Dynamic Signaling Cascades: Reversible Covalent Reaction-Coupled Molecular Switches

ARTICLE *in* JOURNAL OF THE AMERICAN CHEMICAL SOCIETY · OCTOBER 2015

Impact Factor: 12.11 · DOI: 10.1021/jacs.5b09912

JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Article

pubs.acs.org/JACS

Dynamic Signaling Cascades: Reversible Covalent Reaction-Coupled Molecular Switches

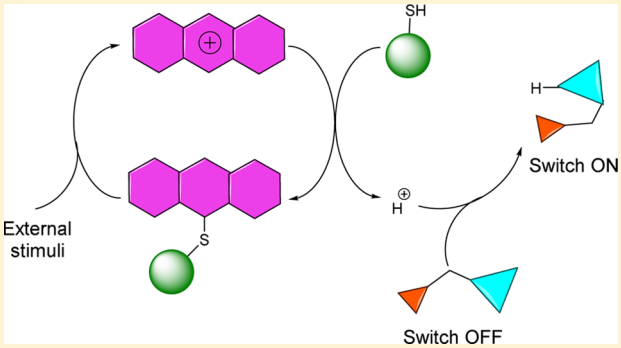
Yulong Ren[†,‡] and Lei You[*,†]

[†]State Key Laboratory of Structural Chemistry, Fujian Institute of Research on the Structure of Matter, Chinese Academy of Sciences, Fuzhou 350002, P. R. China

[‡]College of Chemistry, Fuzhou University, Fuzhou 350116, P. R. China

Supporting Information

ABSTRACT: The research of systems chemistry exploring complex mixtures of interacting synthetic molecules has been burgeoning recently. Herein we demonstrate for the first time the coupling of molecular switches with a dynamic covalent reaction (DCR) and the modulation of created chemical cascades with a variety of inputs, thus closely mimicking a biological signaling system. A novel Michael type DCR of 10-methylacridinium perchlorate and monothiols exhibiting excellent regioselectivity and tunable affinity was discovered. A delicate balance between the unique reactivity of the reactant and the stability of the adduct leads to the generation of a strong acid in a thermodynamically controlled system. The dynamic cascade was next created via coupling of the DCR and a protonation-induced configurational switch (*E*/*Z* isomerization) through a proton relay. Detailed examination of the interdependence of the equilibrium enabled us to rationally optimize the cascade and also shed light on the possible intermediate of the switching process. Furthermore, relative independence of the coupled reactions was verified by the identification of stimuli that are able to facilitate one reaction but suppress the other. To further enhance systematic complexity, a second DCR of electrophilic aldehydes and thiols was employed for the reversible inhibition of the binary system, thus achieving the interplay of multiple equilibria. Finally, a fluorescence switch was turned on through coupling with the DCR, showcasing the versatility of our strategy. The results described herein should pave the way for the exploitation of multifunctional dynamic covalent cascades.



INTRODUCTION

Signal transduction[1] plays a vital role in many biological processes, such as metabolism, transport, and gene expression, and any malfunction within the network can lead to severe human diseases. The signaling pathway generally functions through intricate interactions of a series of large and small molecules. For example, a stimuli or input activates a specific receptor, which in turn triggers a chain of biochemical events and creates a signaling cascade. Due to the reversibility of many interactions involved, the signaling can be switched "on" and "off". In order to mimic as well as understand biological systems, and simultaneously to develop synthetic systems with novel and advanced functions, a field of systems chemistry[2] is emerging recently, which focuses on complex mixtures of interacting molecules. With the use of the classical approach of dynamic combinatorial chemistry[3] which is under thermodynamic control, elegant self-assembling,[4] self-sorting,[5] and adaptive systems[6] have been reported. Furthermore, systems chemistry has also reached into the rich region of kinetically controlled networks, such as self-replicating[7] and autocatalytic systems,[8] oscillating reactions,[9] and molecular machines.[10] Despite significant progress in the field, the exploitation of dynamic synthetic signaling cascades remains underinvestigated.[11] In particular, the interaction of multiple equilibria to achieve multistep cascade is challenging.

One powerful tool for systems chemistry is dynamic covalent chemistry (DCC),[12] which has been flourishing in the past decade. Considered as dynamic interactions complementary to supramolecular bonding forces, the formation and exchange of reversible covalent bonds can lead to systematic complexity with ease and have found important applications in the construction of dynamic combinatorial libraries,[3] creation of molecular sensors,[13] assembly of complex architectures,[4,5] as well as regulation of biological structures and functions.[14] Imine condensation and exchange,[15] acylhydrazone condensation and exchange,[16] disulfide exchange,[17] and cyclic boronate ester formation[18] are among the most exploited dynamic covalent reactions (DCRs), which are the cornerstone of DCC. It is thus of great significance to discover novel DCRs and unravel new applications of DCC.

As a marriage of DCC and intelligent molecules, such as switches[19] and motors,[20] which are an intensive area of research in supramolecular chemistry and nanoscience, herein we achieved

Received: September 21, 2015

DOI: 10.1021/jacs.5b09912
J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

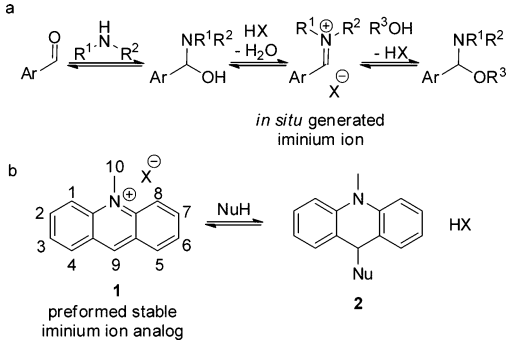
the use of DCRs for driving molecular switches and hence the creation of dynamic signaling cascades. Our strategy allows reversible covalent manipulation of molecular switches within the context of systems chemistry. First, a reversible Michael reaction was discovered on the basis of 10-methylacridinium ion. A unique balance between the reactivity of the reactant and the stability of the product resulted in the generation of a strong acid in a thermodynamically controlled system. The created protons as an input enabled the activation of one configurational switch and one fluorescence switch through a dynamic proton relay. By taking advantage of the stimuli-responsiveness feature of DCRs, the interplay of multiple reactions was also accomplished, thereby further enhancing the complexity and closely mimicking natural signaling network.

RESULTS AND DISCUSSION

Design of a Reactivity Based Dynamic Covalent Reaction. In this work, we chose protonation-induced switches because acid−base equilibrium is one of the most fundamental reactions in nature, and thus require a DCR which is pH sensitive under mild conditions. Many DCRs can be accelerated through acid/base catalysis.[21] The pH dependence of the rate of imine formation is well-documented.[22] Another notable example is the dynamic component exchange of acylhydrazones, in which a strong Brønsted or Lewis acid is generally required.[23] Intramolecular Brønsted acid catalysis was also found to significantly accelerate the rate of hydrazone formation in water.[24] Recently, the strategy of metal coordination-coupled deprotonation[25] and photoacid[26] through light-induced cyclization was developed. However, the generation of a strong acid in a thermodynamically driving homogeneous DCR has not been reported, to the best of our knowledge.

In order to produce protons in a thermodynamically favorable fashion, we conceive that a positive species (i.e., an onium ion) would be needed to maintain the charge balance. Very recently, in an effort to expand the scope of dynamic covalent bonding, we proposed the concept of reactivity based dynamic covalent chemistry and utilized *in situ* generated iminium ions for the reversible binding and chirality discrimination of chiral secondary alcohols (Scheme 1a).[27] Although an apparent

Scheme 1. DCRs Based on Iminium Ion and Its Analogue



equilibrium constant in the magnitude of 10^4 M^{-2} can be afforded, the low stability of these iminium ions renders them infeasible for this work. In contrast, stable yet reactive aromatic iminium ion analogues could provide a promising platform. We postulated that position 9 in the 10-methylacridinium ion (**1**) is highly electrophilic as a result of its resonance interaction with electron-withdrawing nitrogen, and hence the Michael addition with neutral nucleophiles is plausible (Scheme 1b). It is worthwhile to note that Michael reactions of **1** under basic conditions have been reported, but their reversibility has not been explored.[28] Although the aromatic pyridine ring would break upon the reaction of **1** and NuH, the loss of its resonance stabilization could be offset by the formation of two separate aromatic benzene rings. More importantly, due to the low basicity of the created aromatic amine (an analogue of diphenylamine, pK_a = 0.78 in water), a relatively strong acid would be generated, paving the way for the creation of proton-mediated dynamic cascades.

Characterization of the Dynamic Covalent Reaction. With this concept in mind, 10-methylacridinium perchlorate (**1**) was synthesized through methylation of acridine followed by anion exchange,[29] and the addition reaction with a series of monothiols was next examined. The mixture was stirred in deuterated DMSO at room temperature overnight and studied by ^{1}H NMR spectroscopy. The chemical shift for H9 in 10-methylacridinium ion was around 10.2 ppm (Figure 1a),

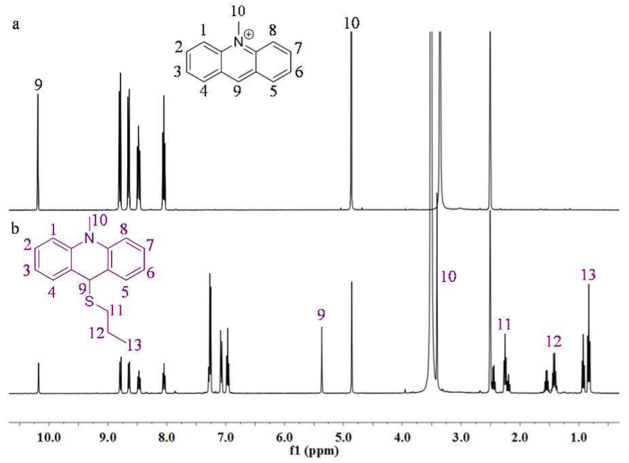


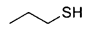
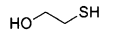
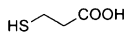
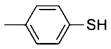
Figure 1. ^{1}H NMR spectra of the reactant **1** (a) and the reaction of **1** (22.9 mM, 1 equiv) with 1-propanethiol (1 equiv) (b) in DMSO-d_6.

which is in close proximity to the aldehyde CHO proton, thus revealing the high electrophilicity of **1**. With 1-propanethiol as an example, the peak at 10.2 ppm from **1** decreased, and one singlet at 5.4 ppm emerged, indicative of the formation of adduct **2a** (Figure 1b and Table 1). Moreover, an upfield shift for methyl protons (H10) further supports the addition reaction. No other products were detected, demonstrating the excellent regioselectivity of the reaction.

The presence of both **1** and its thiol adduct suggests that they are in equilibrium with free thiol, and the equilibrium constant was hence calculated. Because of the acid−base equilibrium involved, the total concentration of **2a** in the form of **2a**·$HClO_4$ was employed to derive the K value as a means of simplifying the system. An equilibrium constant of 227 M^{-1} was found for 1-propanethiol. To expand the substrate scope of the DCR, other thiols were screened (Figures S2−S6), and their corresponding K values are listed in Table 1. For 2-mercaptoethanol, there was an increase in equilibrium constant (333 M^{-1}) compared to 1-propanethiol (227 M^{-1}), probably due to the higher nucleophilicity resulting from enhanced acidity. For 2-propanethiol and *t*-butanethiol, the K value was determined to be 106 and 15.8 M^{-1}, respectively, consistent with their increased sterics. Aromatic 4-methylthiophenol afforded a K value of 48.6 M^{-1}.

DOI: 10.1021/jacs.5b09912
J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

Table 1. Component Distribution as Well as the Equilibrium Constant of the Reaction of 1 and Thiols[a]

Entry	Product	Thiol	1/%	2/%	K/M^{-1}
1	**2a**		35	65	227
2	**2b**		29	71	333
3	**2c**		33	67	232
4	**2d**		57	43	48.6
5	**2e**		47	53	106
6	**2f**		78	22	15.8

[a]The concentration of **1** was 22.9 mM, and 1 equiv of thiol was used.

The kinetic and thermodynamic properties of the reversible covalent reaction were also investigated by monitoring the decrease in the absorbance of 10-methylacridinium perchlorate (Figures S7−S13). The equilibrium was reached around 1 h when 0.2 mM receptor **1** was used, and a rate constant of 257 mol^{-1} L min^{-1} was obtained for 1-propanethiol by using pseudo-first-order kinetics. Binding isotherms from 1-propanethiol, 2-mercaptoethanol, and 4-methylthiophenol were also in agreement with the trend of their equilibrium constant in Table 1. All these results confirm that a DCR with tunable binding affinity as well as rapid equilibrium was discovered.

Modulation of the Dynamic Covalent Reaction. Having verified the feasibility of the Michael type DCR with 10-methylacridinium ion, the next goal was to modulate the reaction with external stimuli. First, triethylamine was employed as a proton scavenger to shift the equilibrium. As expected, quantitative formation of the adduct **2a** was detected when equal amounts of thiol and base were utilized (Figure 2d). The reaction in the presence of base is also supported by previously reported reaction of **1** and thiolate.[30] More importantly, the superposition of ^{1}H NMR resonances of **2** in the absence (Figure 2a) or presence of triethylamine was observed. No apparent upfield shift for **2a** with Et_3N suggests that adduct **2a** exists in the same form as in the case without Et_3N (i.e., neutral amine form). In other words, perchloric acid is generated for the DCR of **1** and monothiols. This is because downfield shifts of ^{1}H NMR peaks are expected if the protonation of **2a** would have taken place. Nevertheless, the trend of equilibrium constants described above is still valid.

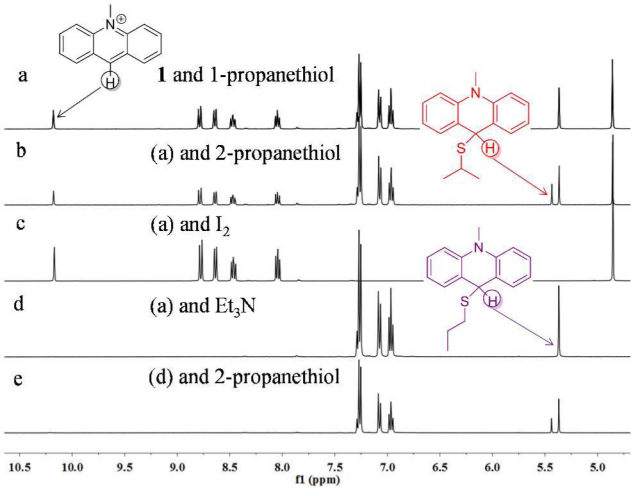
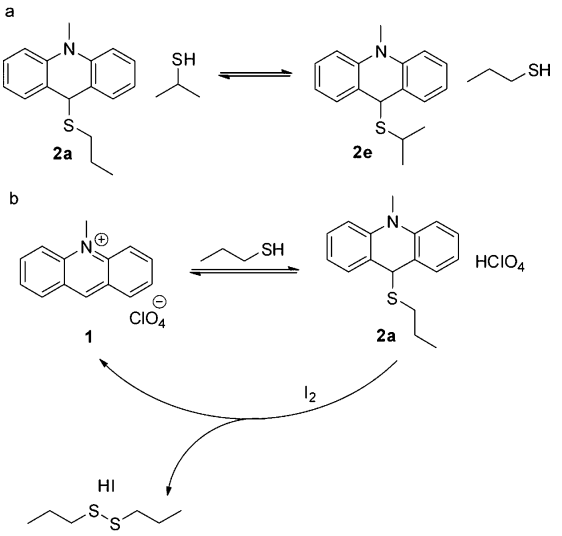


Figure 2. Manipulation of the equilibrium between **1** and thiol. (a) The reaction of **1** (22.9 mM, 1 equiv) and 1-propanethiol (1 equiv). (b) The component exchange of the equilibrium in part a with 2-propanethiol. (c) The addition of I_2 (0.5 equiv) in part a. (d) The reaction of **1** and 1-propanethiol (1 equiv) in the presence of Et_3N (1 equiv). (e) The component exchange of the equilibrium in part d with 2-propanethiol.

To examine the dynamic nature of the system, component exchange was conducted (Scheme 2a). The reaction was initially run with 1-propanethiol, and equal equivalents of 2-propanethiol were subsequently added (Figure 2b). After the equilibrium was reached, ^{1}H NMR revealed a decrease of adduct **2a** incorporating 1-propanethiol with a concomitant increase in 2-propanethiol derived product. Moreover, component exchange was also successful in the presence of triethylamine even though all of the 10-methylacridinium ion (**1**) was consumed (Figure 2e), thus further validating the reversibility of the reaction. In an alternative strategy of proving the reversibility, we took advantage of the facile oxidation of thiols to form the disulfide bond. The reaction was thus conducted with 1-propanethiol, followed by the introduction of iodine, a mild oxidant (Figure 2c). ^{1}H NMR demonstrated that thiols completely transformed to disulfides, with 10-methylacridinium ion **1** fully recovered (Scheme 2b and Figure S14). Hence, the equilibrium of the DCR creating protons described herein can readily be altered by simply adjusting pH, changing components, or adding oxidants, thereby providing ample means for the modulation of dynamic signaling cascades presented below.

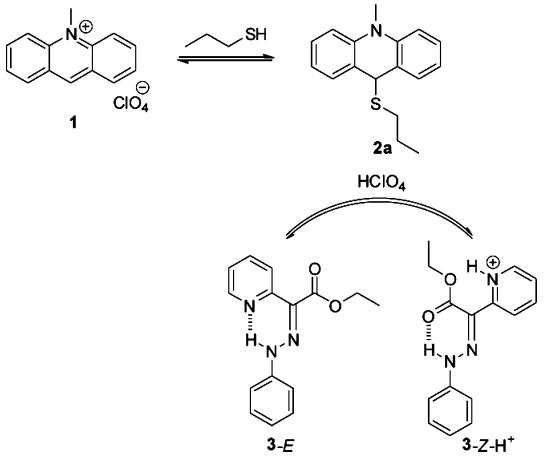
Scheme 2. Modulating the DCR by Dynamic Component Exchange (a) and Addition of I_2 (b)



Driving a Configurational Switch by the Dynamic Covalent Reaction. Having discovered a novel DCR producing protons, we next set out to couple the DCR with protonation activated molecular switches through a proton relay and generate a chemical cascade (Scheme 3). Toward this end, a hydrazone based configurational switch **3** developed by Aprahamian and co-workers was first employed.[31] In acetonitrile, the *E* isomer is the major species (*E*/*Z* = 9.1) in

DOI: 10.1021/jacs.5b09912
J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

Scheme 3. Coupling of the DCR and Switch 3 through a Proton Relay



which an intramolecualr hydrogen bond forms between hydrazone NH and pyridine nitrogen. Upon addition of 2.2 equiv of trifluoroacetic acid (TFA), the *E* isomer converts to the *Z* isomer as the protonation of pyridine nitrogen breaks the original N−H···N hydrogen bond and facilitates the creation of a N−H···O hydrogen bond.

Due to increased solubility of receptor **1** in DMSO, the coupling of its dynamic covalent reaction with switch **3** was investigated in DMSO-d_6. An *E*/*Z* ratio of 4.8 was detected for **3** (Figure 3a), significantly smaller than the value in CD_3CN.

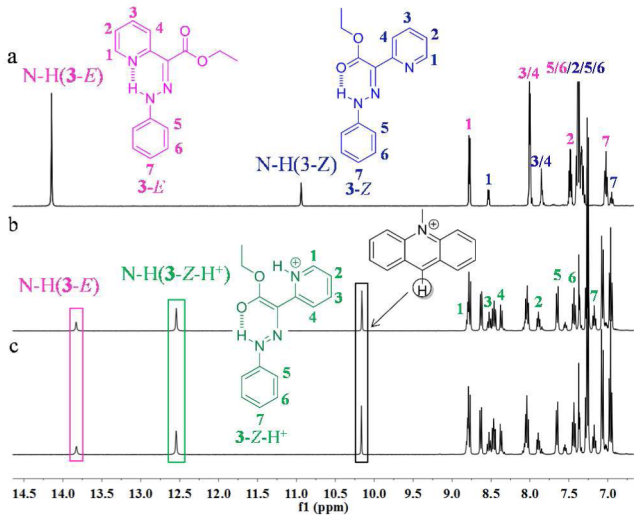


Figure 3. Coupling of the DCR and switch **3** in DMSO-d_6. (a) The distribution of *E* and *Z* isomer of **3**. (b) The addition of **3** (22.9 mM, 0.5 equiv) into the equilibrium mixture of **1** (1 equiv) and 1-propanethiol (1 equiv). (c) The "one pot" reaction of **1**, **3**, and thiol under equilibrium.

It is notable that such a ratio is actually an equilibrium constant, and solvent dependence of configurational isomerization is not uncommon.[32] A control experiment was conducted first with switch **3** and excess 1-propanethiol (a weak acid), and no interference in the ^{1}H NMR spectra of **3** was apparent (Figure S15). As a result, any variation in the distribution and position of *E*/*Z* isomers is indicative of the interaction of the produced acid and switch **3**, and in turn correlates with the equilibrium of the DCR (Scheme 3).

The coupling was then explored in a sequential manner. In detail, the equilibrium for the reaction of equal equivalents of **1** and 1-propanethiol was reached, followed by the addition of 0.5 equiv of **3** (Figure 3b). The proportions of *Z* and *E* isomers were calculated through ^{1}H NMR integrals, and their change in percentage points was followed. A 4-fold increase in the percentage of *Z* isomer (from 17% to 69%) with a concomitant decrease in the amount of *E* isomer (from 83% to 31%) was found, indicating the drive of the isomerization by the strong acid generated from the DCR. Meanwhile, there was a modest increase in the amount of adduct **2a**, supporting the coupling of the DCR and the proton switch. Furthermore, the "one pot" reaction afforded the same results as the sequential addition, thus confirming the reversibility of the signaling cascade (Figure 3c). In comparison, an increase of 26% in the percentage of *Z* isomer (from 17% to 43%) was afforded with 2 equiv of TFA (Table S2). These results validate the formation of a strong acid with a lower pK_a than TFA despite the fact that about only two-thirds of 10-methylacridinium ion **1** reacted with the thiol.

Modulation of the Dynamic Cascade. In order to gain deep insights into interactions of components in the system and hence further optimize the signaling cascade, ^{1}H NMR titration experiments were next performed. The multicomponent reactions were run with varied concentration of receptor **1** and 1-propanethiol (0, 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, and 7.0 equiv) while concentration of **3** stayed intact, and spectral changes are shown in Figure 4a (for component distribution at equilibrium, see Table S1). A gradual increase in the amount of

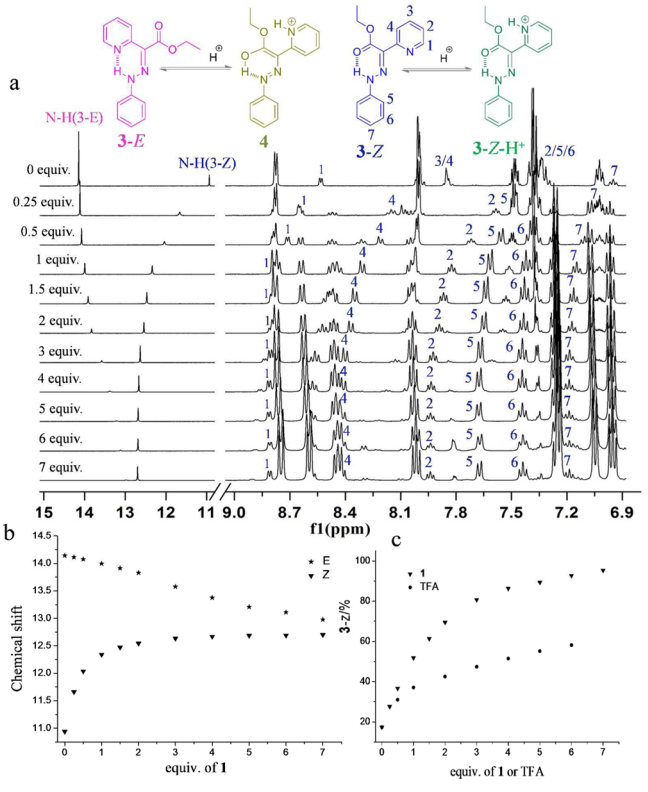
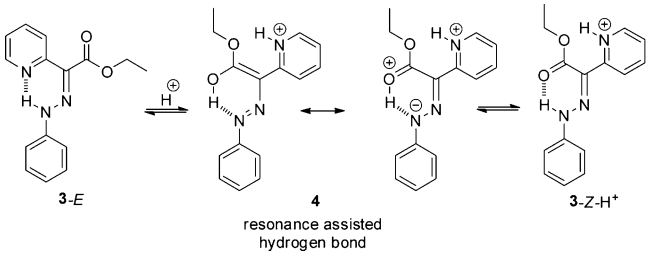


Figure 4. Coupling of switch **3** (22.9 mM) and the DCR with different concentration of **1** and 1-propanethiol in DMSO-d_6. (a) Changes in ^{1}H NMR spectra. (b) The plot of chemical shift (in ppm) of hydrazone NH versus the equivalent of **1**. (c) The plot of percentage of *Z* isomer as a function of the equivalent of **1** or TFA.

DOI: 10.1021/jacs.5b09912
J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

Z isomer was observed (from 17% to 95%). There was also a significant downfield shift of 1.7 ppm (from 11.0 to 12.7 ppm) for hydrazone NH in Z isomer due to the protonation of pyridine nitrogen. One surprising finding is an upfield shift of 1.1 ppm (from 14.1 to 13 ppm) for hydrazone NH from the original E isomer. Moreover, the peak broadened and moved closer to the NH peak from the Z isomer. Additional information was obtained through scrutinization of the binding isotherm (Figure 4b). For the Z isomer, a plateau was reached with increasing concentration of **1** and 1-propanethiol, consistent with the feature of an acid−base equilibrium (i.e., protonation of **3**-Z). In contrast, it appeared that biphasic behavior was present when monitoring the NH peak of the E isomer. Such a finding was rationalized as the protonation and tautomerization of hydrazone and ester π system to create isomer **4** (Scheme 4): in the initial stage of the titration (not enough

Scheme 4. Formation, Stabilization, and Transformation of Intermediate 4



protons), **3**-E converts to **3**-Z-H^+ through **4**, and a significant amount of **3**-E is still present; when the protons are in excess, intermediate **4** is accumulating though it gradually transforms to **3**-Z-H^+. It is also notable that **4** can be stabilized through resonance assisted hydrogen bonding (Scheme 4), and proton transfer within the contributing resonance structure leads to the creation of **3**-Z-H^+, hence explaining the trend of chemical shift change of hydrazone NH. To further exclude that the formation of **4** is due to kinetics, the synthetic cascade was monitored after 24 and 48 h, and the equilibrium was readily reached after 24 h (Figures S16 and S17). These results clearly indicate that the interaction of multiple components and intermediates in the system is under thermodynamic control. Although previous kinetics and computational studies support the tautomerization mechanism,[31b] ours is the first direct spectral evidence. ^{1}H NMR titration of **3** with TFA gave analogous results (Figure S18), but with a lower efficiency for driving the switch (Figure 4c).

As described in the previous section, E/Z equilibrium is solvent dependent. We thus postulated that the chemical cascade could be modulated by simply changing the solvent. To test our hypothesis, ^{1}H NMR titration experiments were conducted in CD_3CN (Figure 5a). No reaction between **1** and 1-propanethiol was detected (Figure S19). In sharp contrast, a mixture of **1** (2 equiv), 1-propanethiol (2 equiv), and switch **3** (1 equiv) afforded an increase in both the percentage of Z isomer (from 10% to 60%) and adduct **2a** (from 0 to 29%), thereby further validating the coupling of the two equilibria. Because the majority of 10-methylacridinium ion remained intact, we set out to manipulate the coupling by varying the concentration of thiol. A net increase of 73% for Z isomer was afforded when 8.0 equiv of thiol was used (Figure 5b). Although a downfield shift of hydrazone NH in Z isomer was still observed, the position of the resonance from hydrazone NH in the original E isomer remained unchanged. This finding is likely due to less stability of

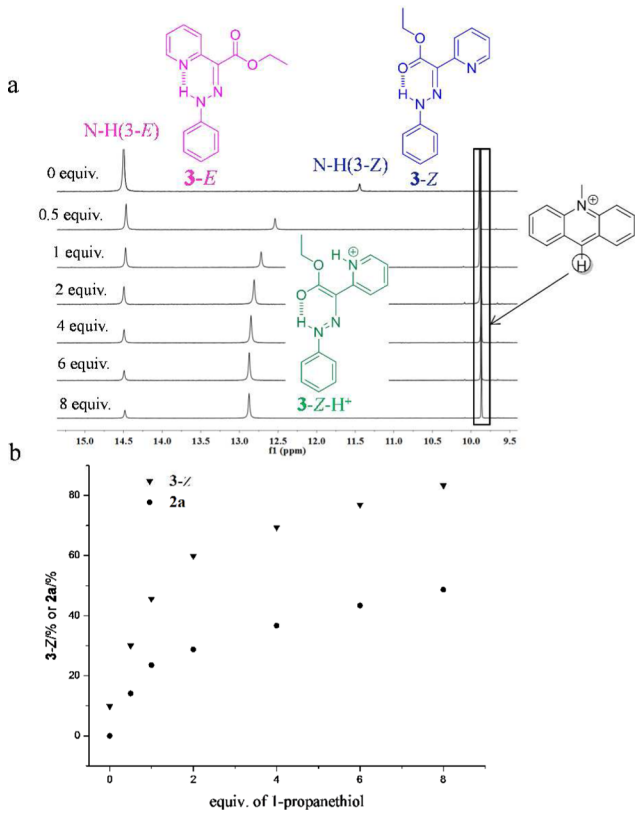


Figure 5. Coupling of DCR and switch **3** (22.9 mM, 1 equiv) with different concentration of 1-propanethiol in CD_3CN. (a) Changes in ^{1}H NMR spectra. (b) The plot of percentage of Z isomer and adduct **2a** versus the equivalent of 1-propanethiol; 2 equiv of **1** was used.

intermediate **4** in CD_3CN. Compared with DMSO-d_6, higher efficiency for the proton relay was obtained in CD_3CN, although the DCR itself favored the reactant (Figure 5b).

Independence of the Coupled Reactions. Although we have demonstrated that the two reversible processes studied are capable of driving each other, their relative independence was then explored. In doing so, the chemical stimuli that are able to facilitate one reaction but suppress the other were identified. First, the signaling cascade of **1** (2 equiv), **3** (1 equiv), and 1-propanethiol (2 equiv) was achieved in DMSO-d_6 (Figure 6b), followed by the addition of triethylamine (2 equiv). ^{1}H NMR indicates complete conversion of **1** to adduct **2a** with concomitant reversal of the switch (from Z to E, Figure 6d). This is because triethylamine neutralizes the created acid due to its high basicity, leading to the regeneration of thermodynamically stable E isomer. Alternatively, 1 equiv of iodine was added to the equilibrium mixture (Figure 6c). As a result of the oxidation of 1-propanethiol to form a disulfide bond, complete dissociation of **2a** was detected. However, with hydroiodic acid produced quantitatively, the percentage of the Z isomer was slightly increased (from 69% to 76%). All these results demonstrate that the two coupled equilibria can be shifted toward opposite directions.

Interplay of Multiple Equilibria. With successful dynamic coupling of the DCR and a proton switch, the next goal was to further enhance the complexity of the signaling cascade. Because the reactions involved are under thermodynamic control, one facile strategy is to introduce a reversible reaction to perturb the binary system (Scheme 5). A second DCR with thiols was thus chosen for this study: the reaction of electron-deficient benzaldehyde (**5a** and **5b**) and 1-propanethiol to

DOI: 10.1021/jacs.5b09912
J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

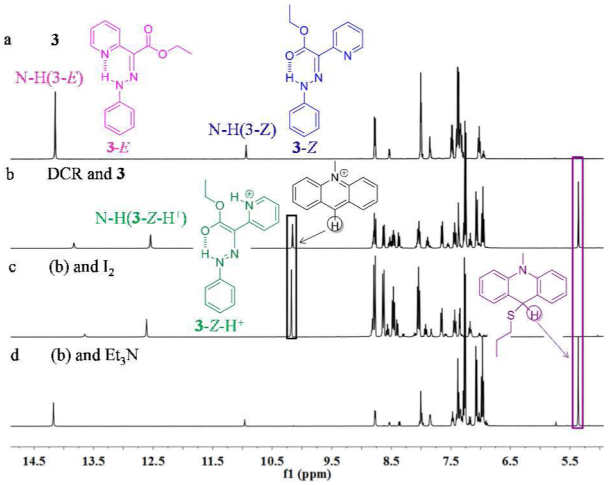
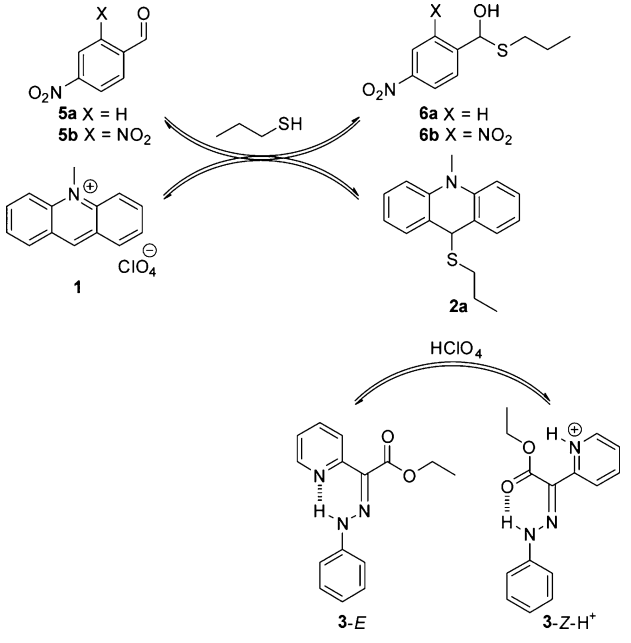


Figure 6. Modulation of the coupling of DCR and switch **3** (22.9 mM, 1 equiv) (b) with I_2 (1 equiv) (c) and Et_3N (2 equiv) (d) in DMSO-d_6. The ^1H NMR of **3** itself is shown in panel a; 2 equiv of **1** and 1-propanethiol were used.

Scheme 5. Interaction of Multiple Reactions



create hemithioacetal (**6a** and **6b**). The acidity of the hydroxyl group in hemithioacetal is very weak, and therefore, this DCR would have no direct interference for switch **3**, instead of modulating the dynamic cascade through its competition with the DCR of 10-methylacridinium ion **1** and 1-propanethiol.

A mixture of **1** (2 equiv), **3** (1 equiv), and 1-propanethiol (2 equiv) was equilibrated first in DMSO-d_6, and different amounts of 4-nitrobenzaldehyde (**5a**) were added (Figure 7, and Figures S20 and S21). The component distribution after reaching equilibrium was calculated using NMR integrals. As listed in Table S4, there was a gradual decrease of adduct **2a** incorporating 1-propanethiol as the amount of **5a** increased, indicating that these two DCRs indeed competed with each other. More importantly, a decrease in the percentage of *Z* isomer was observed, suggesting that *Z* isomer was converting back to *E* isomer. This observation was further supported by

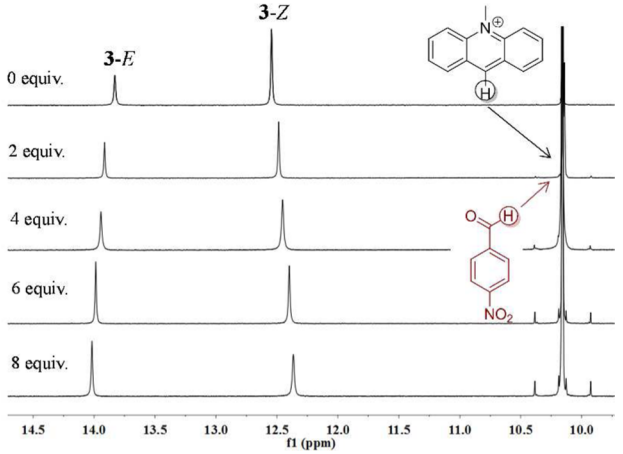


Figure 7. Modulation of the coupling of DCR and switch **3** (22.9 mM, 1 equiv) with different concentration of 4-nitrobenzaldehyde in DMSO-d_6; 2 equiv of **1** and 1-propanethiol were used.

the reversal trend of chemical shift changes of the corresponding hydrazone NH as compared to that in Figure 4. We rationalize these results as follows: the reaction of **1** with thiol is rivaled by 4-nitrobenzaldehyde, and adduct **2a** converts to reactant **1**; with the concentration of created protons decreased, *Z* isomer is reversed back to thermodynamically favorable *E* isomer. Tying it all together, the interaction of three dynamic processes was achieved through the reversible inhibition of the binary system by an external signal (i.e., the aldehyde).

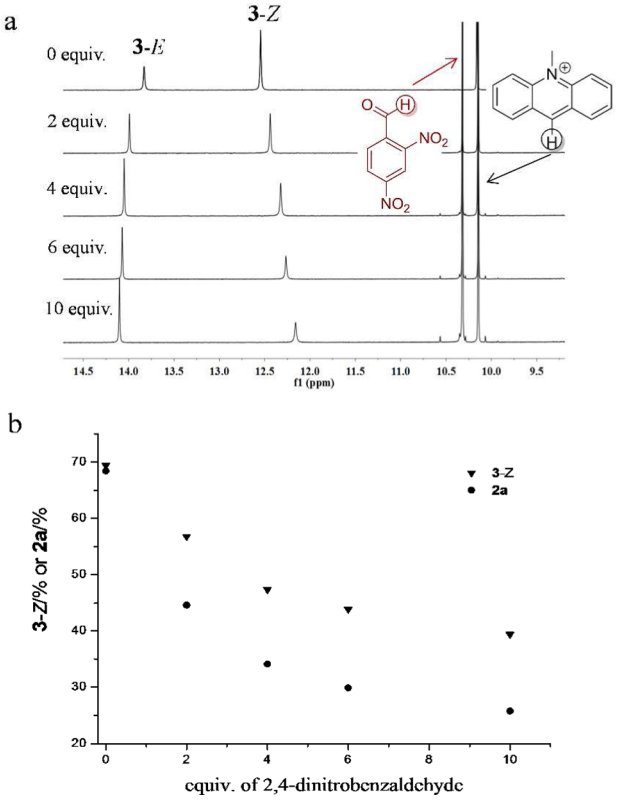
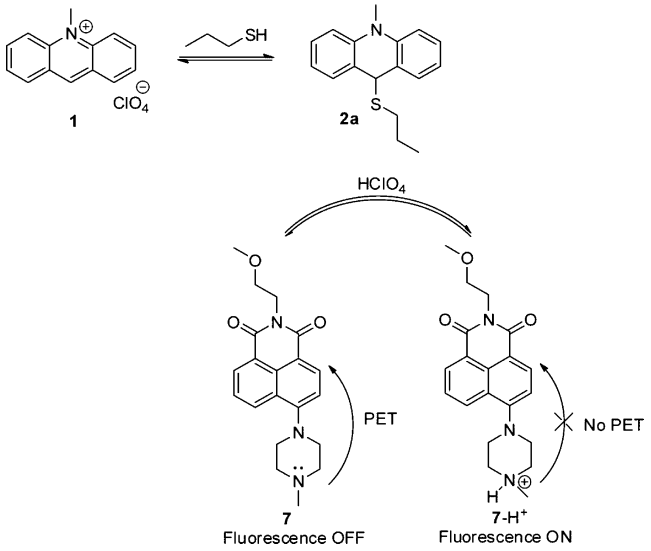


Figure 8. Interaction of multiple reversible reactions. (a) Modulation of the coupling of DCR and switch **3** (22.9 mM, 1 equiv) with different concentration of 2,4-dinitrobenzaldehyde in DMSO-d_6. (b) The plot of percentage of *Z* isomer and adduct **2a** versus the equivalent of 2,4-dinitrobenzaldehyde; 2 equiv of **1** and 1-propanethiol were used.

DOI: 10.1021/jacs.5b09912
J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

Scheme 6. Coupling of the DCR and Fluorophore 7 through a Proton Relay



Although functional, the efficiency of the multicomponent system is less than desirable, as evidenced by a decrease of 17% in the amount of *Z* isomer (from 69% to 52%, Table S4) using 8 equiv of 4-nitrobenzaldehyde. To solve this challenge, more electrophilic 2,4-dinitrobenzaldehyde (**5b**) was employed (Figure 8; for component distribution at equilibrium, see Table S5). Under similar experimental conditions, the *Z*/*E* conversion was more pronounced, as revealed by both the trend of the isomer distribution and changes in chemical shift of hydrazone NH. For example, with 10 equiv of 2,4-dinitrobenzaldehyde, there was a decrease of 30% for the *Z* isomer. It is also worthwhile to note that water addition to **5b** was detected (Figures S22 and S23). In other words, the efficiency for *Z*/*E* switching should be even higher. However, we did not use vigorously dried DMSO-d_6 as a means of further complicating the signaling network as well as enhancing the practicality of the system.

Driving a Fluorescence Switch by the Dynamic Covalent Reaction. To showcase the versatility of the discovered DCR for the generation of dynamic cascades, its coupling with a fluorescence switch was investigated (Scheme 6). A modified dye *N*-(2-methoxyethyl)-4-(4-methylpiperazine-1-yl)-1,8-naphthalimide (**7**) was designed and synthesized (see details in SI).[33] Due to the photoinduced electron transfer (PET) process[34] from the lone pair on the amine nitrogen, the fluorescence is turned off (Scheme 6). A proton relay from the DCR to **7** would suppress the PET and enable the activation of the fluorophore. As a control, the mixture of **7** and 1-propanethiol (3 equiv) did not afford any detectable change in ^{1}H NMR as individual component (Figure S24). However, the addition of 10-methylacridinium ion **1** (1 equiv) led to turning on the fluorescence. ^{1}H NMR revealed that adduct **2a** was formed in almost quantitative yield (Figure 9a). Furthermore, a downfield shift of

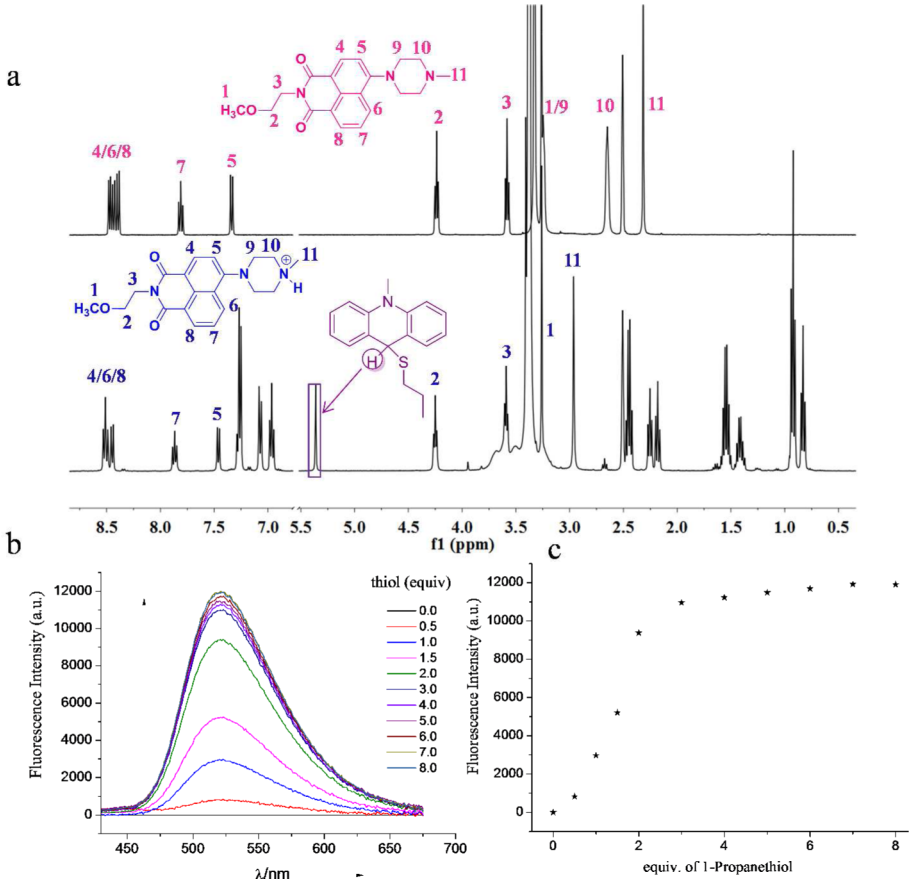


Figure 9. Coupling of DCR and fluorescence switch **7**. (a) ^{1}H NMR of **7** in DMSO-d_6 (top); a mixture of **1** (22.9 mM, 1 equiv), **7** (1 equiv), and 1-propanethiol (3 equiv) at equilibrium in DMSO-d_6 (bottom). (b) The fluorescence change of **7** (0.2 mM) in the presence of a constant concentration of **1** (2 equiv) and an increasing concentration of thiol (0–8 equiv). (c) The corresponding titration curve from part b. The background fluorescence (i.e., without thiol) was subtracted.

DOI: 10.1021/jacs.5b09912
J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

methyl proton (H11) from 2.32 to 2.96 ppm was observed, supporting the protonation of the dye. The protons on the aromatic ring (H5 and H7) also moved downfield slightly. The use of larger equivalents of **1** gave identical results as 1 equiv of **1**. The improved efficiency of the coupling compared to switch **3** is in agreement with high basicity of aliphatic amines. Fluorescence titration of a mixture of **7** and **1** with 1-propanethiol revealed a gradual increase of fluorescence intensity, in agreement with ^{1}H NMR results (Figure 9b,c).

CONCLUSIONS

In summary, dynamic coupling of reversible covalent reactions and proton switches as well as the modulation of these signaling cascades was achieved at the systems level. On the basis of the unique reactivity of 10-methylacridinium perchlorate, a novel Michael type DCR with monothiols exhibiting excellent regioselectivity was discovered. Although the aromaticity of the pyridine ring is lost, the modest loss of resonance stabilization and the weak basicity of the created diphenylamine analogs lead to the production of perchloric acid. The reversibility of the DCR was verified by both dynamic component exchange and complete dissociation of the product upon oxidation of thiols. This DCR was next successfully employed to drive *E*/*Z* isomerization of a configurational switch in both DMSO and acetonitrile through a proton relay, thus creating a dynamic cascade. Although the DCR exhibits better affinity in DMSO, higher efficiency for the coupling was afforded in acetonitrile. Furthermore, chemical stimuli that are able to facilitate one reaction but suppress the other were identified, thus demonstrating the relative independence of the coupled reactions. To further enhance the complexity, a second DCR of electrophilic aldehydes and thiols enabled the reversible inhibition of the signaling cascade, thereby achieving the interaction of multiple equilibria. Finally, the discovered DCR was able to turn on a fluorescence switch by suppressing the PET process. This work provides a new means for the creation and modulation of intelligent dynamic chemical cascades that mimic biological systems.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b09912.

Synthetic procedures, characterization, UV−vis studies, selected NMR spectra, and component distribution at equilibrium (PDF)

AUTHOR INFORMATION

Corresponding Author

*lyou@fjirsm.ac.cn

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank The Recruitment Program of Global Youth Experts and National Natural Science Foundation of China (21403239) for financial support.

REFERENCES

(1) (a) Li, W.; Chen, L.; Li, X.; Jia, X.; Feng, C.; Zhang, L.; He, W.; Lv, J.; He, Y.; Li, W.; Qu, X.; Zhou, Y.; Shi, Y. *Mol. BioSyst.* **2013**, *9*, 3026−3035. (b) Sacchettini, J. C.; Baum, L. G.; Brewer, C. F. *Biochemistry* **2001**, *40*, 3009−3015. (c) Zhang, M.; Li, H.; He, Y.; Sun, H.; Xia, L.; Wang, L.; Sun, B.; Ma, L.; Zhang, G.; Li, J.; Li, Y.; Xie, L. *J. Proteome. Res.* **2015**, *14*, 2745−2757.

(2) (a) Grzybowski, B.; Otto, S.; Philp, D. *Chem. Commun.* **2014**, *50*, 14924−14925. (b) Hunt, R. A. R.; Otto, S. *Chem. Commun.* **2011**, *47*, 847−858. (c) Kindermann, M.; Stahl, I.; Reimold, M.; Pankau, W. M.; von Kiedrowski, G. *Angew. Chem., Int. Ed.* **2005**, *44*, 6750−6755. (d) Ludlow, R. F.; Otto, S. *Chem. Soc. Rev.* **2008**, *37*, 101−108. (e) Brachvogel, R.-C.; Hampel, F.; von Delius, M. *Nat. Commun.* **2015**, *6*, 7129.

(3) (a) Corbett, P. T.; Leclaire, J.; Vial, L.; West, K. R.; Wietor, J.-L.; Sanders, J. K. M.; Otto, S. *Chem. Rev.* **2006**, *106*, 3652−3711. (b) Herrmann, A. *Chem. Soc. Rev.* **2014**, *43*, 1899−1933. (c) Li, J.; Nowak, P.; Otto, S. *J. Am. Chem. Soc.* **2013**, *135*, 9222−9239. (d) Mondal, M.; Hirsch, A. K. H. *Chem. Soc. Rev.* **2015**, *44*, 2455−2488. (e) Moulin, E.; Cormos, G.; Giuseppone, N. *Chem. Soc. Rev.* **2012**, *41*, 1031−1049.

(4) (a) Collier, J. H.; Rudra, J. S.; Gasiorowski, J. Z.; Jung, J. P. *Chem. Soc. Rev.* **2010**, *39*, 3413−3424. (b) Jelfs, K. E.; Wu, X.; Schmidtmann, M.; Jones, J. T. A.; Warren, J. E.; Adams, D. J.; Cooper, A. I. *Angew. Chem., Int. Ed.* **2011**, *50*, 10653−10656. (c) Smith, K. H.; Tejeda-Montes, E.; Poch, M.; Mata, A. *Chem. Soc. Rev.* **2011**, *40*, 4563−4577. (d) Ward, M. D.; Raithby, P. R. *Chem. Soc. Rev.* **2013**, *42*, 1619−1636.

(5) (a) He, Z.; Jiang, W.; Schalley, C. A. *Chem. Soc. Rev.* **2015**, *44*, 779−789. (b) Lin, J.-B.; Xu, X.-N.; Jiang, X.-K.; Li, Z.-T. *J. Org. Chem.* **2008**, *73*, 9403−9410. (c) Ji, Q.; Lirag, R. C.; Miljanic, O. S. *Chem. Soc. Rev.* **2014**, *43*, 1873−1884.

(6) (a) Lehn, J.-M. *Angew. Chem., Int. Ed.* **2013**, *52*, 2836−2850. (b) Lehn, J.-M. *Angew. Chem., Int. Ed.* **2015**, *54*, 3276−3289.

(7) (a) Carnall, J. M. A.; Waudby, C. A.; Belenguer, A. M.; Stuart, M. C. A.; Peyralans, J. J.-P.; Otto, S. *Science* **2010**, *327*, 1502−1506. (b) Nowak, P.; Colomb-Delsuc, M.; Otto, S.; Li, J. *J. Am. Chem. Soc.* **2015**, *137*, 10965−10969. (c) Robertson, A.; Sinclair, A. J.; Philp, D. *Chem. Soc. Rev.* **2000**, *29*, 141−152. (d) Zhang, R.; Walker, D. A.; Grzybowski, B. A.; Olvera de la Cruz, M. *Angew. Chem.* **2014**, *126*, 177−181.

(8) (a) Bissette, A. J.; Fletcher, S. P. *Angew. Chem., Int. Ed.* **2013**, *52*, 12800−12826. (b) Ribó, J. M.; Blanco, C.; Crusats, J.; El-Hachemi, Z.; Hochberg, D.; Moyano, A. *Chem. - Eur. J.* **2014**, *20*, 17250−17271. (c) Todd, M. H. *Chem. Soc. Rev.* **2002**, *31*, 211−222.

(9) (a) Buyukcakir, O.; Yasar, F. T.; Bozdemir, O. A.; Icli, B.; Akkaya, E. U. *Org. Lett.* **2013**, *15*, 1012−1015. (b) Liang, E.; Zhou, H.; Ding, X.; Zheng, Z.; Peng, Y. *Chem. Commun.* **2013**, *49*, 5384−5386. (c) Masuda, T.; Hidaka, M.; Murase, Y.; Akimoto, A. M.; Nagase, K.; Okano, T.; Yoshida, R. *Angew. Chem.* **2013**, *125*, 7616−7619.

(10) (a) Balzani, V.; Credi, A.; Raymo, F. M.; Stoddart, J. F. *Angew. Chem., Int. Ed.* **2000**, *39*, 3348−3391. (b) Coskun, A.; Banaszak, M.; Astumian, R. D.; Stoddart, J. F.; Grzybowski, B. A. *Chem. Soc. Rev.* **2012**, *41*, 19−30. (c) Willner, I.; Basnar, B.; Willner, B. *Adv. Funct. Mater.* **2007**, *17*, 702−717.

(11) (a) Campbell, V. E.; de Hatten, X.; Delsuc, N.; Kauffmann, B.; Huc, I.; Nitschke, J. R. *Nat. Chem.* **2010**, *2*, 684−687. (b) Campbell, V. E.; de Hatten, X.; Delsuc, N.; Kauffmann, B.; Huc, I.; Nitschke, J. R. *Chem. - Eur. J.* **2009**, *15*, 6138−6142. (c) Ray, D.; Foy, J. T.; Hughes, R. P.; Aprahamian, I. *Nat. Chem.* **2012**, *4*, 757−762. (d) Elani, Y.; Law, R. V.; Ces, O. *Nat. Commun.* **2014**, *5*, 5305. (e) Salles, A. G.; Zarra, S.; Turner, R. M.; Nitschke, J. R. *J. Am. Chem. Soc.* **2013**, *135*, 19143−19146. (f) Wang, Z. J.; Clary, K. N.; Bergman, R. G.; Raymond, K. N.; Toste, F. D. *Nat. Chem.* **2013**, *5*, 100−103.

(12) (a) Jin, Y.; Yu, C.; Denman, R. J.; Zhang, W. *Chem. Soc. Rev.* **2013**, *42*, 6634−6654. (b) Lehn, J.-M. *Chem. Soc. Rev.* **2007**, *36*, 151−160. (c) Rowan, S. J.; Cantrill, S. J.; Cousins, G. R. L.; Sanders, J. K. M.; Stoddart, J. F. *Angew. Chem., Int. Ed.* **2002**, *41*, 898−952.

(13) (a) Zhang, X.; Yin, J.; Yoon, J. *Chem. Rev.* **2014**, *114*, 4918−4959. (b) Lim, S. H.; Musto, C. J.; Park, E.; Zhong, W.; Suslick, K. S. *Org. Lett.* **2008**, *10*, 4405−4408. (c) Huang, Y.-J.; Ouyang, W.-J.; Wu, X.; Li, Z.; Fossey, J. S.; James, T. D.; Jiang, Y.-B. *J. Am. Chem. Soc.* **2013**, *135*, 1700−1703.

DOI: 10.1021/jacs.5b09912
J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

(14) (a) Bhat, V. T.; Caniard, A. M.; Luksch, T.; Brenk, R.; Campopiano, D. J.; Greaney, M. F. *Nat. Chem.* **2010**, *2*, 490−497. (b) Leung, I. K. H.; Brown, T., Jr.; Schofield, C. J.; Claridge, T. D. W. *MedChemComm* **2011**, *2*, 390−395. (c) Serafimova, I. M.; Pufall, M. A.; Krishnan, S.; Duda, K.; Cohen, M. S.; Maglathlin, R. L.; McFarland, J. M.; Miller, R. M.; Frödin, M.; Taunton, J. *Nat. Chem. Biol.* **2012**, *8*, 471−476. (d) Ulrich, S.; Dumy, P. *Chem. Commun.* **2014**, *50*, 5810−5825.
(15) (a) Belowich, M. E.; Stoddart, J. F. *Chem. Soc. Rev.* **2012**, *41*, 2003−2024. (b) Ciaccia, M.; Di Stefano, S. *Org. Biomol. Chem.* **2015**, *13*, 646−654. (c) Kovaříček, P.; Lehn, J.-M. *J. Am. Chem. Soc.* **2012**, *134*, 9446−9455.
(16) (a) Folmer-Andersen, J. F.; Lehn, J.-M. *J. Am. Chem. Soc.* **2011**, *133*, 10966−10973. (b) Jiang, Y.; Wu, J.; He, L.; Tu, C.; Zhu, X.; Chen, Q.; Yao, Y.; Yan, D. *Chem. Commun.* **2008**, 6351−6353.
(17) Black, S. P.; Sanders, J. K. M.; Stefankiewicz, A. R. *Chem. Soc. Rev.* **2014**, *43*, 1861−1872.
(18) (a) Nakahata, M.; Mori, S.; Takashima, Y.; Hashidzume, A.; Yamaguchi, H.; Harada, A. *ACS Macro Lett.* **2014**, *3*, 337−340. (b) Sun, X.; James, T. D. *Chem. Rev.* **2015**, *115*, 8001−8037.
(19) (a) Gorodetsky, B.; Branda, N. R. *Adv. Funct. Mater.* **2007**, *17*, 786−796. (b) Petersen, A. U.; Broman, S. L.; Olsen, S. T.; Hansen, A. S.; Du, L.; Kadziola, A.; Hansen, T.; Kjaergaard, H. G.; Mikkelsen, K. V.; Brøndsted Nielsen, M. *Chem. - Eur. J.* **2015**, *21*, 3968−3977. (c) Su, X.; Aprahamian, I. *Chem. Soc. Rev.* **2014**, *43*, 1963−1981. (d) Wang, Y.; Tan, X.; Zhang, Y.-M.; Zhu, S.; Zhang, I.; Yu, B.; Wang, K.; Yang, B.; Li, M.; Zou, B.; Zhang, S. X.-A. *J. Am. Chem. Soc.* **2015**, *137*, 931−939.
(20) (a) Kay, E. R.; Leigh, D. A.; Zerbetto, F. *Angew. Chem., Int. Ed.* **2007**, *46*, 72−191. (b) Pollard, M. M.; Klok, M.; Pijper, D.; Feringa, B. L. *Adv. Funct. Mater.* **2007**, *17*, 718−729.
(21) (a) Fulton, D. A. *Org. Lett.* **2008**, *10*, 3291−3294. (b) Holler, M.; Allenbach, N.; Sonet, J.; Nierengarten, J.-F. *Chem. Commun.* **2012**, *48*, 2576−2578. (c) Kihara, N.; Kidoba, K. *Org. Lett.* **2009**, *11*, 1313. (d) Zhu, N.; Zhang, F.; Liu, G. *J. Comb. Chem.* **2010**, *12*, 531−540.
(22) Larsen, D.; Pittelkow, M.; Karmakar, S.; Kool, E. T. *Org. Lett.* **2015**, *17*, 274−277.
(23) della Sala, F.; Kay, E. R. *Angew. Chem., Int. Ed.* **2015**, *54*, 4187−4191.
(24) Kool, E. T.; Park, D.-H.; Crisalli, P. *J. Am. Chem. Soc.* **2013**, *135*, 17663−17666.
(25) Foy, J. T.; Ray, D.; Aprahamian, I. *Chem. Sci.* **2015**, *6*, 209−213.
(26) Shi, Z.; Peng, P.; Strohecker, D.; Liao, Y. *J. Am. Chem. Soc.* **2011**, *133*, 14699−14703.
(27) Zhou, Y.; Ye, H.; You, L. *J. Org. Chem.* **2015**, *80*, 2627−2633.
(28) Satoh, N.; Fukuzumi, S. *Rev. Heteroatom Chem.* **1999**, *20*, 249−281.
(29) Lee, I.-S. H.; Kil, H. J.; Ji, Y. R. *J. Phys. Org. Chem.* **2007**, *20*, 484−490.
(30) Motoyoshiya, J.; Inoue, H.; Takaguchi, Y.; Aoyama, H. *Heteroat. Chem.* **2002**, *13*, 252−257.
(31) (a) Landge, S. M.; Aprahamian, I. *J. Am. Chem. Soc.* **2009**, *131*, 18269−18271. (b) Landge, S. M.; Tkatchouk, E.; Benítez, D.; Lanfranchi, D. A.; Elhabiri, M.; Goddard, W. A.; Aprahamian, I. *J. Am. Chem. Soc.* **2011**, *133*, 9812−9823.
(32) Ramanathan, S.; Lemal, D. M. *J. Org. Chem.* **2007**, *72*, 1566−1569.
(33) Tian, H.; Gan, J.; Chen, K.; He, J.; Song, Q. L.; Hou, X. Y. *J. Mater. Chem.* **2002**, *12*, 1262−1267.
(34) (a) Akimov, A. V.; Neukirch, A. J.; Prezhdo, O. V. *Chem. Rev.* **2013**, *113*, 4496−4565. (b) Liu, X.; Jiang, L.; Li, J.; Wang, L.; Yu, Y.; Zhou, Q.; Lv, X.; Gong, W.; Lu, Y.; Wang, J. *J. Am. Chem. Soc.* **2014**, *136*, 13094−13097.

DOI: 10.1021/jacs.5b09912
J. Am. Chem. Soc. XXXX, XXX, XXX−XXX