Supporting Information

Dynamic Exchange of Substituents in a Prebiotic Organocatalyst: Initial Steps towards an Evolutionary System

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General Information

All solvents and chemicals were purchased from commercial sources (abcr GmbH, Acros Organics b.v.b.a., Sigma-Aldrich Co. LLC, Strem Chemicals Inc. and TCI Europe N.V.) and stored according to the respective instructions. Argon gas (Ar 5.0) and hydrogen sulfide gas (H₂S 5.0) were purchased from Air Liquide Deutschland GmbH. Propionaldehyde was distilled and stored under argon at -20 °C prior to the organocatalytic reaction.

Isolated compounds were stored under argon at -20 °C.

NMR spectroscopy

NMR spectra were recorded on a 400 MHz Bruker Avance III HD spectrometer and a 600 MHz Varian NMR-System with a CryoProbe Prodigy. Spectra were calibrated using the residual solvent peaks. Chemical shifts δ are reported in ppm and coupling constants J in Hz. The different multiplicities are defined by s (singlet), d (doublet), t (triplet), q (quartet), sext (sextet), m (multiplet), bs (broad singlet) or by combinations of these. The assignment of all signals was realized by two-dimensional NMR spectroscopy (COSY, HSQC, HMBC). Atom numbering for NMR assignments is not based on IUPAC. Numbers separated by “,” refer to an assignment of both atoms, numbers separated by “/” refer to an assignment of one of the atoms. Magnetically inequivalent hydrogen atoms of the same carbon atom are differentiated by a and b.

Mass Spectrometry

Mass spectrometric analysis was performed using a Thermo Scientific Q Exactive Plus mass spectrometer coupled to electrospray ionization (ESI) by direct injection. As injection solvent, 80:20 isopropanol:water with 0.05% formic acid was used. The sample was dissolved in acetonitrile or distilled water and the mass spectrometer was operated in full scan measuring in positive and negative mode.

HPLC-Analysis

HPLC measurements were performed on an Agilent Series 1200 Infinity system equipped with a high-performance autosampler model HiP-ALS SL+ and a G1315D photodiode array detector (DAD) coupled to a 6120 Quadrupole LC/MS detector with atmospheric-pressure chemical ionization (APCI). The separation of different imidazolidine-4-thiones was performed with a 4.6 mm x 250 mm EC Nucleodur 100-5 (Macherey Nagel) at 20 °C and a flow of 1.00 mL/min. 1 µL of the sample was injected and eluted using a gradient elution of n-hexane (A) and isopropanol (B): A/B 99/1 → 95/5 (10 min) → 90/10 (15 min) → 60/40 (25 min). The peaks were detected at 270 nm.
**GC-Analysis**

GC analysis of the one-pot mixture of aldehyde and hydrogen sulfide in ammonia was performed with a Thermo Scientific Trace GC Ultra with split injector (15 mL/min) and a flame ionization detector at 250 °C. The instrument was coupled to an ISQ single quadrupole on electron impact (EI) mode operated at 70 eV. The temperatures of the injection, transfer interface, and ion source were set to 250 °C, 300 °C, and 200 °C, respectively. A SE-30 column (dimethylpolysiloxane, 25 m x 250 μm i.d., 250 nm film thickness) was used as stationary phase. 0.1 μL samples were injected and helium was used as the carrier gas at a constant pressure of 80 kPa. The starting temperature of 70 °C was held for 5 min before raising it with a gradient of 3 K/min until 220 °C which was again held for 5 min.

**UHPLC-QTOF-Analysis**

Liquid Chromatography was performed on an Agilent Technologies 1260 Infinity LC System. An Agilent Extend C18 (2.1x50mm, 1.80 μm particle size) column was used for separation. During analysis, the column was constantly set to 40 °C. The mobile phase was a gradient prepared from highly pure water containing 0.05% (v/v) formic acid (eluent A) and methanol (eluent B). The gradient used for LC-MS was: 0% B held constant for 0.59 min, then increased to 40% B within 2.38 min and held for 0.59 min. Subsequent increase to 100% of eluent B within 0.03 min and held for 0.57 min to flush the column. Subsequent reconstitution of the starting conditions with 0.03 min and re-equilibration with 0% B for 6.81 min resulted in a total analysis time of 11 min. The liquid flow was set to 0.55 mL/min. The highly pure water was obtained from a VWR Puranity PU 15 UV water purification plant. The methanol was purchased in HPLC-MS grade from Aldrich. Consecutive downstream mass analysis was performed by an Agilent Technologies 6550 iFunnel Q-TOF/MS. The ions were analysed in a scan range of 40-1100 m/z. No prior splitting was applied. The following parameters were applied during the analysis: gas temperature: 200 °C, drying gas flow: 14 L/min, nebulizer pressure: 35 psi, sheath gas temperature: 275 °C, sheath gas flow: 11 L/min, capillary voltage: 4 kV, capillary current: 0.0 μA, nozzle voltage: 0 V, fragmentor voltage 130 V; for internal calibration the Agilent Technologies ESI-TOF reference mass solution was used, diluted in acetonitrile:water (95:5). The internal reference masses were set to 122.9855 and 1033.9881.
Experimental Procedures and Characterization Data of Isolated Compounds

Second generation aldehyde (1a’)

3-Methyl-4-oxobutanenitrile (1a’)

Freshly distilled propionaldehyde (1.79 mL, 25 mmol, 5.0 eq.), bromoacetonitrile (349 µL, 5.0 mmol, 1.0 eq.), 2,6-lutidine (1.16 mL, 10 mmol, 2.0 eq.) and 2,2,5,5-tetramethylimidazolidine-4-thione 3dd (158 mg, 1.0 mmol, 0.2 eq.) were dissolved in 2.0 mL DMSO in a flat bottom vial. The sample was stirred and irradiated overnight by a Roschwege Star-UV365-01-00-00 emitter (365 nm) placed below. The reaction mixture was quenched with water and 2 M HCl and extracted with diethyl ether. The combined organic phases were dried over MgSO<sub>4</sub>, filtered and concentrated by evaporation in the nitrogen stream. The title compound was isolated as slightly yellow oil (220 mg, 2.3 mmol, 46%) using column chromatography (SiO<sub>2</sub>, 4:1 n-pentane:diethyl ether).

Retardation factor: \( R_f = 0.15 \) (3:2 n-pentane:diethyl ether).

\( ^1H \) NMR (400 MHz, CDCl<sub>3</sub>) \( \delta = 9.65 \) (s, 1H, H<sub>1</sub>), 2.78 (m, 1H, H<sub>2</sub>), 2.67 (dd, \( J = 16.9 \) Hz, 5.5 Hz, 1H, H<sub>4a/b</sub>), 2.46 (dd, \( J = 16.9 \) Hz, 7.6 Hz, 1H, H<sub>4a/b</sub>), 1.37 (d, \( J = 7.5 \) Hz, 3H, H<sub>3</sub>).

\( ^13C \) NMR (101 MHz, CDCl<sub>3</sub>) \( \delta = 200.0 \) (C<sub>1</sub>), 117.8 (C<sub>5</sub>), 42.8 (C<sub>2</sub>), 18.0 (C<sub>4</sub>), 13.4 (C<sub>3</sub>).

\( ^1H \) NMR (400 MHz, CD<sub>3</sub>CN) \( \delta = 9.58 \) (s, 1H, H<sub>1</sub>), 2.85 – 2.75 (m, 1H, H<sub>2</sub>), 2.63 (dd, \( J = 17.0 \) Hz, 6.1 Hz, 1H, H<sub>4a/b</sub>), 2.53 (dd, \( J = 17.1 \) Hz, 6.5 Hz, 1H, H<sub>4a/b</sub>), 1.25 (d, \( J = 7.5 \) Hz, 3H, H<sub>3</sub>).

\( ^13C \) NMR (101 MHz, CD<sub>3</sub>CN) \( \delta = 202.6 \) (C<sub>1</sub>), 119.4 (C<sub>5</sub>), 43.1 (C<sub>2</sub>), 18.1 (C<sub>4</sub>), 13.1 (C<sub>3</sub>).
Second generation α-aminonitrile (2a’)

The α-aminonitrile was obtained following a procedure by Paventi and Edward.[1]  

1,3-Dicyano-2-methylpropan-1-aminium chloride (2a’)

3-Methyl-4-oxobutanenitrile 1a’ (198 mg, 2.0 mmol, 1.0 eq.) was dissolved in 0.2 mL concentrated aqueous NH₃ and 1.5 mL THF at 0 °C. Ammonium chloride (109 mg, 2.0 mmol, 1.0 eq.) and potassium cyanide (133 mg, 2.0 mmol, 1.0 eq.) were added and the mixture was stirred for 1.5 h. 1.5 g sodium sulfate and 6.0 mL diethyl ether were added and stirred for 30 min. The solution was decanted and the residue washed with diethyl ether. The organic phases were combined and concentrated in vacuo. The product was obtained as colorless liquid.

HRMS (ESI) m/z: calculated for C₆H₁₀N₃ [M+H]⁺: 124.0869, found: 124.0870.

Diastereomer A:

¹H NMR (400 MHz, DMSO-d₆) δ = 6.77 (d, J = 6.0 Hz, 2H, H²), 4.42 – 4.35 (m, 1H, H³), 2.74 – 2.61 (m, 2H, H₆), 2.25 – 2.10 (m, 1H, H⁴), 1.08 (d, J = 6.9 Hz, 3H, H⁵).

¹³C NMR (101 MHz, DMSO-d₆) δ = 119.9 (C¹), 119.1 (C⁷), 63.5 (C³), 34.7 (C⁴), 19.5 (C⁶), 14.8 (C⁵).

Diastereomer B:

¹H NMR (400 MHz, DMSO-d₆) δ = 6.72 (d, J = 5.5 Hz, 2H, H²), 4.61 – 4.56 (m, 1H, H³), 2.74 – 2.61 (m, 1H, H⁶a/b), 2.57 – 2.43 (m, 1H, H⁶a/b), 2.25 – 2.10 (m, 1H, H⁴), 1.09 (d, J = 6.8 Hz, 3H, H⁵).

¹³C NMR (101 MHz, DMSO-d₆) δ = 119.6 (C¹), 118.8 (C⁷), 62.8 (C³), 34.3 (C⁴), 19.3 (C⁶), 14.2 (C⁵).
Second generation imidazolidine-4-thiones (3a’d, 3da’)

3-(2,2-Dimethyl-5-thioxoimidazolidin-4-yl)butanenitrile (3a’d)

The imidazolidine-4-thione was prepared following our previously reported procedure with slight modifications that simplified the workup.[2]

1,3-Dicyano-2-methylpropan-1-aminium chloride 2a'-HCl (399 mg, 2.50 mmol, 1.0 eq.) and potassium hydroxide (140 mg, 2.5 mmol, 1.0 eq.) were dissolved in 4 mL of concentrated aqueous ammonia. After cooling to 0 °C, acetone (185 µL, 2.5 mmol, 1.0 eq.) was added and hydrogen sulfide was bubbled through the solution (3 x 17 bar). The vial was sealed and left stirring at 0 °C for 12 hours before the remaining hydrogen sulfide was purged with nitrogen at 55 °C. The solution was extracted with dichloromethane and the combined organic phases were dried over sodium sulfate, filtered and concentrated in vacuo. Column chromatography (SiO₂, 1:1 n-pentane:ethyl acetate) of the crude product yielded the title compound (17 mg, 0.08 mmol, 3 %) as a white solid.

HRMS (ESI) m/z: calculated for C₉H₁₆N₃S [M+H]+: 198.1059, found: 198.1056.

Retardation factor:  \( R_f = 0.61 \) (1:1 n-pentane:ethyl acetate).

\(^1\)H NMR (400 MHz, CDCl₃) \( \delta = 9.00 \) (s, 1H, H₂), 4.15 (d, \( J = 3.3 \) Hz, 1H, H⁵), 2.78 (sext, \( J = 7.0, 3.2 \) Hz, 1H, H⁸), 2.50 (dd, \( J = 7.4, 1.7 \) Hz, 2H, H¹⁰), 1.94 (bs, 1H, H⁴), 1.50 – 1.46 (m, 3H, H⁶,7), 1.04 (d, \( J = 6.8 \) Hz, 3H, H⁹).

\(^{13}\)C NMR (101 MHz, CDCl₃) \( \delta = 201.1 \) (C¹), 118.7 (C¹¹), 80.0 (C⁵), 72.1 (C⁵), 33.1 (C⁸), 29.4 (C⁶⁻⁷), 28.8 (C⁶⁻⁷), 22.6 (C¹⁰), 13.3 (C⁶).
3-(4,4-Dimethyl-5-thioxoimidazolidin-2-yl)butanenitrile (3da')

The imidazolidine-4-thione 3da' was prepared from 3db by dynamic exchange of the carbonyl residue in ring position 2.

2,5,5-Trimethylimidazolidine-4-thione 3db (101 mg, 0.7 mmol, 1.0 eq.) was dissolved in 5.0 mL distilled water. 3-Methyl-4-oxobutanenitrile 1a' (136 mg, 1.4 mmol, 2.0 eq.) was added and the mixture was stirred for 12 h. The solution was extracted with dichloromethane, the combined organic phases were dried over sodium sulfate, filtered and concentrated in vacuo. Column chromatography (SiO2, 1:1 iso-hexane:ethyl acetate) of the crude product yielded the title compound (3 mg, 15 µmol, 2 %) as a white solid.

HRMS (ESI) m/z: calculated for C9H16N3S [M+H]+: 198.1059, found: 198.1060.

Retardation factor:  \( R_f = 0.24 \) (1:1 iso-hexane:ethyl acetate).

Diastereomer A:

\(^1\)H NMR (400 MHz, CDCl₃) \( \delta = 9.03 \) (b, 1H, H\(^2\)), 4.80 (d, \( J = 4.8 \) Hz, 1H, H\(^3\)), 2.56 (dd, \( J = 16.9, 5.7 \) Hz, 1H, H\(^8a\)), 2.36 (dd, \( J = 16.9, 7.6 \) Hz, 1H, H\(^8b\)), 2.14 - 2.05 (m, 1H, H\(^6\)), 1.45 (s, 3H, H\(^10/11\)), 1.40 (s, 3H, H\(^10/11\)), 1.17 (d, \( J = 7.0 \) Hz, 3H, H\(^7\)).

\(^{13}\)C NMR (101 MHz, CDCl₃) \( \delta = 211.2 \) (C\(^1\)), 118.2 (C\(^9\)), 76.3 (C\(^3\)), 70.4 (C\(^5\)), 35.2 (C\(^6\)), 28.9 (C\(^10/11\)), 27.9 (C\(^10/11\)), 20.1 (C\(^8\)), 14.7 (C\(^7\)).

Diastereomer B:

\(^1\)H NMR (400 MHz, CDCl₃) \( \delta = 8.95 \) (b, 1H, H\(^2\)), 4.60 (d, \( J = 7.1 \) Hz, 1H, H\(^3\)), 2.52 (dd, \( J = 6.0, 4.7 \) Hz, 2H, H\(^8a/b\)), 2.05 - 1.96 (m, 1H, H\(^6\)), 1.90 (b, 1H, H\(^8\)), 1.43 (s, 3H, H\(^10/11\)), 1.41 (s, 3H, H\(^10/11\)), 1.20 (d, \( J = 6.8 \) Hz, 3H, H\(^7\)).

\(^{13}\)C NMR (101 MHz, CDCl₃) \( \delta = 211.2 \) (C\(^1\)), 118.2 (C\(^9\)), 77.1 (C\(^3\)), 70.4 (C\(^5\)), 36.2 (C\(^6\)), 29.5 (C\(^10/11\)), 27.9 (C\(^10/11\)), 20.4 (C\(^8\)), 15.8 (C\(^7\)).
Side products of the one-pot procedure (5, 6, 11)

2-((1-Cyanopropyl)amino)butanethioamide (5)

Potassium cyanide (163 mg, 2.5 mmol, 1.0 eq.), ammonium chloride (134 mg, 2.5 mmol, 1.0 eq.), and propionaldehyde (180 µL, 2.5 mmol, 1.0 eq.) were dissolved in 4.0 mL water at 0 °C. Hydrogen sulfide was bubbled through the solution (3 x 17 bar) and the vial was sealed and left stirring for 5 days. The precipitate was filtered and washed with H2O. Column chromatography (SiO2, 2:1 n-pentane:ethyl acetate) of the crude product yielded the title compound (7 mg, 0.04 mmol, 3 %) as a white solid.

Retardation factor: \( R_f = 0.53 \) (1:1 n-pentane:ethyl acetate).

\( ^1H \text{ NMR} \) (400 MHz, acetone-\( d_6 \)) \( \delta = 8.92 \) (bs, 1H, H1a), 8.81 (s, 1H, H1b), 3.65 (dd, \( J = 7.0 \), 5.5 Hz, 1H, H7), 3.45 (t, \( J = 7.0 \) Hz, 1H, H3), 1.88 – 1.75 (m, 3H, H4, 8a), 1.73 – 1.62 (m, 1H, H8b), 1.08 (t, \( J = 7.4 \) Hz, 3H, H5), 0.98 (t, \( J = 7.5 \) Hz, 3H, H9).

\( ^13C \text{ NMR} \) (101 MHz, DMSO-\( d_6 \)) \( \delta = 212.9 \) (C2), 120.9 (C1b), 69.8 (C3), 51.4 (C3), 27.6 (C4), 10.6 (C5), 10.4 (C9). C8 masked by acetone peak.
Potassium cyanide (163 mg, 2.5 mmol, 1.0 eq.), ammonium chloride (134 mg, 2.5 mmol, 1.0 eq.), and propionaldehyde (180 µL, 2.5 mmol, 1.0 eq.) were dissolved in 4.0 mL water at 0 °C. Hydrogen sulfide was bubbled through the solution (3 x 17 bar) and the vial was sealed and left stirring for 5 days. The precipitate was filtered and washed with H₂O. Column chromatography (SiO₂, 2:1 n-pentane:ethyl acetate) of the crude product yielded the title compound (57 mg, 0.36 mmol, 29 %) as a white solid.

**HRMS** (ESI) m/z: calculated for C₈H₁₇N₃S₂ [M+H]⁺: 220.0937, found: 220.0933.

**Retardation factor:** \( R_f = 0.21 \) (1:1 n-pentane:ethyl acetate).

**¹H NMR** (400 MHz, DMSO-\(d_6\)) \( \delta = 9.73 \) (bs, 2H, \( H_{1a,11a} \)), 9.14 (bs, 2H, \( H_{1b,11b} \)), 3.08 (q, \( J = 6.3 \) Hz, 2H, \( H_{3,7} \)), 1.61 – 1.46 (m, 4H, \( H_{4,8} \)), 0.85 (t, \( J = 7.5 \) Hz, 6H, \( H_{5,9} \)).

**¹³C NMR** (101 MHz, DMSO-\(d_6\)) \( \delta = 209.7 \) (C²,¹⁰), 67.8 (C³,⁷), 29.0 (C⁴,⁸), 10.3 (C⁵,⁹).
2,4,6-Triethyl-1,3,5-dithiazinane (11)

For pure isolation of 11, the one-pot synthesis was performed without potassium cyanide, following a slightly modified procedure by Kawai et al.\textsuperscript{[3]}

Propionaldehyde (179 µL, 2.5 mmol, 3.0 eq.) was dissolved in 4.0 mL concentrated aqueous ammonia at 0 °C. Hydrogen sulfide was bubbled through the solution (2 x 17 bar), the vial was sealed and left stirring for 19 h. The remaining hydrogen sulfide was purged with nitrogen at 55 °C and the mixture was extracted with diethyl ether. The combined organic phases were washed with hot water, dried over sodium sulfate and concentrated \textit{in vacuo}. Without further purification the title compound was yielded as colorless liquid (75 mg, 0.37 mmol, 44 \%).

HRMS (ESI) \textit{m/z}: calculated for C\textsubscript{9}H\textsubscript{20}NS\textsubscript{2} [M+H]\textsuperscript{+}: 206.1032, found: 206.1030.

\textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \textit{δ} = 4.27 (t, \textit{J} = 6.5 Hz, 1H, H\textsubscript{4}), 4.01 (dt, \textit{J} = 12.1, 6.4 Hz, 2H, H\textsubscript{1,8}), 1.89 – 1.81 (m, 2H, H\textsubscript{5}), 1.81 – 1.72 (m, 4H, H\textsubscript{2,9}), 1.57 (bs, 1H, H\textsubscript{7}), 1.11 (t, \textit{J} = 7.4 Hz, 3H, H\textsubscript{6}), 1.06 (t, \textit{J} = 7.5 Hz, 6H, H\textsubscript{3,10}).

\textsuperscript{13}C NMR (101 MHz, CDCl\textsubscript{3}) \textit{δ} = 68.0 (C\textsubscript{1,8}), 51.9 (C\textsubscript{4}), 30.5 (C\textsubscript{2,9}), 30.2 (C\textsubscript{5}), 10.9 (C\textsubscript{6}), 10.5 (C\textsubscript{3,10}).
Phosphorylated imidazolidine-4-thiones

(2,5-Dimethyl-4-thioxoimidazolidin-1-yl)phosphonic acid (3bb-P)

A flame dried flask was charged with 2,5-dimethylimidazolidine-4-thione (651 mg, 5.00 mmol, 1.0 eq.) under argon atmosphere. Dry dichloromethane (25.0 mL) and triethylamine (693 µL, 5.00 mmol, 1.0 eq.) were added before the solution was cooled to 0 °C. Phosphoryl chloride (456 µL, 5.00 mmol, 1.0 equiv.) was added slowly at 0 °C. The solution was stirred for 10 min at 0 °C, then additional 7 days at room temperature under argon atmosphere. Dichloromethane and highly pure water were added, the layers were separated and the aqueous layer was extracted with dichloromethane. The combined organic layers were dried over magnesium sulfate and the solvent was removed in vacuo. The product was obtained as an orange-brown solid (678 mg, 3.22 mmol, 64 %).

HRMS (ESI) m/z: calculated for C₅H₁₀N₂O₃PS- [M-H]-: 209.0155, found: 209.0151.

1H NMR (400 MHz, CDCl₃) δ = 7.95 (s, 1H, H3), 5.50 (dq, J = 11.6 Hz, J = 5.8 Hz, 1H, H4), 4.59 (dq, J = 13.9 Hz, J = 6.9 Hz, 1H, H1), 1.76 (d, J = 6.9 Hz, 3H, H6), 1.65 (d, J = 5.8 Hz, 3H, H7).

13C NMR (101 MHz, CDCl₃) δ = 200.6 (s, C2), 73.7 (d, J = 7.7 Hz, C4), 68.0 (m, C1), 25.2 (d, J = 3.5 Hz, C7), 23.3 (d, J = 3.4 Hz, C6).

31P NMR (162 MHz, CDCl₃) δ = 10.9 (dd, J = 12.7 Hz, P5).

31P NMR {1H} (162 MHz, CDCl₃) δ = 10.9 (P5).
One-pot synthesis

Potassium cyanide (81 mg, 1.25 mmol, 1.0 eq.) and ammonium chloride (67 mg, 1.25 mmol, 1.0 eq.) were dissolved in 2 mL of the respective solvent. After cooling to 0 °C, propionaldehyde (90 µL, 1.25 mmol, 1.0 eq.) was added and hydrogen sulfide was bubbled through the solution (1 x 17 bar). The vial was sealed and left stirring at 0 °C for 24 hours before the remaining hydrogen sulfide was purged with nitrogen at 55 °C. The solution was extracted with dichloromethane and the combined organic phases were dried over sodium sulfate, filtered and concentrated in vacuo. Column chromatography (SiO2, 1:1 iso-hexane:ethyl acetate) of the crude product yielded the title compound as a white solid.

All analytical data is in good agreement with previously reported one.[2]

| Solvent: | water (pH 8.0) | ammonia (pH 10.5) | two-step synthesis[2] |
|----------|----------------|-------------------|-----------------------|
| Yield:   | 18.3 mg, 0.11 mmol, 19 % | 6.4 mg, 0.04 mmol, 6 % | 149.9 mg, 0.95 mmol, 76 % |

In addition, samples were taken after 30 min, 2 h, 4 h, 24 h, and 48 h and were directly analyzed with Orbitrap mass spectrometry (see Figures S1 and S2). The observed m/z values were assigned to intermediates or plausible side products of the investigated reaction as shown in Scheme S1.

Scheme S1. Catalyst 3aa, intermediates, and side-products detected in the mass spectra of the reaction of propionaldehyde 1a with KCN, NH4Cl and H2S.
One-pot procedure with propionaldehyde in ammonia

Figure S1. High resolution mass spectra of the reaction of propionaldehyde 1a with KCN, NH₄Cl and H₂S in ammonia. Samples were taken after 30 min, 2 h, 4 h, 24 h, and 48 h and diluted with acetonitrile before the measurement. The peak that refers to the desired catalyst 3aa is framed. Peaks at m/z = 84.0184, 102.0918, 121.1225, and 282.2791 are also detected in the measurement of pure acetonitrile and are thus not part of the samples.
**One-pot procedure with propionaldehyde in water**

**Figure S2.** High resolution mass spectra of the reaction of propionaldehyde 1a with KCN, NH₄Cl and H₂S in water. Samples were taken after 30 min, 2 h, 4 h, 24 h, and 48 h and diluted with acetonitrile before the measurement. The peak that refers to the desired catalyst 3aa is framed. Peaks at m/z = 84.0184, 102.0918, 121.1225, and 282.2791 are also detected in the measurement of pure acetonitrile and are thus not part of the samples.
**One-pot procedure with acetone in ammonia**

Potassium cyanide (81 mg, 1.25 mmol, 1.0 eq.) and ammonium chloride (67 mg, 1.25 mmol, 1.0 eq.) were dissolved in 2 mL of the respective solvent. After cooling to 0 °C, acetone (93 µL, 1.25 mmol, 1.0 eq.) was added and hydrogen sulfide was bubbled through the solution (1 x 17 bar). Samples were taken after 30 min, 2 h, 4 h, and 24 h and were directly analyzed with Orbitrap mass spectrometry (see Figures S3 and S4).

**Figure S3.** High resolution mass spectra of the reaction of acetone 1d with KCN, NH₄Cl and H₂S in ammonia. Samples were taken after 30 min, 2 h, 4 h, and 24 h and diluted with acetonitrile before the measurement. The peak that refers to the desired catalyst 3dd is framed. Peaks at m/z = 84.0184, 102.0918, 121.1225, and 282.2791 are also detected in the measurement of pure acetonitrile and are thus not part of the samples.
One-pot procedure with acetone in water

Figure S4. High resolution mass spectra of the reaction of acetone 1d with KCN, NH₄Cl and H₂S in water. Samples were taken after 30 min, 2 h, 4 h, and 24 h and diluted with acetonitrile before the measurement. The peak that refers to the desired catalyst 3dd is framed. Peaks at m/z = 84.0184, 102.0918, 121.1225, and 282.2791 are also detected in the measurement of pure acetonitrile and are thus not part of the samples.
One-pot procedure without KCN

Propionaldehyde (180 µL, 2.5 mmol) was dissolved in 4.0 mL concentrated aqueous ammonia. Hydrogen sulfide was bubbled through the solution (2 x 17 mbar) and the mixture was stirred for 20 h. The reaction was extracted with diethyl ether, the organic phases dried over sodium sulfate and analyzed with GC-MS (Figure S5). Additionally, samples taken directly after hydrogen sulfide addition as well as after 1 h and 2 h were diluted in water and analyzed with high resolution Orbitrap mass spectrometry (Figure S6).

Figure S5. GC chromatogram of the reaction of propionaldehyde 1a with hydrogen sulfide in ammonia after 24 h. Separation was performed on a SE-30 column at a constant pressure of 80 kPa. The starting temperature of 70 °C was held for 5 min before raising it with a gradient of 3 K/min until 220 °C which was again held for 5 min.
Figure S6. High resolution mass spectra of the reaction of propionaldehyde 1a with hydrogen sulfide in ammonia. Samples were taken after 0 min, 1 h, and 2 h and diluted with water.
Dynamic Variation

Side chain exchange with a mixture of reactants

5-Ethyl-2-methylimidazolidine-4-thione \(3\text{ab}\) (14.4 mg, 100 µmol, 1.0 eq.) was dissolved in 1.0 mL distilled water (pH 7.5). Propionaldehyde (7.4 µL, 100 µmol, 1.0 eq.), isobutyraldehyde (9.1 µL, 100 µmol, 1.0 eq.) and acetone (7.4 µL, 100 µmol, 1.0 eq.) were added and the mixture was stirred for 20 h. The solution was extracted with dichloromethane, the combined organic phases were dried over sodium sulfate, filtered and concentrated \textit{in vacuo}. The resulting mixture was directly analyzed with NMR spectroscopy.

\[\text{H-NMR spectra of imidazolidine-4-thiones 3aa, 3ab, and 3ac formed from a mixture of 3ab, propionaldehyde 1a, isobutyraldehyde 1c, and acetone 1d in water (blue, top). Enlarged area illustrates the ratio of products (1:1:1). Reference spectra of imidazolidine-4-thiones are shown in black below.}\]
Side chain exchange with acetone
To verify that acetone is not incorporated into the imidazolidine-4-thione skeleton by dynamic exchange, it was added solely and thus as only possible reaction partner. No significant incorporation was detected.

Figure S8. $^1$H-NMR spectra of the mixture of 3aa with acetone in water (black, bottom) and reference spectra of 3ad (gray, top). Enlarged areas illustrate only slight formation of 3ad.
**Side chain exchange dependence on pH value**

The reaction was repeated in water of different pH values, adjusted with 2 M NaOH. Above a pH value of 10, no exchange of the second carbonyl moiety was observed.

5-Ethyl-2-methylimidazolidine-4-thione 3ab (14.4 mg, 100 µmol, 1.0 eq.) was dissolved in 1.0 mL distilled water of different pH values. Propionaldehyde (7.4 µL, 100 µmol, 1.0 eq.), isobutyraldehyde (9.1 µL, 100 µmol, 1.0 eq.) and acetone (7.4 µL, 100 µmol, 1.0 eq.) were added and the mixture was stirred for 20 h. The solution was extracted with dichloromethane, the combined organic phases were dried over sodium sulfate, filtered and concentrated *in vacuo*. The resulting mixture was directly analyzed with NMR spectroscopy.

*Figure S9. 1H-NMR spectra of imidazolidine-4-thiones 3aa, 3ab, and 3ac formed from a mixture of 3ab, propionaldehyde 1a, isobutyraldehyde 1c, and acetone 1d at different pH values.*


**Side chain exchange dependence on reactant equivalents**

To further examine the amount of exchange based on the present ratio of reactants, the previous 1:1 ratio of Figure S7 was extended to 2:1 and 5:1 regarding aldehyde to imidazolidine-4-thione. The observed exchange reflected the used ratio of the reactants.

![Chemical structure and NMR spectra](image)

*Figure S10. *H-NMR spectra of imidazolidine-4-thiones 3ab and 3ac formed from a mixture of 3ab and isobutyaldehyde 1c in a ratio of 1:2 (top) and 1:5 (bottom).*
Selectivity

**Imidazolidine-4-thione formation**

Ammonium chloride (53 mg, 1 mmol, 0.5 eq.) and potassium cyanide (65 mg, 1.0 mmol, 0.5 eq.) were dissolved in distilled water or concentrated aqueous ammonia (28-30%). Acetaldehyde (112 µL, 2.0 mmol, 1.0 eq.), isobutyraldehyde (183 µL, 2.0 mmol, 1.0 eq.) in case of A and additionally acetone (143 µL, 2.0 mmol, 1.0 eq.) in case of B were added at 0 °C and hydrogen sulfide was bubbled through the solution (1 x 17 bar). The vial was sealed and left stirring at 0 °C for 12 hours before the remaining hydrogen sulfide was purged with nitrogen at 55 °C. The solution was extracted with dichloromethane and the combined organic phases were dried over sodium sulfate, filtered and concentrated in vacuo. The crude mixture was directly analyzed with HPLC-MS (see Figures S11 and S12).

![Diagram](image)

**Figure S11.** HPLC separation of imidazolidine-4-thiones formed from a one-pot mixture of acetaldehyde 1b and isobutyraldehyde 1c in water (top) and ammonia (bottom) on a 4.6 mm x 250 mm EC Nucleodur 100-5 with gradient elution of n-hexane (A) / isopropanol (B): A/B 99/1 ➔ 95/5 (10 min) ➔ 90/10 (15 min) ➔ 60/40 (25 min).
Figure S12. HPLC separation of imidazolidine-4-thiones formed from a one-pot mixture of acetaldehyde 1b, isobutyraldehyde 1c, and acetone 1d in water (top) and ammonia (bottom). Acetone containing species are highlighted in blue. Separation was performed on a 4.6 mm x 250 mm EC Nucleodur 100-5 with gradient elution of n-hexane (A) / isopropanol (B): A/B 99/1 → 95/5 (10 min) → 90/10 (15 min) → 60/40 (25 min).
Time dependent imidazolidine-4-thione formation in ammonia

Acetaldehyde 1b, isobutyraldehyde 1c, and acetone 1d were reacted in concentrated aqueous ammonia according to the above described procedure (case B). The reaction was stopped directly after hydrogen sulfide addition, after 10 min, 30 min, 1 h, 2 h, 4 h and 24 h and the mixtures were analyzed with HPLC-MS (see Figure S13). Quantification was realized by relative peak integration (see Table S1).

Figure S13. HPLC separation of imidazolidine-4-thiones formed from a one-pot mixture of acetaldehyde 1b, isobutyraldehyde 1c, and acetone 1d in ammonia after certain reaction times. Product species are assigned in the chromatogram at the bottom. Separation was performed on a 4.6 mm x 250 mm EC Nucleodur 100-5 with gradient elution of n-hexane (A) / isopropanol (B): A/B 99/1 → 95/5 (10 min) → 90/10 (15 min) → 60/40 (25 min). The deviating retention times in the 24 h measurement are due to a new column, but structures could be confirmed with associated MS spectra.
**Time dependent imidazolidine-4-thione formation in water**

Acetaldehyde 1b, isobutyraldehyde 1c, and acetone 1d were reacted in water according to the above described procedure (case B). The reaction was stopped directly after hydrogen sulfide addition, after 10 min, 30 min, 1 h, 2 h, 4 h and 24 h and the mixtures were analyzed with HPLC-MS (see Figure S14). Quantification was realized by relative peak integration (see Table S1).

**Figure S14.** HPLC separation of imidazolidine-4-thiones formed from a one-pot mixture of acetaldehyde 1b, isobutyraldehyde 1c, and acetone 1d in water after certain reaction times. Product species are assigned in the two chromatograms at the bottom. Separation was performed on a 4.6 mm x 250 mm EC Nucleodur 100-5 with gradient elution of n-hexane (A) / isopropanol (B): A/B 99/1 → 95/5 (10 min) → 90/10 (15 min) → 60/40 (25 min).
Table S1. Relative peak integration of HPLC chromatograms presented in Figures S14 and S15. Formation of imidazolidine-4-thiones 3 from a one-pot mixture of acetaldehyde 1b, isobutyraldehyde 1c, and acetone 1d in water or ammonia at certain reaction times.
α-Aminonitrile formation

Acetaldehyde 1b (278 µL, 5.0 mmol, 0.6 eq.), isobutyraldehyde 1c (456 µL, 5.0 mmol, 0.6 eq.), and acetone 1d (368 µL, 5.0 mmol, 0.6 eq.) were dissolved in 4.5 mL THF and 1.0 mL concentrated aqueous ammonia (28-30 %) at 0 °C. Ammonium chloride (401 mg, 7.5 mmol, 1.0 eq.) and potassium cyanide (488 mg, 7.5 mmol, 1.0 eq.) were added and the mixture was stirred for 1.5 h. 4.5 g sodium sulfate and 12 mL diethyl ether were added and the mixture was again stirred for 30 min. The solution was decanted and the residue washed with diethyl ether. The organic phases were combined and concentrated in vacuo. The residual oil was dissolved in diethyl ether and precipitated with hydrogen chloride in diethyl ether (2 M). A ratio of 1:19:0 between 2b-HCl:2c-HCl:2d-HCl was obtained.

Figure S15. ^1H-NMR spectra of α-aminonitrile hydrochlorides 2-HCl formed from a mixture of acetaldehyde 1b, isobutyraldehyde 1c, and acetone 1d (blue, top). Enlarged area illustrates the ratio of the CH₃ group(s) of 2b-HCl and 2c-HCl. Reference spectra of α-aminonitrile hydrochlorides are shown in black.
Adenosine Phosphorylation

Adenosine (26.7 mg, 100 µmol, 1.0 equiv.) and (2,5-Dimethyl-4-thioxoimidazolidin-1-yl)-phosphonic acid (105 mg, 500 µmol, 5.0 equiv.) were dissolved in highly pure water (1.00 mL). Zinc chloride (6.81 mg, 50.0 µmol, 0.5 equiv.) was added. After the reaction mixture was stirred for 10 min the pH-value was adjusted to pH 6. The mixture was stirred for 7 days at room temperature. An aliquot of 10 µL was taken after 7 days and the sample was analysed via LC-QTOF-MS and Orbitrap-MS.

HRMS (ESI) m/z: calculated for C_{10}H_{13}N_{5}O_{7}P⁻ [M-H]⁻ 346.0558, found: 346.0556.

Figure S16: High resolution mass spectra of the reaction of ITO-P with adenosine and zinc chloride in highly pure water. Sample was taken after 7 days and diluted with highly pure water.
**Figure S17:** Extracted Ion Chromatogram of the separation of the phosphorylated adenosine. Applied mass filter: m/z 346.0541-346.0575. The black curve depicts the scan of the reaction mixture, the red curve depicts the bought adenosine 5'-monophosphate reference, and the blue curve depicts the bought adenosine 3'-monophosphate reference. Separation was performed on an Agilent Extend C18 (2.1x50mm, 1.80 µm particle size) column with gradient elution of 0.05% (v/v) formic acid (eluent A) and methanol (eluent B): 0% B held constant for 0.59 min, then increased to 40% B within 2.38 min and held for 0.59 min. Subsequent increase to 100% of eluent B within 0.03 min and held for 0.57 min to flush the column.

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**Author Contributions**

A.C.C. and M.B. performed the experiments. A.C.C. and O.T. designed the experiments, analysed the results and wrote the manuscript.