarbazide and a protected aldehyde, were synthesized (see Supporting Information). Building blocks **A** and **B** were both designed around a central 1*R*,2*R*-diaminocyclohexane scaffold in which one amino group was converted into a thiosemicarbazide, while an amide coupling strategy was used to append an aromatic aldehyde-containing moiety onto the other amino group (Chart 1). Use of the C_2-symmetric 1*R*,2*R*-diaminocyclohexane as a central scaffold ensures that only one stereoisomer can result from this type of modular synthesis.

Chart 1. Catalyst Structures



Building block **C** is based on a simple benzene scaffold with a thiosemicarbazone and a protected aldehyde in a 1,3-substitution pattern. While conventional aldehyde protection schemes proved sufficiently stable for building blocks **A** and **C** (acetals of methanol and ethylene glycol, respectively), successful isolation of building block **B** was achieved only after forming the kinetically stabilized acetal of 2,2-dimethylpropane-1,3-diol in a practical example of applying the Thorpe–Ingold effect.[11]

2.2. Reaching Thermodynamic Equilibrium: The Need for an Equilibrator. With building block **A** in hand, we initiated experiments to determine whether thiosemicarbazone exchange can be used to form dynamic systems. Initially, we dissolved **A** (1.0 mM) in chloroform with TFA (5 vol %), thereby exposing the acetal-protected aldehyde function and starting thiosemicarbazone formation (Figure 2). These initial conditions were similar to those used to achieve equilibration of acylhydrazone DCLs. By use of high-performance liquid chromatography (HPLC) with UV detection coupled with mass spectrometry (MS), we were able to identify several of the expected macrocyclic oligomers, $\mathbf{A}_n$, ranging from dimer $\mathbf{A}_2$ to heptamer $\mathbf{A}_7$ in a distribution pattern systematically favoring the smallest macrocyclic species (black trace in Figure 2). Macrocycle formation was fast, as evidenced by the fact that macrocycles were present in the library in high concentrations immediately after preparation.

To verify whether this distribution was a true dynamic system at thermodynamic equilibrium, we isolated the macrocyclic dimer $\mathbf{A}_2$ by preparative chromatography and set up a library under identical conditions to the library started from monomer building block **A**. In a truly thermodynamic system, the library should equilibrate to give the same distribution, but as is evident from Figure 2, the library started from $\mathbf{A}_2$ showed only minor signs of formation of larger macrocycles after 28 days (compare black and red traces in Figure 2). Thus, the thiosemicarbazone exchange reaction is significantly more resistant to equilibration than acylhydrazone exchange.

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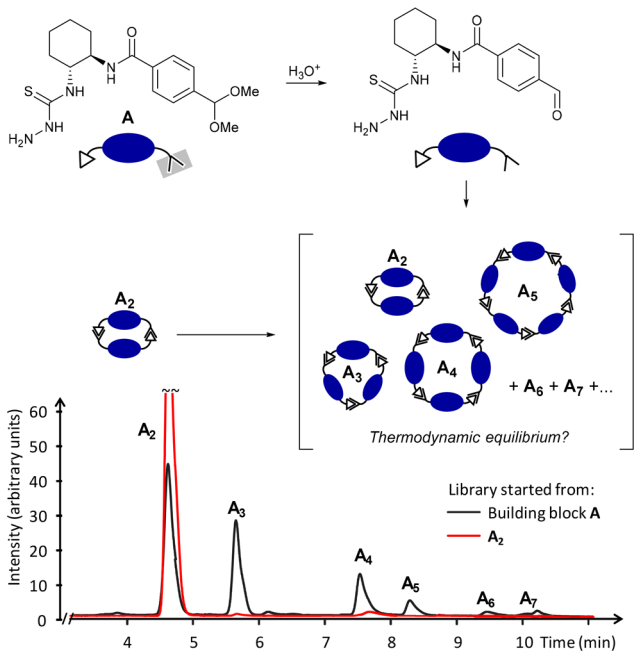
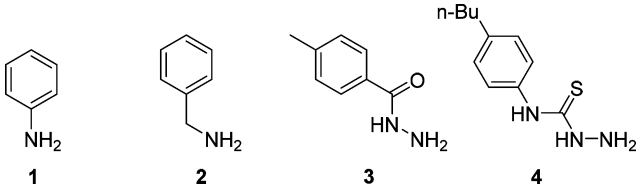


Figure 2. (Top) Schematic representation of acid-catalyzed hydrolysis of the acetal protecting group and subsequent macrocyclization. (Bottom) HPLC-UV chromatograms (at 325 nm) of libraries started from either building block **A** or from isolated macrocycle $\mathbf{A_2}$ after 28 days of equilibration in $CHCl_3$ with 5 vol % TFA.

To overcome this apparent barrier for equilibration, we tested a range of nucleophilic catalysts for their ability to afford efficient exchange between library members and thereby allow the DCL to reach a thermodynamic equilibrium. The catalysts are envisioned to perform a nucleophilic attack on the C−N double bond of the thiosemicarbazones. This leads to a linear thiosemicarbazide, which can then intermolecularly open another macrocyclic library member or perform an intramolecular ring closure, resulting in faster scrambling of the library.[10,12] Because of their specific purpose in this study, these nucleophilic catalysts were termed equilibrators. Several possible equilibrators were screened, ranging in nucleophilicity from aniline **1** up to aromatic thiosemicarbazide **4** (Chart 2).

Chart 2. Equilibrator Structures



Libraries starting from either **A** or $\mathbf{A_2}$ were set up in the presence of equilibrator, and the composition of the resulting libraries was followed by HPLC-UV daily. Use of aniline **1** (100 mM) did speed up the exchange, though the exchange was still too slow to be practically useful. After 28 days in the presence of **1**, the two libraries started from **A** and $\mathbf{A_2}$ had not yet reached identical compositions (Figure S1).

Though benzylamine **2** is orders of magnitude more nucleophilic,[13] use of **2** (100 mM) did not result in faster exchange in comparison to aniline **1**, presumably because of the concomitant higher degree of protonation of the nucleophilic amine. Therefore, we turned our attention to hydrazide and thiosemicarbazide-based equilibrators **3** and **4**.

Both aniline **1** and hydrazide **3** have been employed in previous studies to afford faster exchange in dynamic systems.[10,12] Whereas use of hydrazide **3** in acylhydrazone-based dynamic combinatorial libraries results in formation of linear acylhydrazone species along with the macrocyclic library members, we were delighted to see that this was not the case for our thiosemicarbazone-based system. Thus, use of hydrazide **3** (10 mM) was found to afford efficient exchange without any detectable signs of linear acylhydrazones in the mixture.

Depending on conditions and what building blocks were used, dynamic combinatorial libraries set up by use of equilibrator **3** reached equilibrium within 5−15 days, as illustrated in Figure 3, which shows the composition of two

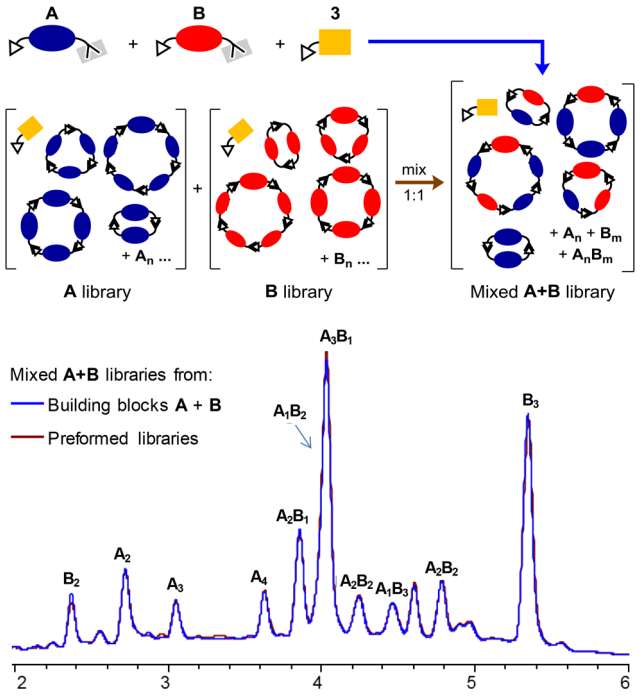


Figure 3. Applying equilibrator **3** afforded DCLs at thermodynamic equilibrium, as evidenced by the fact that mixed **A** + **B** libraries (1:1, 1.0 mM total building block concentration in $CHCl_3$ with 15 vol % methanol, 10 vol % acetonitrile, and 1 vol % TFA) reached the same composition whether they were started by combining the building blocks **A** and **B** or by mixing already formed libraries of **A** and **B**.

different libraries made from **A** and **B** in equimolar amounts (1.0 mM total building block concentration in $CHCl_3$ with 15 vol % methanol, 10 vol % acetonitrile, and 1 vol % TFA). One library was set up simply by mixing the two building blocks. The other library was made by mixing a preformed library of **A** with a preformed library of **B**. A few hours after the two preformed libraries were mixed, macrocycles that contained both **A** and **B** monomer units were detected, and after 15 days, the two libraries had reached the same composition. This observation proves that thiosemicarbazone exchange can be used to form dynamic systems at true thermodynamic equilibrium by use of an equilibrator such as hydrazide **3**.

Use of thiosemicarbazide-based equilibrator **4** (10 mM) also resulted in efficient exchange, giving rise to a dynamic system that reached thermodynamic equilibrium within a matter of 4−6 days. Not surprisingly, libraries with equilibrator **4** contained

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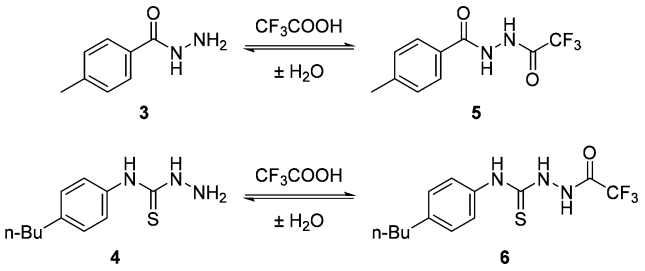
linear thiosemicarbazone species along with the macrocyclic library members (Figure S2). This illustrates that thiosemicarbazone exchange can be applied to dynamic systems containing both macrocyclic and linear library members. Furthermore, along with the notion that these DCLs formed [2]catenanes (as will be discussed), we propose that thiosemicarbazone-based dynamic systems in a future study can be the basis for further exploration of the recently proposed expansion of the Jacobson–Stockmayer cyclization theory to include reversible formation of [2]catenanes.[14]

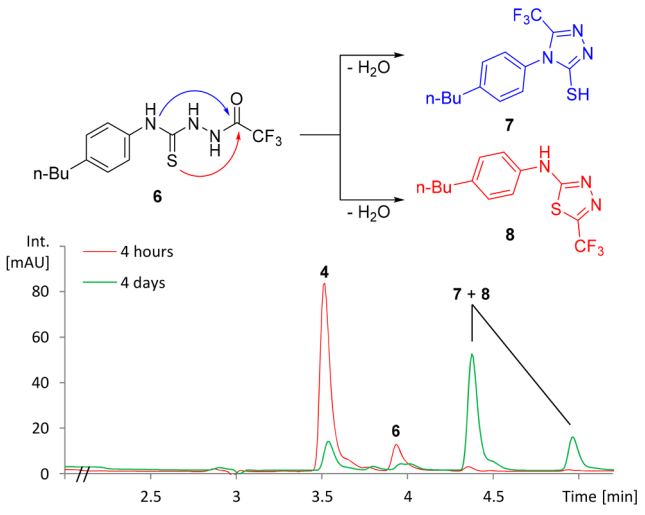
Use of an equilibrator adds another level of control over the dynamic system, as we will exploit further, but it also adds to the overall complexity of the system, and sometimes unexpected side reactions start to occur. We found that using either hydrazide **3** or thiosemicarbazide **4** as equilibrator resulted in formation of unexpected condensation products **5** and **6** with TFA when applied in $CHCl_3$ (Scheme 1).

Scheme 1. Condensation Products 5 and 6



If this condensation reaction is truly reversible, in itself it does not pose a problem for the dynamic system. It was found, however, that when hydrazide **3** was used, the effective concentration of nucleophilic equilibrator became so low that the exchange reaction slowly halted, and for thiosemicarbazide **4** it was found, as evidenced by LC–MS, that condensation product **6** underwent further cyclization reactions to form either of two isomers, triazole **7** or thiadiazole **8** (Scheme 2).

Scheme 2. Cyclization Products 7 and 8[a]



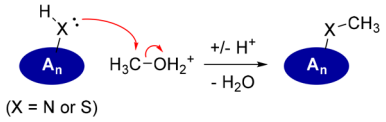
[a]Reaction was performed in $CHCl_3$ with TFA (10 vol %). Chromatogram at 325 nm after 4 h showed formation of condensation product **6**, while the chromatogram taken after 4 days showed predominant presence of cyclization products **7** and **8**.

These aromatic cyclization reactions are, for all practical applications, completely irreversible, and in light of the aforementioned formation of linear library members when equilibrator **4** was used, this side reaction results in trapping the system in an undesired static state.

A range of carboxylic acids were screened to test for this undesired condensation, and the extent of condensation was found to rely on both the pK_a of the acid and the apparent steric bulk of the carboxylic acid (Table S1). It was also found that addition of as little as 0.5 vol % water efficiently prevented the condensation with TFA, and therefore we developed conditions in which up to 1 vol % water was used. This was made possible by adding methanol (15 vol %) and/or acetonitrile (10 vol %) to the otherwise $CHCl_3$-based solvent.

Further examination revealed that sulfonic acids such as trifluoromethanesulfonic acid and *p*-toluenesulfonic acid could also be used to furnish dynamic systems, and inorganic acids such as aqueous HBr or HCl were also applicable when care was taken not to use too high acid concentrations in methanol-containing (15 vol %) solvent mixtures (Tables S2 and S3). Due to their high acidities, use of 1 vol % concentrated aqueous HBr or HCl was found to afford methylation of the library members via protonation of methanol and subsequent nucleophilic attack by a lone pair, presumably from a nitrogen or sulfur atom, of the library member (Scheme 3). This mechanism of methylation was confirmed by setting up a library with 15 vol % CD_3OD in place of nonisotopically labeled methanol (Figures S3 and S4).

Scheme 3. Methylation of Library Members by Protonated Methanol



In summary, we identified suitable conditions under which thiosemicarbazones can exchange to reach a true thermodynamic equilibrium in a matter of days, mediated by use of a nucleophilic hydrazide or thiosemicarbazide equilibrator under acidic conditions in primarily organic solvents. We found that the condensation products that follow from using a hydrazide or a thiosemicarbazide equilibrator in conjunction with a carboxylic acid can be avoided by addition of small amounts of water (0.5–1 vol %) to the reaction mixture. Use of methanol as a cosolvent can lead to unwanted methylation reactions over time, but this occurs only in the presence of high excess of strong mineral acids (HBr or HCl). Alternatively, thiosemicarbazone DCLs can be set up by use of sulfonic acids, such as *p*-toluenesulfonic acid and trifluoromethanesulfonic acid.

2.3. Distinction of Macrocyclic Sequence Isomers by HPLC–MS/MS. When building blocks **A** and **C** were used (1:1, 1.0 mM total building block concentration in $CHCl_3$ with 15 vol % methanol, 10 vol % acetonitrile, and 1 vol % TFA) to form a mixed **A** + **C** library, a complex mixture of both homomeric ($\mathbf{A}_2$, $\mathbf{A}_3$, $\mathbf{C}_3$, $\mathbf{A}_4$, and others) and heteromeric ($\mathbf{A}_1\mathbf{C}_2$, $\mathbf{A}_1\mathbf{C}_3$, $\mathbf{A}_2\mathbf{C}_1$, $\mathbf{A}_2\mathbf{C}_2$, and others) macrocyclic oligomers was formed. A chromatographic method capable of separating most of the peaks in the diverse library was developed, and it was found that two distinct tetrameric species with retention times (RT) of 6.1 and 7.9 min, respectively, both consisted of two **A** units and two **C** units (Figure 4a). Since the two

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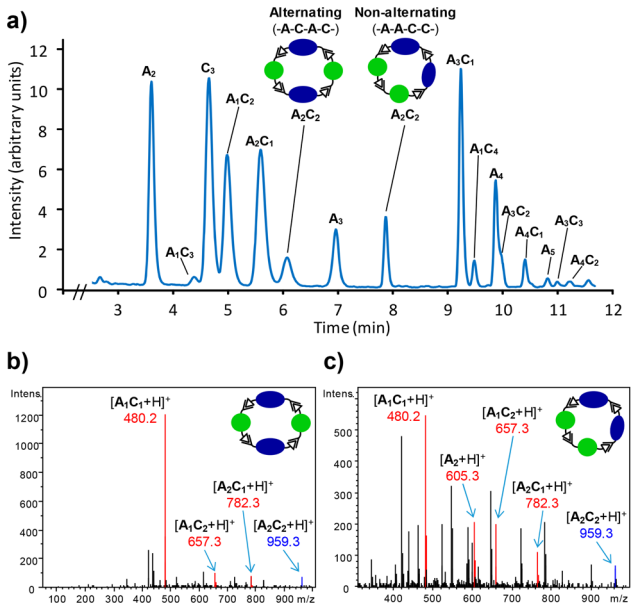

Figure 4. (a) HPLC-UV chromatogram (325 nm) of a library of building blocks **A** and **C**. (b) Tandem mass spectrometry (MS/MS) of the molecular ion (m/z 959.3, shown in blue) of the $\mathbf{A_2C_2}$ species eluting at ca. 6.1 min. (c) MS/MS of the molecular ion (m/z 959.3, shown in blue) of the $\mathbf{A_2C_2}$ species eluting at ca. 7.9 min. Fragment ions corresponding to loss of one or two monomer units are shown in red.

isomeric $\mathbf{A_2C_2}$ oligomers are cyclic (i.e., not linear), only two structural isomers are possible: an alternating isomer (-**A**-**C**-**A**-**C**-) and a nonalternating isomer (-**A**-**A**-**C**-**C**-). All possible [2]catenane structures ($[\mathbf{A_1}]\cdot[\mathbf{A_1C_2}]$, $[\mathbf{A_2}]\cdot[\mathbf{C_2}]$, $[\mathbf{A_1C_1}]\cdot[\mathbf{A_1C_1}]$, and $[\mathbf{A_2C_1}]\cdot[\mathbf{C_1}]$) were ruled out on the basis of the experimental fact that neither $\mathbf{A_1}$, $\mathbf{C_2}$, $\mathbf{A_1C_1}$, nor $\mathbf{C_1}$ was detected experimentally, nor do they seem likely on the basis of simple molecular mechanics.

HPLC-MS/MS study of the two species revealed remarkably different fragmentation patterns, underlining that the two isomers are indeed distinct molecular entities and not two quasi-stable conformations of otherwise identical molecules. The species eluting at 6.1 min had only one large dimeric fragment ion corresponding to $[\mathbf{A_1C_1} + \mathrm{H}]^+$, as well as trimeric fragments corresponding to $[\mathbf{A_1C_2} + \mathrm{H}]^+$ and $[\mathbf{A_2C_1} + \mathrm{H}]^+$ (Figure 4b). The MS/MS spectrum of the peak eluting at 7.9 min also contained all of these fragment ions, albeit at lower intensities, but a dimeric fragment ion corresponding to $[\mathbf{A_2} + \mathrm{H}]^+$ was also evident (Figure 4c). If the unlikely scenario of ion−ion crossover is ruled out,[15] the only isomer that can produce $[\mathbf{A_2} + \mathrm{H}]^+$ fragment ions is the nonalternating isomer (-**A**-**A**-**C**-**C**-).

Similar MS/MS analyses were also used to distinguish two isomers of $\mathbf{A_2B_2}$. Thus, a nonalternating version (-**A**-**A**-**B**-**B**-) was found to elute after 4.2 min, while the alternating isomer (-**A**-**B**-**A**-**B**-) eluted after 4.8 min (peaks marked $\mathbf{A_2B_2}$ in Figure 3; further details in Figure S5).

2.4. Identification of a [2]Catenane from Dynamic System of Macrocyclic Thiosemicarbazones. It was found that dynamic libraries of building block **A** contained two distinct hexameric species (Figure 5a). To identify the structures of these two hexamers, we performed a HPLC-MS/MS study. The molecular ion corresponding to a hexamer of **A** (m/z 1814.9, $[\mathrm{M} + \mathrm{H}]^+$) was selected by a hexapole mass

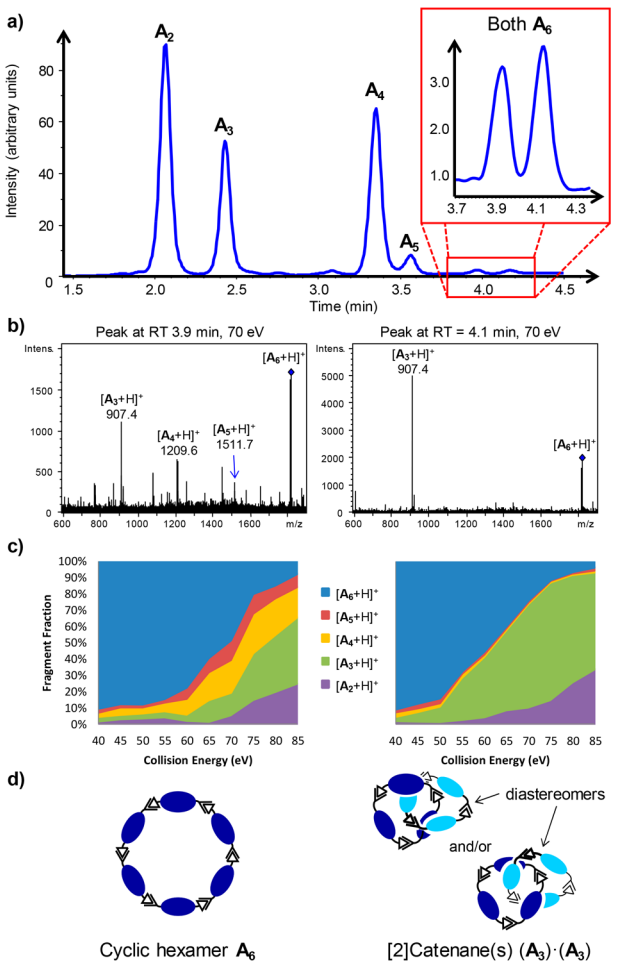

Figure 5. (a) HPLC-UV chromatogram (325 nm) of a library of building block **A** with peaks assigned on the basis of MS. (b) MS/MS at a collision cell energy of 70 eV of hexameric species at RT = 3.9 min (left) and RT = 4.1 min (right). (c) Distribution of intensities of hexameric, pentameric, tetrameric, trimeric, and dimeric ions in MS/MS analysis of both hexameric species as a function of applied collision cell energy. (d) Structural representations of distinct hexameric species corresponding to the peak at RT = 3.9 min (left) and 4.1 min (right).

filter and fragmented in a hexapole collision cell, and finally the intensity of daughter ions (and surviving molecular ion) was measured by passing the ions through a time-of-flight mass spectrometer. The two hexamers showed clearly divergent ion breakdown patterns. The species in the early peak (RT 3.9 min) produced several identifiable daughter ions (at a collision cell energy of 70 eV) corresponding to loss of exactly one, two, or three monomer units, with each daughter ion at lower intensity than the molecular ion (Figure 5b). On the other hand, at the same collision cell energy, the later-eluting hexamer (RT 4.1 min) produced only one daughter ion corresponding to loss of exactly three monomer units, and the intensity of this daughter ion was twice that of the molecular ion.

Upon sweeping the collision cell energy from 40 to 85 eV in a series of consecutive runs, a clear pattern emerged: the hexamer at 3.9 min produced pentameric, tetrameric, and trimeric daughter ions, while the hexamer at 4.1 min produced only trimeric daughter ions (Figure 5c). In both cases, dimeric daughter ions were also seen at higher collision cell energies. This evidence strongly suggests that the species eluting at 3.9 min is a macrocyclic hexamer (i.e., a cyclic $\mathbf{A_6}$ species), while

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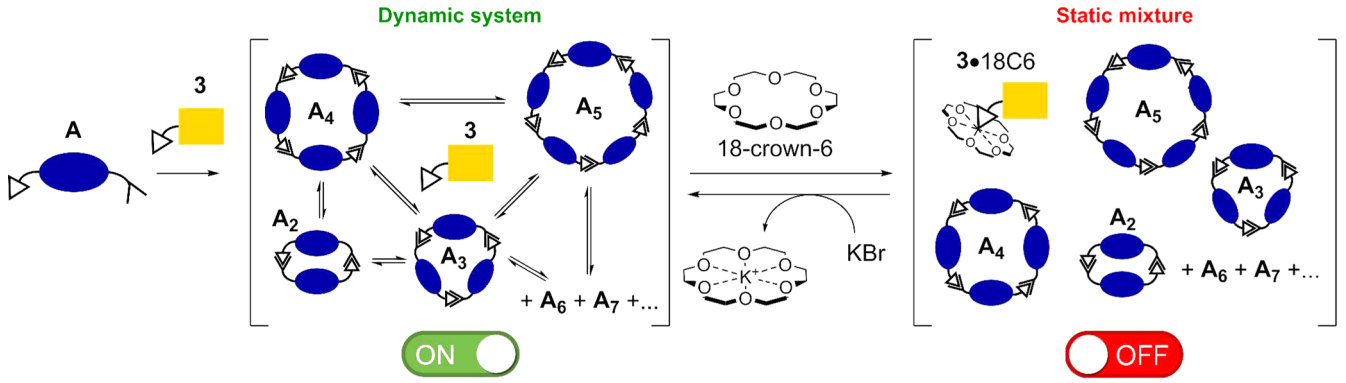

Figure 6. A dynamic mixture of macrocyclic oligomers of **A** is formed by aid of equilibrator **3**. The dynamic exchange of this mixture is then turned off by addition of 18-crown-6, which binds the equilibrator to form nonnucleophilic complex **3**·18C6. Turning the dynamic state of the system back on can be achieved by addition of a potassium salt, since the K^+ ion will competitively bind to 18-crown-6 and thereby liberate the equilibrator.

the species eluting at 4.1 min consists of two mechanically interlocked macrocyclic trimers (i.e., two $\mathbf{A}_3$ species forming a [2]catenane, $[\mathbf{A}_3]\cdot[\mathbf{A}_3]$ (Figure 5d). The HPLC-UV−MS/MS data do not allow for the distinction between the two diastereotopic $[\mathbf{A}_3]\cdot[\mathbf{A}_3]$ [2]catenanes, nor is it possible to verify whether the library produces both diastereomers or only one. The formation of this [2]catenane, which is the first thiosemicarbazone-based catenane reported, illustrates the propensity for dynamic systems to build up molecular complexity.

2.5. From Dynamic to Static and Back: Supramolecular On/Off Switch. The ability to reach a thermodynamic equilibrium is a defining trait of a true dynamic combinatorial library, but the ability to stop the equilibration process can be of equal importance in dynamic systems. For instance, in order to study a specific transient composition or isolate a short-lived library member, it is useful, and sometimes required, to be able to turn off the dynamic exchange reaction at any given time. Ideally, the method to stop the dynamic exchange should be simple, work instantaneously, and be reversible, in the sense that it is possible to turn back on the dynamic exchange, that is, reinitiate the exchange reaction to re-establish a dynamic state.

The fact that the thiosemicarbazone dynamic system developed herein requires a nucleophilic catalyst equilibrator was exploited to implement such an on/off protocol. Addition of 18-crown-6 (1.6-fold excess compared to equilibrator) to a library of building block **A** resulted in trapping of the equilibrator, as it forms an ammonium ion−crown ether complex, **3**·18C6 (Figure 6). This effectively stopped the dynamic exchange reaction as evidenced by the lack of change in composition (Figure 7). In essence, 18-crown-6 turns off the dynamic state of the system by preventing the dynamic exchange reaction from taking place.

Finally, by exploiting 18-crown-6's high affinity for potassium ions (log K = 6.10, compared to log K = 4.27 for ammonium ions in methanol),[16] it was possible to restart the exchange reaction by shaking the DCL with KBr (ca. 3-fold excess compared to equilibrator **3** was added, though not all of the KBr dissolved). Thus the dynamic system, which was deliberately frozen long before it had reached its thermodynamic equilibrium, started adjusting composition immediately after equilibrator **3** was released from its 18-crown-6 trap (Figure 7).

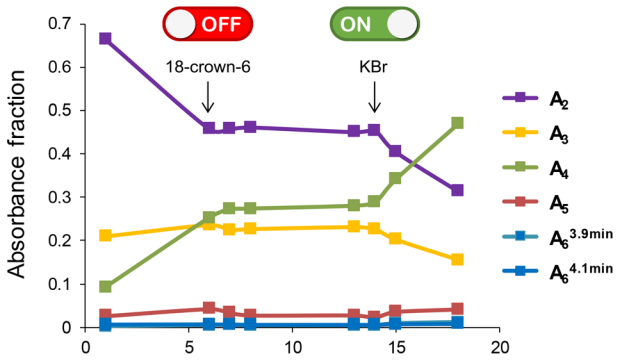

Figure 7. Composition of dynamic library of **A** (chromatograms at 325 nM) as a function of time (days). Addition of 18-crown-6 completely stops library evolution by effectively removing equilibrator **3** from the mixture. Library evolution is started again immediately upon addition of KBr to release **3** by competitive binding of 18-crown-6 by K^+ ions.

3. CONCLUSIONS

We have developed the first example of a thiosemicarbazone-based dynamic combinatorial library. With the set of building blocks produced so far, we showcase that a nucleophilic catalyst, dubbed an equilibrator, is necessary to facilitate the exchange reaction, allowing us to obtain a dynamic system of covalently bound oligomers at thermodynamic equilibrium.

We have shown that the dynamic system produces complex mixtures of macrocyclic oligomers, including formation of a [2]catenane, which was identified by HPLC−MS/MS. Finally, we have demonstrated how the dynamic state of the system can be turned off by addition of 18-crown-6, which binds the equilibrator in an ammonium ion−crown ether complex. The equilibration between oligomers can be reinitiated by addition of potassium ions, which preferentially bind to 18-crown-6 and therefore liberate the equilibrator.

4. EXPERIMENTAL SECTION

Unless stated otherwise, all starting materials and solvents were purchased from commercial suppliers and used as received. HPLC-grade solvents were used for synthesis and library setup, and when needed they were dried prior to synthesis by standing over molecular sieves. Water content of dried solvents and reagents was measured on a Metrohm 737 KF coulometer. Dry column vacuum chromatography (DCVC) was performed with 60 Å, 400−800 mesh silica and solvents of technical or HPLC grade. Melting points are uncorrected.

NMR spectra were recorded on a Bruker Ultrashield Plus 500 spectrometer, operating at 500 MHz (^{1}H) and 126 MHz (^{13}C). All ^{19}F

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spectra, however, were recorded on an Oxford NMR 300 spectrometer, operating at 282 MHz. Unless specified otherwise, all spectra were obtained at 298 K and are referenced to the internal solvent residue for ^{1}H and ^{13}C and to TFA in a sealed tube for ^{19}F. $CDCl_3$ was dried by standing over molecular sieves (4 Å) and treated with oven-dried basic aluminum oxide when the presence of water and/or acid sensitivity was an issue. Chemical shifts (δ) are quoted in parts per million (ppm), and coupling constants (J) are listed in hertz. The following abbreviations are used for convenience in reporting the multiplicities of NMR resonances: s, singlet; d, doublet; t, triplet; q, quartet; p, pentet; m, multiplet; br s, broad singlet. The NMR data were processed by use of MestReNova v8.0.0 from MestreLab Research S.L. Assignment of ^{1}H and ^{13}C resonances was achieved by standard one- and two-dimensional NMR techniques: correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY), ^{13}C attached proton test (APT), heteronuclear single quantum coherence (HSQC; ^{1}H and ^{13}C), and heteronuclear multiple-bond correlation (HMBC; ^{1}H and ^{13}C).

Mass spectrometry, including high-resolution mass spectrometry, was performed on the LC−MS apparatus described below. Isotopic patterns were calculated and visualized by use of IsotopePattern, a part of the LC−MS software package DataAnalysis developed by Bruker Daltonics GmbH. This software package was also used to visualize HPLC chromatograms.

HPLC analysis was performed on a Dionex UltiMate 3000 system, which incorporates an UltiMate 3000 diode-array UV−vis detector capable of measuring absorbance of light in the 190−800 nm range. LC−MS was carried out by connecting the HPLC apparatus to a Bruker MicrOTOF-QII system equipped with an electrospray ionization (ESI) source with nebulizer gas at 1.2 bar, dry gas at 8 L/min, dry temperature at 200 °C, capillary at 4500 V, and end plate offset at −500 V. For m/z values in the 100−1000 range, ion transfer was conducted with funnel 1 and funnel 2 voltage at 200.0 peak-to-peak voltage (Vpp) and hexapole voltage at 100.0 Vpp while the quadrupole ion energy was set at 5.0 eV with a low mass cutoff at 100.00 m/z. In the collision cell, collision energy was set at 8.0 eV and collision voltage at 100.0 Vpp, and a transfer time of 80.0 μs and prepulse storage of 1.0 μs were used. For detection of m/z values in the 500−2500 range, the following parameters were changed: transfer was conducted with funnel 1 voltage at 300.0 Vpp, funnel 2 voltage at 400.0 Vpp, and hexapole voltage at 400.0 Vpp, while the low mass cutoff in the quadrupole was changed to 300.00 m/z. The collision cell energy was raised to 10.0 eV and the collision voltage to 630.0 Vpp. Generally, both detection ranges were used when analyzing libraries to ensure identification of all peaks, with low-range settings used during the first minutes and high-range settings used for the remainder of the analysis.

Solvents and additives of LC−MS grade were purchased from commercial suppliers and used as received. Water was purified on a Millipore Milli-Q Integral 5 system.

Separations were achieved by use of one of four columns I−IV, all maintained at 20 °C unless stated otherwise: (I) Dionex Acclaim rapid separation liquid chromatography (RSLC) 120 C18, 2.2 μm, 120 Å, 2.1 × 50 mm column; (II) same as I except a C8 stationary phase and dimensions of 2.1 × 150 mm; (III) Dionex Acclaim RSLC PolarAdvantage II (PA2) C18, 2.2 μm, 120 Å, 2.1 × 50 mm column; (IV) Waters Acquity ultraperformance liquid chromatography (UPLC) high-strength silica (HSS) C18, 1.8 μm, 100 Å, 2.1 × 50 mm column. The mobile-phase solutions prepared were (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. Injection volumes were generally between 0.10 and 0.30 μL. The column was conditioned to the starting eluent with at least five column volumes prior to each injection.

4.1. Tetramethylfluoroformadinium Hexafluorophosphate (TFFH). Tetramethylurea (100 mL, 0.85 mol) and phosphorus oxychloride (90 mL, 0.96 mol) were stirred at 100 °C under N_2 atmosphere for 1 h before the solution was allowed to reach room temperature. The solution was transferred to a 5 L three-necked round-bottomed flask equipped with a mechanical stirrer, an addition funnel, and a thermometer, and CH_2Cl_2 (2 L) was added. While the solution was cooled in an ice bath, a 60% aqueous solution of hexafluorophosphoric acid (125 mL, 0.85 mol) were added at such a rate that the solution temperature did not exceed 5 °C (approximately 10 min). After addition of water (0.5 L), the organic phase was separated, dried over Na_2SO_4, filtered, and concentrated in vacuo. The remaining crude tetramethylchloroformadinium hexafluorophosphate salt was dissolved in acetonitrile (200 mL), and KF (50 g, 0.86 mol; predried at 125 °C overnight) was added. The solution was stirred at room temperature for approximately 20 h before the precipitate was filtered off. The mother liqueur was concentrated in vacuo, and the resulting precipitate was recrystallized by redissolving it in acetonitrile (50 mL), adding diethyl ether (100 mL), and stirring the resulting two-phase system vigorously at ÷42 °C (dry ice/acetonitrile bath). Overall yield: 111 g (50%) of white crystals.

Melting point 98−101 °C (lit. 108−110 °C).[17] ^{1}H NMR (500 MHz, CD_3CN) δ 3.14 (s). ^{13}C NMR (126 MHz, CD_3CN) δ 157.6 (d, J = 270.4), 40.4.

4.2. 4-Dimethoxymethylbenzoic Acid. To a solution of 4-formylbenzoic acid (20.2 g, 0.13 mol) in methanol (380 mL, 0.35 M) was added ammonium chloride (30.0 g, 0.56 mol) in one portion. The solution, which contained some undissolved ammonium chloride, was heated to reflux for 24 h before the methanol was evaporated in vacuo. The remaining solid was dissolved in diethyl ether (250 mL) and filtered to remove undissolved ammonium chloride. The ethereal phase was washed with water (100 mL), dried over Na_2SO_4, and filtered before the solvent was removed in vacuo to give 28.75 g (83%) of a white powder.

Melting point 119−120 °C (lit. 118−119 °C).[18] ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.30 (br s, 1H), 8.12 (d, J = 8.3 Hz, 2H), 7.58 (d, J = 8.2 Hz, 2H), 5.46 (s, 1H), 3.34 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.8, 144.0, 130.3, 129.3, 127.1, 102.4, 52.9. Anal. Calcd for $C_{10}H_{12}O_4$: C, 61.22; H, 6.16. Found: C, 61.41; H, 6.03. HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{10}H_{12}O_4Na^+$ 219.0628, found 219.0632; $[M - H]^-$ calcd for $C_{10}H_{11}O_4^-$ 195.0663, found 195.0666.

4.3. *N*-[(1*R*,2*R*)-2-Aminocyclohexyl]-4-(dimethoxymethyl)-benzamide. 4-Dimethoxymethylbenzoic acid (1.34 g, 6.83 mmol) and TFFH (1.81 g, 6.85 mmol) were dissolved in CH_2Cl_2 (200 mL, 0.03 M) at 0 °C, and triethylamine (4.8 mL, 35 mmol) was added. After 5 min, (1*R*,2*R*)-1,2-diaminocyclohexane (0.82 g, 7.2 mmol) was added and the reaction mixture was stirred for 3 days at room temperature. The reaction mixture was washed with water (200 mL), and the resulting aqueous phase was washed with CH_2Cl_2 (3 × 60 mL) before the combined organic phases were dried (Na_2SO_4) and the product was purified by DCVC. A column of 5 cm diameter packed with 6 cm of silica was used, and all fractions contained 0.1% triethylamine and had a volume of 50 mL. Gradient: pure heptane to pure EtOAc in 25% increments and then to 30% methanol in EtOAc in 5% increments. Product coeluted with an unidentified impurity (as observed by thin-layer chromatography) in fractions containing 5−25% methanol. Therefore, these fractions were combined and subjected to further purification by DCVC, this time first eluting the byproduct by running four fractions of EtOAc on a column of the same dimensions and finally eluting the pure product by addition of methanol to the EtOAc fractions in 10% increments until all the product had eluted. Evaporation of solvent yielded 1.12 g (60%) of pale yellow crystals.

Melting point 168−169 °C (decomp). ^{1}H (500 MHz, $CDCl_3$) δ 7.78 (d, J = 8.4 Hz, 2H), 7.52 (d, J = 8.0 Hz, 2H), 6.16−6.00 (m, 1H), 5.44 (s, 1H), 3.78−3.67 (m, 1H), 3.32 (s, 6H), 2.55−2.44 (m, 1H), 2.21−2.11 (m, 1H), 2.06−1.97 (m, 1H), 1.81−1.72 (m, 2H), 1.47−1.17 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.4, 141.4, 134.7, 126.9, 126.7, 102.2, 56.6, 55.7, 52.5, 35.7, 32.5, 25.1, 25.0. HRMS (ESI-TOF) m/z $[M + H]$ calcd for $C_{16}H_{25}N_2O_3^+$ 293.1860, found 293.1866.

4.4. Building Block A. *N*-[(1*R*,2*R*)-2-Aminocyclohexyl]-4-(dimethoxymethyl)benzamide (1.17 g, 4.00 mmol) was dissolved in anhydrous methanol (35 mL, 30 ppm). Carbon disulfide (2.42 mL, 40 mmol) and triethylamine (0.73 mL, 4.0 mmol) were added and the resulting solution was stirred for 20 min before the reaction mixture was cooled in an ice bath. Di-*tert*-butyl dicarbonate (840 mg, 3.85

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mmol) dissolved in methanol (5 mL) and 1,4-diazabicyclo[2.2.2]-octane (DABCO; 4.4 mg, 1 mol %) were added, and after the mixture was stirred for 5 min, the ice bath was removed. The solution was stirred for an additional 15 min before the solvent was removed in vacuo. The remaining raw isothiocyanate was redissolved in CH_2Cl_2 (40 mL) and cooled in an ice bath before hydrazine hydrate (60%, 1.94 mL, 40 mmol) was added dropwise over 5 min. After addition, the ice bath was removed and the solution was stirred for an additional 15 min before the reaction mixture was quenched with water (30 mL). The organic phase was collected, and the aqueous phase was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated on Celite in vacuo for purification by DCVC. A column of 4 cm diameter packed with 6 cm of silica was used, and all fractions were 50 mL and contained 0.5% triethylamine. A gradient of 20% ethyl acetate in heptane, followed by a gradient of 2% methanol in ethyl acetate, was used. Fractions 6−9 were collected and concentrated in vacuo to yield 1.17 g (80%) of a slightly yellow powder.

Melting point 176−178 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.91 (d, J = 8.3 Hz, 2H), 7.53 (s, 1H), 7.50 (d, J = 8.3 Hz, 2H), 7.46 (s, 1H), 6.87 (s, 1H), 5.41 (s, 1H), 4.56−4.45 (m, 1H), 3.98−3.89 (m, 1H), 3.67 (s, 2H), 3.32 (s, 6H), 2.32−2.26 (m, 1H), 2.17−2.11 (m, 1H), 1.86−1.77 (m, 2H), 1.48−1.32 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 182.6, 167.2, 141.4, 134.5, 127.4, 127.0, 102.6, 56.6, 56.0, 52.8, 32.6, 32.6, 25.1, 24.7. Anal. Calcd for $C_{17}H_{26}N_4O_3S$: C, 55.71; H, 7.15; N, 15.29. Found: C, 55.90; H, 7.14; N, 15.08. HRMS (ESI-TOF) m/z $[M + H]^+$ calcd for $C_{17}H_{27}N_4O_3S^+$ 367.1798, found 367.1801; $[M + Na]^+$ calcd for $C_{17}H_{26}N_4NaO_3S^+$ 389.1618, found 389.1618.

4.5. Dimer A_2. Building block **A** (100.0 mg) was dissolved in CH_2Cl_2 (2 L), and TFA (2.5 mL) was added. After the mixture was stirred for 20 h at room temperature, the bulk of the solvent was removed by rotary evaporation before the crude product was thoroughly dried in high vacuum. The crude product was redissolved in CH_2Cl_2 and evaporated onto Celite for purification by DCVC. A column of 3 cm diameter packed with 6 cm of silica was used. All fractions were 30 mL and contained 4% triethylamine. A gradient of 10% ethyl acetate in heptane was used, and 18.6 mg of the pure dimer was obtained as a slightly yellow powder from fractions 9 and 10. Fractions 11 and 12 were combined and another column (same parameters) was run on the crude product from these fractions to yield another 21.3 mg of the pure dimer. Yield 40.3 mg (49%).

Melting point 248−255 °C (decomp). ^{1}H NMR (500 MHz, CD_3CN) δ 9.38 (s, 2H, NH), 7.98 (d, J = 4.6 Hz, 2H, NH), 7.82 (d, J = 8.3 Hz, 4H), 7.71 (s, 2H, imine-CH), 7.52 (d, J = 8.3 Hz, 4H), 7.24 (d, J = 8.4 Hz, 2H, NH), 4.38 (qd, J = 11.8, 3.7 Hz, 2H), 3.77 (tt, J = 10.9, 4.3 Hz, 2H), 2.99−2.93 (m, 2H), 2.01 (d, J = 12.0 Hz, 2H), 1.88−1.75 (m, 4H), 1.63 (qd, J = 12.5, 3.6 Hz, 2H), 1.56−1.36 (m, 4H), 1.35−1.23 (m, 2H). ^{13}C NMR (126 MHz, CD_3CN) δ 180.0 (C═S), 167.9 (C═O), 141.8 (imine-C), 137.8, 134.9, 128.9, 127.7, 61.4, 53.0, 32.9, 32.9, 26.3, 25.0. HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{30}H_{36}N_8NaO_2S_2^+$ 627.2295, found 627.2301.

4.6. Methyl 5-Formyl-2-hydroxybenzoate. 5-Formyl-2-hydroxybenzoic acid (2.22 g, 0.134 mol) was suspended in methanol, and concentrated sulfuric acid (1 mL) was added dropwise over ca. 5 min. The mixture was heated to boiling and kept at refluxing temperature for 24 h. After cooling to room temperature, the mixture was concentrated by rotary evaporation. The crude product was dissolved in ethyl acetate and filtered through a plug of silica. Ethyl acetate was removed by rotary evaporation. The crude product was dissolved in methanol (35 mL), and CH_2Cl_2 was added. The solution was washed with water and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic phases were dried (Na_2SO_4) and concentrated to reveal the desired product. Yield 2.31 g (96%).

Melting point 79−80 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.36 (s, 1H), 9.89 (s, 1H), 8.39 (d, J = 2.0 Hz, 1H), 8.01 (dd, J = 8.6, 2.0 Hz, 1H), 7.11 (d, J = 8.6 Hz, 1H), 4.01 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 190.0, 170.1, 166.5, 135.8, 134.0, 128.8, 118.9, 112.8, 53.0. MS: (EI) 180.1. Anal. Calcd for $C_9H_8O_4$: C, 60.00; H, 4.48. Found: C, 60.08; H, 4.53.

4.7. Methyl 2-(Benzyloxy)-5-formylbenzoate. Methyl 5-formyl-2-hydroxybenzoate (2.31 g, 12.8 mmol) was dissolved in CH_2Cl_2/methanol (1:1, 90 mL), and benzyl bromide (7 mL, 0.06 mol) and K_2CO_3 (9.56 g, 69 mmol) were added. The mixture was heated to boiling and kept at refluxing temperature for 2 days before it was allowed to reach room temperature. Excess K_2CO_3 was removed by filtration, and the solvent was removed by rotary evaporation. The crude product was purified by DCVC with a 5% gradient of ethyl acetate in heptane (column diameter 6 cm packed with ca. 6 cm silica, fraction volume 100 mL). Fractions 5−8 were combined and concentrated and the resulting residue was purified once more by DCVC with the same parameters as before. Fractions 7 and 8 were combined and concentrated to reveal the title compound as a clear light yellow oil. Yield 2.20 g (64%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.91 (s, 1H), 8.36 (d, J = 2.2 Hz, 1H), 7.98 (dd, J = 8.7, 2.2 Hz, 1H), 7.49 (d, J = 7.5 Hz, 2H), 7.41 (t, J = 7.5 Hz, 2H), 7.34 (d, J = 7.5 Hz, 1H), 7.14 (d, J = 8.7 Hz, 1H), 5.29 (s, 2H), 3.94 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 190.0, 165.5, 162.6, 135.7, 134.6, 134.3, 129.3, 128.7, 128.2, 126.8, 121.1, 113.7, 70.8, 52.3. HRMS (ESI-TOF) m/z $[M + H]^+$ calcd for $C_{16}H_{15}O_4^+$ 271.0965, found 271.0967.

4.8. Methyl 2-(Benzyloxy)-5-(5,5-dimethyl-1,3-dioxan-2-yl)-benzoate. Methyl 2-(benzyloxy)-5-formylbenzoate (2.20 g, 8.15 mmol) was dissolved in toluene (125 mL), and 2,2-dimethyl-1,3-propanediol (4.97 g, 48 mmol) and *p*-toluenesulfonic acid (0.15 g, 0.87 mmol) were added. The mixture was heated to boiling, and water was removed by azeotropic distillation with a Dean−Stark trap. After 5 h, no more water distilled over, and the reaction mixture was allowed to reach room temperature before the solvent was removed by rotary evaporation. The product was purified by DCVC with a 1% gradient of ethyl acetate in toluene with 1% triethylamine (column diameter 6 cm, packed with ca. 6 cm silica, fraction volume 100 mL). Fractions 3−6 were combined and concentrated to reveal the desired product as light yellow crystals. Yield 1.39 g (48%).

Melting point 114−115 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (d, J = 2.3 Hz, 1H), 7.59 (dd, J = 8.7, 2.3 Hz, 1H), 7.49−7.47 (m, 2H), 7.38−7.35 (m, 2H), 7.31−7.29 (m, 1H), 7.00 (d, J = 8.6 Hz, 1H), 5.36 (s, 1H), 5.20 (s, 2H), 3.90 (s, 3H), 3.77−3.74 (m, 2H), 3.65−3.62 (m, 2H), 1.28 (s, 3H), 0.80 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.5, 158.6, 136.8, 131.3, 131.2, 130.1, 128.7, 127.9, 126.9, 120.5, 113.9, 101.0, 77.8, 70.8, 52.1, 30.3, 23.2, 22.0. MS (EI) 356.2. Anal. Calcd for $C_{21}H_{24}O_5$: C, 70.77; H, 6.79. Found: C, 70..86; H, 6.66.

4.9. 2-(Benzyloxy)-5-(5,5-dimethyl-1,3-dioxan-2-yl)benzoic Acid. Methyl 2-(benzyloxy)-5-(5,5-dimethyl-1,3-dioxan-2-yl)benzoate (1.11 g, 3.11 mmol) was dissolved in tetrahydrofuran (50 mL), and aqueous LiOH (1 M, 4 mL, 4 mmol) was added. The mixture was stirred at ambient temperature for ca. 60 h, after which an additional amount of aqueous LiOH (1 M, 4 mL, 4 mmol) was added. After stirring for 5 h, aqueous NaOH (2 M, 3 mL, 6 mmol) was added, and the mixture was stirred at room temperature for 22 h before it was heated to boiling and kept at refluxing temperature for ca. 4 h. After the mixture was cooled to room temperature, aqueous HCl (2 M, 35 mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 100 mL). The organic extracts were dried (Na_2SO_4) and concentrated to reveal the desired product as a light yellow oil. Yield 1.03 g (97%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.31 (d, J = 2.2 Hz, 1H), 7.72 (dd, J = 8.6, 2.2 Hz, 1H), 7.43−7.38 (m, 5H), 7.11 (d, J = 8.6 Hz, 1H), 5.38 (s, 1H), 5.29 (s, 2H), 3.77−3.73 (m, 2H), 3.65−3.62 (m, 2H), 1.27 (s, 3H), 0.79 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.5, 157.7, 134.4, 133.2, 132.9, 132.2, 129.2, 127.9, 117.9, 113.3, 100.6, 77.7, 72.4, 30.3, 23.2, 22.0. HRMS (ESI-TOF) m/z $[M + H]^+$ calcd for $C_{20}H_{23}O_5^+$ 343.1540, found 343.1543.

4.10. *N*-(1*R*,2*R*)-2-Aminocyclohexyl-2-(benzyloxy)-5-(5,5-dimethyl-1,3-dioxan-2-yl)benzamide. 2-(Benzyloxy)-5-(5,5-dimethyl-1,3-dioxan-2-yl)benzoic acid (1.03 g, 3.0 mmol) was dissolved in CH_2Cl_2 and cooled to 0 °C (ice bath). Tetramethylfluoroformadinium hexafluorophosphate (0.88 g, 7.4 mmol) and triethylamine (2.0 mL, 14 mmol) were added, and the mixture was stirred for 10 min before (1*R*,2*R*)-1,2-diaminocyclohexane (0.38 g, 3.3 mmol) was added. The mixture was stirred for ca. 20 h at room temperature. CH_2Cl_2 (50 mL)

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and water (100 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic phases were dried (Na_2SO_4) and concentrated. The product was purified by DCVC in three consecutive runs with a 20−25% gradient of ethyl acetate in heptane, followed by a 1−5% gradient of methanol in ethyl acetate. The final compound was a light yellow solid. Yield 0.27 g (21%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.28 (d, J = 2.2 Hz, 1H), 7.70 (d, J = 8.5 Hz, 1H), 7.63 (d, J = 8.5 Hz, 1H), 7.44−7.39 (m, 5H), 7.05 (d, J = 8.5 Hz, 1H), 5.38 (s, 1H), 5.13 (s, 2H), 3.75−3.71 (m, 2H), 3.68−3.60 (m, 3H), 2.10−2.00 (m, 1H), 1.89−1.84 (m, 2H), 1.68−1.57 (m, 4H), 1.27 (s, 3H), 1.15−1.08 (m, 2H), 0.78 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.0, 156.9, 135.4, 132.2, 130.8, 130.2, 128.9, 128.4, 121.8, 112.5, 101.1, 77.6, 71.6, 56.1, 55.8, 34.6, 32.2, 30.2, 25.0, 24.9, 23.1, 21.9. HRMS (ESI-TOF) m/z $[M + H]^+$ calcd for $C_{26}H_{35}N_2O_4^+$ 439.2591, found 439.2610.

4.11. *N*-(1*R*,2*R*)-2-Aminocyclohexyl-5-(5,5-dimethyl-1,3-dioxan-2-yl)-2-hydoxybenzamide. *N*-(1*R*,2*R*)-2-Aminocyclohexyl 2-(benzyloxy)-5-(5,5-dimethyl-1,3-dioxan-2-yl)benzamide (0.27 g, 0.62 mmol) was dissolved in methanol (30 mL), and Pd/C (10%, 27 mg) suspended in methanol (10 mL) was added. By use of a three-way valve equipped with a H_2 balloon, three vacuum/H_2 cycles were performed to exchange the air in the flask for H_2, and the reaction mixture was stirred for ca. 18 h. The mixture was filtered through a plug of Celite, and the solvent was removed to reveal the desired product as a white solid. Yield 0.18 g (86%).

Melting point 134−135 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56 (s, 1H), 7.49 (d, J = 8.5 Hz, 1H), 6.95 (d, J = 8.5 Hz, 1H), 6.65 (s, 1H), 5.33 (s, 1H), 3.76 (d, J = 11.1 Hz, 2H), 3.73−3.69 (m, 1H), 3.64 (d, J = 11.1 Hz, 2H), 2.56−2.49 (m, 1H), 2.11−2.06 (m, 1H), 2.02−1.99 (m, 1H), 1.78−1.73 (m, 2H), 1.40−1.32 (m, 2H), 1.27 (s, 3H), 1.25 (s, 2H), 0.80 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.0, 162.2, 132.0, 128.8, 124.1, 118.7, 114.8, 101.2, 77.7, 56.1, 55.2, 35.6, 32.5, 30.3, 25.1, 23.3, 22.0. HRMS (ESI-TOF) m/z $[M + H]^+$ calcd for $C_{19}H_{29}N_2O_4^+$ 349.2122, found 349.2122.

4.12. Building Block B as Its Acetone Condensation Product. *N*-(1*R*,2*R*)-2-Aminocyclohexyl 5-(5,5-dimethyl-1,3-dioxan-2-yl)-2-hydoxybenzamide (185 mg, 0.53 mmol) was dissolved in dry methanol (20 mL), and CS_2 (0.35 mL) and triethylamine (0.08 mL) were added. After stirring for ca. 20 min at room temperature, the reaction mixture was cooled in an ice bath before di-*tert*-butyl dicarbonate (120 mg, 0.55 mmol) in methanol (1 mL) and 4-dimethylaminepyridine (3 mg, 0.025 mmol) were added. After stirring for 5 min, the reaction mixture was removed from the ice bath and allowed to reach room temperature before it was concentrated by rotary evaporation. The remainder was dissolved in CH_2Cl_2 (20 mL), and the mixture was cooled in an ice bath. Aqueous hydrazine (50−60%, 0.033 mL, 0.53 mmol) was added, and the mixture was stirred for 5 min before it was removed from the ice bath and allowed to reach room temperature. The mixture was stirred for ca. 1 h at room temperature. Water (25 mL) was added, and the resulting mixture was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phases were dried (Na_2SO_4) and concentrated by rotary evaporation. The compound was purified by DCVC with a 20% gradient of ethyl acetate in heptane, followed by a 1% gradient of methanol in ethyl acetate (column diameter 4 cm, packed with ca. 4 cm silica; fraction volume 50 mL, all fractions contained 1% triethylamine). Fractions 7 and 8 were combined and concentrated to reveal a light yellow oil. To this was added acetone (10 mL), and the resulting solution was stirred for ca. 10 min before it was concentrated to reveal the desired product as a white solid. Yield 73.9 mg (30%).

Melting point 159−160 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.67 (s, 1H), 8.21 (s, 1H), 8.05 (d, J = 6.8 Hz, 1H), 7.85 (d, J = 1.9 Hz, 1H), 7.54−7.50 (m, 2H), 6.92 (d, J = 8.5 Hz, 1H), 5.33 (s, 1H), 4.59−4.52 (m, 1H), 3.92−3.85 (m, 1H), 3.76 (d, J = 11.1 Hz, 2H), 3.65 (d, J = 11.1 Hz, 2H), 2.34−2.28 (m, 1H), 2.17 (s, 1H), 1.99 (s, 3H), 1.87−1.79 (m, 5H), 1.51−1.38 (m, 4H), 1.32 (s, 3H), 0.79 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 177.8, 170.2, 162.3, 150.2, 132.0, 129.3, 125.3, 118.3, 113.9, 101.5, 77.8, 56.5, 56.1, 32.5, 32.4, 25.3, 25.1, 24.5, 23.4, 22.0. HRMS (ESI-TOF) m/z $[M + H]^+$ calcd for $C_{23}H_{35}N_4O_4S^+$ 463.2374, found 463.2370.

4.13. 2-(3-Nitrophenyl)-1,3-dioxolane. 3-Nitrobenzaldehyde (15 mmol, 2.27 g, 1 equiv), *p*-toluenesulfonic acid (1.5 mmol, 285.3 mg, 0.1 equiv), and ethylene glycol (37.5 mmol, 2.1 mL, 2.5 equiv) were mixed in toluene (0.5 M, 30 mL), and the mixture was heated to reflux overnight with a Dean−Stark apparatus fitted to the setup. The solution was cooled to room temperature, and saturated $NaHCO_3$ solution (40 mL) and EtOAc (40 mL) were added. The phases were separated, and the aqueous phase was extracted with EtOAc (2 × 20 mL). The combined organic extracts were filtered through a plug of silica and evaporated to dryness to yield a yellow oil. This was further purified by dry column chromatography. A column of 2 cm diameter was packed with 5 cm of silica. A gradient of 20% CH_2Cl_2 in heptane, followed by a gradient of 1% MeOH in CH_2Cl_2, was used to yield the target compound as a yellow solid (2.9 g, 99%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.35 (s, 1H), 8.22 (d, J = 8.2, 1H), 7.801 (d, J = 7.6, 1H), 7.56 (t, J = 7.9, 1H), 5.89 (s, 1H), 4.18−4.11 (m, 2H), 4.11−4.04 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 148.4, 140.5, 132.8, 129.5, 124.1, 121.8, 102.4, 65.6. HRMS (ESI-TOF) m/z $[M + H]^+$ calcd for $C_9H_{10}NO_4^+$ 196.0604, found 196.0600.

4.14. 2-(1,3-Dioxolan-2-yl)aniline. 2-(3-Nitrophenyl)-1,3-dioxolane (14.3 mmol, 2.8 g, 1 equiv) was dissolved in MeOH (0.2 M, 70 mL), and 10% Pd/C (10 wt %, 280 mg) suspended in methanol (5 mL) was added. Hydrazine hydrate (71.5 mmol, 4.5 mL, 5 equiv) was added, and the reaction mixture was stirred under N_2 overnight. The reaction mixture was filtered through a plug of Celite and concentrated for analysis. NMR confirmed conversion to the desired product. The crude material was dissolved in 1:1 CH_2Cl_2/hexane (90 mL) and filtered through a short plug of silica. The product was eluted with additional 1:1 CH_2Cl_2/hexane (3 × 90 mL) and concentrated to give a colorless oil (1.32 g), which was used without further purification.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.11 (t, J = 7.8 Hz, 1H), 6.81 (d, J = 7.6 Hz, 1H), 6.77 (s, 1H), 6.65 (d, J = 8.0 Hz, 1H), 5.68 (s, 1H), 4.10−4.02 (m, 2H), 4.01−3.93 (m, 2H), 3.78 (br s, 2H, NH_2). ^{13}C NMR (126 MHz, $CDCl_3$) δ 145.5, 138.9, 129.4, 116.8, 116.2, 113.1, 103.8, 65.3.

4.15. Building Block C. 2-(1,3-Dioxolan-2-yl)aniline (11.9 mmol, 1.96 g, 1 equiv) was dissolved in CH_2Cl_2 (0.2m, 60 mL) and placed under N_2 atmosphere. Et_3N (77.1 mmol, 10.7 mL, 6.5 equiv) and CS_2 (77.1 mmol, 4.6 mL, 6.5 equiv) were added via syringe, and the reaction mixture was stirred overnight at room temperature. TFFH (23.7 mmol, 6.3 g, 2 equiv) was added in one portion while a stream of N_2 was kept over the reaction mixture. The mixture was stirred at room temperature for 4 h. Then the reaction mixture was cooled to 0 °C, hydrazine hydrate (0.28 mol, 17.4 mL, 24 equiv) was added via syringe, and the reaction mixture was stirred for a further 4 h at room temperature. Water (100 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 200 mL). The solution was concentrated. The residue was dissolved in CH_2Cl_2/heptane (3:1, 80 mL) and directly subjected to dry column chromatography. A column of 3 cm diameter was packed with 6 cm of silica. A gradient of 0.5% MeOH in CH_2Cl_2 was used, and all fractions were 50 mL. Fractions 5−8 were collected, concentrated, and stirred with a mixture of CH_2Cl_2/Et_2O (1:2, 200 mL) to obtain 1.56 g (55%) of the product **C** as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.67 (br s, 1H, NH), 9.13 (br s, 1H, NH), 7.72 (br s, 1H), 7.65 (br s, 1H), 7.29 (t, J = 7.8 Hz, 1H), 7.14 (d, J = 7.4 Hz, 1H), 5.69 (s, 1H), 4.05−3.99 (m, 2H), 4.76 (br s, 2H, NH_2), 3.96−3.90 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 179.4, 139.3, 138.2, 127.9, 124.3, 122.3, 121.7, 102.7, 65.8. HRMS (ESI-TOF) m/z $[M + H]^+$ calcd for $C_{10}H_{14}N_3O_2S^+$ 240.0801, found 240.0803.

4.16. 4-Methylbenzohydrazide (3). Ethyl 4-methylbenzoate (3.28 g, 3.21 mL, 20.0 mmol) was dissolved in ethanol (50 mL, 0.4 M), and hydrazine hydrate (25%, 12 mL, 60 mmol, 3 equiv) was added. The mixture was refluxed under stirring for 20 h before the solvent was removed in vacuo. The remnant was redissolved in CH_2Cl_2 (100 mL) and washed with water (80 mL). The organic phase was separated, dried (Na_2SO_4), and concentrated to give a white solid, which was recrystallized from absolute ethanol to yield 1.00 g (34%) of white crystals.

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Melting point 114−115 °C (lit. 116−118 °C).[19] ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.64 (d, J = 8.2 Hz, 2H), 7.32 (br s, 1H), 7.25 (d, J = 8.2 Hz, 2H), 4.08 (br s, 2H), 2.40 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.7, 142.4, 129.8, 129.4, 126.8, 21.5. Anal. Calcd for $C_8H_{10}N_2O$: C, 63.98; H, 6.71; N, 18.65. Found: C, 63.89; H, 6.80; N, 18.64. HRMS (ESI-TOF) m/z $[M + H]^+$ calcd for $C_8H_{11}N_2O^+$ 151.0866, found 151.0868.

4.17. 4-(4-Butylphenyl)thiosemicarbazide (4). 4-Butylaniline (1.58 mL, 10 mmol) was dissolved in CH_2Cl_2 (20 mL), and triethylamine (2.77 mL, 20 mmol) and CS_2 (1.22 mL, 20 mmol) were added. The solution was stirred at room temperature for 5 h before it was cooled in an ice bath, and TFFH (2.64 g, 10 mmol) was added. After the addition, the ice bath was removed and the solution was stirred for 1.5 h at room temperature. Once again the solution was cooled in an ice bath, and hydrazine hydrate (5 mL, 0.1 mol) was added carefully over 5 min. The ice bath was removed and the solution was stirred for 2 days at room temperature before it was quenched with water (50 mL). The aqueous phase was extracted with ethyl acetate (3 × 50 mL), and the combined organic phases were backwashed once with water (50 mL) before they were dried (Na_2SO_4) and concentrated in vacuo. The crude product was recrystallized from ethanol and air-dried to yield 1.62 g (73%) of white crystals.

Melting point 113−115 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 9.15 (br s, 1H), 7.94 (br s, 1H), 7.52−7.33 (m, 2H), 7.24−7.12 (m, 2H), 3.97 (br s, 2H), 2.63−2.56 (m, 2H), 1.62−1.54 (m, 2H), 1.40−1.30 (m, 2H), 0.95−0.90 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 180.1, 129.6, 129.0, 125.5, 124.5, 35.3, 33.7, 22.5, 14.1. Anal. Calcd for $C_{11}H_{17}N_3S$: C, 59.16; H, 7.67; N, 18.81. Found: C, 59.45; H, 7.54; N, 18.89. HRMS (ESI-TOF) m/z $[M + H]^+$ calcd for $C_{11}H_{18}N_3S^+$ 224.1216, found 224.1213.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.7b01161.

Additional text, five figures, one scheme, and three tables describing dynamic combinatorial libraries; ^{1}H and ^{13}C NMR spectra of synthesized compounds (PDF)

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Notes

The authors declare no competing financial interest.

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