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Scope and Limitations of Supramolecular Autoregulation

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Received: November 24, 2015; Accepted: December 14, 2015; Web Released: March 15, 2016

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Abstract

Recently, our group has reported on the ureidopyrimidinone (UPy) induced buffering of 2,7-diamido-1,8-naphthyridine (NaPy) and used this phenomenon to regulate the catalysis of the Michael addition of 2,4-pentanedione to *trans*-β-nitrostyrene. We now show that the observed catalytic activity of NaPy is the result of a strong synergy between NaPy and trace amounts of K_2CO_3, resulting in a more than 100-fold increase in reaction rate compared to the two compounds separately. By keeping the concentration of K_2CO_3, as well as NaPy, constant, an improved regulation of the catalytic activity is achieved. We show that the catalytic activity can be precisely regulated in a noncovalent manner via the addition of the UPy motif. Finally, different salts and Michael substrates are screened to assess the selectivity of this catalytic couple and to provide a platform for future research in molecular buffering, regulation, and chemical networks.

Introduction

In order for biological systems to remain functional, it is often vital that a stable working environment is maintained. To achieve this, regulatory systems have evolved to minimize the effects of varying inputs on the functional activity of the system. Such an adjustment to change is often termed autoregulation. This phenomenon is found both on a macroscopic level, for example to control cerebral[1] and renal[2] blood flow, as well as on a molecular level in the control of gene expression via the use of riboswitches.[3,4] A very simple chemical analogue of autoregulation is a pH-buffer, in which the influence of acid on the proton concentration is minimized by the presence of a conjugated base, resulting in a relatively constant pH. A synthetic system capable of buffering functional molecules, instead of protons, will provide the opportunity to develop more stable and life-like chemical networks.

Recently, our group has presented the concept of supramolecular buffering, based on the equilibria between a ditopic supramolecular moiety that can homo-dimerize, and a chain stopper that is unable to dimerize, but can associate with the other supramolecular moiety (Figure 1).[5,6] Ditopic supramolecular compounds have the ability to form either rings (K_{intra}) or linear polymers (K_{inter}), where the stability of the rings can be defined by the effective molarity ($EM = K_{intra}/K_{inter}$).[7,8] Since the EM represents a local concentration, the formation of intramolecular contacts, i.e. rings, is more favorable at low concentrations, while polymerization is favored at high concentrations. The exact value of the EM depends largely on the flexibility and length of the spacer connecting the binding motifs and their relation is relatively well understood.[7,8] When a chain stopper is added to a ditopic compound an equilibrium is established between cycles with free chain stopper and end-capped linear polymers. Since at high concentrations polymerization is more favorable, the system will in such cases consist

of end-capped polymers and a relatively low fraction of free chain stopper. When the system gets diluted the formation of rings becomes more favorable, resulting in an increase in the fraction of free chain stopper compared to the fraction bound to the polymer. It has been shown that, under certain conditions, such a dilution-induced release of chain stopper results in buffering of the chain stopper i.e., the total concentration of the system can be altered while the concentration of free chain stopper remains nearly constant (Figure 1).

It has been shown that buffering of 2,7-diamido-1,8-naphthyridine (NaPy **1**) chain stoppers can be achieved in practice using the ring-chain equilibrium of a ditopic ureido-pyrimidinone (UPy **2**).[6] In this case, the concentration of unbound **1** is buffered over a broad concentration range (c = 10–80 mM). Additionally, it has been found that free NaPy **1** is able to catalyze the Michael addition of 2,4-pentanedione

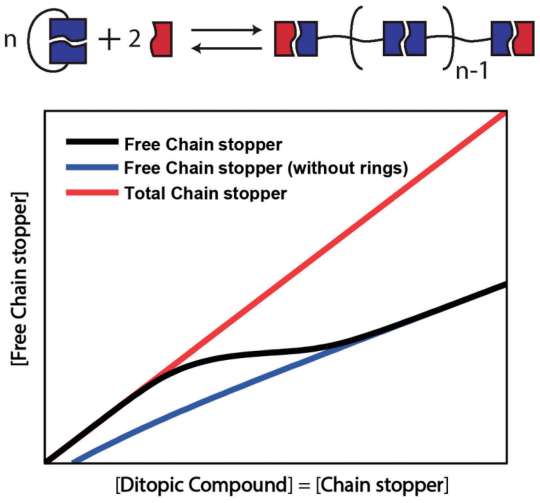

Figure 1. The equilibria between a ditopic supramolecular compound that is able to homo-dimerize and a chain stopper that is unable to dimerize. The graph depicts the concentration of free end-capper as a function of the total concentration, the image was adapted from Ref. 5.

to *trans*-β-nitrostyrene (Figure 2).[6,9] Moreover, the addition of UPy **2** to NaPy decreases the rate of the Michael addition. This has been ascribed to the formation of UPy–NaPy heterodimers, thereby lowering the free NaPy concentration.[10,11] As a result of the buffering described above, the turnover frequency (TOF) of the Michael addition in the presence of NaPy **1** and UPy **2** displays a nearly constant value over a wide concentration range. This buffering phenomenon results in a system with characteristics similar to autoregulated natural systems, therefore this principle has been termed supramolecular autoregulation.[6]

In our search to optimize the autoregulation and to understand the catalytic functions, we have now discovered that the catalytic activity of NaPy is in fact the result of a strong synergy between NaPy and K_2CO_3.[9] Here, we show that this finding results in an improvement of the autoregulatory principle reported originally. The buffering of the TOF can be optimized provided that the concentration of both NaPy and K_2CO_3 are controlled. We complement these findings with an experimental study on the scope and limitations of the catalytic couple. To gain more insight into the observed TOF, the influence of UPy on the reaction rate is examined in more detail. Finally, various salts and substrate combinations are screened to illustrate the scope of this catalytic system and broaden the applicability of the supramolecular autoregulatory concept.

Results and Discussion

Continued research on the UPy–NaPy based autoregulatory system described above required the synthesis of an additional batch of NaPy (See Figures S1–S3 for details). Traditionally, NaPy was purified by recrystallization in ethanol and toluene.[12] For practical reasons, we decided to purify NaPy using column chromatography, leading to a batch of NaPy that surprisingly was catalytically inactive in the Michael addition of 2,4-pentanedione to *trans*-β-nitrostyrene. Since K_2CO_3 is known to catalyze certain Michael additions[13,14] and was previously used in the final step in the synthesis of NaPy, we hypothesized that the work-up procedure via recrystallization might have resulted

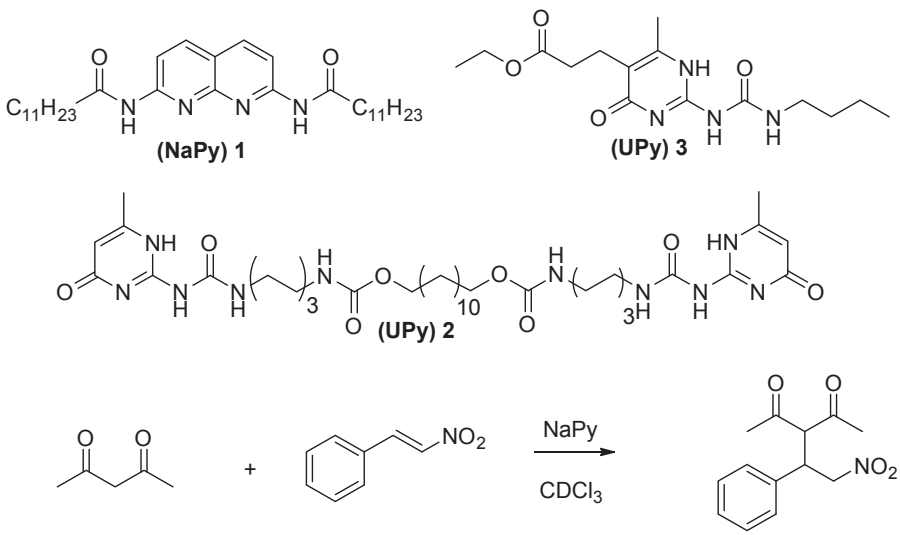

Figure 2. Chemical structures of NaPy and UPys, and the reported NaPy catalyzed Michael addition of 2,4-pentanedione to *trans*-β-nitrostyrene.

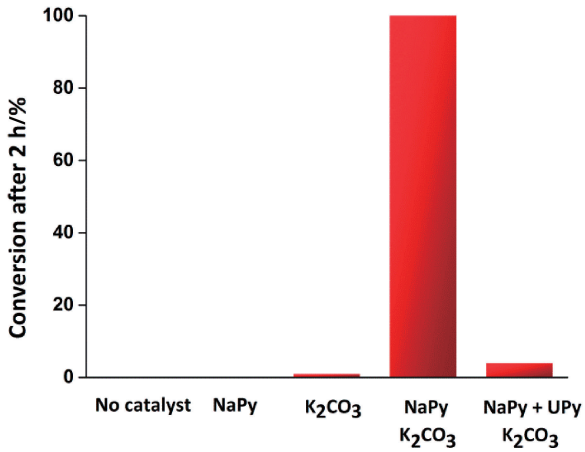

Figure 3. The conversion of the Michael acceptor in the Michael addition between 2,4-pentanedione ($c = 500$ mM) and *trans*-β-nitrostyrene ($c = 100$ mM) upon the addition of combinations of NaPy ($c = 20$ mM), K_2CO_3 ($c = 5$ mM), and UPy ($c = 20$ mM). The conversion was measured by ^{1}H NMR using the relative ratio of Michael acceptor and product. The reaction time is 2 h, the solvent is $CDCl_3$. All components were combined directly.

in incomplete removal of K_2CO_3, thereby affecting the catalytic results. To compare the reaction rates of the Michael addition of 2,4-pentanedione to *trans*-β-nitrostyrene in the presence of either NaPy, K_2CO_3 or a mixture thereof, several reactions were performed (Figure 3). Since the dimerization of NaPy and UPy is typically studied in chloroform,[6,12,15] all studies were performed in $CDCl_3$ at room temperature.

Upon addition of either NaPy or K_2CO_3 (ground before use) to 2,4-pentanedione and *trans*-β-nitrostyrene only trace amounts of Michael adduct were formed, while the combined presence of NaPy and K_2CO_3 gave a quantitative conversion after ca. 60 min, as revealed by additional measurements. The addition of 1 equivalent of UPy **2**, which complexed to most of the free NaPy, resulted in a dramatic decrease of the conversion. These results show that there is only significant catalytic activity when both K_2CO_3 and free Napy are present. Hence, it is likely that the previously used NaPy contained traces of K_2CO_3.[6] The necessity of using both NaPy and K_2CO_3 to obtain efficient catalysis is most likely the result of NaPy acting as a kind of phase-transfer catalyst for K_2CO_3, thereby forming an active NaPy·K_2CO_3 complex. This can either be via a "crown-ether like" mechanism, where NaPy complexes K_2CO_3 as a whole and thereby facilitates its transfer into the liquid phase; or by NaPy reacting with K_2CO_3, resulting in $KHCO_3$ and soluble K^+NaPy^- which acts as the active base for the reaction.[16] Hence, the nature of the ground K_2CO_3 can be of influence in this heterogeneous process to make the active catalyst. The presence of such a complex also explains why the recrystallization step to purify NaPy has been ineffective in removing all K_2CO_3.

In the original autoregulation article,[6] the equilibria between ditopic UPy **2** and NaPy **1** afforded a system in which the concentration of free NaPy (i.e., the active catalyst in combination with K_2CO_3), remained constant over a broad concentration range. As a result, the reaction rates of the Michael addition

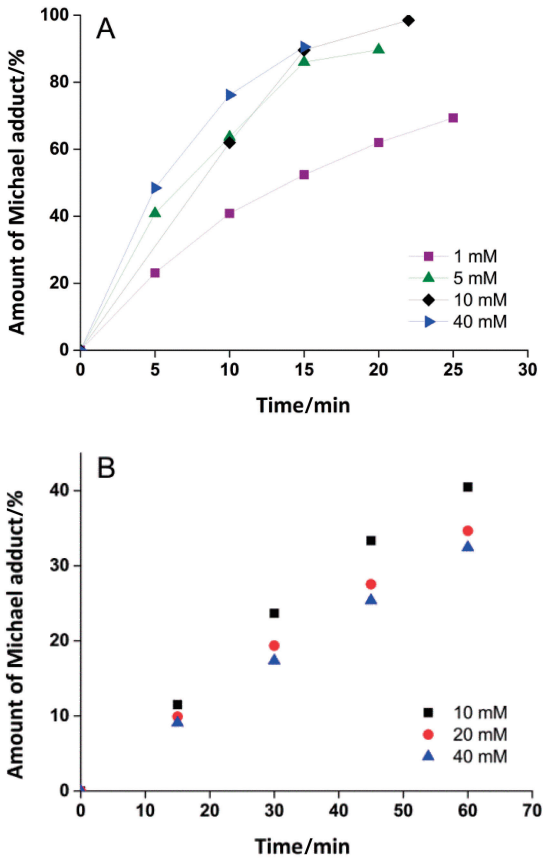

Figure 4. (A) Michael adduct formation for a system containing 2,4-pentanedione ($c = 500$ mM), *trans*-β-nitrostyrene ($c = 100$ mM), K_2CO_3 ($c = 1$ mM), and different amounts of NaPy, lines are to guide the eye. (B) The same experiment as depicted in A, but with 0.5 equivalents of ditopic UPy **2** (with respect to NaPy) present to buffer the free NaPy concentration (See ESI Figure S5).

increased less strongly with the total concentration of NaPy in the presence of ditopic UPy **3** than when no UPy was added (See Figures S17 and S18 in Ref. 6 for more details). According to our new results, the amount of NaPy·K_2CO_3 influenced the rate of the Michael addition. Thus, it should be possible to construct a system with constant reaction rates at different concentrations of NaPy–UPy as long as the concentration of K_2CO_3 is kept constant. To test this, we repeated the original measurements in the presence of a constant concentration of K_2CO_3 and different amounts of NaPy without and with ditopic UPy **3** present. Solutions containing K_2CO_3 ($c = 1$ mM), 2,4-pentanedione ($c = 500$ mM), *trans*-β-nitrostyrene ($c = 100$ mM), NaPy ($c = 1$–50 mM), and ditopic UPy **2** (0.5 equiv with respect to NaPy) were prepared in $CDCl_3$ (see ESI Figures S4 and S5 for details). The formation of Michael adduct was monitored over time by ^{1}H NMR (Figure 4).

Figure 4A shows that the reaction rate of the Michael addition in non-buffered conditions (i.e., no UPy **3** present) increases with roughly a factor 2 when the concentration of NaPy is increased from 1 to 5 mM. A further increase of the NaPy concentration does not lead to a significant increase in the reaction rates; the conversion profiles for 10 and 40 mM NaPy are similar to those observed for 5 mM NaPy concentration.

This is the result of the much smaller and constant amount of K_2CO_3 ($c = 1$ mM) becoming rate limiting. In other words, the addition of more NaPy will no longer increase the NaPy·K_2CO_3 concentration and hence the reaction rate is not enhanced.

In contrast, if the reaction is carried out under the same conditions but with 0.5 equivalent of ditopic UPy **2** present (Figure 4B), the free NaPy concentration is buffered at approximately 1 mM. This buffering of the concentration of active phase-transfer catalyst results in conversion profiles that are independent of the total NaPy–UPy concentration. This indicates that the reaction rates are similar and have become independent of the total NaPy concentration. While in our studies the concentration of K_2CO_3 is kept at a constant level, it has been unintentionally increased simultaneously with the total NaPy concentration in the experiments reported previously.[6] This has no significant effect on the UPy–NaPy equilibria since the concentration at which free NaPy is buffered (≈1 mM) is very similar in both cases. However, the reaction rates observed are much more constant in this study compared to the experiments reported previously (See Ref. 6, Figure S18). This is the consequence of keeping both the K_2CO_3 and NaPy concentration constant, instead of only the latter. Thus, we hereby confirmed that the previously reported supramolecular autoregulation as a result of a buffered free NaPy concentration is indeed possible and becomes even better when the concentration of K_2CO_3 is kept constant.

Although there are many, potentially more efficient, catalysts for the Michael addition, our NaPy–K_2CO_3 system has the unique possibility of exploiting the UPy–NaPy dimerization to regulate the free NaPy concentration. This provides a handle to control catalytic activity in a noncovalent and reversible manner, thereby creating a direct link between noncovalent and covalent chemistry. In light of future applications, we set out to study the scope and limitations of the NaPy–K_2CO_3 catalytic couple. In order to determine the influence of the amount of UPy on the reaction rate in more detail, the coupling of 2,4-pentanedione ($c = 40$ mM) to *trans*-β-nitrostyrene ($c = 40$ mM) was performed in the presence of NaPy ($c = 5$ mM), K_2CO_3 ($c = 5$ mM), and various amounts of monotopic UPy **3**. At all UPy concentrations, >99% of UPy was present as UPy–NaPy heterodimer. When all components were combined directly, an initial lag-phase of approximately 40 min was observed, which we attributed to the time required to form a catalytically active NaPy·K_2CO_3 complex (Figures S6 and S7 for details). As expected, when NaPy **1**, UPy **3**, and K_2CO_3 were stirred overnight before adding the Michael substrates, the lag-phase is not observed. Our results show that there is an exponential decay in the reaction rate upon increasing the amount of UPy (Figure 5). This nonlinearity shows that the reaction order of NaPy is larger than unity, which suggests that more than one NaPy is present in the catalytically active species.

Next the influence of the base and substrate combination on the catalytic activity was investigated. The concentration of K_2CO_3 was varied and other salts were tested, as well as the replacement of NaPy by 18-crown-6, a known phase-transfer catalyst for K_2CO_3.[17] Increasing the amount of K_2CO_3 relative to NaPy resulted in a roughly linear increase in conversion, with slightly lower values at higher K_2CO_3 concentrations (Figures 6A, S8, and S9). This is attributed to K_2CO_3 becoming rate limiting. Additionally, high K_2CO_3 concentrations result in the formation of a poorly soluble yellow powder and the disappearance of part of the *trans*-β-nitrostyrene from the solution; as evidenced by NMR spectroscopy. Most likely, this precipitate is the result of complexation between K^+ and the nitro-moiety,[18] which is corroborated by the fact that this precipitate dissolves again when 18-crown-6 is added. The catalytic activity of NaPy in the presence of other salts such as $CaCO_3$, Na_2CO_3, and KCl is low (Figure 6B), with the exception of Cs_2CO_3 (see ESI Figure S10 for ^{1}H NMR data). In addition, the reaction still occurs when NaPy is replaced by catalyst 18-crown-6, albeit less efficiently.

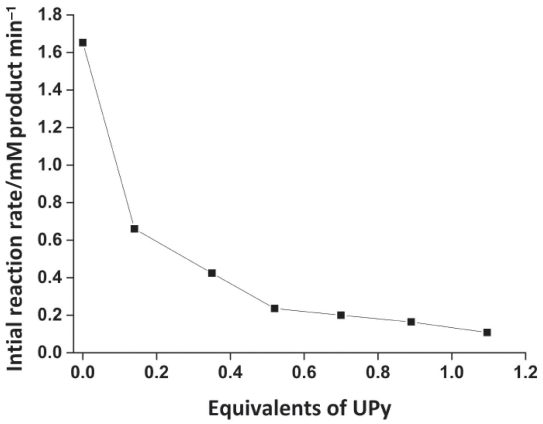

Figure 5. The initial reaction rates of the Michael addition versus the equivalents of UPy **3** in relation to NaPy **1**. Solutions of NaPy **1** (5 mM), K_2CO_3 (5 mM), and different amounts of UPy **3** in $CDCl_3$ were allowed to stir for 24 h before 2,4-pentanedione (40 mM) and *trans*-β-nitrostyrene (40 mM) were added. The measurements were started directly after the addition of the Michael substrates. See ESI (Figures S6 and S7) for additional experimental details and results.

Finally, the scope of substrate pairs suitable for the autoregulatory system were investigated; we selected sixteen Michael donors and acceptors in the screening for their ability to form Michael adducts in the presence of NaPy and K_2CO_3 (See ESI Table S1 and Figures S11–S14). Interestingly, only four combinations showed any conversion after the addition of NaPy and K_2CO_3 (Figure 7). Of these, the synergy between NaPy and K_2CO_3 is only observed for the coupling of 2,4-pentanedione to either *trans*-β-nitrostyrene or 1-(*tert*-butyl)-1*H*-pyrrole-2,5-dione, highlighting the high sensitivity of the catalytic system towards the substrates used. While the reaction with 1-(*tert*-butyl)-1*H*-pyrrole-2,5-dione was slower, it repeatedly afforded the product in quantitative yields. In contrast to the coupling of 2,4-pentanedione to *trans*-β-nitrostyrene, which gave some precipitates at high K_2CO_3 concentrations (vide supra).

Conclusion

Here, we have shown that there is a high degree of synergy between NaPy and K_2CO_3 towards the catalysis of selected Michael additions in $CDCl_3$. The synergy is proposed to be the result of NaPy acting as a kind of phase-transfer catalyst for K_2CO_3 to generate an active catalytic base. We demonstrate that the observed buffering of the TOF of the NaPy-catalyzed

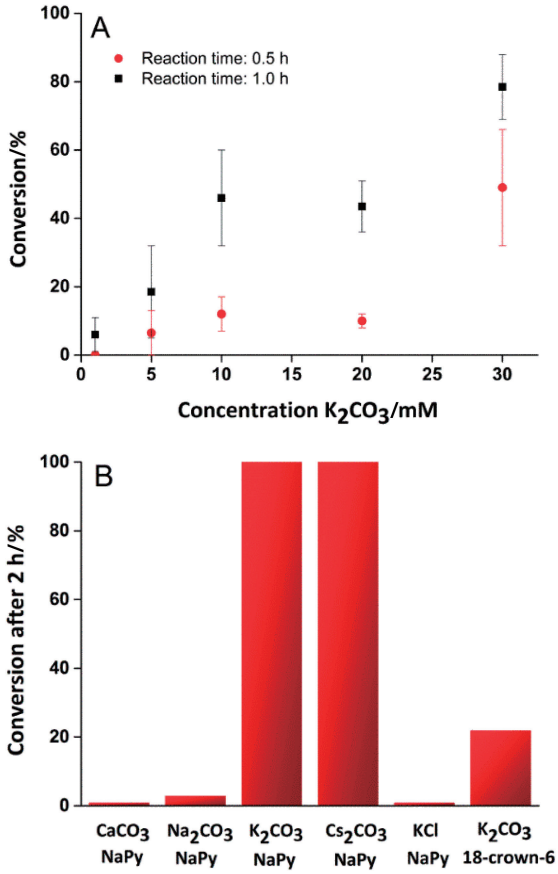

Figure 6. (A) The conversion of the Michael addition between 2,4-pentanedione ($c = 100$ mM) and *trans*-β-nitrostyrene ($c = 100$ mM) in the presence of NaPy ($c = 10$ mM) and different amounts of K_2CO_3. The conversion was measured by ^{1}H NMR using the relative ratio of Michael acceptor and product. The measurements were performed in duplo, error bars depict the standard deviation. Note that we reduced the concentration of 2,4-pentanedione from the usual 500 mM to 100 mM in order to slow down the kinetics. (B) The conversion of Michael adduct formed of 2,4-pentanedione ($c = 500$ mM) to *trans*-β-nitrostyrene ($c = 100$ mM) in the presence of NaPy or 18-crown-6 ($c = 20$ mM) and different salts ($c = 5$ mM), for KCl $c = 10$ mM to keep the potassium concentration equal to that of the K_2CO_3 measurements.

Michael addition reported previously by our group is the result of the unintentional presence of K_2CO_3. Repeating several of the experiments reported earlier shows that constant reaction rates can indeed be achieved at different NaPy concentrations, as long as the K_2CO_3 concentration is kept constant. Only unbound NaPy is able to act as a catalyst, allowing the catalytic activity to be modulated by the addition of the complementary UPy motif. As a result, we obtain a system that shows supramolecular autoregulation behavior. The NaPy–K_2CO_3 catalytic couple is highly selective towards the used Michael substrates and the used salt. Also, only small amounts of K_2CO_3 suffice to generate an active system. We believe that this work will contribute to the further development of supramolecular organocatalytic systems and the construction of synthetic chemical networks.

Experimental

General. Unless noted otherwise all chemicals were purchased from Sigma Aldrich, K_2CO_3 was ground before use. Michael additions were performed on 1–2 mL scale in 45 × 27 mm vials using $CDCl_3$ from Cambridge Isotope Laboratories. NMR spectra were taken on a 400 or 500 MHz (^{1}H) Varian Spectrometer and results were processed using VNMRJ 3.2a software. All reactions in the synthesis of NaPy and UPy were performed under argon. Unless otherwise noted, the Michael substrates were combined directly with the NaPy and K_2CO_3.

Measuring the Initial Reaction Rates of the Michael Addition in the Presence of Various Amounts of UPy. In a typical experiment NaPy **1** (5 mM), K_2CO_3 (5 mM), and various amounts of UPy **3** were dissolved in 15 mL of $CDCl_3$. The mixture was stirred for 24 h to allow for the catalytically active complex to form. Subsequently *trans*-β-nitrostyrene (40 mM) and 2,4-pentanedione (40 mM) were added. Samples of 0.5 mL were taken in roughly 5 min intervals and analyzed directly using ^{1}H NMR. To prevent concentration of K_2CO_3, the samples were returned to the reaction mixture directly after analysis (≈2–3 min later). Conversions were determined using the standard procedure described in the materials and method section. The exact amount of UPy was determined by ^{1}H NMR. In the "direct start" measurement, only NaPy **1** was stirred overnight in $CDCl_3$, after which K_2CO_3 and the Michael substrates were added and the measurement started. The measurement was performed on the same scale and concentration as described above.

Repeating the Supramolecular Autoregulation Experiments with Constant K_2CO_3 Concentration. The measurements without UPy where carried out by preparing a 5 mL solution of NaPy and K_2CO_3 ($c = 1$ mM) which was stirred overnight. A 5 mL solution of 1 M 2,4-pentanedione and 0.2 M *trans*-β-nitrostyrene was then added after which the solution was stirred and 0.5 mL NMR samples were taken at regular intervals. To minimize concentrating the solid K_2CO_3 over time, the samples were poured back into the reaction mixture immediately after measurement. The measurements with UPy were carried out using the ditopic UPy **2**. Ditopic UPy was synthesized according to a literature procedure.[6] The reactions were carried out in an identical manner as for the measurements without UPy, with the only difference that they were performed at 20 mL scale to minimize weighing errors between the NaPy and UPy.

Synthesis of 2,7-Diamido-1,8-naphthyridine (NaPy 1). 7-Amino-1,8-naphthyridin-2-ol (4): Malic acid (9.6 g, 70.4 mmol) and pyridine-2,6-diamine (7.0 g, 64.0 mmol) were grinded to a fine powder and cooled in an ice bath. Subsequently, concentrated sulfuric acid (32 mL) was dropwise added. The solution was heated under Ar to 110 °C for 3 h, poured over ice and made alkaline with concentrated ammonium hydroxide until a pH of 8 was reached. Subsequently, the precipitate was filtrated and washed with H_2O. Removal of the solvent in vacuo yielded the product (11.9 g, quantitative). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.60 (bs, 1H, OH), 7.61 (d, 2H, $J = 9.0$ Hz, Ar), 6.80 (s, 2H, NH_2), 6.33 (d, 1H, $J = 8.5$ Hz, Ar), 6.10 (d, 1H, $J = 9.2$ Hz, Ar). ^{13}C NMR (100 MHz,

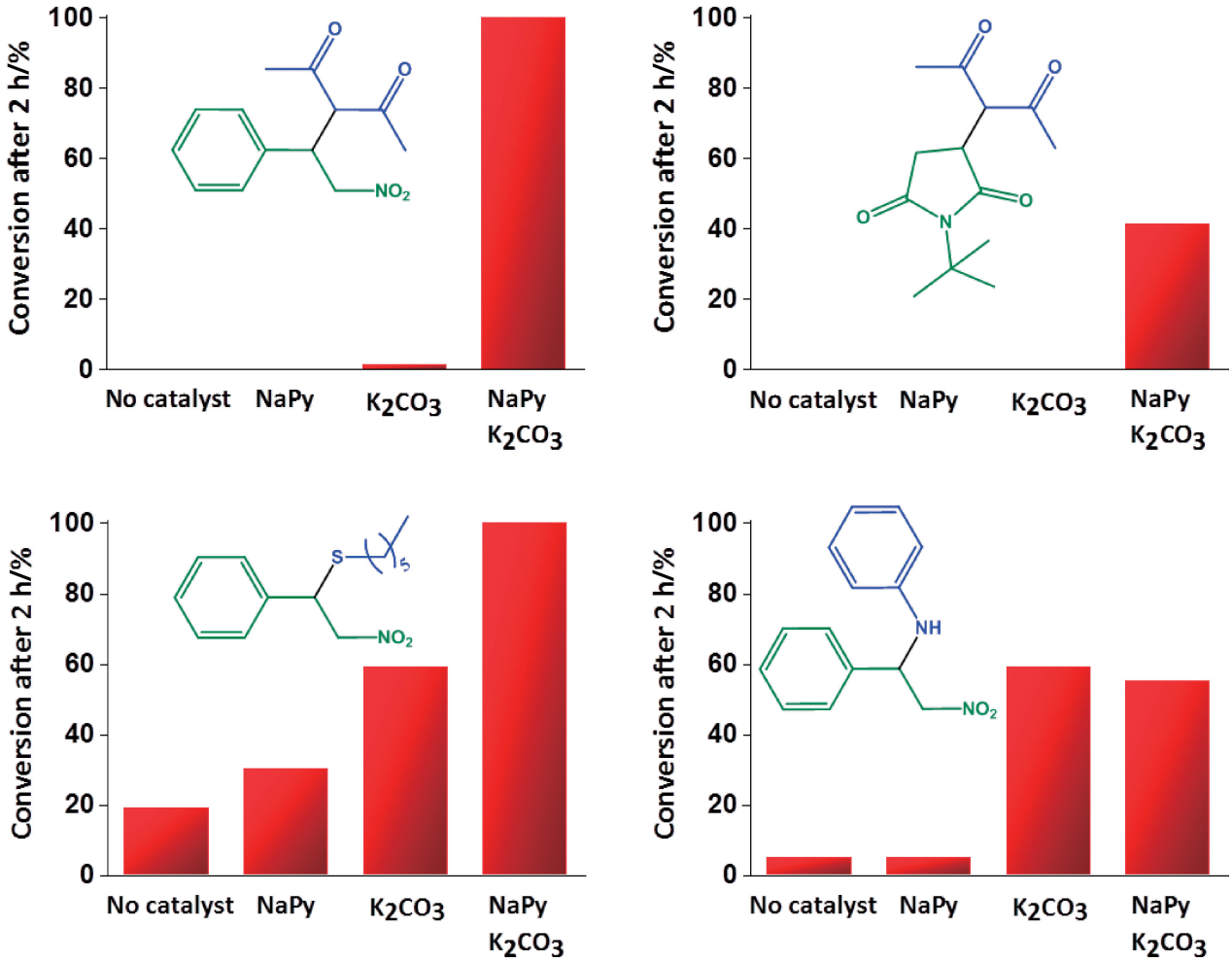

Figure 7. The conversion of the Michael acceptor in the Michael addition between various substrate combinations in $CDCl_3$ after 2 h; Michael donor ($c = 500$ mM), Michael acceptor ($c = 100$ mM), NaPy ($c = 20$ mM), and/or K_2CO_3 ($c = 5$ mM). The conversion was measured by ^{1}H NMR using the relative ratio of Michael acceptor and product. All components were combined directly.

DMSO-d_6): δ 168.69, 165.58, 155.61, 144.70, 142.34, 120.13, 110.11, 110.09.

***N*-(7-Oxo-7,8-dihydro-1,8-naphthyridin-2-yl)dodecanamide (5):** A mixture of 7-amino-1,8-naphthyridin-2-ol (**4**) (14.0 g, 87.0 mmol) and dodecanoyl chloride (34.4 g, 147.9 mmol) in dry pyridine (36 mL) was stirred overnight under Ar at 110 °C and subsequently allowed to cool to room temperature. The precipitate was filtered off and recrystallized from toluene. The product was obtained as a brown solid (18.2 g, 61%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.87 (bs, 1H, NH), 11.84 (bs, 1H, NH), 8.43 (d, 1H, $J = 8.7$ Hz, Ar), 7.99 (d, 1H, $J = 8.7$ Hz, Ar), 7.72 (d, 1H, $J = 9.4$ Hz, Ar), 6.63 (d, 1H, $J = 9.4$ Hz, Ar), 2.67 (t, 2H, $J = 7.4$ Hz, O=C-CH_2), 1.76 (m, 2H, $J = 7.6$, 7.4 Hz, O=C-CH_2-CH_2), 1.25 (m, 16H, alkyl-CH_2), 0.87 (t, 3H, alkyl-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.52, 165.27, 154.29, 148.62, 139.79, 139.05, 119.79, 111.17, 110.75, 37.10, 31.92, 29.66, 29.25, 25.38, 22.69, 14.13.

***N*-(7-Chloro-7,8-dihydro-1,8-naphthyridin-2-yl)dodecanamide (6):** A mixture of *N*-(7-oxo-7,8-dihydro-1,8-naphthyridin-2-yl)dodecanamide (**5**) (18.2 g, 50.1 mmol) in $POCl_3$ (150 mL) was stirred at 95 °C for 4 h under Ar and subsequently allowed to cool to room temperature. The remaining solution was slowly poured into vigorously stirred iced water (2 L), followed by neutralization with concentrated aqueous NH_3 solution to pH 8. The product was obtained as a white powder by filtration of the precipitate (17.2 g, 89%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.58 (d, 1H, $J = 8.9$ Hz, Ar), 8.32 (bs, 1H, NH), 8.18 (d, 1H, $J = 8.9$ Hz, Ar), 8.09 (d, 1H, Ar), 7.41 (d, 1H, $J = 8.4$ Hz, Ar), 2.47 (t, 2H, $J = 7.6$ Hz, O=C-CH_2), 1.76 (m, 2H, $J = 7.6$, 7.4 Hz, O=C-CH_2-CH_2), 1.26 (m, 16H, alkyl-CH_2), 0.88 (t, 3H, $J = 6.7$ Hz, alkyl-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.42, 154.12, 139.14, 138.74, 122.05, 115.18, 76.67, 38.07, 31.89, 29.58, 29.42, 29.31, 29.11, 25.21, 22.67, 14.11. MALDI-ToF MS: calculated for $C_{20}H_{30}ClN_3O$ 361.91, observed m/z 362.25 [M + H$^+$].

***N*,*N*′-(1,8-Naphthyridine-2,7-diyl)didodecanamide (1):** A flask was charged with *N*-(7-chloro-7,8-dihydro-1,8-naphthyridin-2yl)dodecanamide (**6**) (15.6 g, 42.8 mmol), dodecanamide (10.3 g, 51.3 mmol), $Pd(OAc)_2$ (593 mg, 2.6 mmol), Xantphos (3.3 g, 5.8 mmol), K_2CO_3 (5.9 g, 42.8 mmol), and dry dioxane (250 mL). Oven-dried molecular sieves (4 Å) were added and the mixture was stirred overnight at room temperature under Ar. Subsequently, the mixture was heated to 80 °C and stirred overnight under Ar. After cooling to room

temperature, the mixture was filtered and the residue was rinsed with $CHCl_3$. Recrystallization in ethanol and purification by flash chromatography (80% $CHCl_3$/20% EtOAc) yielded the product as a white solid (16.5 g, 73%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.42 (d, 2H, $J = 8.8$ Hz, Ar), 8.14 (d, 2H, $J = 8.8$ Hz, Ar), 8.12 (bs, 2H, NH), 2.47 (t, 4H, $J = 7.6$ Hz, O=C-CH_2), 1.56 (m, 4H, $J = 8.0$, 7.6 Hz, O=C-CH_2-CH_2), 1.26 (m, 32H, alkyl-CH_2), 0.88 (t, 6H, alkyl-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.2, 153.74, 138.98, 113.34, 110.01, 76.75, 38.13, 31.90, 29.60, 29.44, 29.33, 29.15, 25.29, 22.68, 14.12. MALDI-ToF MS: calculated for $C_{32}H_{52}N_4O_2$ 524.78, observed m/z 525.43 [M + H$^+$], 1087.79 [dimer + K$^+$]. Elemental analysis: calculated for $C_{32}H_{52}N_4O_2$: C, 73.24; H, 9.99; N, 10.68; O, 6.10%. Found: C, 73.35; H, 9.59; N, 10.26%.

Synthesis of Ethyl 3-[2-(3-Butylureido)-6-methyl-4-oxo-1,4-dihydropyrimidin-5-yl]propanoate (UPy 3). Ethyl 3-(2-Amino-6-methyl-4-oxo-1,4-dihydropyrimidin-5-yl)propanoate (7): A mixture of diethyl 2-acetylpentanedioate (1.9 mL, 8.7 mmol) and guanidine carbonate (784 mg, 8.7 mmol) in ethanol (20 mL) was refluxed for 18 h under Ar and subsequently allowed to cool to room temperature. Precipitation at 0 °C yielded the product as a white solid (690 mg, 35%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.09 (q, 2H, O-CH_2-CH_3), 2.70 (t, 2H, O=C-CH_2-CH_2), 2.51 (t, 2H, O=C-CH_2-CH_2), 2.21 (s, 3H, C-CH_3), 1.26 (t, 3H, CH_2-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 175.74, 62.90, 35.98, 24.08, 17.35. Remaining ^{13}C signals and J-coupling constans could not be identified with certainty due to the poor solubility of this compound in common NMR solvents. MALDI-ToF MS: calculated for $C_{10}H_{15}N_3O_3$ 225.24, observed m/z 226.29 [M + H$^+$], 248.24 [M + Na$^+$].

Ethyl 3-[2-(3-Butylureido)-6-methyl-4-oxo-1,4-dihydropyrimidin-5-yl]propanoate (3): Ethyl 3-(2-amino-6-methyl-4-oxo-1,4-dihydropyrimidin-5-yl)propanoate (**7**) (1.0 g, 4.6 mmol) in dry DMF (25 mL) was heated to 70 °C. 1-Isocyanatobutane (770 µL, 6.9 mmol) was added and the mixture was stirred for 6 h at 70 °C under Ar. After removal of the solvent and excess 1-isocyanatobutane in vacuo, the product was obtained as a white solid (1.4 g, 95%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.88 (bs, 1H, NH), 11.89 (bs, 1H, NH), 10.11 (bs, 1H, NH), 4.08 (q, 2H, $J = 7.2$ Hz, O-CH_2-CH_3), 3.24 (q, 2H, $J = 6.1$ Hz, NH-(C=O)-NH-CH_2), 2.68 (t, 2H, $J = 7.1$ Hz, O-(C=O)-CH_2-CH_2), 2.60 (t, 2H, $J = 7.1$ Hz, O-(C=O)-CH_2-CH_3), 2.30 (s, 3H, C-CH_3), 1.57 (m, 2H, alkyl-CH_2), 1.41 (m, 2H, CH_2-CH_2-CH_3), 1.24 (t, 3H, $J = 7.1$ Hz, O-CH_2-CH_3), 0.94 (t, 3H, $J = 6.6$ Hz, CH_3). ^{13}C NMR (126 MHz, $CDCl_3$): δ 173.29, 172.06, 156.63, 153.34, 143.98, 116.07, 60.31, 39.64, 32.12, 31.34, 21.14, 20.11, 17.13, 14.20, 13.73. MALDI-ToF MS: calculated for $C_{15}H_{24}N_4O_4$ 324.38, observed m/z 325.30 [M + H$^+$], 347.27 [M + Na$^+$].

We would like to thank Tim Paffen for useful discussions and providing bifunctional UPy **3**. This work is financed by the Dutch Organisation for Scientific Research (NWO–TOP grant: No. 10007851), the Dutch Ministry of Education, Culture and Science (Gravity program 024.001.035), and the European Research Council (FP7/2007–2013, ERC Advanced Grant No. 246829).

Supporting Information

Characterization data including ^{1}H and ^{13}C NMR spectra and kinetic experiments of the Michael additions. This material is available on http://dx.doi.org/10.1246/bcsj.20150407.

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