Supporting Information

A light-releasable potentially prebiotic nucleotide activating agent

Angelica Mariani[‡], David A. Russell[‡], Thomas Javelle, John D. Sutherland[*]

MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge Biomedical Campus, Cambridge CB2 0QH, UK.

Materials and methods. Reagents and solvents were obtained from *Acros Organics*, *Alfa Aesar*, *Sigma-Aldrich*, or *Synthon Chemicals*, and were used without any further purification. $Na_3[Fe(CN)_5NH_3]\cdot 3H_2O$ was prepared by the method of Kenney, Flynn and Gallini.[1] All photochemical reactions were carried out in quartz cuvettes, using a *Ted Pella, Inc.* high intensity mercury lamp with a principal emission at 365 nm. A *Mettler Toledo* SevenEasy pH Meter S20 was used to monitor the pH of the solutions, adjusted with either NaOH or HCl solutions as appropriate. Deoxygenation of H_2O/D_2O (9:1) mixtures was achieved by sparging with N_2 gas for 0.5 hours prior to use. ^1H, ^{13}C and ^{31}P NMR spectra were acquired using a *Bruker* Avance-III spectrometer operating at 400.1 MHz, 100.6 and 162.0 MHz respectively. Samples consisting of H_2O/D_2O mixtures were analyzed using HOD suppression to collect ^1H NMR data. Chemical shifts (δ) are shown in ppm. Coupling constants (*J*) are given in Hertz (Hz) and the notations s, d, t, m, and bs represent the multiplicities of singlet, doublet, triplet, multiplet, and broad singlet signals, respectively. The yields of conversion were determined by relative integration of the signals in the ^1H or ^{31}P NMR spectra. Data analysis was performed using MestReNova (version 7.0) and GraphPad Prism (version 7.0b).

General procedure for the synthesis of nucleoside 5′-phosphorimidazolides (ImpN). The nucleoside 5′-monophosphate (0.05 mmol) and imidazole (**2**, Im, 3.4 mg, 0.05 mmol) were dissolved in H_2O/D_2O (9:1, 0.5 mL) and the pH was adjusted to the reported value with NaOH/HCl. Acetaldehyde (**3**, 11.2 μL, 0.2 mmol) was added, followed by methyl isocyanide (**1**, 11.9 μL, 0.2 mmol). The reaction was allowed to stand at room temperature and its progress was monitored by ^1H and ^{31}P NMR over the course of 3 d. The chemical shifts of ImpN are reported as they appear in the ^1H NMR of each reaction mixture. Spectroscopic data are in agreement with previous literature.[2] (See Supplementary Table 1 for pH values, reaction times and yields).

^1H NMR (400.1 MHz, H_2O/D_2O, 9:1): *ImpA* δ 8.17 (s, 1H), 8.09 (s, 1H), 7.91 (bs, 1H), 7.14 (bs, 1H), 6.94 (bs, 1H), 5.97 (d, J = 5.2 Hz, 1H), 4.70 (t, J = 5.2 Hz, 1H), 4.39 (t, J = 4.8 Hz, 1H) 4.29-4.26 (m, 1H), 4.14-4.01 (m, 2H); ^{31}P NMR (162.0 MHz, H_2O/D_2O, 9:1, ^1H-decoupled): *ImpA* δ -8.47.

^{1}H NMR (400.1 MHz, H_2O/D_2O, 9:1): *ImpC* δ 8.01 (bs, 1H), 7.65 (d, *J* = 7.6 Hz, 1H), 7.32 (bs, 1H), 7.15 (bs, 1H), 6.00 (d, *J* = 7.6 Hz, 1H), 5.88 (d, *J* = 4.0 Hz, 1H), 4.19-4.05 (m, 5H); ^{31}P NMR (162.0 MHz, H_2O/D_2O, 9:1, ^{1}H-decoupled): *ImpC* δ -8.50.

^{1}H NMR (400.1 MHz, H_2O/D_2O, 9:1): *ImpU* δ 8.01 (bs, 1H), 7.68 (d, *J* = 8.0 Hz, 1H), 7.33 (bs, 1H), 7.16 (bs, 1H), 5.89 (overlapping d, 1H), 5.87 (d, *J* = 4.8 Hz, 1H), 4.31-4.12 (m, 5H); ^{31}P NMR (162.0 MHz, H_2O/D_2O, 9:1, ^{1}H-decoupled): *ImpU* δ -8.43.

^{1}H NMR (400.1 MHz, H_2O/D_2O, 9:1): *ImpG* δ 7.94 (bs, 1H), 7.87 (s, 1H), 7.17 (bs, 1H), 6.98 (bs, 1H), 5.81 (d, *J* = 5.2 Hz, 1H), 4.73 (overlapping t, 1H), 4.40 (t, *J* = 4.8 Hz, 1H) 4.11-4.07 (m, 2H), the signal for one of the sugar protons is obscured by a signal corresponding to 2-hydroxy-*N*-methylpropanamide **13**; ^{31}P NMR (162.0 MHz, H_2O/D_2O, 9:1, ^{1}H-decoupled): *ImpG* δ -8.40.

General procedure for the synthesis of adenosine 5′-phosphoro-2-aminoimidazolides ($2NH_2$ImpA). Adenosine 5′-monophosphate monohydrate (AMP, 18.3 mg, 0.05 mmol) and 2-aminoimidazole hemisulfate (**6**, $2NH_2$Im, 10.8 mg, 0.05 mmol) were dissolved in H_2O/D_2O (9:1, 0.5 mL) and the pH was adjusted to the reported value with NaOH/HCl. Acetaldehyde (**3**, 11.2 μL, 0.2 mmol) was added, followed by methyl isocyanide (**1**, 11.9 μL, 0.2 mmol). The reaction was allowed to stand at room temperature and its progress was monitored by ^{1}H and ^{31}P NMR over the course of 3 d. The chemical shifts of $2NH_2$ImpA are reported as they appear in the ^{1}H NMR of the reaction mixture. Spectroscopic data are in agreement with previous literature.[3] (See Supplementary Table 1 for pH values, reaction times and yields). ^{1}H NMR (400.1 MHz, H_2O/D_2O, 9:1): *$2NH_2$ImpA* δ 8.19 (s, 1H), 8.07 (s, 1H), 6.62 (m, 1H), 6.48 (m, 1H), 5.96 (d, *J* = 4.8 Hz, 1H), 4.70 (t, *J* = 4.8 Hz, 1H), 4.40 (t, *J* = 4.8 Hz, 1H) 4.29-4.25 (m, 1H), 4.04-3.98 (m, 2H); ^{31}P NMR (162.0 MHz, H_2O/D_2O, 9:1, ^{1}H-decoupled): *$2NH_2$ImpA* δ -8.70.

Synthesis of ImpA or $2NH_2$ImpA in diluted conditions. The reactions were carried out as described in the general procedures, but 10-fold diluted with respect to AMP (1.8 mg, 0.005 mmol). The pH of the solution was adjusted to either 6.5 (ImpA) or 7.0 ($2NH_2$ImpA) with NaOH/HCl. Yield (ImpA): 89% (0.5 h); yield ($2NH_2$ImpA): 76% (5 h).

Synthesis of ImpA in the presence of Mg^{2+}. AMP (18.3 mg, 0.05 mmol) and imidazole (**2**, 3.4 mg, 0.05 mmol) were dissolved in H_2O/D_2O (9:1, 0.45 mL). 50 □L of a 200 mM $MgCl_2$ aqueous solution were added and the pH of the final mixture was adjusted to 6.5 with NaOH/HCl. Acetaldehyde (**3**, 11.2 μL, 0.2 mmol) was added, followed by methyl isocyanide (**1**, 11.9 μL, 0.2 mmol). The reaction was allowed to stand at room temperature and its progress was monitored by ^{1}H and ^{31}P NMR over the course of 3 d. Yield: 71% (0.5 h).

Synthesis of ImpA in the presence of CN^-. The reaction was carried out as described in the general procedure, adding KCN (3.2 mg, 0.05 mmol) to the initial mixture, before adjusting the pH of the solution to 6.5 with NaOH/HCl. Yield: 69% (0.5 h).

Synthesis of ImpA under recycling conditions. AMP (1.8 mg, 0.005 mmol) and imidazole (**2**, 1.7 mg, 0.025 mmol) were dissolved in H_2O/D_2O (9:1, 0.5 mL) and the pH was adjusted to 6.5 with NaOH/HCl. Acetaldehyde (**3**, 11.2 μL, 0.2 mmol) was added, followed by methyl isocyanide (**1**, 3.0 μL, 0.05 mmol). The reaction was allowed to stand at room temperature and every 24 h a new portion of methyl isocyanide (**1**, 3.0 μL, 0.05 mmol) was added, for a total of three cycles of ImpA generation and hydrolysis.

Synthesis of methylamine (9). NaCN (4.9 mg, 0.1 mmol) and $NaH_2PO_2 \cdot H_2O$ (42.4 mg, 0.4 mmol) were dissolved in deoxygenated H_2O/D_2O (9:1, 0.9 mL). The pH of the mixture was adjusted to 9.8 with NaOH/HCl before sponge nickel (100 μL, slurry in H_2O) was added carefully (vigorous evolution of hydrogen gas!). The mixture was allowed to stand at room temperature for 2 h, at which point analysis of the mixture by ^1H and ^{13}C NMR spectroscopy showed methylamine **9** to be the only organic product (yield: 42%, based on integration relative to a non-volatile internal standard: sodium acetate). ^1H NMR (400.1 MHz, H_2O/D_2O, 9:1): *methylamine* **9**: δ 2.62 (s); ^{13}C NMR (100.6 MHz, H_2O/D_2O, 9:1): *methylamine* **9**: δ 24.8.

Synthesis of $[Fe(CN)_5NO]^{2-}$ (8, nitroprusside ion) from $[Fe(CN)_6]^{4-}$ (7). $K_4[Fe(CN)_6] \cdot 3H_2O$ (**7**, 42.2 mg, 0.1 mmol), Na_2HPO_4 (14.2 mg, 0.1 mmol), and $NaNO_2$ (6.9 mg, 0.1 mmol) were dissolved in deoxygenated H_2O/D_2O (9:1, 1.0 mL). The pH of the mixture was adjusted to 9.8 with NaOH/HCl before the solution was transferred to a sealed quartz cuvette and irradiated for 2 h. Analysis by ^{13}C NMR spectroscopy showed a mixture of unreacted $[Fe(CN)_6]^{4-}$ **7** and $[Fe(CN)_5NO]^{2-}$ **8** (nitroprusside ion, yield: approximately 29%, based on quantitative ^{13}C NMR spectroscopy and integration relative to an internal standard: sodium formate). ^{13}C NMR (100.6 MHz, H_2O/D_2O, 9:1): *$[Fe(CN)_6]^{4-}$* **7**: δ 177.4; *$[Fe(CN)_5NO]^{2-}$* **8**: δ 134.5, 132.3. Spectroscopic data are in agreement with previous literature.[4]

Synthesis of $[Fe(CN)_5(CNCH_3)]^{3-}$ (12) starting from a mixture of $[Fe(CN)_5NO]^{2-}$ (8) and methylamine (9). NaCN (4.9 mg, 0.1 mmol) and Na_2HPO_4 (14.2 mg, 0.1 mmol) were dissolved in H_2O/D_2O (9:1, 1.0 mL). Methylamine (**9**, 7.8 μL, 40%/wt aqueous solution, 0.1 mmol) was added and the pH was adjusted to 9.8 with NaOH/HCl before $Na_2[Fe(CN)_5NO]$ (**8**, 26.2 mg, 0.1 mmol) was added to initiate the reaction. The mixture was allowed to stand at room temperature for 20 h. Analysis of the mixture by ^1H NMR spectroscopy at this time point showed 31% conversion of methylamine to a mixture of products consisting of: $[Fe(CN)_5(CNCH_3)]^{3-}$ **12** (yield: 4%, based on conversion of methylamine), acetonitrile (yield: 16%), methanol (yield: 61%), methyl phosphate (yield: 14%), and methyl chloride (yield: 5%). The formation of **12** occurs as **7** is produced in the reaction due to trapping of $[Fe(CN)_5]^{3-}$ (generated *in situ* following reaction of **8**) by CN^-. The formation of

methyl chloride occurs due to addition of Cl^- (in the form of HCl) when adjusting the pH of the mixture prior to the addition of $Na_2[Fe(CN)_5NO]$ **8** and the initiation of the reaction. ^{1}H NMR (400.1 MHz, H_2O/D_2O, 9:1): *$[Fe(CN)_5(CNCH_3)]^{3-}$* **12** δ 3.36 (s); *acetonitrile* δ 2.06 (s); *methanol* δ 3.34 (s); *methyl phosphate* δ 3.44 (d, J_{HP} = 10.0 Hz); *methyl chloride* δ 3.02 (s); ^{31}P NMR (162.0 MHz, H_2O/D_2O, 9:1, ^{1}H-decoupled) *methyl phosphate* δ 3.66. Spectroscopic data are in agreement with previous literature for known standards.[5,6]

Synthesis of $[Fe(CN)_5(CNCH_3)]^{3-}$ (12) starting from a mixture of $[Fe(CN)_6]^{4-}$ (7), $[Fe(CN)_5NO]^{2-}$ (8), and methylamine (9). $K_4[Fe(CN)_6]\cdot 3H_2O$ (**7**, 42.2 mg, 0.1 mmol), NaCN (4.9 mg, 0.1 mmol), and Na_2HPO_4 (14.2 mg, 0.1 mmol) were dissolved in H_2O/D_2O (9:1, 1.0 mL). Methylamine (**9**, 7.8 μL, 40%/wt aqueous solution, 0.1 mmol) was added and the pH was adjusted to 9.8 with NaOH/HCl before $Na_2[Fe(CN)_5NO]$ (**8**, 26.2 mg, 0.1 mmol) was added to initiate the reaction. The mixture was allowed to stand at room temperature for 20 h. Analysis of the mixture by ^{1}H NMR spectroscopy at this time point showed 31% conversion of methylamine to a mixture of products consisting of: $[Fe(CN)_5(CNCH_3)]^{3-}$ **12** (yield: 26%, based on conversion of methylamine), acetonitrile (yield: 13%), methanol (yield: 46%), methyl phosphate (yield: 13%), and methyl chloride (yield: 2%). Analysis of the same reaction mixture after 4 days showed 63% conversion of methylamine to the same mixture of products: $[Fe(CN)_5(CNCH_3)]^{3-}$ **12** (yield: 27%, based on conversion of methylamine), acetonitrile (yield: 12%), methanol (yield: 46%), methyl phosphate (yield: 14%), and methyl chloride (yield: 1%). The product distribution changes slightly over time as the pH of the mixture drops as the reaction proceeds (leading to increasing protonation of certain nucleophiles).

When the above experiment was repeated at pH 7.0 analysis of the reaction mixture after 20 h showed 2% conversion of methylamine to a mixture of products consisting of: $[Fe(CN)_5(CNCH_3)]^{3-}$ **12**, methanol, methyl phosphate, and methyl chloride (yield: 17%). Analysis of the same reaction mixture after 4 days showed 6% conversion of methylamine to the same mixture of products. Acetonitrile was not produced in this reaction as little CN^- was available (pK_a of hydrogen cyanide = 9.2).

Synthesis of $[Fe(CN)_5(CNCH_3)]^{3-}$ (12) in the presence of NH_3. In order to confirm that ammonia did not interfere with the diazotization reaction, we repeated the synthesis of the isocyanide complex **12** adding 1 equiv of NH_4Cl. $K_4[Fe(CN)_6]\cdot 3H_2O$ (**7**, 42.2 mg, 0.1 mmol), NaCN (4.9 mg, 0.1 mmol), Na_2HPO_4 (14.2 mg, 0.1 mmol), and NH_4Cl (5.4 mg, 0.1 mmol) were dissolved in H_2O/D_2O (9:1, 1.0 mL). Methylamine (**9**, 7.8 μL, 40%/wt aqueous solution, 0.1 mmol) was added and the pH was adjusted to 9.8 with NaOH/HCl before $Na_2[Fe(CN)_5NO]$ (**8**, 26.2 mg, 0.1 mmol) was added to initiate the reaction. The mixture was allowed to stand at room temperature for 20 h. Analysis of the mixture by ^{1}H NMR spectroscopy at this time point showed 70% conversion of methylamine to a mixture of products consisting of: $[Fe(CN)_5(CNCH_3)]^{3-}$ **12** (yield: 26%, based on conversion of methylamine), acetonitrile (yield: 16%), methanol (yield: 42%), methyl phosphate (yield: 14%), and methyl chloride (yield: 2%). Analysis of the same reaction mixture after 4 days showed 85% conversion of methylamine to

the same mixture of products: $[Fe(CN)_5(CNCH_3)]^{3-}$ **12** (yield: 27%, based on conversion of methylamine), acetonitrile (yield: 14%), methanol (yield: 44%), methyl phosphate (yield: 14%), and methyl chloride (yield: 1%). The product distribution changes slightly over time as the pH of the mixture drops as the reaction proceeds (see above).

Synthesis of $[Fe(CN)_5(CNCH_3)]^{3-}$ (12) in the presence of Cl^-. In order to determine the effect of chloride on the diazotization reaction, we repeated the synthesis of the isocyanide complex **12** adding 1 or 10 equiv of NaCl. $K_4[Fe(CN)_6]\cdot 3H_2O$ (**7**, 42.2 mg, 0.1 mmol), NaCN (4.9 mg, 0.1 mmol), Na_2HPO_4 (14.2 mg, 0.1 mmol), and NaCl (5.8 mg, 0.1 mmol) were dissolved in H_2O/D_2O (9:1, 1.0 mL). Methylamine (**9**, 7.8 μL, 40%/wt aqueous solution, 0.1 mmol) was added and the pH was adjusted to 9.8 with NaOH/HCl before $Na_2[Fe(CN)_5NO]$ (**8**, 26.2 mg, 0.1 mmol) was added to initiate the reaction. The mixture was allowed to stand at room temperature for 20 h. Analysis of the mixture by ^1H NMR spectroscopy at this time point showed 35% conversion of methylamine to a mixture of products consisting of: $[Fe(CN)_5(CNCH_3)]^{3-}$ **12** (yield: 25%, based on conversion of methylamine), acetonitrile (yield: 16%), methanol (yield: 42%), methyl phosphate (yield: 13%), and methyl chloride (yield: 4%).

When the above experiment was repeated with the addition of a 10-fold excess of NaCl (58.5 mg, 1.0 mmol), analysis of the reaction mixture after 20 h showed 32% conversion of methylamine to the same mixture of products: $[Fe(CN)_5(CNCH_3)]^{3-}$ **12** (yield: 22%, based on conversion of methylamine), acetonitrile (yield: 15%), methanol (yield: 35%), methyl phosphate (yield: 11%), and methyl chloride (yield: 17%).

Synthesis of methyl isocyanide (1) starting from a mixture of $[Fe(CN)_6]^{4-}$ (7), $[Fe(CN)_5NO]^{2-}$ (8), and methylamine (9). NaCN (4.9 mg, 0.1 mmol) and Na_2HPO_4 (14.2 mg, 0.1 mmol) were dissolved in H_2O/D_2O (9:1, 1.0 mL). Methylamine (**9**, 7.8 μL, 40%/wt aqueous solution, 0.1 mmol) was added and the pH was adjusted to 9.8 with NaOH/HCl before $Na_2[Fe(CN)_5NO]$ (**8**, 26.2 mg, 0.1 mmol) was added to initiate the reaction, as before. The mixture was allowed to stand at room temperature for 20 h, at which point 100 μL of the mixture was removed and added to a deoxygenated solution of NaCN (4.9 mg, 0.1 mmol) in H_2O/D_2O (9:1, 0.9 mL). The pH of this mixture was adjusted to 9.8 with NaOH/HCl before it was transferred to a sealed quartz cuvette and irradiated for 2 h. Analysis of the mixture by ^1H NMR spectroscopy showed 86% conversion of methylamine to a mixture of products consisting of: $[Fe(CN)_5(CNCH_3)]^{3-}$ **12** (yield: 13%, based on conversion of methylamine), acetonitrile (yield: 22%), methanol (yield: 37%), methyl phosphate (yield: 15%), and methyl isocyanide (yield: 13%). We suggest that the greater conversion of methylamine, observed after irradiation, may be due to radical nitrosation taking place, following photolysis of residual nitroprusside (and release of $NO^{\cdot}$).[7]

Synthesis of $[Fe(CN)_5(CNCH_3)]^{3-}$ (12) from $Na_3[Fe(CN)_5NH_3]\cdot 3H_2O$ and methyl isocyanide (1) (0.1 M solution). $Na_3[Fe(CN)_5NH_3]\cdot 3H_2O$ (32.6 mg, 0.1 mmol) was dissolved in deoxygenated H_2O/D_2O (9:1, 1.0 mL) in the dark and nitro-

gen gas was bubbled through the solution for 2 h to remove NH_3. The resulting solution of $[Fe(CN)_5H_2O]^{3-}$ was cooled to 0 ^oC before methyl isocyanide (**1**, 5.9 μL, 0.1 mmol) was added. The mixture was immediately allowed to warm to room temperature and stand for 2 h, at which point analysis by ^1H and ^{13}C NMR spectroscopy showed complete formation of $[Fe(CN)_5(CNCH_3)]^{3-}$ **12**. ^1H NMR (400.1 MHz, H_2O/D_2O, 9:1): *$[Fe(CN)_5(CNCH_3)]^{3-}$* **12** δ 3.36 (s); ^{13}C NMR (1006 MHz, H_2O/D_2O, 9:1): *$[Fe(CN)_5(CNCH_3)]^{3-}$* **12** δ 172.3, 172.2, 163.7, 29.3.

Synthesis of $[Fe(CN)_5(CNCH_3)]^{3-}$ (12) by photoaquation of $[Fe(CN)_6]^{4-}$ (7) in the presence of methyl isocyanide (1). $K_4[Fe(CN)_6]\cdot 3H_2O$ (**7**, 42.2 mg, 0.1 mmol) was dissolved in deoxygenated H_2O/D_2O (9:1, 1.0 mL). Methyl isocyanide (**1**, 5.9 μL, 0.1 mmol) was then added before the mixture was transferred to a sealed quartz cuvette and irradiated for 4 h. Analysis by ^1H NMR spectroscopy showed almost complete association of methyl isocyanide **1** to $[Fe(CN)_5]^{3-}$ (generated *in situ* from **7**) affording $[Fe(CN)_5(CNCH_3)]^{3-}$ **12** (yield: 84%) together with two minor species, tentatively assigned as *cis*-$[Fe(CN)_4(CNCH_3)_2]^{2-}$ and *trans*-$[Fe(CN)_4(CNCH_3)_2]^{2-}$ (combined yield: 15%, signals unassigned), and unreacted methyl isocyanide **1** (yield: 1%). ^1H NMR (400.1 MHz, H_2O/D_2O, 9:1): *$[Fe(CN)_5(CNCH_3)]^{3-}$* **12** δ 3.36 (s); *cis-$[Fe(CN)_4(CNCH_3)_2]^{2-}$* and *trans-$[Fe(CN)_4(CNCH_3)_2]^{2-}$* δ 3.41 (s) and 3.37 (s) (signals unassigned); *methyl isocyanide* **1** δ 3.16 (1:1:1 t, J_{HN} = 2.36 Hz).

Release of methyl isocyanide (1) by photolysis of $[Fe(CN)_5(CNCH_3)]^{3-}$ (12) in the presence of CN^-. NaCN (4.9 mg, 0.1 mmol) was dissolved in deoxygenated H_2O/D_2O (9:1, 0.9 mL) and the pH of the solution was adjusted to 9.8 with NaOH/HCl. $[Fe(CN)_5(CNCH_3)]^{3-}$ (**12**, 100 μL of a 0.1 M solution prepared as described above, 0.01 mmol) was then added and the mixture was transferred to a sealed quartz cuvette and irradiated for 2 h. Analysis by ^1H NMR spectroscopy after 2 h showed a mixture of free methyl isocyanide **1** (50%) and the isocyanide complexes $[Fe(CN)_5(CNCH_3)]^{3-}$ **12**, *cis*-$[Fe(CN)_4(CNCH_3)_2]^{2-}$, and *trans*-$[Fe(CN)_4(CNCH_3)_2]^{2-}$ (50% in total). Interestingly, the formation of such diisonitrile complexes, presumably by association of methyl isocyanide to the coordinately unsaturated complex $[Fe(CN)_4(CNCH_3)]^{2-}$, indicates that irradiation of $[Fe(CN)_5(CNCH_3)]^{3-}$ **12** can also induce loss of a cyanide ligand. Spectroscopic data are in agreement with previous literature for methyl isocyanide.[8] ^1H NMR (400.1 MHz, H_2O/D_2O, 9:1): *methyl isocyanide* **1** δ 3.16 (1:1:1 t, J_{HN} = 2.36 Hz); *$[Fe(CN)_5(CNCH_3)]^{3-}$* **12** δ 3.36 (s); *cis-$[Fe(CN)_4(CNCH_3)_2]^{2-}$* and *trans-$[Fe(CN)_4(CNCH_3)_2]^{2-}$* δ 3.41 (s) and 3.37 (s) (signals unassigned).

Supplementary Figures and Tables:

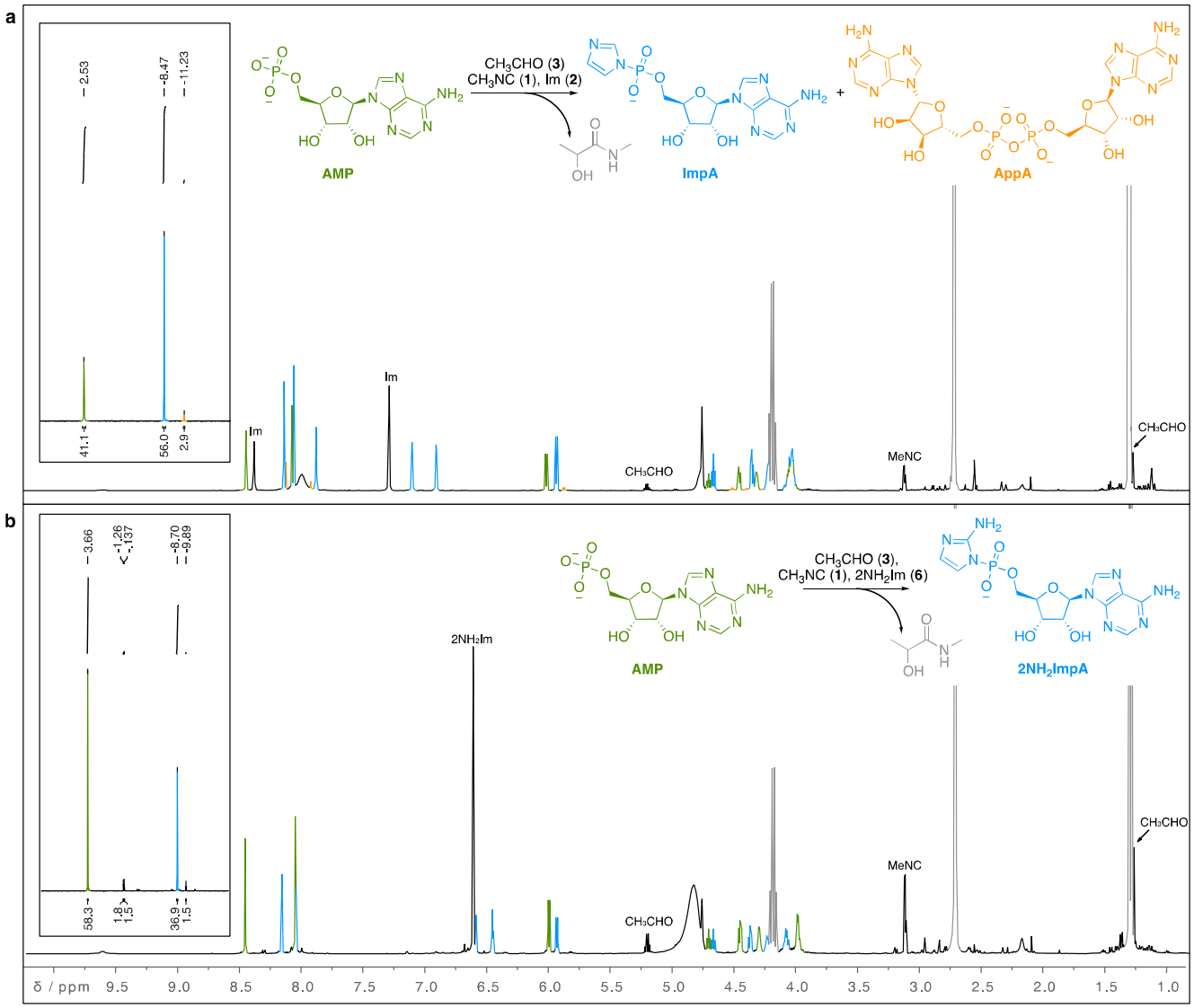

Figure S1. Representative ^{1}H and ^{31}P NMR spectra for the synthesis of ImpA and $2NH_2$ImpA. a, ^{1}H, ^{31}P (insert) NMR Spectra and reaction scheme showing the formation of ImpA and AppA from AMP (100 mM AMP, pH 6.5, 2.5 h). **b,** ^{1}H, ^{31}P (insert) NMR Spectra and reaction scheme showing the formation of $2NH_2$ImpA from AMP (100 mM AMP, pH 7.0, 2.5 h); AppA was not detected under these conditions, while unknown peaks were detected at δ ≈ -1.3 and -9.9 in the ^{31}P NMR spectrum (<5%). The chemical shifts are influenced by the different pHs of the reaction mixtures.

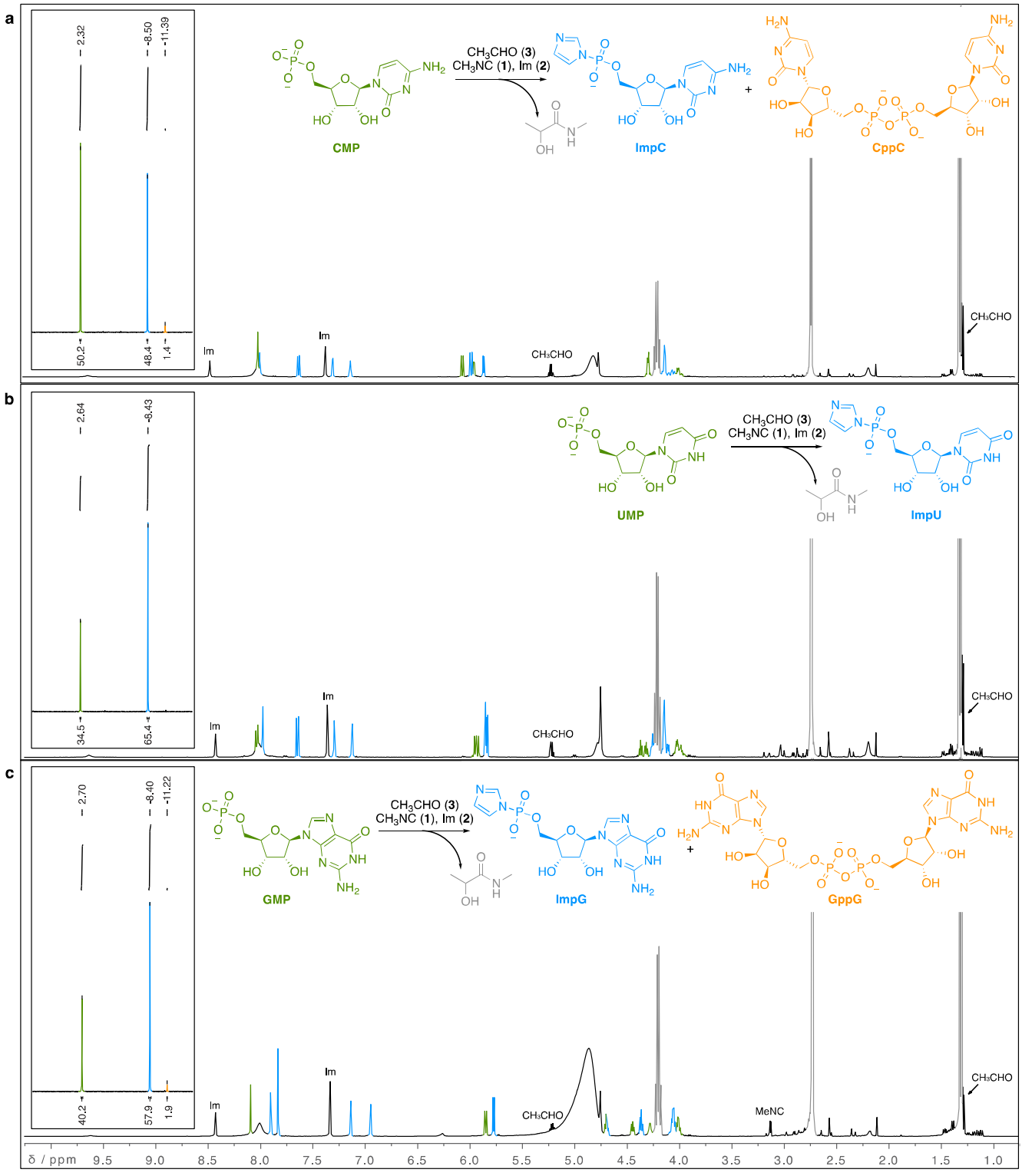

Figure S2. Representative ^{1}H and ^{31}P NMR spectra for the synthesis of ImpN. ^{1}H, ^{31}P (insert) NMR Spectra and reaction schemes showing the formation of **a,** ImpC, **b,** ImpU and **c,** ImpG and their corresponding pyrophosphates (100 mM NMP, pH 6.5, 2.5 h). For UMP, UppU was not detected under these conditions.

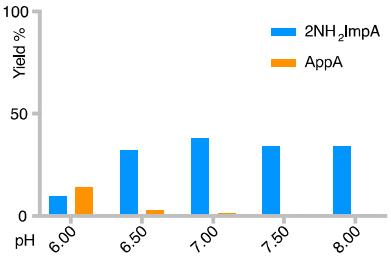

Figure S3. pH profile for the synthesis of $2NH_2$ImpA. Plot of the maximum % yield vs. pH of the reaction mixture for the synthesis of $2NH_2$ImpA. (Blue: $2NH_2$ImpA, orange: AppA).

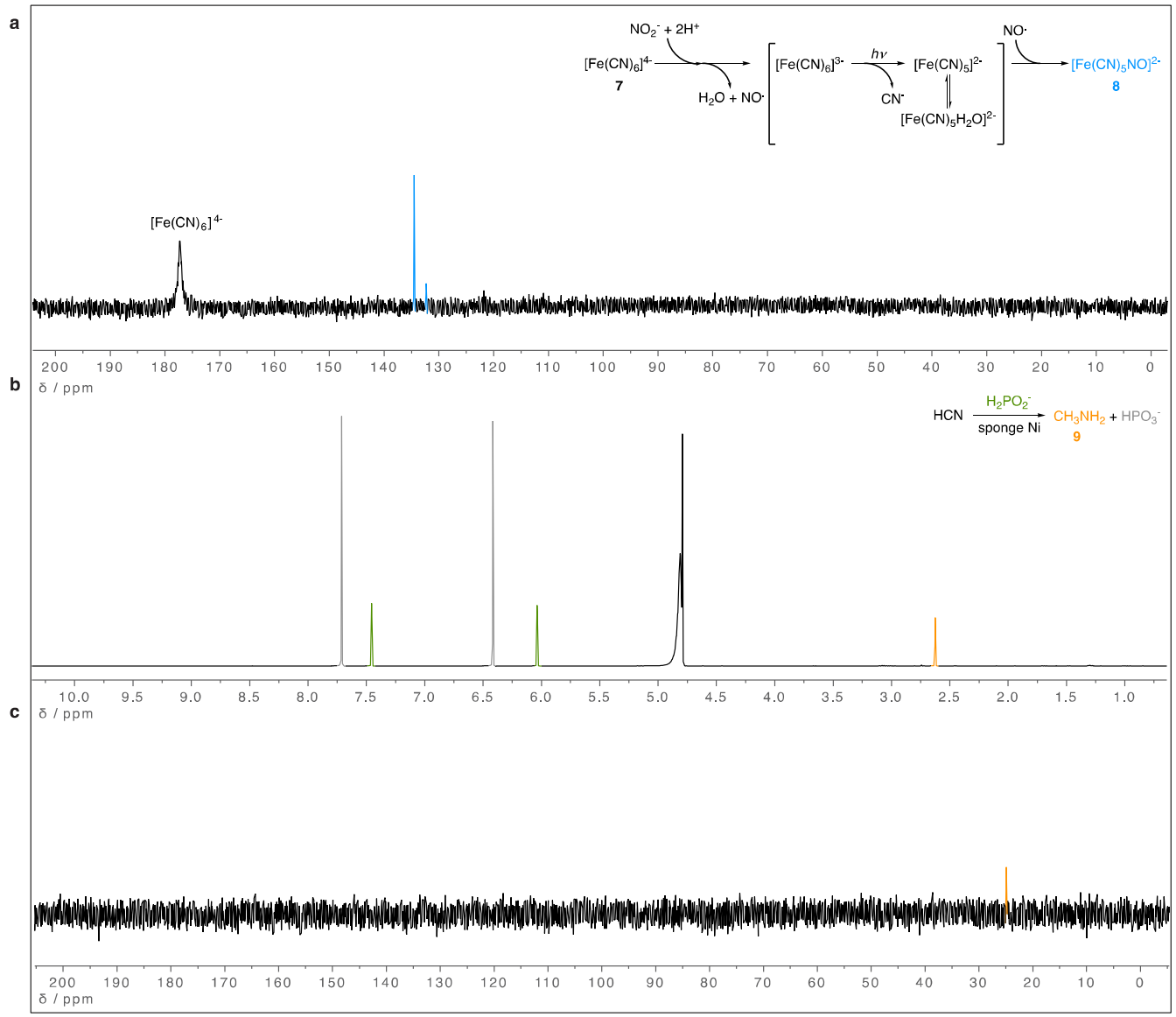

Figure S4. Representative ^{1}H and ^{13}C NMR spectra for the synthesis of $[Fe(CN)_5NO]^{2-}$ (8) and CH_3NH_2 (9). a, ^{13}C NMR spectrum and reaction scheme showing the synthesis of $[Fe(CN)_5NO]^{2-}$ **8** from $[Fe(CN)_6]^{4-}$ **7** (reaction mixture after 2 h). Suggested reaction mechanism: oxidation of **7** by NO_2^- gives $[Fe(CN)_6]^{3-}$ and $NO^·$. Irradiation of $[Fe(CN)_6]^{3-}$ gives $[Fe(CN)_5]^{2-}$, which is in equillibrium with the aqua complex[9-12] $[Fe(CN)_5H_2O]^{2-}$. Association of $NO^·$ to $[Fe(CN)_5]^{2-}$ provides $[Fe(CN)_5NO]^{2-}$. The broad signal corresponding to unreacted **7** is due to redox chemistry taking place, producing $[Fe(CN)_6]^{3-}$ (a paramagentic Fe^{III} species undetectable by ^{13}C NMR spetroscopy). **b,** ^{1}H and **c,** ^{13}C NMR spectra and reaction scheme showing the synthesis of CH_3NH_2 **9** from HCN (reaction mixture after 2h). (Green: $H_2PO_2^-$, grey: HPO_3^-, blue: $[Fe(CN)_5NO]^{2-}$, orange: CH_3NH_2).

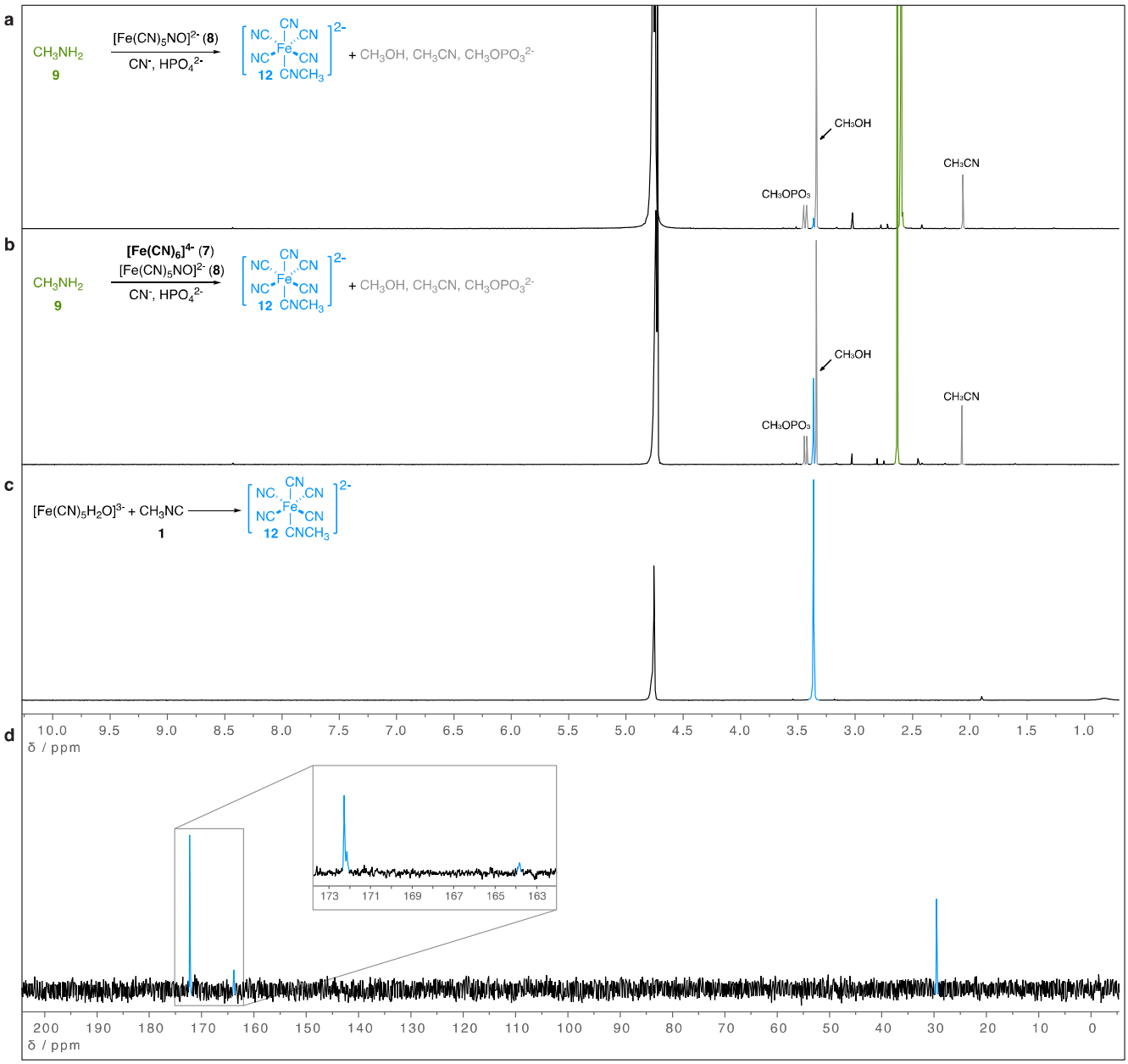

Figure S5. Representative ^{1}H and ^{13}C NMR spectra for the synthesis of $[Fe(CN)_5CNCH_3]^{3-}$ (12). a, ^{1}H NMR spectrum and reaction scheme showing the synthesis of $[Fe(CN)_5CNCH_3]^{3-}$ **12**, CH_3OH, CH_3CN and $CH_3PO_3^{2-}$ from $[Fe(CN)_5NO]^{2-}$ **8** and CH_3NH_2 **9** (reaction mixture after 20 h). Traces of CH_3Cl ($\delta \approx 3$) are also present due to addition of Cl^- in the form of HCl when adjusting the pH of the mixture at the outset. **b,** as **a,** with 1 equivalent of added **7** (reaction mixture after 20 h). **c,** ^{1}H and **d,** ^{13}C NMR spectra and reaction scheme showing the synthesis of **12** from $[Fe(CN)_5H_2O]^{3-}$ and methyl isocyanide **1** (insert: magnification showing the δ 173-163 region). (Green: CH_3NH_2, blue: $[Fe(CN)_5CNCH_3]^{3-}$).

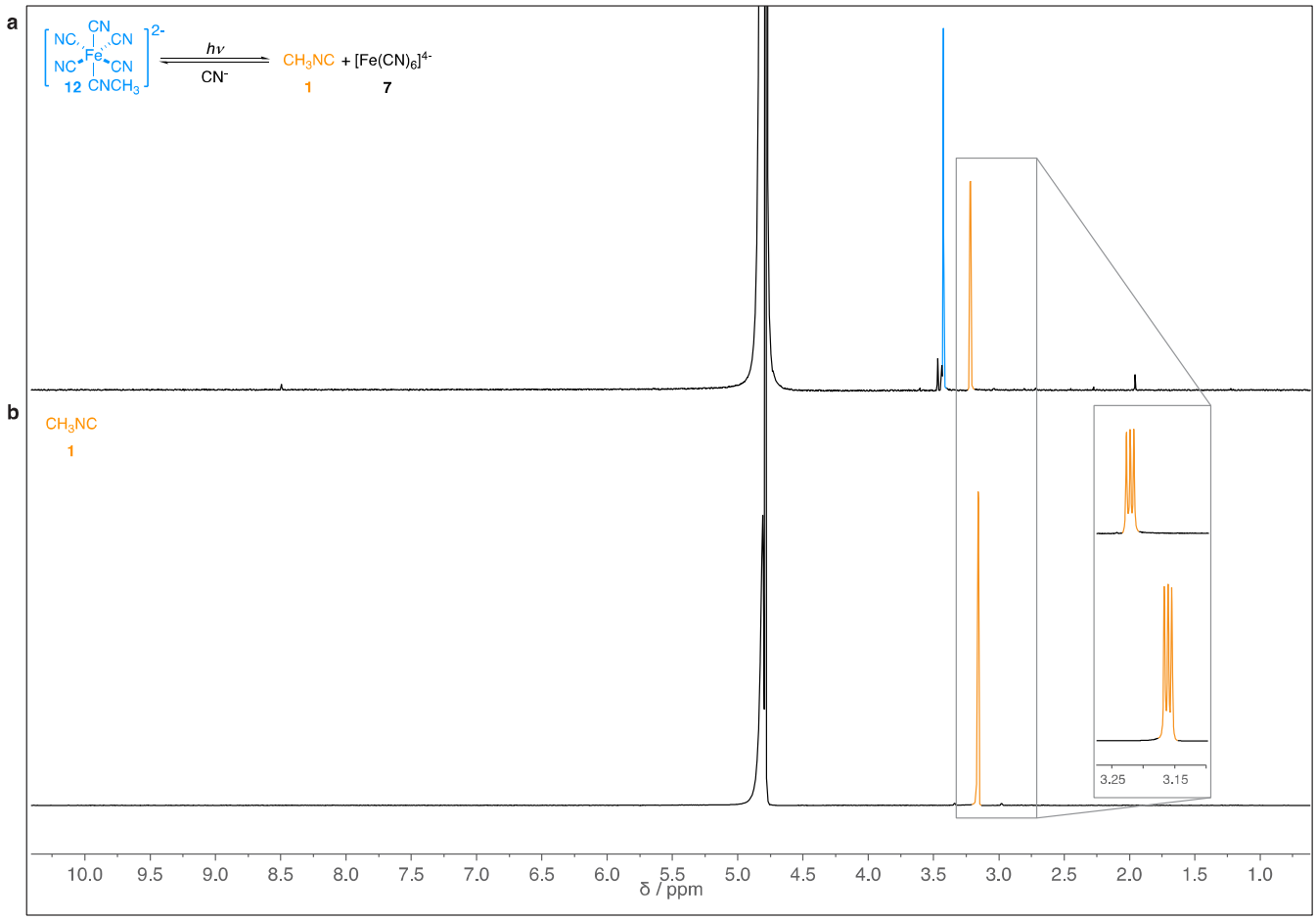

Figure S6. Representative ^{1}H NMR spectra for the photorelease of methyl isocyanide (1). a, ^{1}H NMR Spectrum and reaction scheme showing the photorelease of methyl isocyanide **1** from $[Fe(CN)_5CNCH_3]^{3-}$ **12** (reaction mixture after 2 h). **b,** Reference ^{1}H NMR spectrum of **1** (insert: magnification showing the δ 3.25-3.10 region). (Blue: $[Fe(CN)_5CNCH_3]^{3-}$, orange: methyl isocyanide). The chemical shifts are influenced by the different pHs of the mixtures.

Table S1. Reaction conditions and yields for the synthesis of ImpN and $2NH_2$ImpA.

Imidazolide	NMP (mM)	pH	Additive	Max yield (%)	t (h)
ImpA	100	6.0	-	54	0.5
	100	6.25	-	62	0.5
	100	6.5	-	**71**	0.5
	100	6.75	-	56	0.5
	100	7.0	-	38	1.5
	100	7.5	-	24	5
	100	8.0	-	17	5
	100	6.5	Mg^{2+} (20 mM)	71	0.5
	100	6.5	CN^- (100 mM)	69	0.5
	10	6.5	-	**89**	0.5
$2NH_2$ImpA	100	6.0	-	9.5	15
	100	6.5	-	32	15
	100	7.0	-	**38**	5
	100	7.5	-	34	5
	100	8.0	-	34	5
	10	7.0	-	**76**	5
ImpC	100	6.5	-	69	0.5
ImpU	100	6.5	-	75	0.5
ImpG	100	6.5	-	69*	0.5

*The actual yield of ImpG might be affected by the low solubility of GMP under these conditions.

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