Reaction Rates of Various N-Acylenamines in the Inverse-Electron-Demand Diels-Alder Reaction

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Reaction Rates of Various *N*-Acylenamines in the Inverse-Electron-Demand Diels–Alder Reaction

Sander B. Engelsma,[a] Thomas C. van den Ende,[a] Hermen S. Overkleeft,[a] Gijsbert A. van der Marel,*[a] and Dmitri V. Filippov*[a]

Abstract: In light of the bioorthogonal inverse-electron-demand Diels–Alder strategy, an extended investigation into the effects of ring strain and electron inductive effects on the reactivity of the *N*-acylenamine core towards tetrazine has been carried out. Through a comparative study between *N*-acylazetines, *N*-vinylcarbamates and an *N*-vinylamide it was shown that ring strain has a more significant effect on reaction rate than electron donation. A significantly improved synthetic route is reported for the preparation of an *N*-acylazetine bioorthogonal tag we have invented previously.

Introduction

The inverse-electron-demand Diels–Alder (IEDDA) reaction between (cyclic) alkenes and tetrazines has been well-studied for several decades.[1–7] Renewed interest arose in 2008, due to the breakthrough of tetrazine ligation in the field of bioorthogonal chemistry.[8,9] Bioorthogonal ligation handles require an intricate balance between several physical properties; preferably the handle should be sterically small in size and be stable under physiological conditions, while maintaining high reactivity towards tetrazines. An additional beneficial property is the moderate-to-high hydrophilicity to assist the water solubility of the functionalized chemical probe. By now, a strong positive correlation had been established between the ring strain within cyclic alkenes and the enhanced reactivity towards tetrazines.[5] Contrary, significantly less effort has been directed towards the effects of the alkene electron density on reactivity. Recently we reported[10] a new ligation handle featuring an *N*-acylazetine as the reactive IEDDA partner (e.g. **1** in Figure 1), which was designed to utilize both ring strain and electron-donating properties to enhance reactivity towards tetrazines. An *N*-acylazetine tag functionalized as *p*-nitrophenyl active ester was synthesized and successfully applied in an activity-based protein profiling experiment. These advancements warranted further investigation into the structure–reactivity relationship of the *N*-alkene structure.

We here describe the synthesis of two *N*-acylazetines (**1**, **2**, Figure 1) and two *N*-vinylcarbamates (**3**, **4**) and the assessment of their stability in water and reactivity towards tetrazines. By comparing the reaction-rate constants of the IEDDA reactions
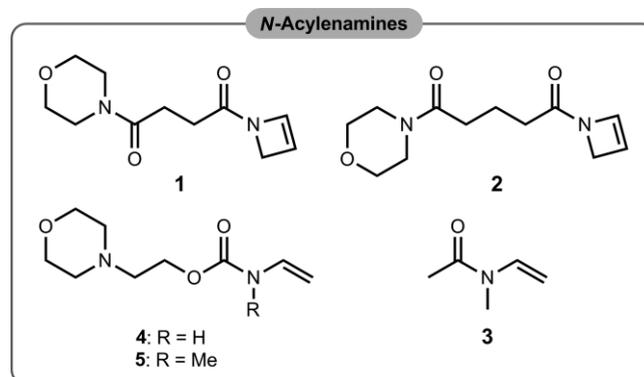
Figure 1. The *N*-acylazetines **1** and **2** and *N*-vinyl derivatives **3**–**5**.

of *N*-acylazetines **1** and **2** with *N*-vinyl derivatives (**3**–**5**) insight can be gained about the contribution of both ring strain and electron-donating effects on their reactivity towards tetrazines. Ultimately this knowledge may lead to improved probes for the tetrazine ligation strategy.

N-vinylcarbamates (**3** and **4**) and vinylamide (**5**) can be considered as linear analogues of the original cyclic *N*-acylazetine (**1**) and thereby potentially suitable for IEDDA reactions with tetrazine derivatives. A possible tautomerization equilibrium between the enamine and imine forms, as in **3**, makes *N*-vinyl groups both electrophilic at the α-carbon and nucleophilic at β-carbon.[11] This property make vinylamides susceptible to hydrolysis, or polymerization in either acid-catalyzed or radical-mediated processes.[12,13] N-methylation of *N*-vinyl derivatives suppresses tautomerization and makes the corresponding *N*-methyl-*N*-vinylamides more stable (i.e. **4**). In addition, disconnection of the *N*-acylazetine core between the N-CH$_2$ and the *N*-vinyl-β-CH leads to commercially available *N*-methyl-*N*-vinylamide (**5**). To ensure the necessary water solubility in the kinetic experiments, morpholine was incorporated in the *N*-acylazetines (**1**–**2**) and *N*-vinylcarbamates (**3**–**4**).

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Results and Discussion

The earlier described synthesis of *N*-acylazetine **1** consists of six reaction steps and lacks overall efficiency and scalability.[10] Therefore, another method to synthesize **1** was explored (Scheme 1). Commercially available 3-hydroxyazetidine **6** was selected as the starting compound, and the tosyl group was used instead of the mesyl group as a more potent and UV-detectable leaving group. Boc-protection of the amine followed by tosylation of the hydroxyl group in **6** provided protected azetidine **7** in 72 % yield over two steps. To prevent potential displacement of the tosyl group the Boc-group in **7** was cleaved using *p*-toluenesulfonic acid.
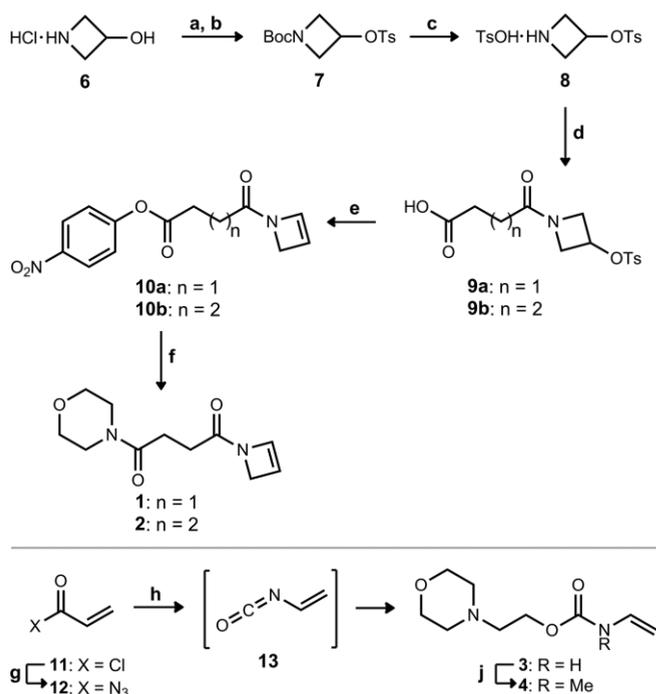
Scheme 1. Synthesis of *N*-acylazetines **1**, **2** and *N*-vinylcarbamates **3**, **4**. Reagents and conditions: [a] i: Boc$_2$O, TEA, MeOH, 0 °C, 2 h, used crude. [b] TsCl, TEA, DCM, 2 h, 72 % over 2 steps. [c] *p*TsOH, DCE, reflux, 16 h, 79 %. [d] Succinic- or glutaric anhydride, K$_2$CO$_3$, MeCN, reflux, 6 h, 50 % **9a**, 70 % **9b**. [e] KO*t*Bu, DMF, 2 h. ii: bis(*p*-nitrophenyl)carbonate, 16 h, 80 % **10a**, 69 % **10b**. [f] morpholine, DCM, 1 h, 84 % **1**, 100 % **2**. [g] NaN$_3$, H$_2$O/toluene (1:1), 6 h. [h] 2-morpholinoethanol, pyridine, hydroquinone, toluene, 100 °C, 1.5 h, 29 % [j] NaH, THF, MeI, 0 °C, 1.5 h, 75 %.

Subsequent purification by crystallization from MeOH yielded azetidine **8** in 80 %. Treatment of **8** with both succinic and glutaric anhydride was carried out in the presence of potassium carbonate in acetonitrile at reflux temperature, to give the four- and five-carbon spacer **9a**, and **9b** respectively. Next, the key elimination was initiated by the addition of a solution of KO*t*Bu in THF. The presence of the tosyl group in **9a** and **9b** improved solubility while the reaction proceeded readily at room temperature. The respective *N*-acylazetine intermediates were treated in situ with bis(*p*-nitrophenol)carbonate to provide linkable handles **10a** (80 %) and **10b** (69 %) in good yields over two steps. Initially, the *p*-nitrophenol was introduced using DIC or EDC as coupling reagents. However, switching to bis(*p*-nitrophenyl)carbonate resulted in cleaner and more consistent con-

versions. Treatment of *p*-nitrophenyl esters **10a** and **b** with morpholine gave target *N*-acylazetines **1** and **2** in high yield. Alternatively, one could consider converting the *N*-acylazetine-containing carboxylic acids to the amides in a "one-pot" procedure. We considered this approach to be impractical and did not investigate it.

The route of synthesis toward *N*-vinylcarbamates **3** and **4** makes use of the Curtius rearrangement of acryl azide (**12**), generated in situ from acryloyl chloride (**11**) and sodium azide. Upon heating, azide **12** rearranges into vinyl isocyanate (**13**), which can be transformed into *N*-vinylcarbamates by coupling with an alcohol of choice. A small amount of base and radical scavenger, such as hydroquinone or phenothiazine, is needed to suppress dimerization and radical polymerization. In practice, a solution of acryl azide **12** in toluene was prepared and slowly added to a reaction mixture containing 2-morpholinoethanol, pyridine and hydroquinone in toluene at 100 °C, providing *N*-vinylcarbamate **3** in 29 % isolated yield. The vinyl nitrogen atom was methylated with sodium hydride and methyl iodide in THF at 0 °C, to give *N*-methyl-*N*-vinylcarbamate **4**.

The instability of *N*-vinylcarbamates brings about the question of whether these compounds would survive the conditions of the kinetic experiments and future bioorthogonal tagging reactions. The aqueous stability of *N*-acylazetine **2** and *N*-vinylcarbamates **3** and **4** was evaluated by dissolving the respective compounds in deuterated water and monitoring the solution over a period of 13 hours at 37 °C with ^{1}H NMR spectroscopy. After the full duration, secondary *N*-vinylcarbamate **3** showed 4 % hydrolysis of the vinyl group, as determined by integration of the formyl hydrogen (δ = 9.74 ppm) resulting from the formed acetaldehyde. Fortunately, this rate of decomposition proved to be insignificant within the 30 minutes timeframe required for the kinetic experiments (Table 1). *N*-acylazetine **2** and *N*-methyl-*N*-vinylcarbamate **4** proved to be stable during the experiment. In addition, the *N*-acylazetine group stability was evaluated in the presence of 100 mᴍ of ethanethiol and 2-aminoethanol, to mimic the physiological conditions used during in vivo activity-based protein profiling experiments. Again, no decomposition occurred.

With the *N*-acylazetines **1**, **2** and *N*-vinyl derivatives **3**, **4**, **5** in hand, the stage was set for determining the rates of IEDDA reactions with tetrazine **14** (Table 1). The conditions we opted to apply for the kinetic experiments were similar to those used by Devaraj et al. in their work featuring the 1-methylcyclopropene ligation handle.[14] Pseudo-first-order and second-order reaction rates were assessed by reacting a 20-fold excess of the respective dienophile (**1**–**5**) with tetrazine **14** in 12 % aqueous DMSO. The rate of tetrazine consumption was measured by monitoring the characteristic tetrazine absorption at 517 nm. Each experiment was conducted thrice both at room temperature (20 °C) and at body temperature (37 °C). The results are summarized in Table 1. The availability of *N*-acylazetine **1** and **2** allows the determination of the influence of the spacer length on the IEDDA reaction rate. The outcome indicates that shortening the spacer length by one carbon as in **1** results in a minor decrease of the reaction rate. This result could be explained by the increased proximity of the electron-withdrawing carbonyl

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Table 1. Reaction rates for addition of dienophiles **1**–**5** to tetrazine **14**. The first-order reaction rates were calculated from three data sets each using all measurement intervals up until $t_{1/2}$. The error ranges are the standard deviations derived from the generated first-order rate data. Half-life values are calculated from first-order rate constant: $t_{1/2} = \ln(2)/k$.

14: R = p-PhCH_2OH

	Dienophile	T [°C]	1st Order [s^{-1}]	2nd Order [M^{-1}·s^{-1}]	Half-life [s]
1		20	$4.10 \cdot 10^{-3} \pm 3.71 \cdot 10^{-4}$	$6.88 \cdot 10^{-1} \pm 6.23 \cdot 10^{-2}$	169
		37	$7.11 \cdot 10^{-3} \pm 8.95 \cdot 10^{-5}$	$1.21 \pm 1.42 \cdot 10^{-2}$	97
2		20	$4.40 \cdot 10^{-3} \pm 1.57 \cdot 10^{-4}$	$7.50 \cdot 10^{-1} \pm 2.62 \cdot 10^{-2}$	157
		37	$7.78 \cdot 10^{-3} \pm 4.25 \cdot 10^{-5}$	$1.32 \pm 7.40 \cdot 10^{-3}$	89
3		20	$1.27 \cdot 10^{-3} \pm 2.04 \cdot 10^{-5}$	$2.16 \cdot 10^{-1} \pm 3.48 \cdot 10^{-3}$	544
		37	$1.97 \cdot 10^{-3} \pm 9.34 \cdot 10^{-5}$	$3.34 \cdot 10^{-1} \pm 1.59 \cdot 10^{-2}$	351
4		20	$7.53 \cdot 10^{-4} \pm 8.54 \cdot 10^{-6}$	$1.27 \cdot 10^{-1} \pm 1.44 \cdot 10^{-3}$	920
		37	$1.63 \cdot 10^{-3} \pm 4.65 \cdot 10^{-5}$	$2.76 \cdot 10^{-1} \pm 7.90 \cdot 10^{-3}$	424
5		20	$3.12 \cdot 10^{-4} \pm 2.16 \cdot 10^{-6}$	$5.27 \cdot 10^{-2} \pm 3.67 \cdot 10^{-4}$	2223
		37	$5.05 \cdot 10^{-4} \pm 1.68 \cdot 10^{-6}$	$8.54 \cdot 10^{-2} \pm 2.88 \cdot 10^{-4}$	1372

group to the azetine moiety. However, given the small size of this effect and a relatively big measurement uncertainty, this result has to be treated with caution. Comparison of the reaction rates for N-acylazetine **2** and N-vinylamide **5** shows the influence of ring strain on the IEDDA reaction rate. Evidently, ring strain contributes significantly to the reaction rate, as the acyclic N-vinylamines show (both at 20 °C and 37 °C) a 15-fold increase in half-life time. A similar comparison between **4** and **5** reflects the increase in electron density by progressing from an N-vinylamide to an N-vinylcarbamate. Here the results show an approximate 2.8-fold decrease in half-life time, considerably less impactful than the effects induced by ring strain. Methylation at the nitrogen atom of **3** to form secondary N-vinylamide **4** only induces a minor (1.5-fold) increase in half-life time. Additional support for our assertion that the electronic-density affects make only a minor contribution to the reactivity differences has been provided by the calculated[15] energies of occupied frontier molecular orbitals (HOMO's) for model N-acylenamines (Supporting Information, pages S32–S36).

Conclusions

An investigation into the contribution of ring strain and electron-donating effects upon the IEDDA reactivity of N-acylen-amines towards tetrazines is described. To this end, an improved synthesis toward N-acylazetines **1**, **2** is presented while N-vinylcarbamates **3**, **4** could be accessed through the Curtius rearrangement of acryl azide. The reaction rate constants were determined for N-acylazetine **1** and **2** and N-vinyl compounds

3–**5** at 20 °C and 37 °C. Comparison between the reaction rates of N-acylazetine **2** and N-vinylamide **5** shows a 15-fold higher reaction rate for the four-membered ring. The influence of the differences in electron donation between a N-vinylamide and a N-vinylcarbamate was significantly less, resulting in a 2.8-fold increase in reaction rate for the latter.

Experimental Section

All solvents and reagents were obtained commercially and used as received. Reactions were executed at ambient temperatures unless stated otherwise. Reactions were monitored by TLC analysis, spraying with varying stains; an aqueous solution of cerium molybdate [(NH$_4$)$_6$Mo$_7$O$_{24}$·4H$_2$O 25 g/L], an aqueous solution of potassium permanganate (5 g of KMnO$_4$, 25 g of K$_2$CO$_3$ per L) or an ethanolic solution of bromocresol [0.4 g in 1 L, addition of 0.1 M NaOH$_{(aq)}$ until the solution turns blue]. Column chromatography was performed on silica gel (40–63 μm). ^{1}H and ^{13}C-APT spectra were recorded on a Bruker AV-400 (400 MHz), Bruker DMX-600 (600 MHz) or Bruker BioSpin (850 MHz). All present ^{13}C-APT spectra are proton decoupled. High-resolution mass spectrometry was recorded by direct injection (2 μL of a 2 μM solution in water/acetonitrile; 50:50; v/v and 0.1 % formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250 °C) with resolution R = 600000 at m/z 400 (mass range m/z = 120–400).

***tert*-Butyl 3-(Tosyloxy)azetidine-1-carboxylate (7):** A solution of 3-hydroxyazetidine hydrochloride (115 mmol, 10.55 g, 1 equiv.) and Et$_3$N (161 mmol, 22.5 mL, 1.4 equiv.) in MeOH (115 mL) was pre-

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pared at 0 °C. Boc$_2$O (126.5 mmol, 27.6 g, 1.1 equiv.) was added and the ice bath was removed. After 5 h of stirring, the reaction mixture was concentrated in vacuo, redissolved in DCM and washed twice with water. The water layers were combined and extracted twice with DCM. The organic layers were combined, dried with magnesium sulfate, filtered and concentrated in vacuo. The intermediate Boc-hydroxyazetidine was used without further purification. An ice-cooled solution of the Boc-protected intermediate and Et$_3$N (172.5 mmol, 24 mL, 1.5 equiv.) in dry DCM (100 mL) was prepared under argon atmosphere. p-Toluenesulfonyl chloride (138 mmol, 26.3 g, 1.2 equiv.) was added in eight portions over 2 hours and the reaction mixture was stirred overnight. The reaction mixture was washed with water twice and the combined aqueous layers were extracted thrice with DCM. The organic layers were combined, dried with magnesium sulfate, filtered and concentrated in vacuo. The crude product was purified by column chromatography (5 % → 10 % EtOAc in pentane), yielding tosylate **7** as a yellow oil. (82.6 mmol, 27.7 g, 72 % over two steps). ^{1}H NMR (300 MHz, CDCl$_3$): δ = 7.75 (d, J = 8.1 Hz, 2 H), 7.34 (d, J = 8.1 Hz, 2 H), 4.97 (ddd, J = 10.8, 6.6, 4.3 Hz, 1 H), 4.14–4.01 (m, 2 H), 3.97–3.82 (m, 2 H), 2.43 (s, 3 H), 1.38 (s, 9 H) ppm. ^{13}C NMR (75 MHz, CDCl$_3$): δ = 155.86, 145.61, 132.91, 130.17, 127.92, 80.23, 67.84, 56.28, 28.30, 21.74 ppm. HRMS: Calculated for C$_{15}$H$_{22}$NO$_5$S$^+$ 328.12132 [M + H]$^+$, found 328.12128.

Azetidin-3-yl 4-Methylbenzenesulfonate (8): A solution of compound **7** (82.6 mmol, 27.1 g, 1 equiv.) in DCE (165 mL) was charged with p-toluenesulfonic acid (90.9 mmol, 17.3 g, 1.1 equiv.) and refluxed for 20 h. The reaction mixture was concentrated in vacuo. The crude product was crystalized from MeOH, yielding compound **8** as a white crystalline substance (65 mmol, 25.9 g, 79 %). ^{1}H NMR (400 MHz, CDCl$_3$): δ = 9.05 (br. s, 1 H), 8.94 (br. s, 1 H), 7.70 (d, J = 8.2 Hz, 4 H), 7.29 (d, J = 8.1 Hz, 2 H), 7.19 (d, J = 7.8 Hz, 2 H), 5.06 (t, J = 6.2 Hz, 1 H), 4.25 (br. s, 1 H), 4.13 (br. s, 1 H), 2.42 (s, 3 H), 2.39 (s, 3 H) ppm. ^{13}C NMR (101 MHz, CDCl$_3$): δ = 146.07, 141.26, 132.03, 130.38, 129.39, 128.17, 125.92, 67.85, 53.37, 21.86, 21.55 ppm.

4-Oxo-4-[3-(tosyloxy)azetidin-1-yl]butanoic Acid (9a): Compound **8** (20.0 mmol, 5.58 g, 1.1 equiv.) was co-evaporated with dioxane, redissolved in MeCN (200 mL), and put under argon atmosphere. Succinic anhydride (18.2 mmol, 1.82 g, 1 equiv.) was added to the reaction mixture, followed by potassium carbonate (45.5 mmol, 6.3 g, 2.5 equiv.) and the reaction mixture was refluxed for 6 h. Reaction progression was monitored by TLC, using a bromocresol stain to visualize the produced carboxylic acid. The reaction mixture was diluted with water (200 mL) and Amberlite-H$^+$ (IR120, ±70 g) was added until the pH fell below 3. The solution was filtered and the residual MeCN was removed in vacuo. The water layer was extracted twice with EtOAc. The organic layers were combined, dried with magnesium sulfate, filtered and concentrated in vacuo. The crude product was purified by column chromatography. The crude product was purified by column chromatography (5 % → 10 % EtOH in DCM), yielding compound **9a** as a white crystalline substance (9.1 mmol, 3.09 g, 50 %). ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.80 (d, J = 8.3 Hz, 2 H), 7.39 (d, J = 8.2 Hz, 2 H), 5.08 (ddd, J = 11.1, 6.9, 4.3 Hz, 1 H), 4.42 (dd, J = 9.4, 7.5 Hz, 1 H), 4.30–4.08 (m, 2 H), 3.94 (dd, J = 11.5, 4.0 Hz, 1 H), 2.71–2.61 (m, 2 H), 2.48 (s, 3 H), 2.35 (t, J = 6.8 Hz, 2 H) ppm. ^{13}C NMR (101 MHz, CDCl$_3$): δ = 171.99, 145.99, 130.37, 128.07, 76.84, 67.22, 57.38, 55.23, 28.81, 26.20 ppm. HRMS: Calculated for C$_{14}$H$_{18}$NO$_6$S$^+$ 328.08548 [M + H]$^+$, found 328.08477.

4-Nitrophenyl 4-[Azet-1(2H)-yl]-4-oxobutanoate (10a): Compound **9a** (9.1 mmol, 3.09 g, 1 equiv.) was co-evaporated with diox-

ane, redissolved in dry DMF (45.5 mL) and put under argon atmosphere. Next, a 1 M solution of potassium tert-butoxide in THF (20 mL, 2.1 equiv.) was added to the reaction mixture and the reaction was stirred for 1 h. Subsequently the reaction mixture was charged with bis(p-nitrophenol)carbonate (10 mmol, 3.01 g, 1.1 equiv.) and left stirring for an additional 3 h. The reaction mixture was diluted with EtOAc and washed twice with 10 % aqueous sodium hydrogen carbonate, twice with water and once with brine. The combined organic layers were dried with MgSO$_4$, filtered and concentrated in vacuo. The crude product was purified with column chromatography (50 % → 100 % EtOAc in pentane), yielding compound **10a** as a yellow crystalline substance (7.3 mmol, 2.0 g, 80 %). ^{1}H NMR (400 MHz, CDCl$_3$): δ = 8.26 (d, J = 9.2 Hz, 2 H), 7.31 (d, J = 9.1 Hz, 2 H), 6.91 (s, 0.5 H), 6.71 (s, 0.5 H), 5.75 (d, J = 5.3 Hz, 1 H), 4.61 (s, 1 H), 4.48 (s, 1 H), 2.97 (t, J = 6.6 Hz, 2 H), 2.75 (t, J = 6.5 Hz, 1 H), 2.66 (t, J = 6.5 Hz, 1 H) ppm. ^{13}C NMR (101 MHz, CDCl$_3$): δ = 170.76, 165.26, 164.88, 155.48, 145.32, 137.43, 136.64, 125.22, 122.55, 114.19, 113.93, 77.16, 58.67, 56.91, 29.13, 29.03, 26.74, 25.76 ppm. HRMS: Calculated for C$_{13}$H$_{13}$N$_2$O$_5$$^+$ 277.08245 [M + H]$^+$, found 277.08191.

1-[Azet-1(2H)-yl]-4-morpholinobutane-1,4-dione (1): A solution of compound **10a** (1.03 mmol, 232 mg, 1 equiv.) and morpholine (3.09 mmol, 0.27 mL, 3 equiv.) in DCM (2.6 mL) was prepared and left stirring for 3 hours. The reaction mixture was directly purified by column chromatography (79 %/1 % → 50 %/5 % acetone/EtOH in DCM), yielding compound **1** as off-white crystals (0.86 mmol, 193 mg, 84 %). ^{1}H NMR (400 MHz, CDCl$_3$): δ = 6.87 (s, 0.5 H), 6.72 (s, 0.5 H), 5.70 (d, J = 13.9 Hz, 1 H), 4.61 (s, 1 H), 4.42 (s, 1 H), 3.65 (dt, J = 8.9, 4.9 Hz, 4 H), 3.61–3.55 (m, 2 H), 3.54–3.43 (m, 2 H), 2.67 (t, J = 6.2 Hz, 2 H), 2.66–2.58 (m, 1 H), 2.52 (t, J = 6.6 Hz, 1 H) ppm. ^{13}C NMR (101 MHz, CDCl$_3$): δ = 170.27, 166.58, 166.25, 137.27, 137.10, 113.71, 113.48, 66.80, 66.53, 58.72, 56.57, 45.73, 42.04, 27.73, 27.59, 26.91, 25.97 ppm. HRMS: Calculated for C$_{11}$H$_{17}$N$_2$O$_3$$^+$ 225.12392 [M + H]$^+$, found 225.12336.

5-Oxo-5-[3-(tosyloxy)azetidin-1-yl]pentanoic Acid (9b): Compound **8** (14.0 mmol, 5.58 g, 1.1 equiv.) was co-evaporated with dioxane, redissolved in MeCN (140 mL) and put under argon atmosphere. Glutaric anhydride (12.7 mmol, 1.45 g, 1 equiv.) was added to the reaction mixture, followed by potassium carbonate (31.8 mmol, 4.48 g, 2.5 equiv.) and the reaction mixture was refluxed for 6 hours. Reaction progression was monitored by TLC, using a bromocresol stain to visualize the produced carboxylic acid. The reaction mixture was diluted with water (200 mL) and Amberlite-H$^+$ (IR120, ±50 g) was added until the pH fell below 3. The solution was filtered and the residual MeCN was removed in vacuo. The water layer was extracted twice with EtOAc. The organic layers were combined, dried with magnesium sulfate, filtered and concentrated in vacuo. The crude product was purified by column chromatography (3 % → 5 % EtOH in DCM), yielding compound **9b** as a white crystalline substance (8.9 mmol, 3.06 g, 70 %). ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.81 (d, J = 8.3 Hz, 2 H), 7.40 (d, J = 8.1 Hz, 2 H), 5.08 (tt, J = 6.8, 4.2 Hz, 1 H), 4.45–4.33 (m, 1 H), 4.26–4.13 (m, 2 H), 3.93 (dd, J = 11.5, 4.3 Hz, 1 H), 2.49 (s, 3 H), 2.42 (t, J = 7.0 Hz, 2 H), 2.17 (t, J = 7.3 Hz, 2 H), 1.92 (p, J = 7.2 Hz, 2 H) ppm. ^{13}C NMR (101 MHz, CDCl$_3$): δ = 177.76, 172.54, 145.84, 132.58, 130.24, 127.93, 67.11, 57.23, 54.93, 32.93, 30.29, 21.78, 19.57 ppm. HRMS: Calculated for C$_{15}$H$_{20}$NO$_6$S$^+$ 342.10058 [M + H]$^+$, found 342.10045.

4-Nitrophenyl 5-Oxo-5-[3-(tosyloxy)azetidin-1-yl]pentanoate (10b): Compound **9b** (8.9 mmol, 3.04 g, 1 equiv.) was co-evaporated with dioxane, redissolved in dry DMF (44.5 mL), and put under argon atmosphere. Next, a 1 M solution of potassium tert-butoxide in THF (18.7 mL, 2.1 equiv.) was added to the reaction mixture and

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left stirring for 1 hour. Subsequently the reaction mixture was charged with bis(p-nitrophenol)carbonate (9.8 mmol, 2.95 g, 1.1 equiv.) and left stirring for another 3 hours. The reaction mixture was diluted with EtOAc and washed twice with 10 % aqueous sodium hydrogen carbonate, twice with water and once with brine. The combined organic layers were dried with magnesium sulfate, filtered and concentrated in vacuo. The crude product was purified by column chromatography (50 % → 100 % EtOAc in pentane), yielding compound 10b[10] as a yellow crystalline substance (6.1 mmol, 1.78 g, 69 %). ^{1}H NMR (400 MHz, CDCl$_3$): δ = 8.26 (d, J = 9.2 Hz, 2 H), 7.31 (d, J = 9.1 Hz, 2 H), 6.91 (s, 0.5 H), 6.71 (s, 0.5 H), 5.75 (d, J = 5.3 Hz, 1 H), 4.61 (s, 1 H), 4.48 (s, 1 H), 2.97 (t, J = 6.6 Hz, 2 H), 2.75 (t, J = 6.5 Hz, 1 H), 2.66 (t, J = 6.5 Hz, 1 H) ppm. ^{13}C NMR (101 MHz, CDCl$_3$): δ = 170.76, 165.26, 164.88, 155.48, 145.32, 137.43, 136.64, 125.22, 122.55, 114.19, 113.93, 77.16, 58.67, 56.91, 29.13, 29.03, 26.74, 25.76 ppm.

1-(5-Morpholino-5-oxopentanoyl)azetidin-3-yl 4-Methylbenzenesulfonate (2): A solution of compound 10b (0.68 mmol, 198 mg, 1 equiv.) in DCM (1.7 mL), was charged with morpholine (2.04 mmol, 0.18 mL, 3 equiv.) and left stirring for 2 hours. The reaction mixture was directly purified by column chromatography (20 %/1 % → 45 %/5 % acetone/EtOH in DCM), yielding compound 2 as a yellow oil (0.68 mmol, 158 mg, 100 %). ^{1}H NMR (400 MHz, CDCl$_3$): δ = 6.87 (s, 0.5 H), 6.67 (s, 0.5 H), 5.74–5.63 (m, 2 H), 4.55–4.49 (m, 1 H), 4.41 (s, 1 H), 3.68–3.60 (m, 4 H), 3.58 (d, J = 5.0 Hz, 2 H), 3.50–3.44 (m, 2 H), 2.39 (t, J = 7.1 Hz, 3 H), 2.29 (t, J = 6.8 Hz, 1 H), 2.01–1.90 (m, 2 H) ppm. ^{13}C NMR (101 MHz, CDCl$_3$): δ = 171.16, 167.25, 166.73, 137.41, 137.18, 113.53, 66.96, 66.76, 58.72, 56.59, 46.01, 41.95, 32.15, 32.11, 31.24, 30.08, 20.61, 20.49 ppm. HRMS: Calculated for C$_{12}$H$_{19}$N$_2$O$_3^+$ 239.13902 [M + H]$^+$, found 239.13900.

Acryloyl Azide (12): Acryloyl chloride (10 mmol, 0.81 mL, 1 equiv.) in toluene (1.3 mL) was added to an ice-cooled solution of NaN$_3$ (683 mg, 10.5 mmol, 1.05 equiv.) in water (2.5 mL). The ice bath was removed and the two-layered reaction mixture was stirring vigorously for 4 hours. Toluene was added (1.3 mL) and the mixture was poured into a separation funnel and washed twice with saturated aqueous sodium hydrogen carbonate, twice with water and once with brine. The organic layer was dried with magnesium sulfate, filtered and the solution was stored overnight at 4 °C. The product was used in solution without further purification.

2-Morpholinoethyl Vinylcarbamate (3): A solution of hydroquinone (55 mg, 0.5 mmol, 0.05 equiv.), pyridine (36 µL, 0.5 mmol, 0.5 equiv.), N-ethoxymorpholine (20 mmol, 2.4 mL, 2 equiv.) in toluene (2 mL) was put under argon atmosphere and heated to 100 °C. The prepared solution of acryloyl azide in toluene was added dropwise over one hour. After complete addition, the reaction mixture was stirred for an additional 30 min at 100 °C, before being cooled to room temp. The mixture was filtered and concentrated in vacuo. The crude product was purified by column chromatography (0 % → 5 % EtOH/DCM), yielding vinylcarbamate 3 as a yellow oil. (2.85 mmol, 570 mg, 29 % over two steps). ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.19 (s, 1 H), 6.62 (dt, J = 15.8, 10.0 Hz, 1 H), 4.41 (d, J = 15.8 Hz, 1 H), 4.21 (d, J = 8.9 Hz, 1 H), 4.20–4.13 (m, 2 H), 3.71–3.61 (m, 4 H), 2.60–2.52 (m, 2 H), 2.44 (s, 4 H) ppm. ^{13}C NMR (101 MHz, CDCl$_3$): δ = 153.68, 129.99, 93.23, 66.69, 61.80, 57.33, 53.68 ppm.

HRMS: Calculated for C$_6$H$_{17}$N$_2$O$_3^+$ 201.12392 [M + H]$^+$, found 201.12337.

2-Morpholinoethyl Methyl(vinyl)carbamate (4): Sodium hydride (60 % in mineral oil, 2.4 mmol, 96 mg, 1 equiv.) was added potionwise to a solution of vinylcarbamate 3 (2.4 mmol, 480 mg, 1 equiv.) in dry THF (8 mL), under argon atmosphere. The reaction mixture was sonicated until hydrogen evolution was complete. The reaction mixture was cooled to 0 °C and methyl iodide (2.52 mmol, 0.16 mL, 1.05 equiv.) was added. The reaction mixture was stirred for 1.5 h, quenched with Et$_3$N•HCl and concentrated in vacuo. The crude product was purified by column chromatography (2 % → 5 % EtOH in DCM), yielding compound 4 as a yellow liquid. (1.81 mmol, 387 mg, 75 %). ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.24–7.01 (m, 1 H), 4.37–4.15 (m, 4 H), 3.76–3.59 (m, 4 H), 3.01 (s, 2 H), 2.65 (t, J = 5.8 Hz, 2 H), 2.54–2.38 (m, 4 H) ppm. ^{13}C NMR (101 MHz, CDCl$_3$): δ = 134.20, 133.51, 91.95, 66.86, 63.42, 57.25, 53.80, 30.23 ppm. HRMS: Calculated for C$_7$H$_{19}$N$_2$O$_3^+$ 215.13957 [M + H]$^+$, found 215.13911.

Acknowledgments

[1] J. Sauer, D. K. Heldmann, J. Hetzenegger, J. Krauthan, H. Sichert, J. Schuster, Eur. J. Org. Chem. **1998**, 2885–2896.
[2] H. Schuster, H. Sichert, J. Sauer, Tetrahedron Lett. **1983**, 24, 1485–1488.
[3] A. C. Knall, M. Hollauf, C. Slugovc, Tetrahedron Lett. **2014**, 55, 4763–4766.
[4] D. R. Soenen, J. M. Zimpleman, D. L. Boger, J. Org. Chem. **2003**, 68, 3593–3598.
[5] F. Thalhammer, U. Wallfahrer, J. Sauer, Tetrahedron Lett. **1990**, 31, 6851–6854.
[6] L. Törk, G. Jiménez-Osés, C. Doubleday, F. Liu, K. N. Houk, J. Am. Chem. Soc. **2015**, 137, 4749–4758.
[7] W. Chen, D. Wang, C. Dai, D. Hamelberg, B. Wang, Chem. Commun. **2012**, 48, 1736–1738.
[8] M. L. Blackman, M. Royzen, J. M. Fox, J. Am. Chem. Soc. **2008**, 130, 13518–13519.
[9] N. K. Devaraj, R. Weissleder, S. A. Hilderbrand, Bioconjugate Chem. **2008**, 19, 2297–2299.
[10] S. B. Engelsma, L. I. Willems, C. E. van Paaschen, S. I. van Kasteren, G. A. van der Marel, H. S. Overkleeft, D. V. Filippov, Org. Lett. **2014**, 16, 2744–2747.
[11] D. J. am Ende, K. M. DeVries, P. J. Clifford, S. J. Brenek, Org. Process Res. Dev. **1998**, 2, 382–392.
[12] D. J. Dawson, R. D. Gless, R. E. Wingard, J. Am. Chem. Soc. **1976**, 98, 5996–6000.
[13] R. W. Stackman, R. H. Summerville, Ind. Eng. Chem. Prod. Res. Dev. **1985**, 24, 242–246.
[14] J. Yang, J. Šečkute, C. M. Cole, N. K. Devaraj, Angew. Chem. Int. Ed. **2012**, 51, 7476–7479; Angew. Chem. **2012**, 124, 7594.
[15] F. Liu, Y. Liang, K. N. Houk, J. Am. Chem. Soc. **2014**, 136, 11483–11493.