

# Common origins of RNA, protein and lipid precursors in a cyanosulfidic protometabolism

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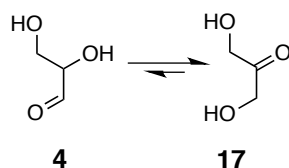
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## 1. General methods

Reagents and solvents were bought from Sigma-Aldrich, VWR International, and Acros Organics and were used without further purification unless otherwise stated.  $^{13}\text{C}$ -Labelled potassium cyanide had an isotopic incorporation of 99%. A *Mettler Toledo* SevenEasy pH Meter S20 was used to monitor pH/pD and deoxygenation of  $\text{H}_2\text{O}$  was achieved by sparging argon through the solution for 15 min before use. All reactions were carried out at room temperature unless otherwise stated. All irradiations were carried out in a Rayonet reactor using lamps with principal emission at 254 nm, and with the cooling fan turned on resulting in an internal reactor temperature of *ca.* 35 °C, unless otherwise stated.  $^1\text{H}$ -NMR spectra were acquired using a *Bruker* Ultrashield 400 Plus machine operating at 400.1 MHz  $^1\text{H}$  frequency, and a *Bruker* DRX spectrometer operating at 600 MHz  $^1\text{H}$  frequency, equipped with cryogenic inverse probe, and with a sample temperature of 298 K.  $^{31}\text{P}$ -NMR spectra were acquired using a *Bruker* Avance III spectrometer operating at 162 MHz  $^{31}\text{P}$  frequency. Samples consisting of  $\text{H}_2\text{O}/\text{D}_2\text{O}$  mixtures were analysed using HOD suppression to collect  $^1\text{H}$ -NMR data. Chemical shifts ( $\delta$ ) are shown in ppm. The yields of conversion were determined by relative integrations of the signals in the  $^1\text{H}$ -NMR spectrum, or by spiking with internal standards calcium formate, pentaerythritol, and dimethyl sulfone, unless otherwise stated. Solid IR spectra were recorded on a *Thermo* Nicolet iS5 with an iD5 ATR diamond attachment with resolution of  $< 0.8\text{ cm}^{-1}$ . Elemental analysis was carried out by the London Metropolitan Elemental Analysis Service (LMEAS) and the University of Cambridge Microanalysis Department (UCMD).

## 2. Synthesis of glycerol-1-phosphate (21)

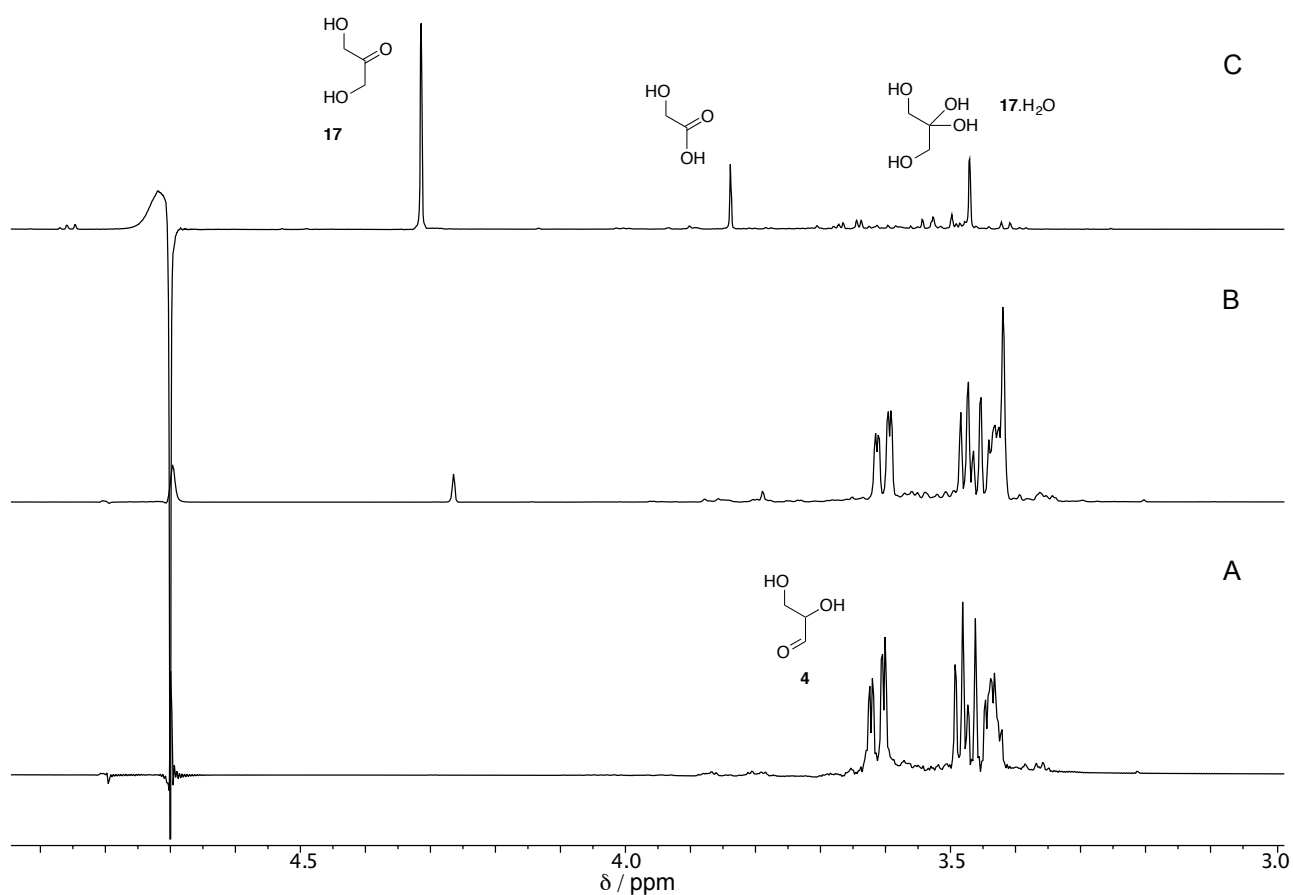
*Conversion of glyceraldehyde 4 to dihydroxyacetone 17*



Glyceraldehyde dimer (22.5 mg, 0.125 mmol as dimer, 0.250 mmol of monomer) and  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  (78.0 mg, 0.5 mmol) were dissolved in a solution of  $\text{H}_2\text{O}/\text{D}_2\text{O}$  (9:1, 5 mL). The pH was adjusted to 8 and the solution was allowed to stir at room temperature for 20 d in a sealed vessel without rigorously excluding oxygen. The reaction outcome was monitored by  $^1\text{H}$ -NMR spectroscopy. After 20 d, **4** was converted to dihydroxyacetone **17** (ketonic form: 40.7%; hydrated form: 18.3%; total 59%) together with the oxidation by-products glycolate (14.1%) and formate (12.7%). These would not be produced prebiotically because of the lack of oxygen in the atmosphere of the early Earth.

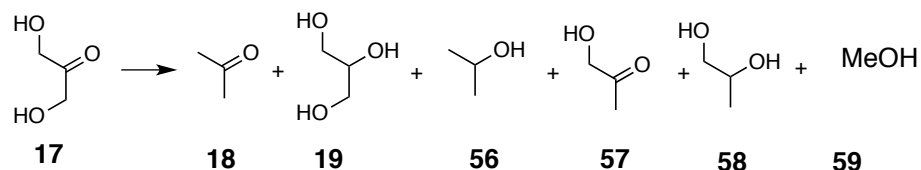
Yields were calculated using an internal standard, i.e. calcium formate (certified reference material from Sigma, suitable for quantitative NMR (qNMR) spectroscopy of water soluble samples) at a known concentration taking into account the quantity of formate already present in the sample and that its purity is 99.92%. Specifically, the sample (1 mL) was spiked with a standard solution of  $\text{Ca}(\text{HCOO})_2$  (1.0 M; 20  $\mu\text{l}$ ). The yields are considered conservative as the spectrum was collected using HOD suppression, which partly suppress the dihydroxyacetone ketonic form signal (4.3 ppm).

$^1\text{H}$ -NMR (600 MHz,  $\text{H}_2\text{O}/\text{D}_2\text{O}$ , 9:1) *glyceraldehyde 4*:  $\delta$  3.61 (dd,  $J = 2.5, 11.6$  Hz, 1H), 3.46-3.45 (m, 1H), 3.45-3.42 (m, 1H); *dihydroxyacetone 17*:  $\delta$  4.30 (s, 4H, ketonic form), 3.45 (s, 4H, hydrate form); *glycolate*:  $\delta$  3.82 (s, 2H); *formate*:  $\delta$  8.3 (s, 1H).



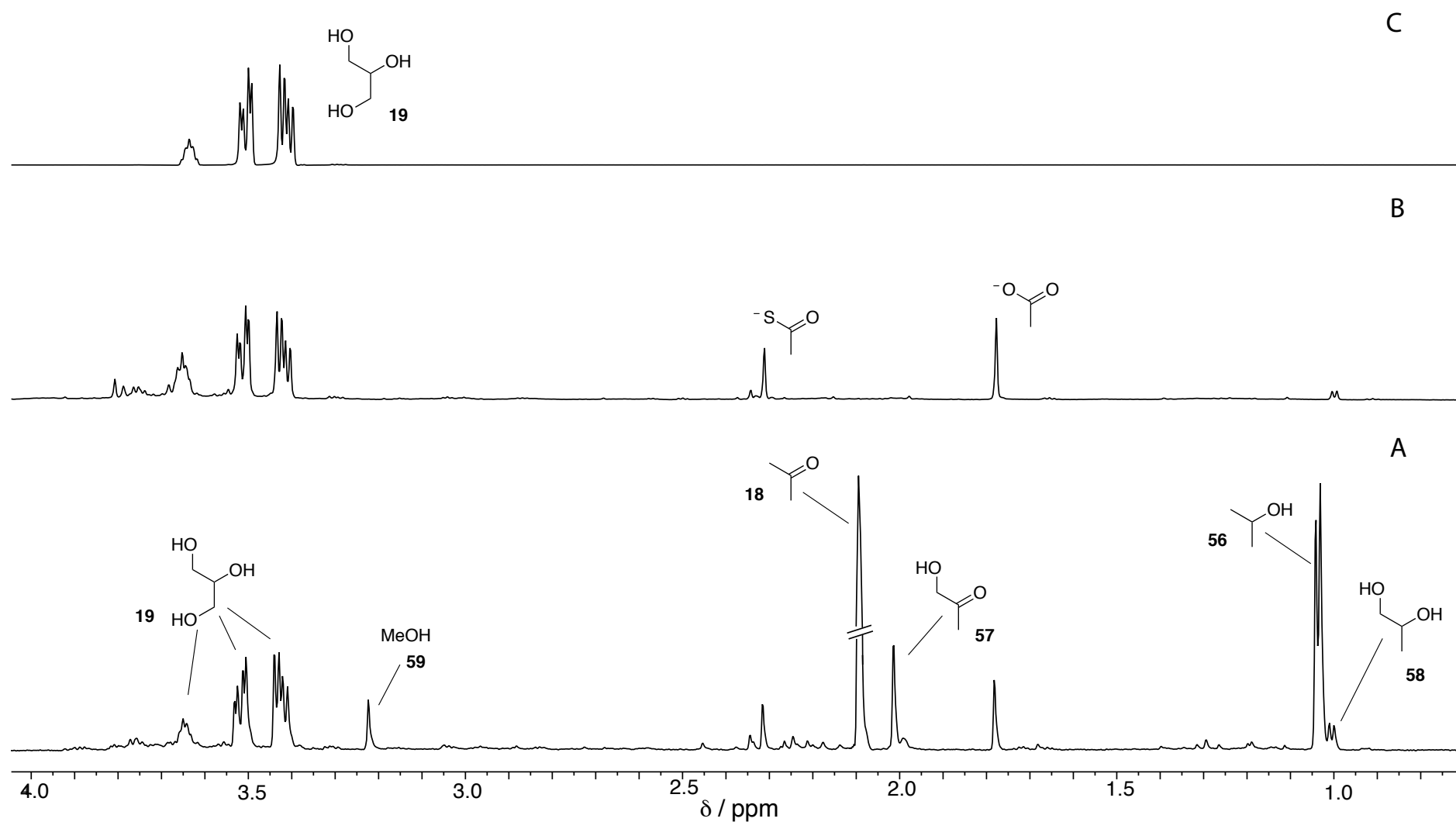
**Figure S1.** <sup>1</sup>H-NMR analysis (H<sub>2</sub>O/D<sub>2</sub>O, 9:1) of the conversion of glyceraldehyde **4** to dihydroxyacetone **17** by enolisation-ketonisation. A – glyceraldehyde at t = 0; B – reaction after 3 d showing the formation of glycolate and dihydroxyacetone (ketonic and hydrate form); C – reaction after 20 d showing the near complete conversion of glyceraldehyde to dihydroxyacetone (ketonic and hydrate form, 59 %), glycolate (14.1 %) and formate (12.7%). The formate peak at 8.3 ppm is not shown.

Formation of glycerol **19** and acetone **18** from dihydroxyacetone **17** by photoredox chemistry



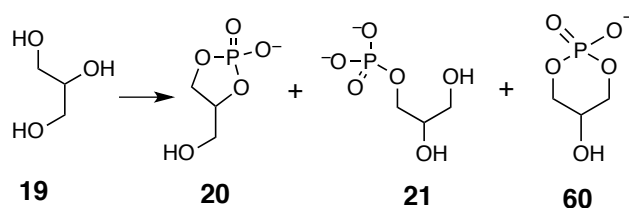
Dihydroxyacetone (8.0 mg, 0.089 mmol) and  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  (45 mg, 0.288 mmol) were dissolved in  $\text{H}_2\text{O}/\text{D}_2\text{O}$  (85:15, 8 mL) and the resultant solution degassed for 15 min.  $\text{NaSH} \cdot x\text{H}_2\text{O}$  (60 mg, assume 60% NaSH, 0.642 mmol) was added and the solution turned yellow. When NaSH was dissolved completely, the pH was adjusted to 7 using degassed NaOH/HCl. The solution was then transferred to a quartz tube containing CuCN (4.5 mg, 0.050 mmol) and immediately sealed, whereupon a black precipitate formed. The tube was placed in a Rayonet reactor and then irradiated for 6 h. After this time an aliquot (0.6 mL) was removed and examined by  $^1\text{H}$ -NMR spectroscopy after the addition of a known amount of calcium formate ( $\text{Ca}(\text{HCO}_2)_2$ ) to serve as internal standard for quantitative  $^1\text{H}$ -NMR spectroscopy. Yields were calculated referring integrals to the internal standard singlet at 8.3 ppm. Glycerol **19** was obtained in 34% yield together with acetone **18** in 29% yield. The by-products were hydroxyacetone **57** (10%), 1,2-propandiol **58** (4%), isopropanol **56** (20%) and, through concurrent Norrish type I photochemistry, methanol **59** (3%). Spiking with authentic standards showed their identity. The mixture contained also traces of thioacetate and acetic acid that we proved to be contaminants of NaSH (data not shown). The remaining mixture was evaporated to dryness without heating and the crude residue was dissolved in  $\text{H}_2\text{O}/\text{D}_2\text{O}$  (9:1, 1 mL). The mixture was centrifuged and the supernatant was then analysed by  $^1\text{H}$ -NMR spectroscopy. After evaporation the percentage composition of glycerol **19** in the mixture increased to 60% (Figure S2).

$^1\text{H}$ -NMR (600 MHz,  $\text{H}_2\text{O}/\text{D}_2\text{O}$ , 9:1) *dihydroxyacetone* **17**:  $\delta$  4.30 (s, 4H, ketonic form), 3.45 (s, 4H, hydrate form); *glycerol* **19**:  $\delta$  3.65-3.62 (m, 1H), 3.50 (dd,  $J = 4.5, 11.7$  Hz, 2H), 3.41 (dd,  $J = 6.3, 11.4$ , 2H); *acetone* **18**:  $\delta$  2.09 (s, 6H); *hydroxyacetone* **57**:  $\delta$  4.23 (s, 2H), 2.01 (s, 3H); *1,2-propandiol* **58**:  $\delta$  3.78-3.72 (m, 1H), 3.42 (dd,  $J = 4.2, 11.7$  Hz, 1H), 3.31 (dd,  $J = 6.7, 11.7$  Hz, 1H), 1.01 (d,  $J = 6.4$  Hz, 3H); *isopropanol* **56**:  $\delta$  3.89 (sept,  $J = 6.2$  Hz, 1H), 1.05 (d,  $J = 6.2$  Hz, 6H); *methanol* **59**:  $\delta$  3.2 (s, 3H); *thioacetate*:  $\delta$  2.31 (s, 3H); *acetate*:  $\delta$  1.78 (s, 3H).



**Figure S2.** <sup>1</sup>H-NMR analysis (H<sub>2</sub>O/D<sub>2</sub>O, 9:1) of the conversion of dihydroxyacetone **17** into acetone **18** and glycerol **19**. A – spectrum of a sample from the photochemical reaction after 6 h; B – spectrum of the same sample after evaporation to dryness and redissolution in H<sub>2</sub>O/D<sub>2</sub>O (9:1, 1 mL); C – spectrum of a reference sample of glycerol.

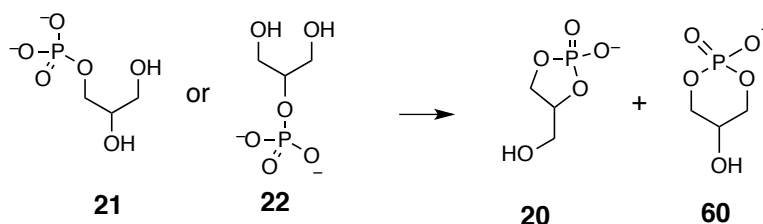
## Phosphorylation of glycerol **19**



Glycerol **19** (92 mg, 1.0 mmol) was dissolved in formamide (10 mL) and ammonium dihydrogen phosphate (23 mg, 0.2 mmol) and urea (600 mg, 10 mmol) were added. The reaction mixture was heated to 120 °C with stirring. After 7 d, the formamide was removed in vacuo and the residue was analysed by  $^{31}\text{P}$ -NMR spectroscopy (Figure S3). Yields were determined by integration of signals in the  $^{31}\text{P}$ -NMR spectrum: glycerol-1,2-cyclic phosphate **20** (53%), glycerol-1-phosphate **21** (20%) and glycerol-1,3-cyclic phosphate **60** (27%). The presence of glycerol-1,2- and 1,3-cyclic phosphates was confirmed by comparison with synthetic standards (Figure S4).

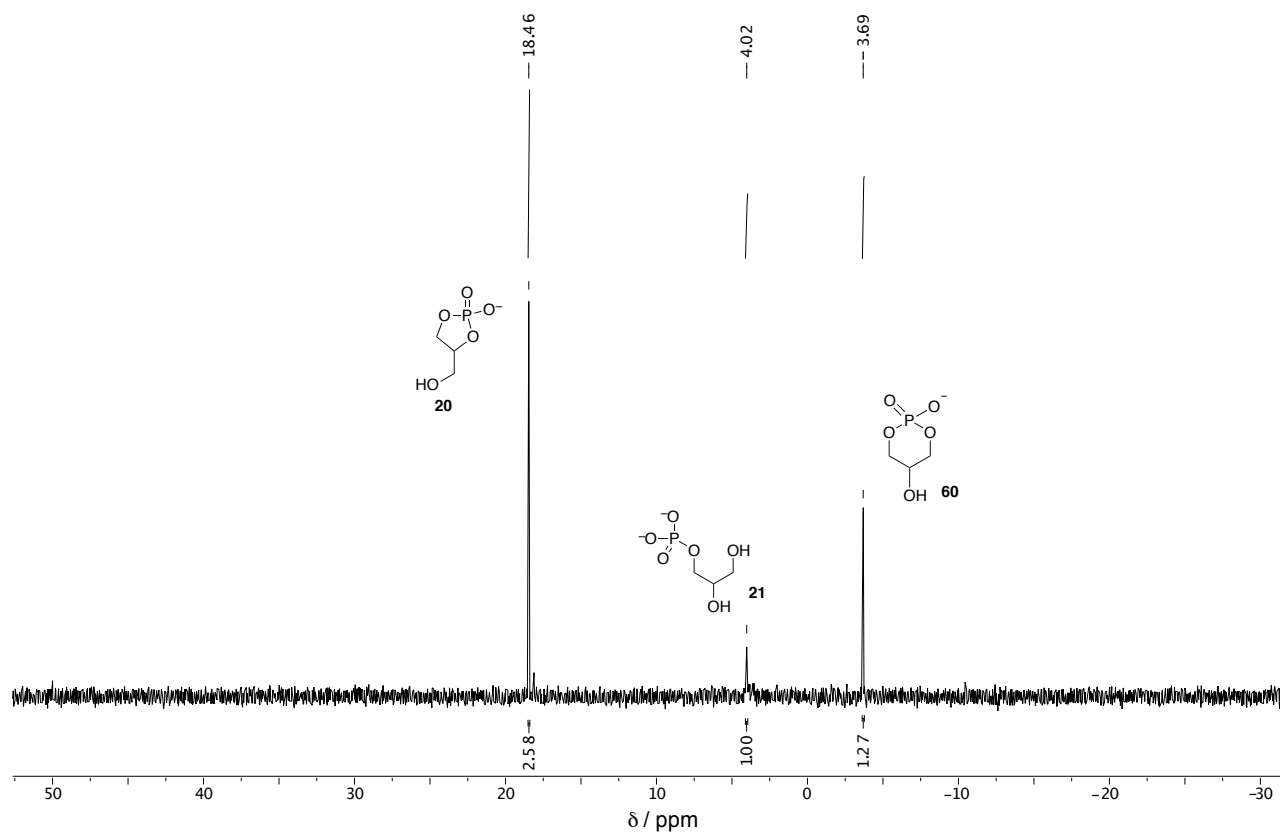
*Glycerol-1,2-cyclic phosphate 20*:  $^{31}\text{P}$  NMR (162 MHz,  $\text{D}_2\text{O}$ ,  $^1\text{H}$ -decoupled)  $\delta$  18.5 (s); m/z ES-API, Neg: 153.1 [M-H]; *glycerol-1-phosphate 21*:  $^{31}\text{P}$  NMR (162 MHz,  $\text{D}_2\text{O}$ ,  $^1\text{H}$ -decoupled)  $\delta$  3.9 (s); m/z ES-API, Neg: 171.1 [M-H]; *glycerol-1,3-cyclic phosphate 60*:  $^{31}\text{P}$  NMR (162 MHz,  $\text{D}_2\text{O}$ ,  $^1\text{H}$ -decoupled)  $\delta$  -3.7 (s); m/z ES-API, Neg: 153.1 [M-H].

*General procedure for the synthesis of glycerol 1,2-cyclic phosphate 20 and 1,3-cyclic phosphate 60 as synthetic standards*



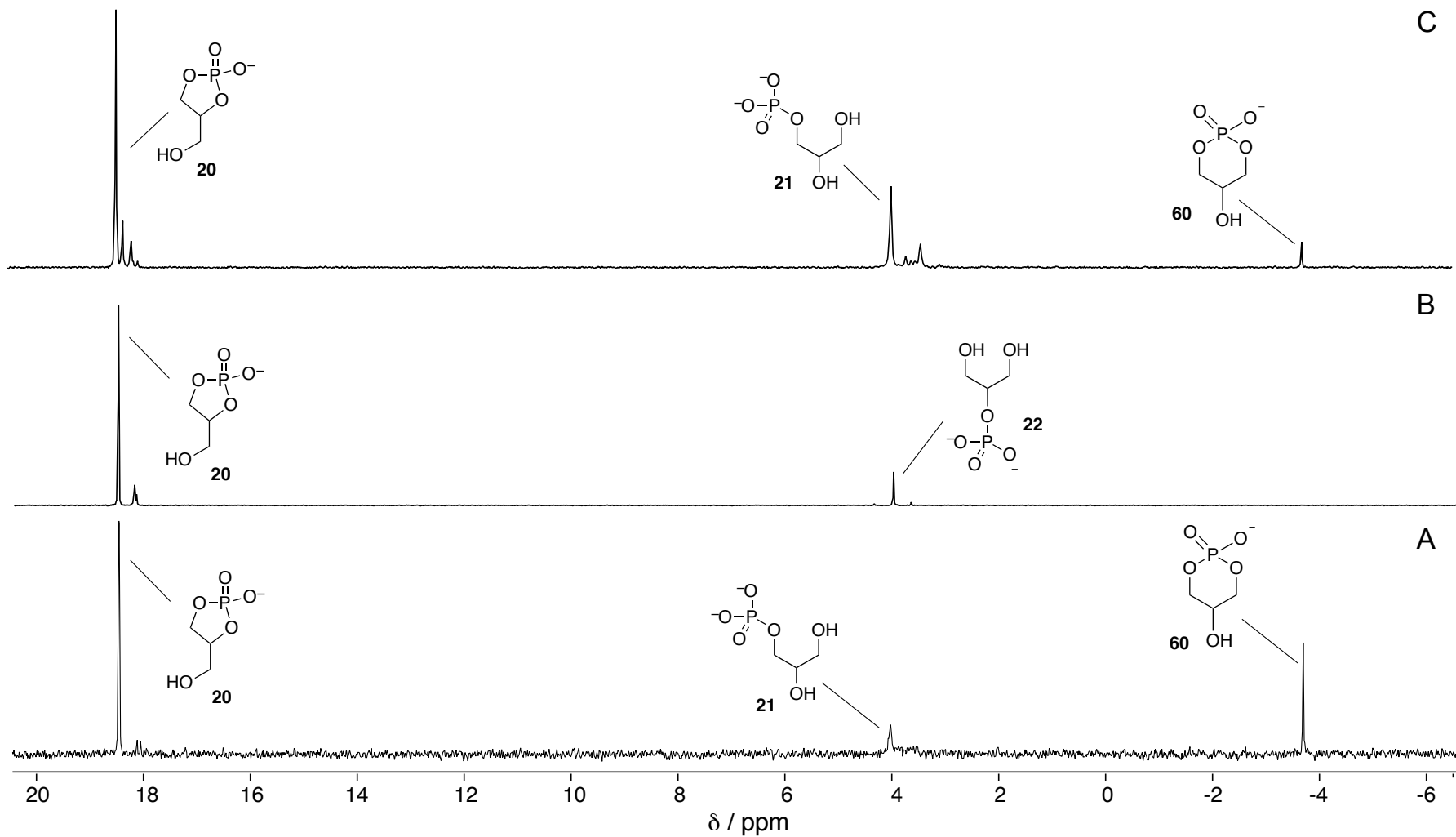
The synthetic standards of **20** and **60** were synthesised starting from glycerol-2-phosphate disodium salt and DL-glycerol-1-phosphate magnesium salt hydrate, respectively. The latter was subjected to ion-exchange treatment with Dowex resin ( $\text{Na}^+$  form) prior to use. Either glycerol phosphate disodium salt (30 mg, 0.138 mmol) was dissolved in  $\text{D}_2\text{O}$  (400  $\mu\text{L}$ ) and the pD was adjusted to 7.4. *N*-cyanoimidazole (NCI, 18.6 mg, 0.200 mmol) was added and the solution was made up to 1 mL using  $\text{D}_2\text{O}$ . The reaction was stirred for 24 h and analysed by  $^{31}\text{P}$ -NMR spectroscopy.

$^{31}\text{P}$ -NMR (162 MHz,  $\text{D}_2\text{O}$ ,  $^1\text{H}$ -decoupled) *glycerol-1,2-cyclic phosphate 20*:  $\delta$  18.5 (s); *glycerol-2-phosphate 22*:  $\delta$  4.0 (s); *glycerol-1,3-cyclic phosphate 60*:  $\delta$  -3.7 (s); *glycerol-1-phosphate 21*:  $\delta$  4.2 (s).



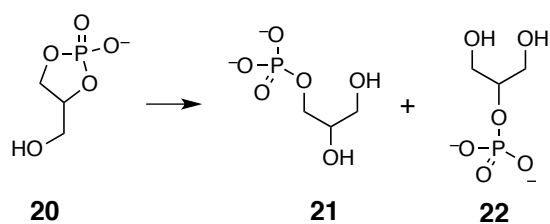
**Figure S3.**  $^{31}\text{P}$ -NMR ( $\text{D}_2\text{O}$ ) analysis of the products of phosphorylation of glycerol **19** after 7 d.





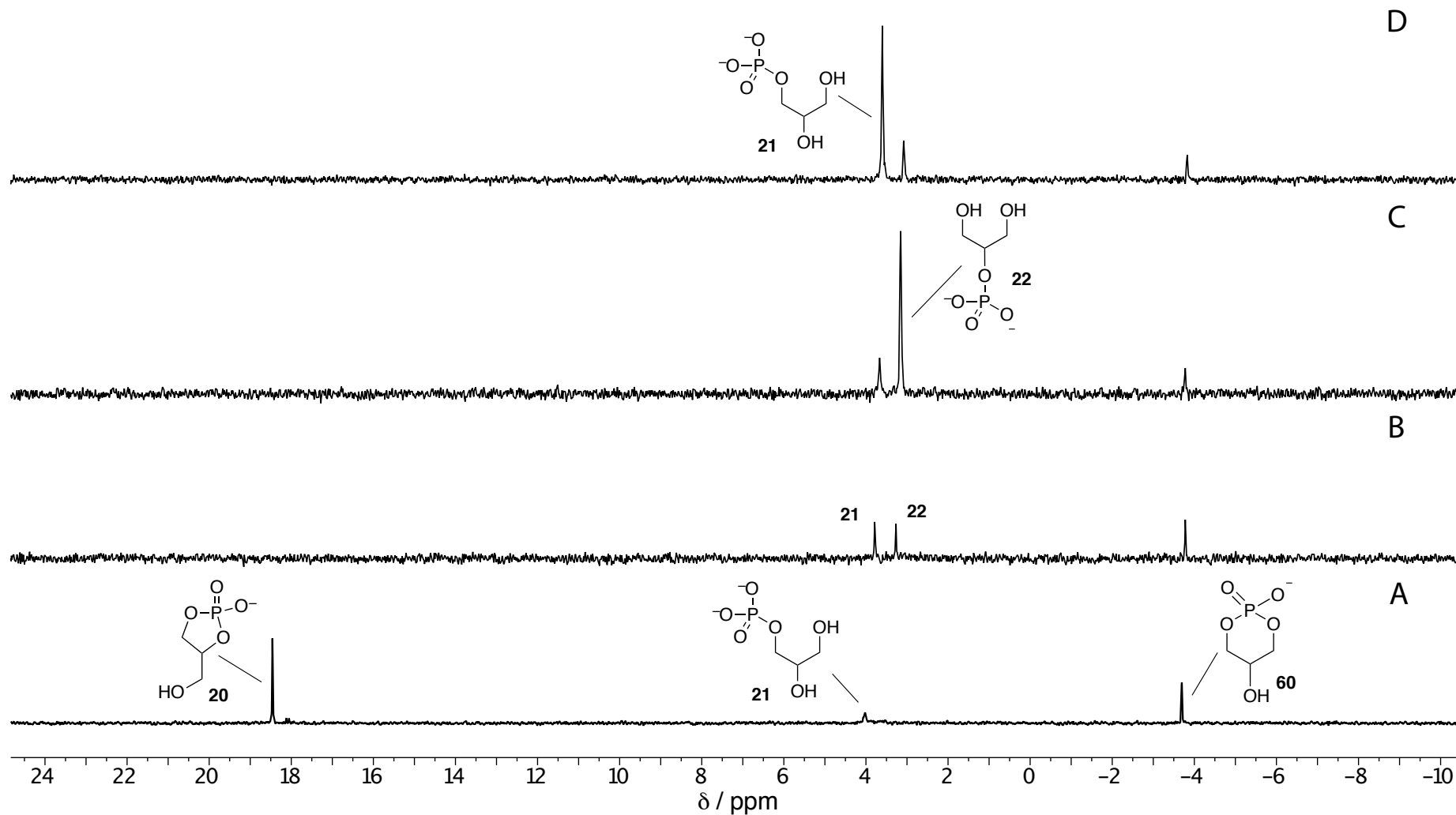
**Figure S4.**  $^{31}\text{P}$ -NMR ( $\text{D}_2\text{O}$ ) analysis of the synthetic standards glycerol-1,2-cyclic phosphate **20** and glycerol-1,3-cyclic phosphate **60** obtained by the intramolecular cyclisation of glycerol-2-phosphate **22** and glycerol-1-phosphate **21**. A – spectrum of the products of phosphorylation of glycerol **19**; B – spectrum of the products of cyclisation of glycerol-2-phosphate **22** with NCI after 24 h; C – spectrum of the products of cyclisation of glycerol-1-phosphate **21** with NCI after 24 h.

### Hydrolysis of the crude glycerol phosphorylation products



The crude solid (50 mg) obtained from the glycerol phosphorylation reaction was dissolved in D<sub>2</sub>O (1 mL). Zinc nitrate (107 mg, 0.360 mmol) was added and the reaction mixture was heated to 60 °C with stirring. After 72 h, the reaction mixture was directly analysed by <sup>31</sup>P-NMR spectroscopy. The presence of glycerol-1-phosphate **21** and glycerol-2-phosphate **22** was confirmed by spiking with the commercially available standards (Figure S5). Yields were calculated assuming that the amount of glycerol-1,3-cyclic phosphate **60** does not vary during the hydrolysis reaction, and knowing that 1-glycerol phosphate **21** was present as 20% of the crude solid before being subjected to hydrolysis. The final composition of the mixture after hydrolysis was glycerol-1-phosphate **21** 31 % (11% derived by hydrolysis of **20**), glycerol-2-phosphate **22** 40% (all from hydrolysis of **20**) and glycerol-1,3-cyclic phosphate **60** 29% (~unchanged by hydrolysis). The reactant glycerol-1,2-cyclic phosphate **20** (53% in the mixture before hydrolysis) was thus quantitatively converted to glycerol-1-phosphate **21** and glycerol-2-phosphate **22** as the sum of the percentages of the two compounds after hydrolysis was 51% and no signal for **20** was observable in the <sup>31</sup>P-NMR spectrum. According to this calculation, the yields of hydrolysis of **20** to give glycerol-1-phosphate **21** and glycerol-2-phosphate **22** were 21% and 75%, respectively.

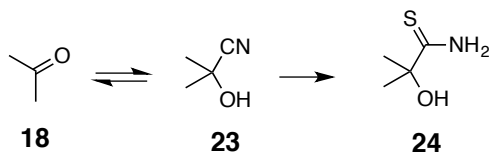
*Glycerol-1-phosphate 21*: <sup>31</sup>P-NMR (162 MHz, D<sub>2</sub>O, <sup>1</sup>H-decoupled) δ 4.19 (s); m/z ES-API, Neg: 171.1 [M-H]; *glycerol-2-phosphate 22*: <sup>31</sup>P-NMR (162 MHz, D<sub>2</sub>O, <sup>1</sup>H-decoupled) δ 3.64 (s); m/z ES-API, Neg: 171.1 [M-H]; *glycerol-1,3-cyclic phosphate 60*: <sup>31</sup>P-NMR (162 MHz, D<sub>2</sub>O, <sup>1</sup>H-decoupled) δ -3.24 (s); m/z ES-API, Neg: 153.1 [M-H].



**Figure S5.**  $^{31}\text{P}$ -NMR ( $\text{D}_2\text{O}$ ) analysis of the hydrolysis reaction of the crude products of phosphorylation of glycerol **19** (containing glycerol-1,2-cyclic phosphate **20** in 53% yield) to give glycerol-2-phosphate **22** and glycerol-1-phosphate **21**. A – spectrum of the products of phosphorylation of glycerol **19**; B – spectrum of the hydrolysis reaction products; C – spectrum of the hydrolysis reaction products spiked with glycerol-2-phosphate **22**; D – spectrum of the hydrolysis reaction products spiked with glycerol-1-phosphate **21**.

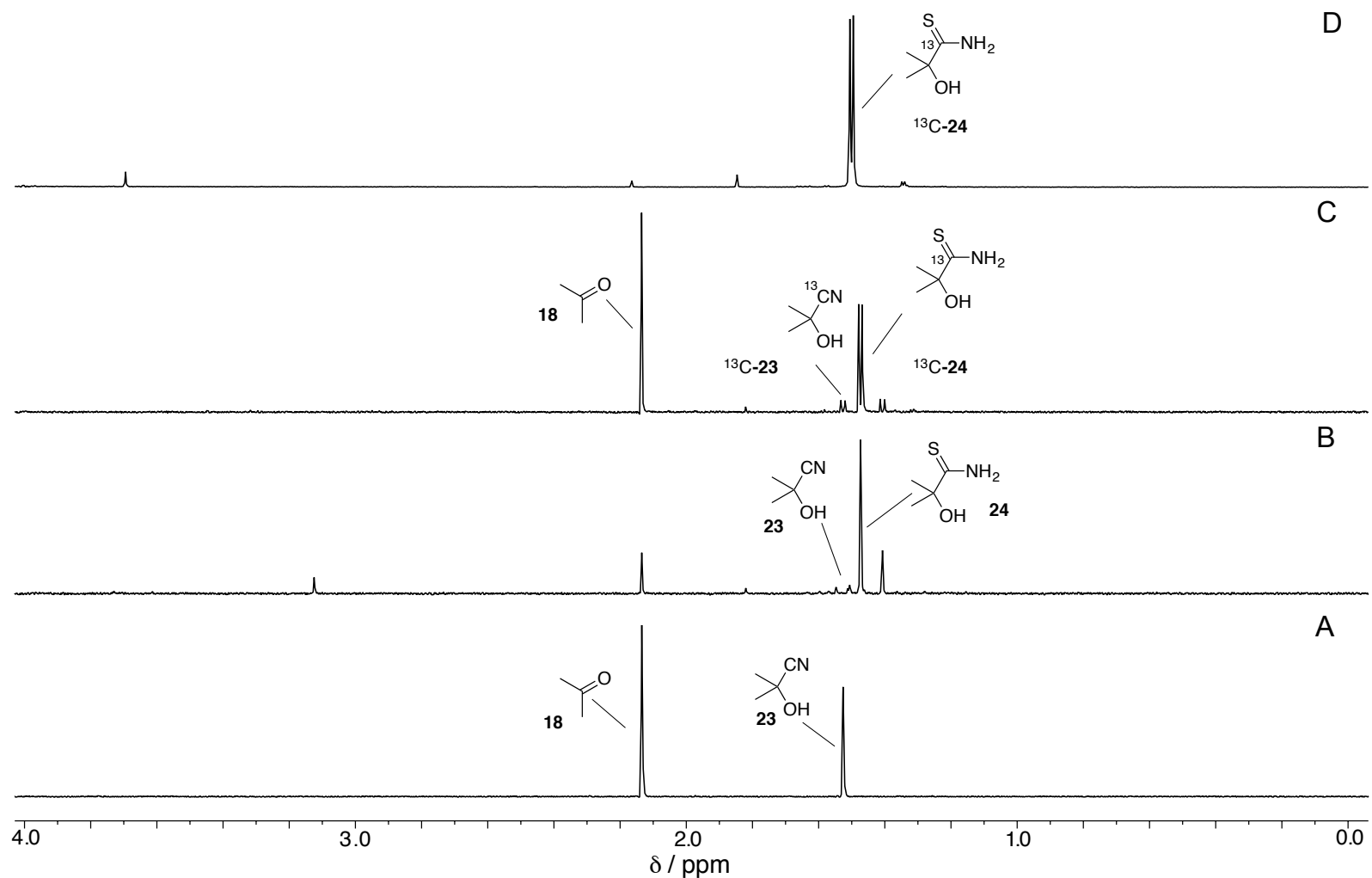
### 3. Synthesis of Val and Leu $\alpha$ -aminonitriles (27 & 31)

Conversion of acetone **18** into  $\alpha$ -hydroxythioamide **24** by way of cyanohydrin **23**



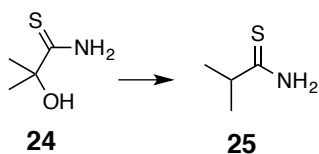
Acetone **18** (7.35  $\mu$ l, 0.1 mmol),  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  (78 mg, 0.5 mmol) and KCN (65.11 mg, 1 mmol) were dissolved in a degassed solution of  $\text{H}_2\text{O}/\text{D}_2\text{O}$  (9:1, 1 mL) and the pH was adjusted to 7 using degassed NaOH/HCl. After stirring for 3 h the sample was analysed by  $^1\text{H}$ -NMR spectroscopy, which revealed that the cyanohydrin **23** had been formed in 41% yield, with 59% of acetone **18** left unreacted (based on relative integration of **18** and **23** in the  $^1\text{H}$ -NMR spectrum).  $\text{NaSH} \cdot x\text{H}_2\text{O}$  (47 mg, assume 60% NaSH, 0.5 mmol) was added and the pH of the solution adjusted to 9. The orange solution was stirred under an argon atmosphere and the formation of the  $\alpha$ -hydroxythioamide **24** was checked periodically by  $^1\text{H}$ -NMR spectroscopy. The yields were calculated by relative integrations of the NMR signals. After 48 h, **24** was present in 62% yield (1.47 ppm), with 15% of acetone **18** (2.13 ppm) and 6% of cyanohydrin **23** (1.53 ppm) remaining. The pH was reduced to 7 and the solution sparged with  $\text{N}_2$  in order to purge HCN,  $\text{H}_2\text{S}$ , and the residual acetone. After 1 h, the solvent was removed in vacuo and the dark/brown residual solid was dissolved in  $\text{D}_2\text{O}$  and analysed by NMR spectroscopy. The evaporation procedure provided an enrichment of the  $\alpha$ -hydroxythioamide **24** up to 90% of the residual mixture. Thioamide formation can also be followed by  $^{13}\text{C}$ -NMR spectroscopy using  $^{13}\text{C}$ -labelled  $\text{K}^*\text{CN}$ , the thiocarbonyl group of the  $\alpha$ -hydroxythioamide **24** showing a characteristic signal at 214.6 ppm. Cyanide (143.2 ppm) and thiocyanate (133.2 ppm) could also be detected in the mixture. After evaporation only the thiocyanate and the  $\alpha$ -hydroxythioamide signals are still visible (data not shown).

$^1\text{H}$ -NMR (400 MHz,  $\text{H}_2\text{O}/\text{D}_2\text{O}$ , 9:1) acetone **18**:  $\delta$  2.13 (s, 6H); 2-hydroxy-2-methylpropanenitrile (unlabelled) **23**:  $\delta$  1.53 (s, 6H); 2-hydroxy-2-methylpropanethioamide **24**:  $\delta$  1.47 (s, 6H).



**Figure S6.**  $^1\text{H}$ -NMR analysis ( $\text{H}_2\text{O}/\text{D}_2\text{O}$ , 9:1) of the conversion of acetone **18** into cyanohydrin **23** and  $\alpha$ -hydroxythioamide **24**. A – spectrum of the reaction products 3 h after the addition of KCN; B – spectrum of the reaction products 48 h after the addition of NaSH; C – spectrum of the corresponding reaction using  $^{13}\text{C}$ -labelled  $\text{K}^*\text{CN}$  for the cyanohydrin formation 24 h after the addition of NaSH; D – as C after removal of excess cyanide and acetone by sparging with  $\text{N}_2$  followed by evaporation to dryness.

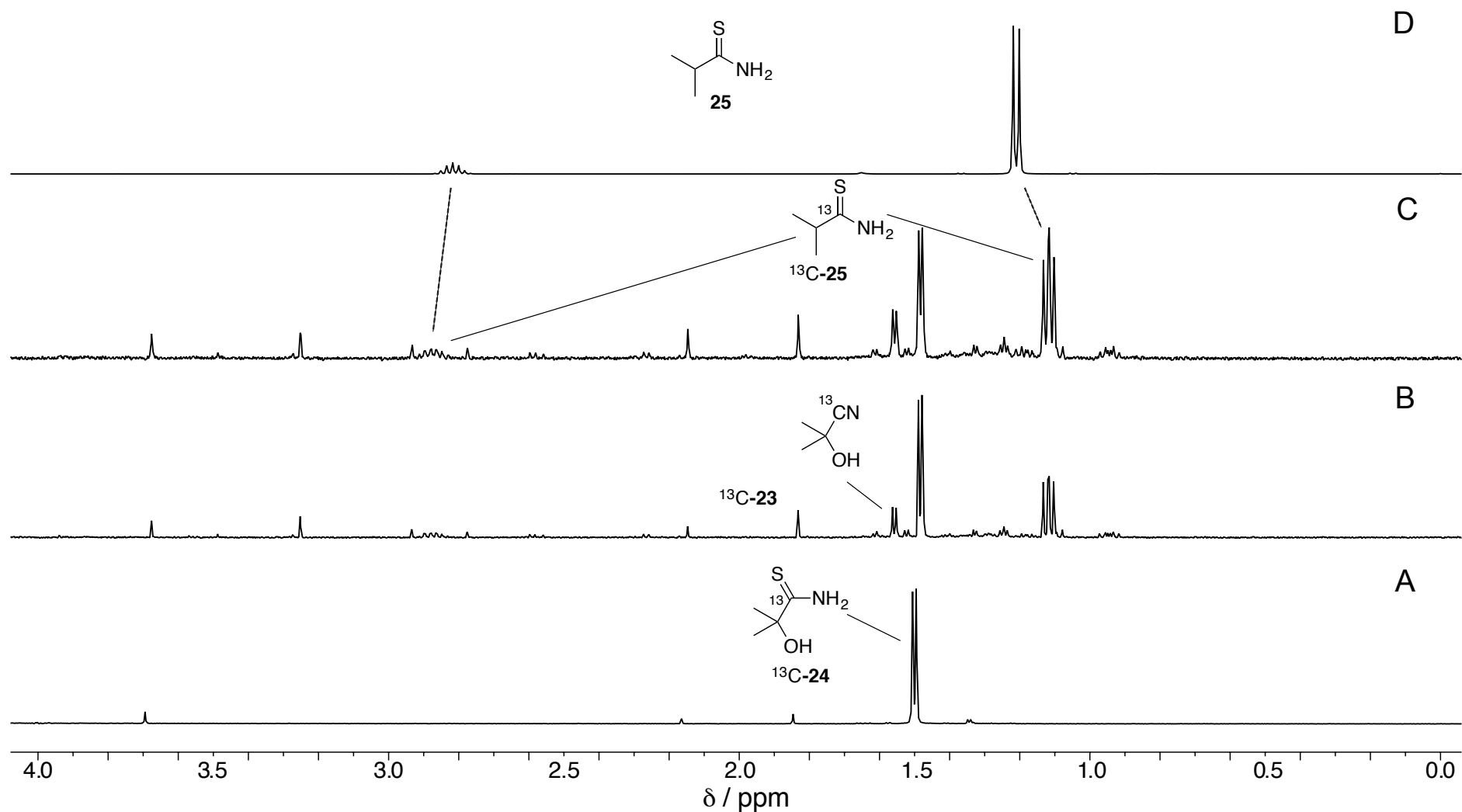
Conversion of  $\alpha$ -hydroxythioamide **24** into thioamide **25**



The crude solid (130 mg) obtained from the previous reaction and containing  $^{13}\text{C}$ -labelled  $\alpha$ -hydroxythioamide **24** (0.0265 mmol, 62% calculated yield) was dissolved in  $\text{H}_2\text{O}/\text{D}_2\text{O}$  (9:1, 3 mL) and the resultant solution was degassed for 15 min.  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  (16 mg, 0.1 mmol) and  $\text{NaSH} \cdot x\text{H}_2\text{O}$  (7 mg, assume 60% NaSH, 0.075 mmol) were added to the solution and the pH was adjusted to 7 using degassed NaOH/HCl. The solution was then transferred to a sealed quartz cuvette containing CuCN (1.5 mg, 0.017 mmol) whereupon a black precipitate formed. The cuvette was placed in the Rayonet reactor and irradiated with stirring for 6 h. Yields were calculated by relative integrations of the  $^1\text{H}$ -NMR signals. The thioamide **25** was obtained with 41% yield with 29% of **24** remaining. Longer irradiation resulted in some  $\alpha$ -hydroxythioamide **24** undergoing elimination of  $\text{H}_2\text{S}$  regenerating cyanohydrin **23**, and hence acetone **18** at equilibrium, and reduction of the latter to isopropanol **56** as by-product. The presence of the thioamide **25** was confirmed by comparison with a synthetic standard (Figure S7).

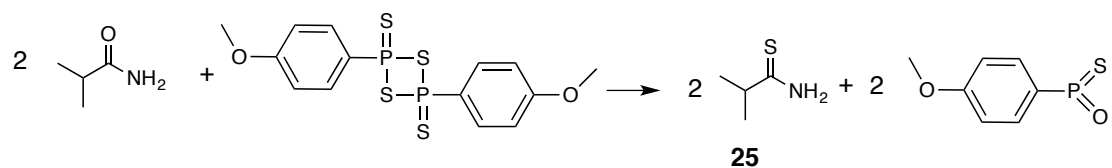
The same procedure has been conducted with the crude solid from the previous reaction containing unlabelled  $\alpha$ -hydroxythioamide **24** to afford the unlabelled thioamide **25**.

*2-Methylpropanethioamide 25 (unlabelled)*:  $^1\text{H}$ -NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  2.93-2.86 (m, 1H), 1.13 (d,  $J = 6.8$  Hz, 6H).



**Figure S7.**  $^1\text{H-NMR}$  analysis of the conversion of the  $\alpha$ -hydroxythioamide **24** into thioamide **25**. A ( $\text{D}_2\text{O}$ ) –  $^{13}\text{C}$ -labelled  $\alpha$ -hydroxythioamide starting material; B ( $\text{H}_2\text{O}/\text{D}_2\text{O}$ , 9:1) – formation of  $^{13}\text{C}$ -labelled thioamide **25** after 3 h of irradiation; C ( $\text{H}_2\text{O}/\text{D}_2\text{O}$ , 9:1) – formation of  $^{13}\text{C}$ -labelled thioamide **25** after 6 h of irradiation; D ( $\text{CDCl}_3$ ) – the unlabelled thioamide **25** synthesised by standard synthetic methods as described in the next section.

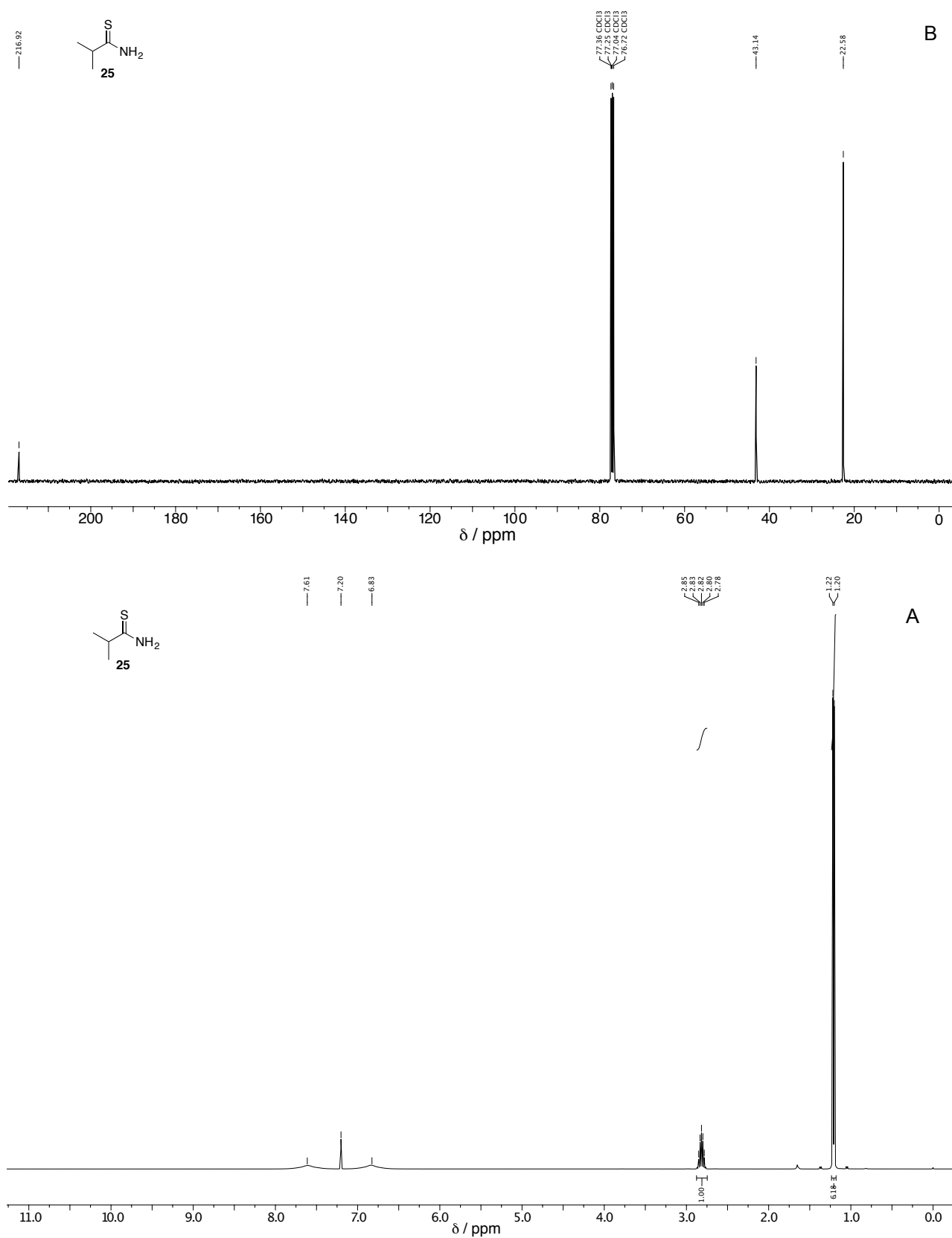
Synthesis of a synthetic standard of thioamide **25**



A solution of 2-methylpropanamide (0.5 g, 5.75 mmol) and 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide (1.16 g, 2.87 mmol) in THF (25 mL) was heated to reflux. After 4 h, the reaction mixture was cooled to room temperature and poured into saturated aqueous NaHCO<sub>3</sub> solution (50 mL). The resultant mixture was extracted with diethyl ether (4 x 50 mL), the organic layers were combined and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and the solvent removed in vacuo. Purification by column chromatography (hexane/ethyl acetate, 8:2) afforded the synthetic standard **25** in 50% yield.

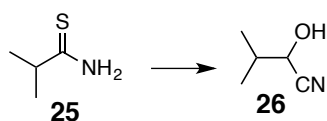
*2-Methylpropanethioamide 25*: <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>): δ 7.61 (br s, 1H), 6.83 (br s, 1H), 2.85-2.78 (set, 1H, *J* = 6.8 Hz), 1.21 (d, 6H, *J* = 6.8 Hz). <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>): δ 216.9, 43.1, 22.6. The <sup>1</sup>H-NMR spectrum is in agreement with published data.<sup>1</sup>





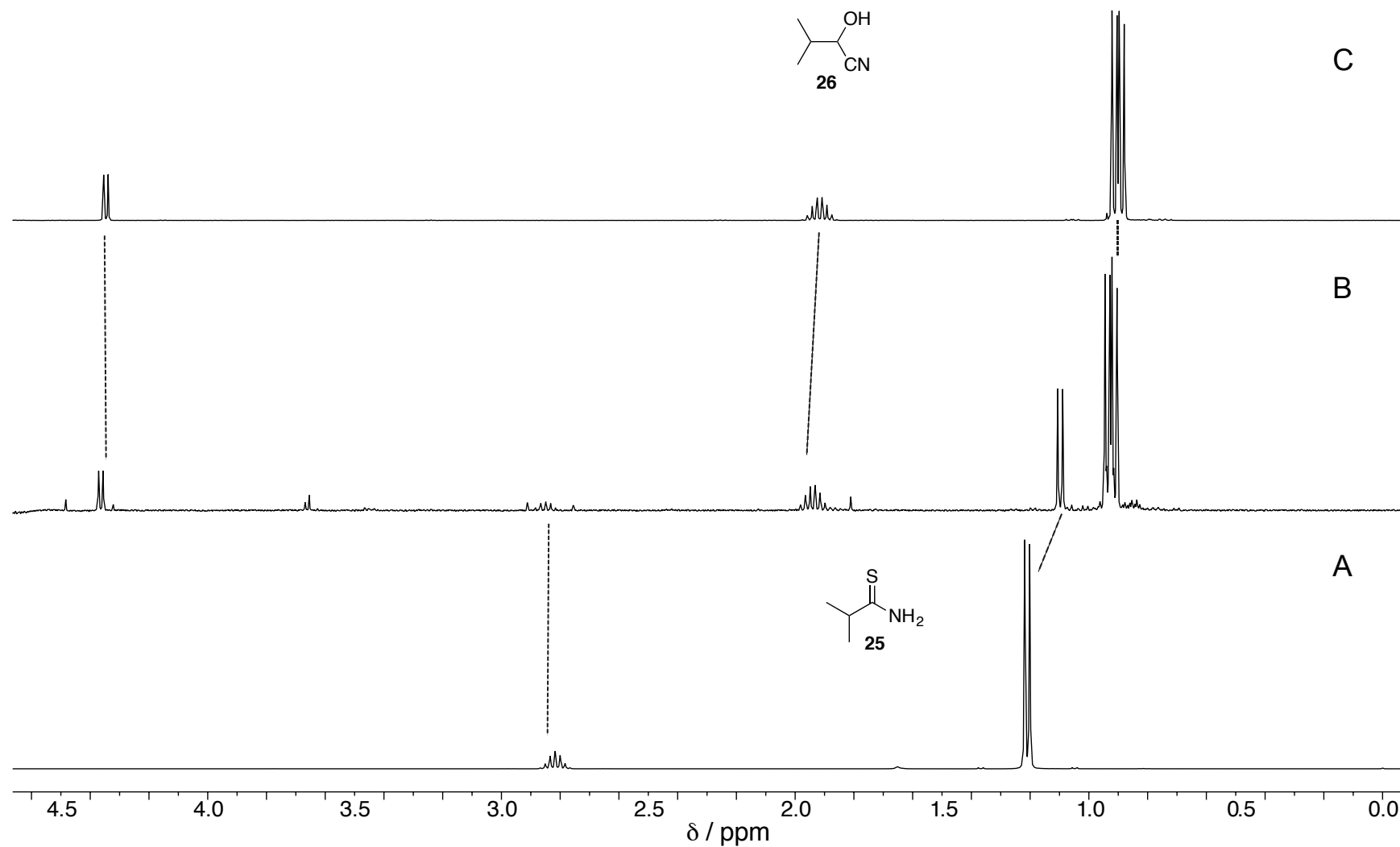
**Figure S8.** Analysis of the synthetic thioamide **25**: A –  $^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ ); B –  $^{13}\text{C-NMR}$  (100 MHz,  $\text{CDCl}_3$ ).

*Conversion of thioamide 25 to cyanohydrin 26 by photoredox chemistry*



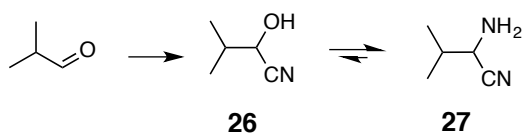
Conventionally synthesised thioamide **25** (3.0 mg, 0.03 mmol) was dissolved in H<sub>2</sub>O/D<sub>2</sub>O (9:1, 3 mL) and the resultant solution degassed for 15 min. NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O (46.8 mg, 0.3 mmol) and NaSH.xH<sub>2</sub>O (21 mg, assume 60% NaSH, 0.22 mmol) were added to the solution and the pH was adjusted to 7 using degassed NaOH/HCl. The solution was then transferred to a sealed quartz cuvette containing CuCN (3.5 mg, 0.039 mmol) whereupon a black precipitate formed. The cuvette was placed in a Rayonet reactor and then irradiated with stirring for 3 h. Yields were calculated by relative integrations of the <sup>1</sup>H-NMR signals. Cyanohydrin **26** was obtained in 78% yield with 22% of the thioamide **25** remaining. The presence of the cyanohydrin **26** was confirmed by comparison with a synthetic standard (Figure S9).

*2-Hydroxy-3-methylbutanenitrile 26*: <sup>1</sup>H-NMR (400 MHz, H<sub>2</sub>O/D<sub>2</sub>O, 9:1): δ 4.34 (d, 1H, *J* = 6.1 Hz), 1.98-1.86 (m, 1H), 0.94-0.88 (m, 6H).



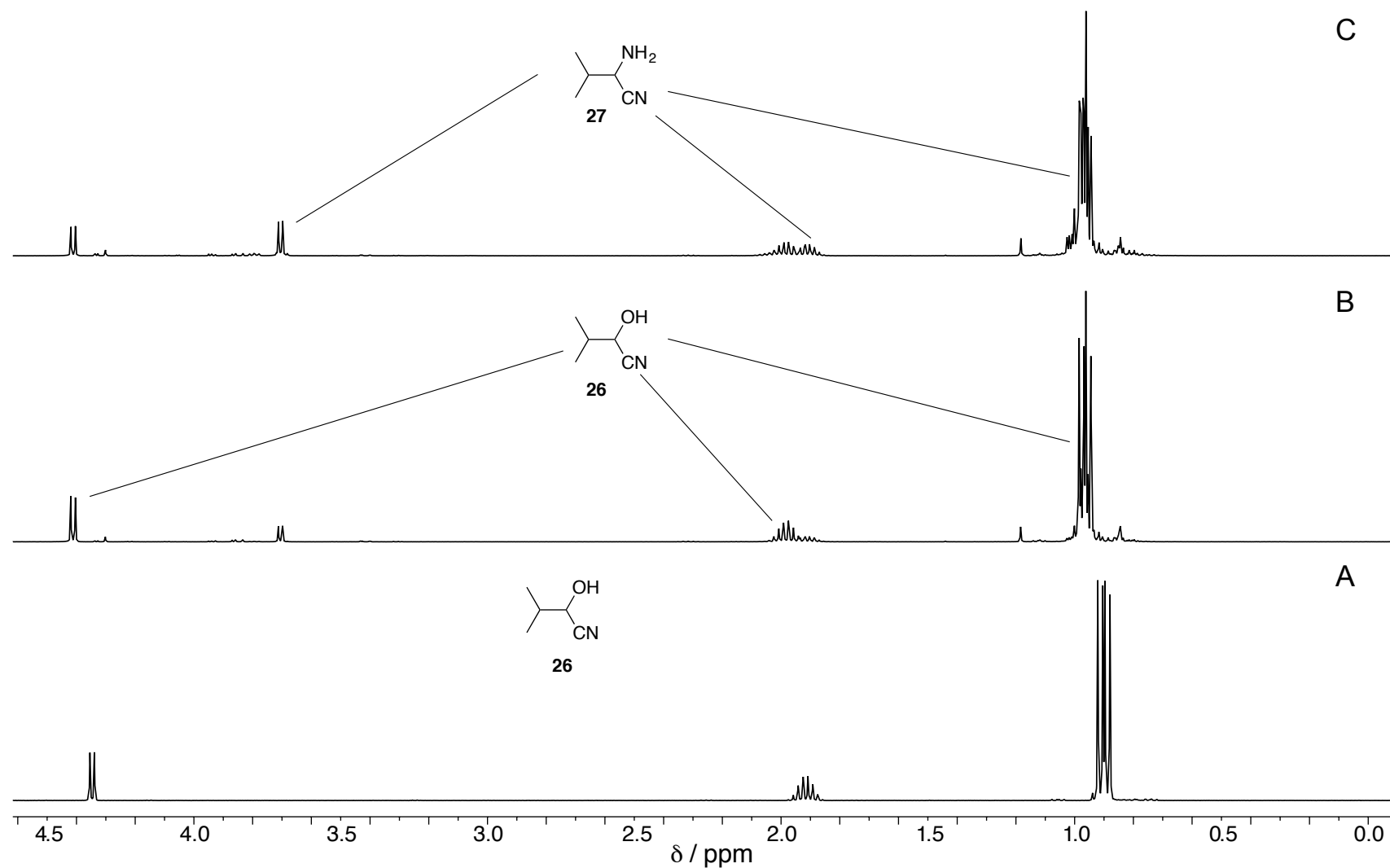
**Figure S9.**  $^1\text{H-NMR}$  analysis of the conversion of the thioamide **25** into cyanohydrin **26**. A ( $\text{CDCl}_3$ ) – pure synthetic standard of thioamide **25**; B ( $\text{H}_2\text{O}/\text{D}_2\text{O}$ , 9:1) – the products of the photoreaction after 3 h of irradiation; C  $^1\text{H-NMR}$  ( $\text{H}_2\text{O}/\text{D}_2\text{O}$ , 9:1) – pure synthetic standard of cyanohydrin **26** prepared as described in the following section.

Conversion of cyanohydrin **26** to  $\alpha$ -aminonitrile **27**



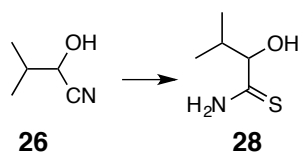
The  $\alpha$ -aminonitrile **27** could be produced from cyanohydrin **26** as produced from thioamide **25**, but, to simplify the handling procedures, the cyanohydrin **26** actually used to demonstrate the conversion to **27** was synthesised quantitatively from the commercially available isobutyraldehyde. Isobutyraldehyde (9.13  $\mu$ l, 0.1 mmol) was dissolved in H<sub>2</sub>O/D<sub>2</sub>O (9:1, 0.5 mL) and KCN (9.75 mg, 0.15 mmol) was added with stirring. After 1 h the quantitative formation of the corresponding cyanohydrin **26** was monitored by <sup>1</sup>H-NMR spectroscopy. Then, 5 equivalents of NH<sub>3</sub> (39  $\mu$ l of a 13 M buffered solution of NH<sub>3</sub>/NH<sub>4</sub><sup>+</sup>) were added and the pH adjusted to 9.2 using NaOH/HCl. The solution was stirred for 13 d after which the  $\alpha$ -aminonitrile **27** was formed in a 42% yield with 58% of **26** remaining. The yields were calculated by relative integrations of the <sup>1</sup>H-NMR signals.

<sup>1</sup>H-NMR (400 MHz, H<sub>2</sub>O/D<sub>2</sub>O, 9:1) *2-hydroxy-3-methylbutanenitrile* **26**:  $\delta$  4.34 (d, 1H,  $J$  = 6.1 Hz), 1.98-1.86 (m, 1H), 0.94-0.88 (m, 6H); *2-amino-3-methylbutanenitrile* **27**:  $\delta$  3.70 (1H, d,  $J$  = 5.7), 1.95-1.86 (m, 1H), 0.98-0.94 (m, 6H). The <sup>1</sup>H-NMR spectrum for the  $\alpha$ -aminonitrile **27** is in agreement with published data.<sup>2</sup>



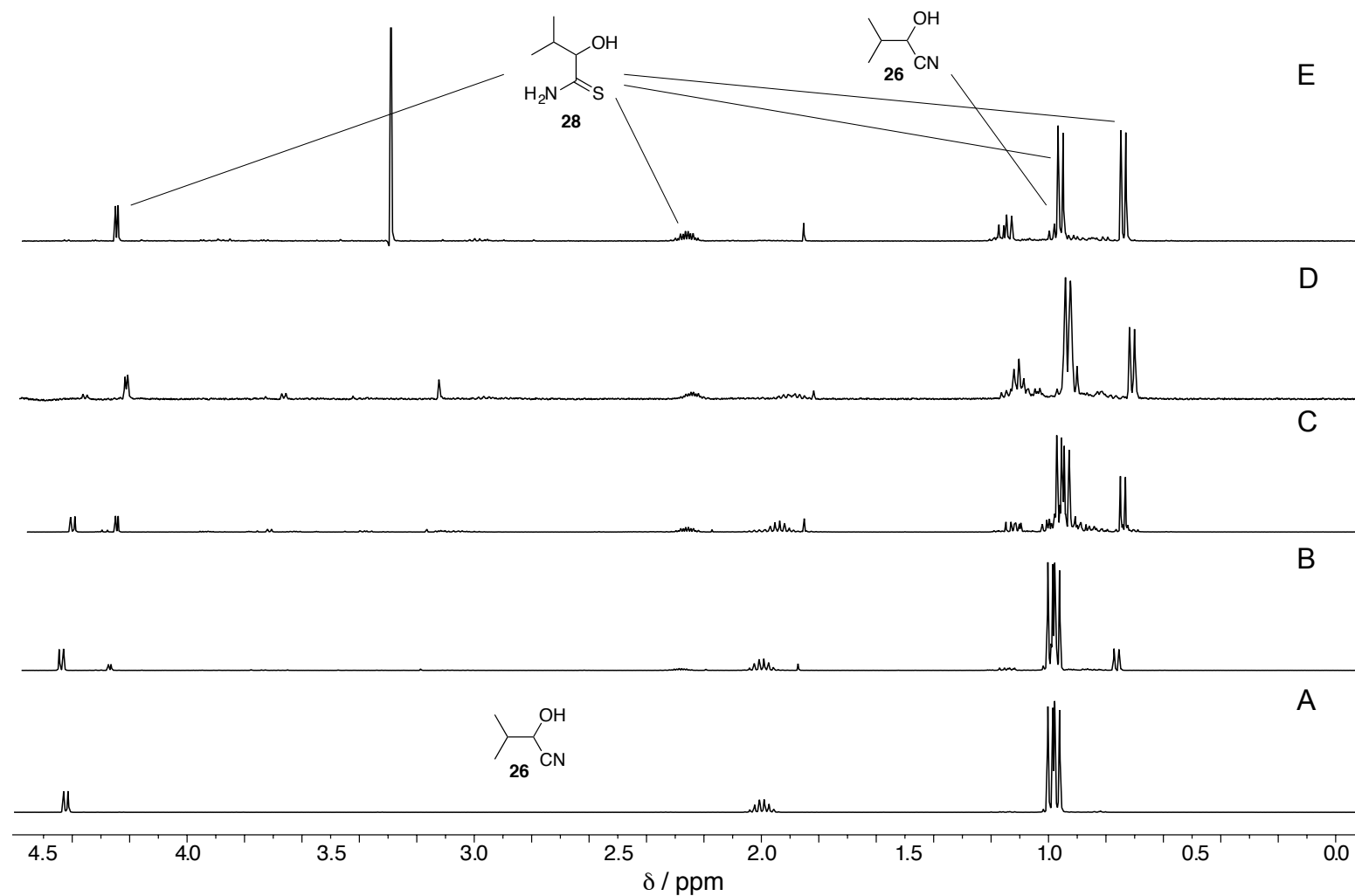
**Figure S10.**  $^1\text{H-NMR}$  analysis ( $\text{H}_2\text{O}/\text{D}_2\text{O}$ , 9:1) of the conversion of cyanohydrin **26** into  $\alpha$ -aminonitrile **27**. A – spectrum of the reaction mixture before addition of ammonia; B – spectrum of the reaction mixture 4 d after ammonia addition; C – spectrum of the reaction mixture 13 d after ammonia addition.

Conversion of cyanohydrin **26** to  $\alpha$ -hydroxythioamide **28**



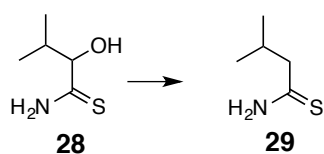
To a solution (0.5 mL) of the cyanohydrin **26** (synthesised as described in the preceding section) NaSH.xH<sub>2</sub>O (118 mg, assume 60% NaSH, 1.26 mmol) was added and the pH of the solution adjusted to 9. The orange solution was stirred and the formation of the  $\alpha$ -hydroxythioamide **28** was assessed by <sup>1</sup>H-NMR spectroscopy. After 9 d, **28** had been formed in ~57 % yield with ~28% of cyanohydrin residual and an unknown compound in 15% yield. The yields were calculated by relative integrations of the <sup>1</sup>H-NMR signals. The product was used without further purification in the following step.

<sup>1</sup>H-NMR (400 MHz, H<sub>2</sub>O/D<sub>2</sub>O, 9:1) *2-hydroxy-3-methylbutanenitrile* **26**:  $\delta$  4.41 (d, 1H,  $J$  = 6.1 Hz), 2.03-1.95 (m, 1H), 1.00-0.96 (m, 6H); *2-hydroxy-3-methylbutanthioamide* **28**:  $\delta$  4.24 (d, 1H,  $J$  = 3.8 Hz), 2.29-2.22 (m, 1H), 0.96 (d, 3H,  $J$  = 6.9 Hz), 0.74 (d, 3H,  $J$  = 7.0 Hz).



**Figure S11.** <sup>1</sup>H-NMR analysis (H<sub>2</sub>O/D<sub>2</sub>O, 9:1) of the conversion of cyanohydrin **26** into  $\alpha$ -hydroxythioamide **28**. A – spectrum of the reaction mixture before addition of NaSH showing the cyanohydrin **26** as the only product; B – spectrum of the reaction mixture 2 d after NaSH addition; C – as B after 3 d; D – as B after 7 d; E – as B after 9 d. The appearance of methanol after 9 d (signal at 3.25 ppm) is thought to be due to a contamination during sample handling.

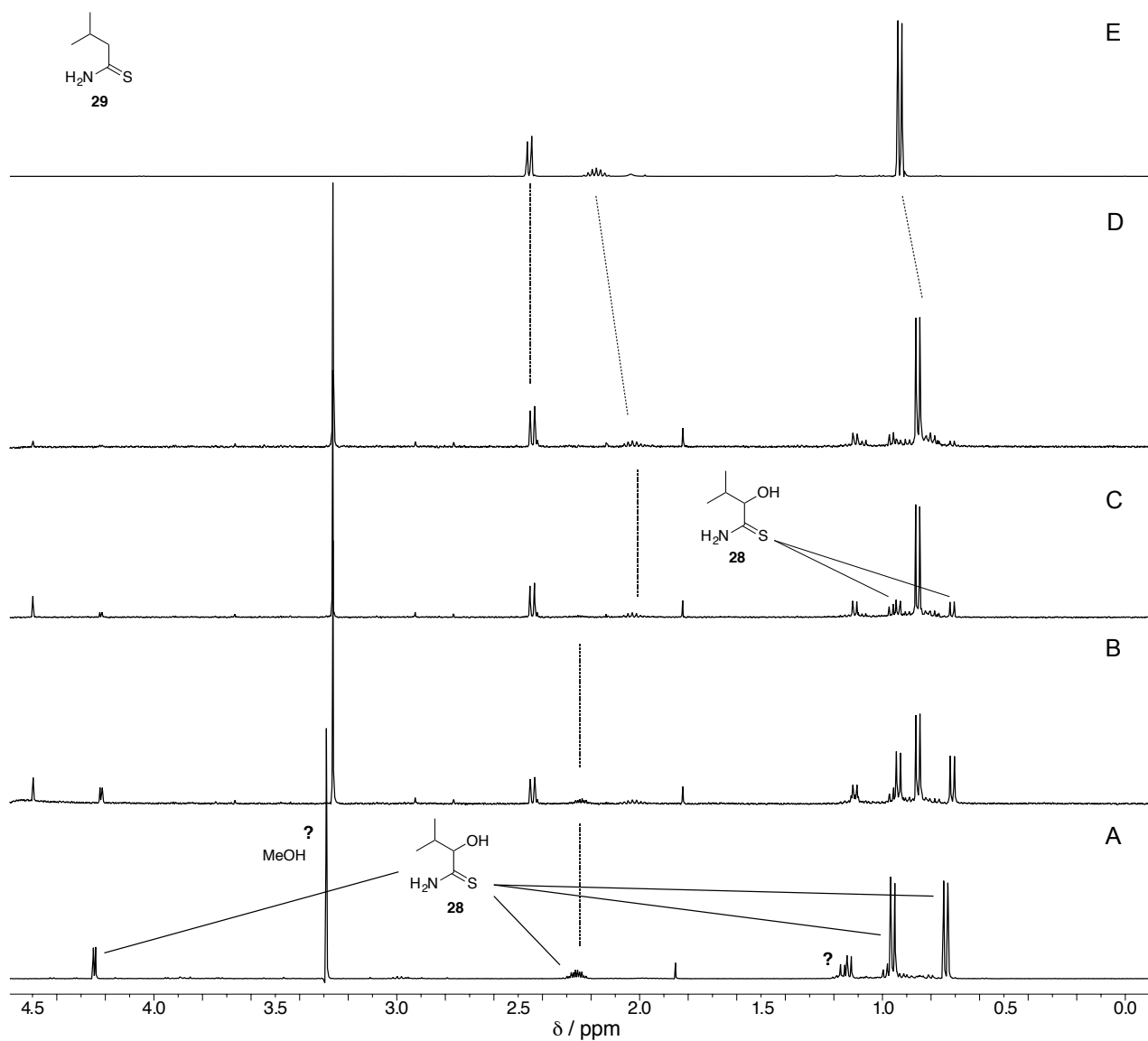
Conversion of  $\alpha$ -hydroxythioamide **28** to thioamide **29**



A portion of the solution containing the crude  $\alpha$ -hydroxythioamide **28** in 57% yield (0.4 mL, 0.048 mmol) was diluted to 3 mL with a degassed H<sub>2</sub>O/D<sub>2</sub>O solution (9:1). NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O (67.4 mg, 0.432 mmol) and Na<sub>2</sub>S·9H<sub>2</sub>O (80 mg, 0.336 mmol) were added to the solution and the pH was adjusted to 7 using degassed NaOH/HCl. The solution was then transferred to a sealed quartz cuvette containing CuCN (1.5 mg, 0.017 mmol), whereupon a black precipitate formed. The cuvette was placed in the Rayonet reactor and then irradiated with stirring for 5h. The yield for **29** was ~ 75% with 11% of **28** remaining along with 15% of the unknown compound. The yields were calculated by relative integrations of the <sup>1</sup>H-NMR signals. The product was used without further purification in the following step. The presence of the thioamide **29** was confirmed by comparison with a synthetic standard.

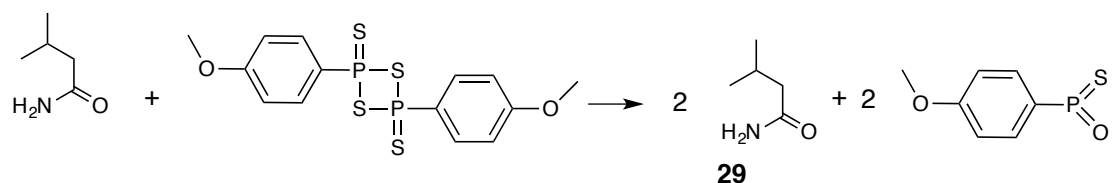
<sup>1</sup>H-NMR (400 MHz, H<sub>2</sub>O/D<sub>2</sub>O, 9:1) *2-hydroxy-3-methylbutanethioamide 28*:  $\delta$  4.24 (d, 1H,  $J = 3.8$  Hz), 2.29-2.22 (m, 1H), 0.96 (d, 3H,  $J = 6.9$  Hz), 0.74 (d, 3H,  $J = 7.0$  Hz); *3-methylbutanethioamide 29*:  $\delta$  2.44 (d, 2H,  $J = 7.2$  Hz), 2.06-1.98 (m, 1H), 0.85 (d, 6H,  $J = 7.0$  Hz)





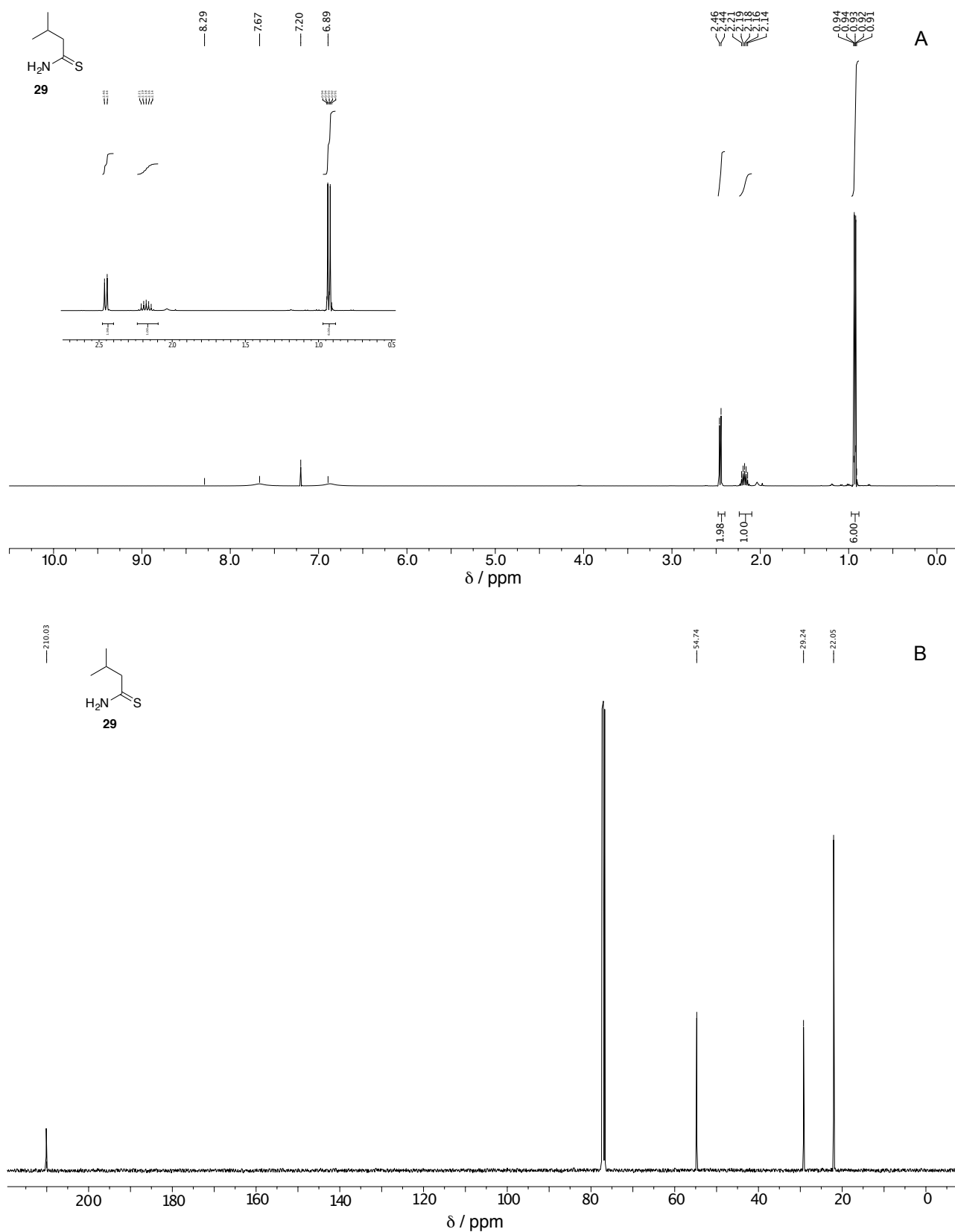
**Figure S12.** <sup>1</sup>H-NMR analysis of the conversion of α-hydroxythioamide **28** into thioamide **29**. A (H<sub>2</sub>O/D<sub>2</sub>O, 9:1) – reaction mixture before irradiation; B (H<sub>2</sub>O/D<sub>2</sub>O, 9:1) – reaction mixture after irradiation for 1.5 h; C – as B after irradiation for 3 h; D – as B after irradiation for 5 h. E (CDCl<sub>3</sub>) – the synthetic standard of thioamide **29** synthesised as described in the next section. The signal at 3.25 ppm in all spectra corresponds to a methanol contamination in the starting sample – see Figure S11.

*Synthesis of a synthetic standard of thioamide 29*



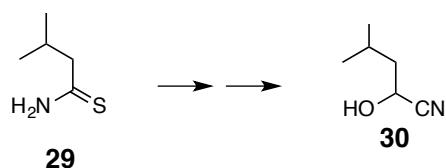
A solution of 3-methylbutanamide (0.5 g, 4.94 mmol) and 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide (1.0 g, 2.5 mmol) in THF (20 mL) was heated to reflux. After 4 h, the reaction mixture was cooled to room temperature and poured into saturated aqueous NaHCO<sub>3</sub> (50 mL). The resultant mixture was extracted with diethyl ether (4 x 50 mL). The organic layers were combined and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, the solvent removed in vacuo. Purification by column chromatography (hexane/ethyl acetate 8:2) afforded the synthetic standard **29** in 70% yield.

*3-Methylbutanethioamide 29*: <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) δ 7.67 (br s, 1H), 6.89 (br s, 1H), 2.65 (d, 2H, *J* = 7.1 Hz), 2.23-2.13 (m, 1H), 0.93 (d, 6H, *J* = 6.3 Hz). <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>) δ 210.0, 54.74, 29.24, 22.05



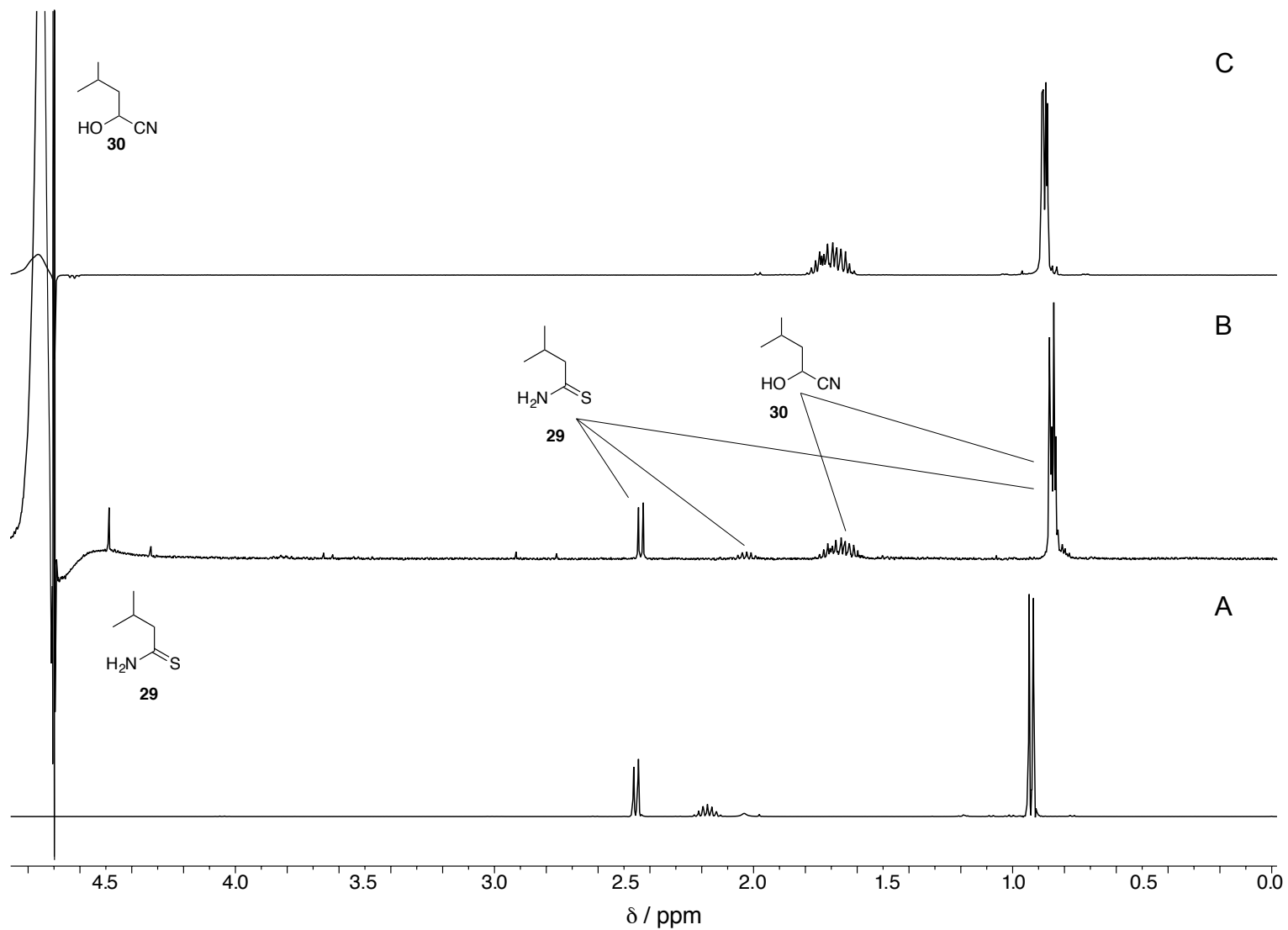
**Figure S13.** Analysis of the synthetic standard of thioamide **29**: A –  $^1\text{H}$ -NMR (400 MHz,  $\text{CDCl}_3$ ).  
 Insert: expanded section between 0.5-3 ppm; B –  $^{13}\text{C}$ -NMR (100 MHz,  $\text{CDCl}_3$ ).

*Conversion of thioamide 29 to cyanohydrin 30 by photoredox chemistry*



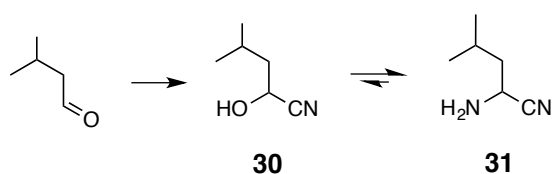
Conventionally synthesised thioamide **29** (3.5 mg, 0.03 mmol) was dissolved in H<sub>2</sub>O/D<sub>2</sub>O (9:1, 3 mL) and the resultant solution degassed for 15 min. NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O (31.2 mg, 0.2 mmol) and Na<sub>2</sub>S·9H<sub>2</sub>O (36 mg, 0.15 mmol) were added to the solution and the pH was adjusted to 7 using degassed NaOH/HCl. The solution was then transferred to a sealed quartz cuvette containing CuCN (3.2 mg, 0.036 mmol), whereupon a black precipitate was formed. The cuvette was placed in the Rayonet reactor and then irradiated with stirring for 1.5 h. Yields were calculated by relative integrations of the <sup>1</sup>H-NMR signals, **30** 66% and unreacted **29** 34%. The presence of the cyanohydrin **30** was confirmed by comparison with a synthetic standard.

*2-Hydroxy-4-methylpentanenitrile 30*: <sup>1</sup>H-NMR (400 MHz, H<sub>2</sub>O/D<sub>2</sub>O, 9:1) δ 1.79-1.61 (m, 3H), 0.88-0.86 (m, 6H); other 1H not assigned due to HOD suppression of the signal.



**Figure S14.** <sup>1</sup>H-NMR analysis of the conversion of thioamide **29** to cyanohydrin **30**. A (CDCl<sub>3</sub>) –starting material, the pure synthetic standard of thioamide **29**; B (H<sub>2</sub>O/D<sub>2</sub>O, 9:1) –reaction products after 1.5 h of irradiation; C (H<sub>2</sub>O/D<sub>2</sub>O, 9:1) – pure synthetic standard of cyanohydrin **30**.

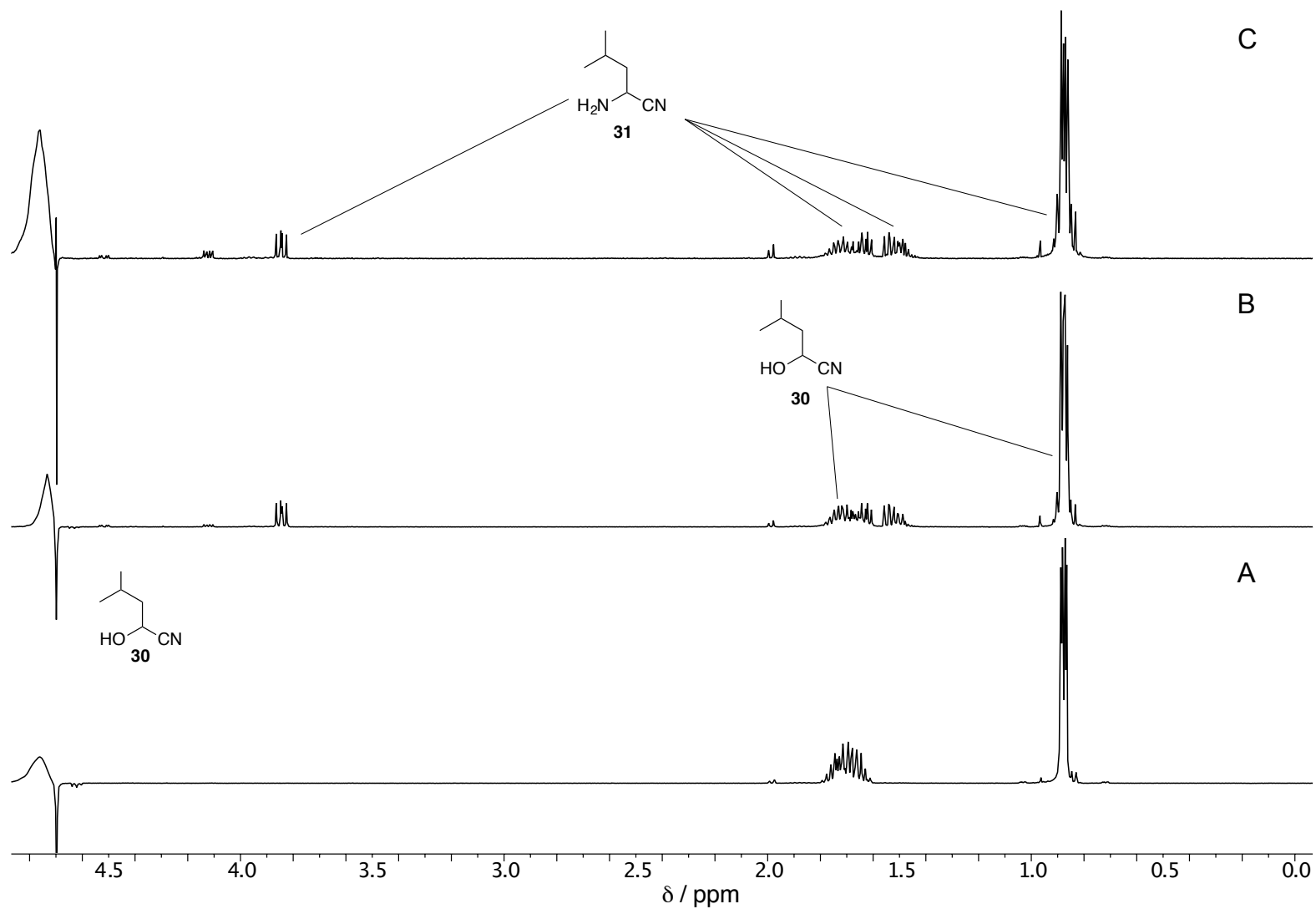
*Conversion of cyanohydrin 30 to  $\alpha$ -aminonitrile 31*



The  $\alpha$ -aminonitrile **31** could be produced from cyanohydrin **30** as produced from thioamide **29**, but, to simplify the handling procedures, the cyanohydrin **30** actually used to demonstrate the conversion to **31** was synthesised quantitatively from the commercially available aldehyde. Isovaleraldehyde (10.7  $\mu$ l, 0.1 mmol) was dissolved in H<sub>2</sub>O/D<sub>2</sub>O (9:1, 5 mL) and KCN (9.75 mg, 0.15 mmol) was added with stirring. After 1 h, the quantitative formation of the corresponding cyanohydrin **30** was monitored by <sup>1</sup>H-NMR spectroscopy. Then 5 equivalents of NH<sub>3</sub> (39  $\mu$ l of a 13 M buffered solution of NH<sub>3</sub>/NH<sub>4</sub><sup>+</sup>) were added and the pH adjusted to 9.2 using NaOH/HCl. The solution was stirred for 13 d and monitored by <sup>1</sup>H-NMR spectroscopy. The conversion of cyanohydrin **30** into the  $\alpha$ -aminonitrile **31** was observed to have proceeded in ~42% yield with ~58% of **30** remaining. The yields were calculated by relative integrations of the <sup>1</sup>H-NMR signals.

*2-Amino-4-methylpentanenitrile 30*: <sup>1</sup>H-NMR (400 MHz, H<sub>2</sub>O/D<sub>2</sub>O, 9:1)  $\delta$  3.84 (dd, 1H,  $J = 7.0, 8.7$  Hz), 1.61-1.77 (m, 1H), 1.57-1.46 (m, 2H), 0.89-0.86 (m, 6H).

The <sup>1</sup>H-NMR spectrum is consistent with the <sup>1</sup>H-NMR spectrum of the  $\alpha$ -aminonitrile hydrochloride, which is reported in the literature.<sup>3</sup>



**Figure S15.**  $^1\text{H-NMR}$  analysis ( $\text{H}_2\text{O}/\text{D}_2\text{O}$ , 9:1) of the conversion of cyanohydrin **30** into  $\alpha$ -aminonitrile **31**. A – spectrum of the reaction mixture before addition of ammonia showing the pure cyanohydrin **30**; B – spectrum of the reaction mixture 4 d after ammonia addition. C – spectrum of the reaction mixture 13 d after ammonia addition.

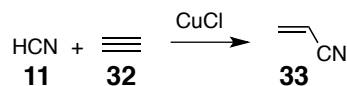
**Table S1. Yields for that part of the reaction network shown in Figure 1a**

Conversion	No. steps	Yield %	Conversion	No. steps	Yield %
<b>4 → 17</b>	1	59	<b>26 → 28</b>	1	57
<b>17 → 18 +</b>	1	29	<b>28 → 29</b>	1	75
<b>19 +</b>		34	<b>26 → 29</b>	2	
<b>56 +</b>		20	<b>29 → 30</b>	2	43
<b>57 +</b>		10	<b>30 → 31</b>	1	66
<b>58 +</b>		4			42
<b>59</b>		3			
<b>18 → 24</b>	2	62	<b>19 → 20 +</b>	1	53
			<b>21 +</b>		20
			<b>60</b>		27
<b>24 → 25</b>	1	41	<b>20 → 21 +</b>	1	21
			<b>22</b>		75
<b>25 → 26</b>	2	78	<b>19 → 21 +</b>	2	31
			<b>22</b>		40
<b>26 → 27</b>	1	42			



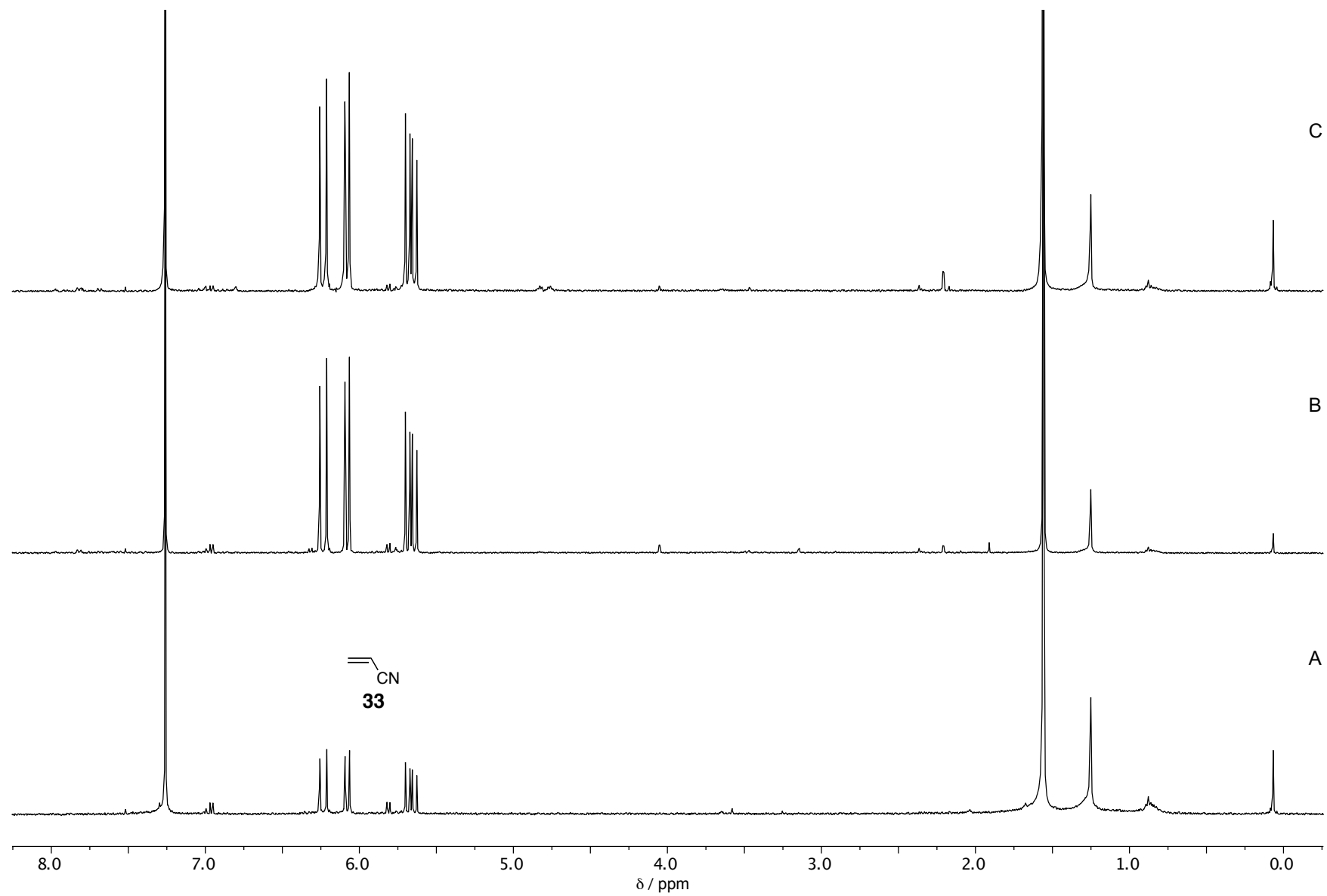
#### 4. Synthesis of Pro and Arg $\alpha$ -aminonitriles (46 & 47)

Formation of acrylonitrile **33** from acetylene **32** and HCN **11**



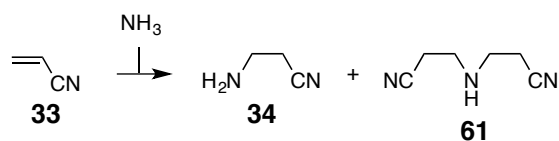
Copper(I) chloride (0.69 g, 7.0 mmol) and potassium chloride (0.50 g, 6.7 mmol) were suspended in H<sub>2</sub>O (0.6 mL) and the resultant suspension heated at 70 °C for 1 h to generate the *Nieuwland* catalyst mixture, K[CuCl<sub>2</sub>]. The temperature was then lowered to 50 °C. Acetylene **32** (generated from addition of CaC<sub>2</sub> to H<sub>2</sub>O) was bubbled into the mixture with simultaneous addition (syringe pump) of KCN (65 mg, 1.0 mmol) in H<sub>2</sub>O (0.5 mL) at pH 7.0, over 15 min. The reaction mixture changed from a brown suspension to first, a brown solution, and then a yellow/orange solution. The reaction was stirred at 50 °C and samples were taken at specific time intervals. The reaction was monitored by <sup>1</sup>H-NMR spectroscopy by extraction of reaction aliquots with CDCl<sub>3</sub> (Figure S16).

*Acrylonitrile 33*: <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  6.23 (dd,  $J = 17.9, 0.9$  Hz, 1H), 6.08 (dd,  $J = 11.7, 0.9$  Hz, 1H), 5.66 (dd,  $J = 17.9, 11.7$  Hz, 1H).



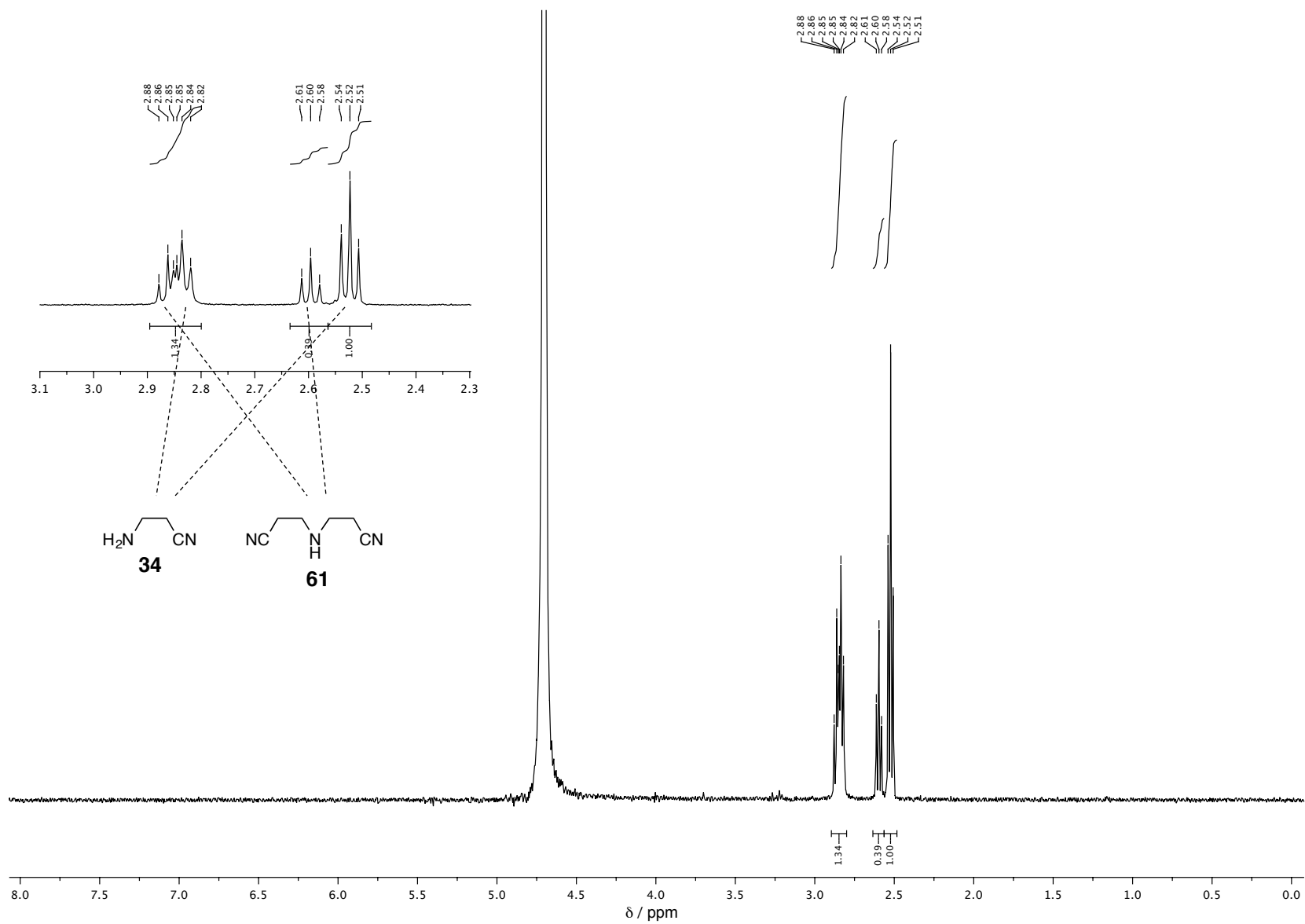
**Figure S16.**  $^1\text{H-NMR}$  analysis ( $\text{CDCl}_3$ ) of the formation of acrylonitrile **33**. A – extract after 1 h; B – extract after 3 h; C – extract after 22 h.

Formation of aminopropionitrile **34** from acrylonitrile **33**



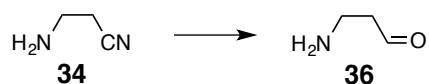
A mixture of  $\text{NH}_3/\text{NH}_4\text{Cl}$  (1.0 M; 8.0 mL, 8.0 mmol) and  $\text{H}_2\text{O}/\text{D}_2\text{O}$  (1:1, 2 mL) was adjusted to pH 9.2 using NaOH. Acrylonitrile **33** (0.13 mL, 2.0 mmol) was added slowly and the reaction mixture stirred at room temperature. The reaction was monitored by  $^1\text{H}$ -NMR spectroscopy by diluting aliquots in  $\text{D}_2\text{O}$ . After 6 h, the reaction was complete giving a mixture of aminopropionitrile **34** (83%) and 3,3'-iminodipropionitrile **61** (17%) (Figure S17).

*Aminopropionitrile 34*:  $^1\text{H}$ -NMR (400 MHz,  $\text{H}_2\text{O}/\text{D}_2\text{O}$ , 9:1)  $\delta$  2.84 (t,  $J = 6.4$  Hz, 2H), 2.52 (t,  $J = 6.5$  Hz, 2H). *3,3'-Iminodipropionitrile 61*:  $^1\text{H}$ -NMR (400 MHz,  $\text{H}_2\text{O}/\text{D}_2\text{O}$ , 9:1)  $\delta$  2.86 (t,  $J = 6.7$  Hz, 4H), 2.60 (t,  $J = 6.7$  Hz, 4H).



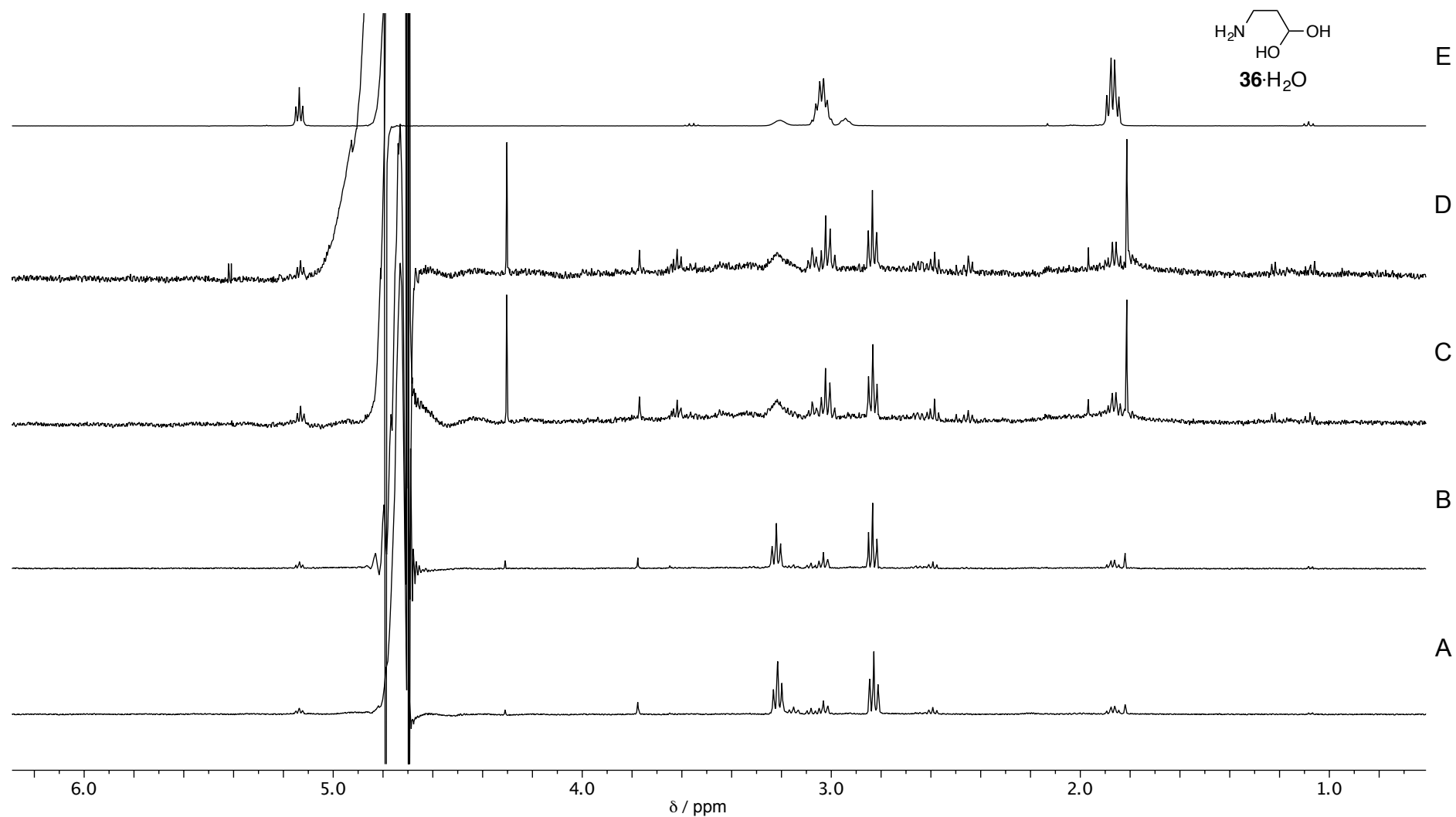
**Figure S17.**  $^1\text{H-NMR}$  analysis (D<sub>2</sub>O) of the formation of aminopropionitrile **34** along with 3,3'-iminodipropionitrile **61**, from acrylonitrile **33** after 6 h.

*Conversion of aminopropionitrile 34 to aminopropionaldehyde 36 by photoredox chemistry*



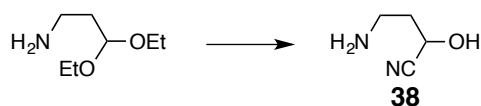
Aminopropionitrile **34** (2.0  $\mu\text{L}$ , 0.03 mmol) and  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  (47 mg, 0.30 mmol) were dissolved in degassed  $\text{H}_2\text{O}/\text{D}_2\text{O}$  (6:1, 2.8 mL) and the solution was adjusted to pH 6.5 using degassed  $\text{NaOH}/\text{HCl}$ .  $\text{NaSH} \cdot x\text{H}_2\text{O}$  (21 mg, assumed 60%  $\text{NaSH}$ , 0.23 mmol) was added and upon dissolution of the  $\text{NaSH}$  the mixture was adjusted to pH 6.9 using degassed  $\text{NaOH}/\text{HCl}$ . The solution was then added to a quartz cuvette containing  $\text{CuCN}$  (ca. 0.5 mg), whereupon a black precipitate formed, and the cuvette was immediately sealed. The cuvette was placed in a Rayonet reactor and irradiated. Samples of the reaction were taken at different time points (4, 6, 16, 22 h) and analysed by  $^1\text{H}$ -NMR spectroscopy (Figure S18). The yield of the conversion to **36** $\cdot\text{H}_2\text{O}$  after 22 h was 45%.

$^1\text{H}$ -NMR (400 MHz,  $\text{H}_2\text{O}/\text{D}_2\text{O}$ , 9:1) *aminopropionitrile 34*:  $\delta$  3.22 (t,  $J = 6.7$  Hz, 2H), 2.83 (t,  $J = 6.7$  Hz, 2H); *aminopropionaldehyde hydrate 36* $\cdot\text{H}_2\text{O}$ :  $\delta$  5.14 (t,  $J = 5.5$  Hz, 1H), 3.04 (m, 2H), 1.87 (td,  $J = 7.0, 5.4$  Hz, 2H).



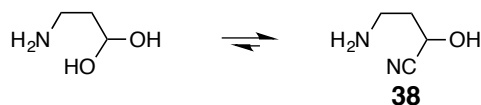
**Figure S18.** <sup>1</sup>H-NMR analysis (H<sub>2</sub>O/D<sub>2</sub>O, 9:1) of the photochemical conversion of aminopropionitrile **34** to aminopropionaldehyde hydrate **36**·H<sub>2</sub>O. A – reaction mixture after 4 h; B – reaction mixture after 6 h; C – reaction mixture after 16 h; D – reaction mixture after 22 h; E – synthetic standard of aminopropionaldehyde hydrate **36**·H<sub>2</sub>O prepared as described in the next section.

*Procedure for the synthesis of a standard sample of cyanohydrin 38*



1-Amino-3,3-diethoxypropane (0.48 mL, 3.0 mmol) was added to aqueous HCl (3.0 M; 3.0 mL, 9.0 mmol) at 5 °C and the resultant mixture gradually warmed to room temperature over 1 h. After further stirring at room temperature for 2 h, an aliquot was taken to obtain a reference sample of the aldehyde hydrate **36**·H<sub>2</sub>O (Figs. S18-E & S19-A). The remaining mixture was diluted with ice-cold H<sub>2</sub>O (10 mL) and KCN (0.19 g, 2.9 mmol) was added. The solution was adjusted to pH 3 using 3.0 M HCl and stirred at room temperature for 6 h. The mixture was degassed for 2 h and the water was evaporated to leave a residual white solid (0.20 g, 67%) confirmed to be the desired cyanohydrin **38** by <sup>1</sup>H-NMR spectroscopy.

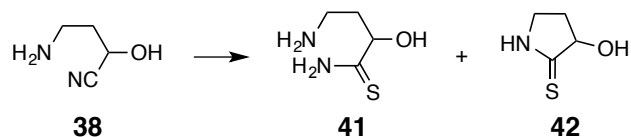
*Conversion of aminopropionaldehyde hydrate 36·H<sub>2</sub>O to the corresponding cyanohydrin 38*



Aminopropionaldehyde hydrate **36**·H<sub>2</sub>O in H<sub>2</sub>O (1.0 M; 0.1 mL, 0.1 mmol) and NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O (16 mg, 0.1 mmol) were dissolved in H<sub>2</sub>O/D<sub>2</sub>O (8:1, 0.9 mL) and the solution was adjusted to pH 7.0 using NaOH/HCl. KCN (7.8 mg, 0.12 mmol) in H<sub>2</sub>O (0.1 mL) at pH 7.0 was then added and conversion to the cyanohydrin was analysed by <sup>1</sup>H-NMR spectroscopy (Figure S19-B). The yield of the conversion to **38** was ~100%.

<sup>1</sup>H-NMR (400 MHz, H<sub>2</sub>O/D<sub>2</sub>O, 9:1) *aminopropionaldehyde hydrate 36*·H<sub>2</sub>O: δ 5.07 (t, *J* = 5.5 Hz, 1H), 2.96 (m, 2H), 1.81 (td, *J* = 7.0, 5.4 Hz, 2H); *4-amino-2-hydroxybutanenitrile 38*: δ 4.80 (dd, *J* = 7.4, 5.2 Hz, 1H), 3.19 (m, 2H), 2.22 (m, 2H).

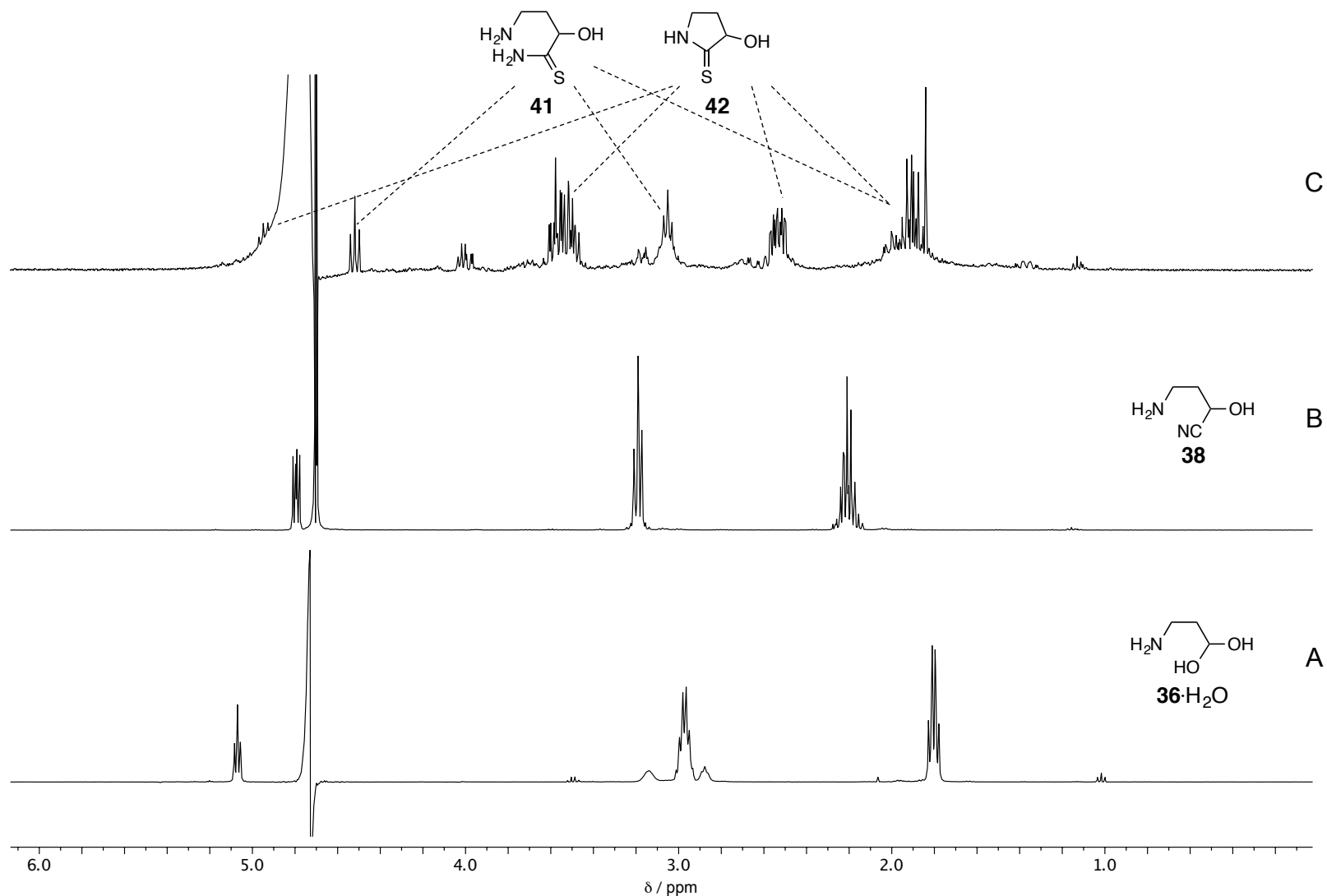
Conversion of cyanohydrin **38** to  $\alpha$ -hydroxythioamides **41** and **42**



Aminopropionaldehyde cyanohydrin **38** (10 mg, 0.1 mmol), NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O (39 mg, 0.25 mmol), and NaSH·xH<sub>2</sub>O (23 mg, assumed 60% NaSH, 0.25 mmol) were dissolved in H<sub>2</sub>O/D<sub>2</sub>O (9:1, 0.5 mL) and the solution was adjusted to pH 8.0 using NaOH/HCl. The reaction mixture was stirred at room temperature and conversion to the  $\alpha$ -hydroxythioamide mixture was analysed by <sup>1</sup>H-NMR spectroscopy (see Figure S19-C). The yields of conversion to **41** and **42** were 30% and 60%, respectively, with ~10% of unidentified by-products.

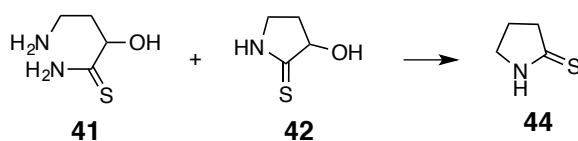
<sup>1</sup>H-NMR (400 MHz, H<sub>2</sub>O/D<sub>2</sub>O, 9:1) 4-amino-2-hydroxybutanethioamide **41**:  $\delta$  4.52 (t,  $J$  = 8.2 Hz, 1H), 3.05 (br t,  $J$  = 7.4 Hz, 2H), 1.93 (m, 2H); 3-hydroxypyrrolidine-2-thione **42**:  $\delta$  3.54 (m, 2H), 2.52 (m, 1H), 1.93 (m, 1H), other 1H not assigned due to HOD suppression of the signal.





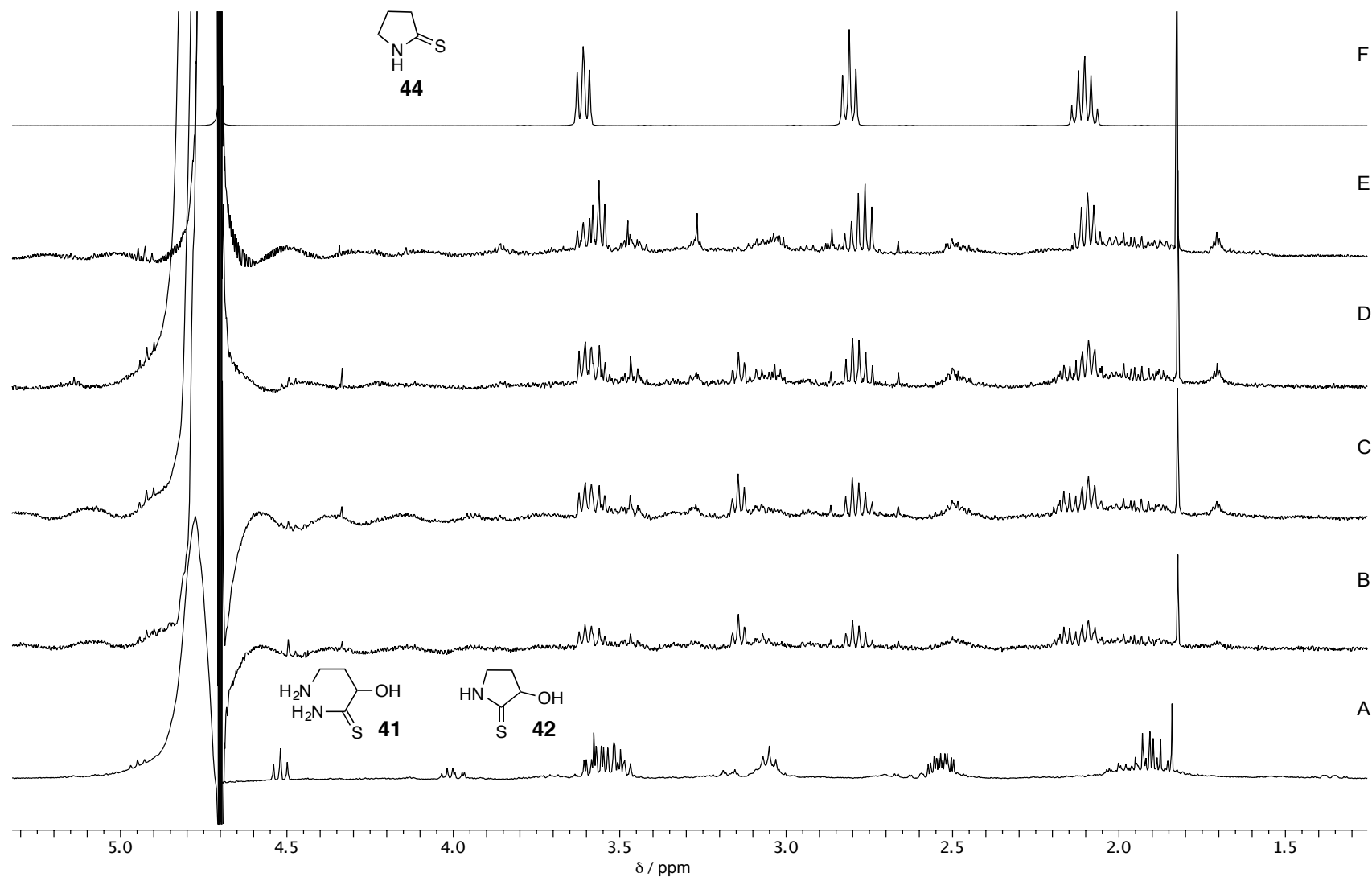
**Figure S19.** <sup>1</sup>H-NMR analysis of the conversion of aminopropionaldehyde **36** to α-hydroxythioamides **41** and **42** by way of cyanohydrin **38**. A (H<sub>2</sub>O/D<sub>2</sub>O, 9:1) – synthetic standard of aminopropionaldehyde hydrate **36·H<sub>2</sub>O**; B (D<sub>2</sub>O) – isolated aminopropionaldehyde cyanohydrin **38**; C (H<sub>2</sub>O/D<sub>2</sub>O, 9:1) – reaction mixture of aminopropionaldehyde cyanohydrin **38** and NaSH at pH 8.0 giving a mixture of linear **41** and cyclic **42** α-hydroxythioamide.

Conversion of  $\alpha$ -hydroxythioamides **41** and **42** to pyrrolidine-2-thione **44** by photoredox chemistry



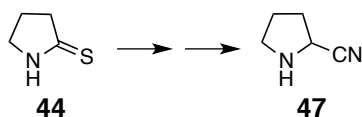
The mixture of  $\alpha$ -hydroxythioamides **41** and **42** (0.1 M; 0.3 mL, 0.03 mmol) and  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  (47 mg, 0.30 mmol) were dissolved in degassed  $\text{H}_2\text{O}/\text{D}_2\text{O}$  (5:1, 2.4 mL) and the solution was adjusted to pH 6.5 using degassed  $\text{NaOH}/\text{HCl}$ .  $\text{NaSH} \cdot x\text{H}_2\text{O}$  (14 mg, assumed 60%  $\text{NaSH}$ , 0.15 mmol) was added and upon dissolution of the  $\text{NaSH}$  the mixture was adjusted to pH 6.9 using degassed  $\text{NaOH}/\text{HCl}$ . The solution was then added to a quartz cuvette containing  $\text{CuCN}$  (ca. 0.5 mg), whereupon a black precipitate formed, and the cuvette was immediately sealed. The cuvette was placed in a Rayonet reactor and irradiated. A sample of the reaction was taken at different time points (3, 5, 7, 12 h) and analysed by  $^1\text{H}$ -NMR spectroscopy (Figure S20). The yield of the conversion to **44** after 12 h was 78%.

$^1\text{H}$ -NMR (400 MHz,  $\text{H}_2\text{O}/\text{D}_2\text{O}$ , 9:1) 4-amino-2-hydroxybutanethioamide **41**:  $\delta$  4.52 (t,  $J = 8.2$  Hz, 1H), 3.05 (br t,  $J = 7.4$  Hz, 2H), 1.93 (m, 2H); 3-hydroxypyrrolidine-2-thione **42**:  $\delta$  3.54 (m, 2H), 2.52 (m, 1H), 1.93 (m, 1H), other 1H not assigned due to HOD suppression of the signal; pyrrolidine-2-thione **44**:  $\delta$  3.61 (t,  $J = 7.4$  Hz, 2H), 2.81 (t,  $J = 8.0$  Hz, 2H), 2.11 (app p, 2H).



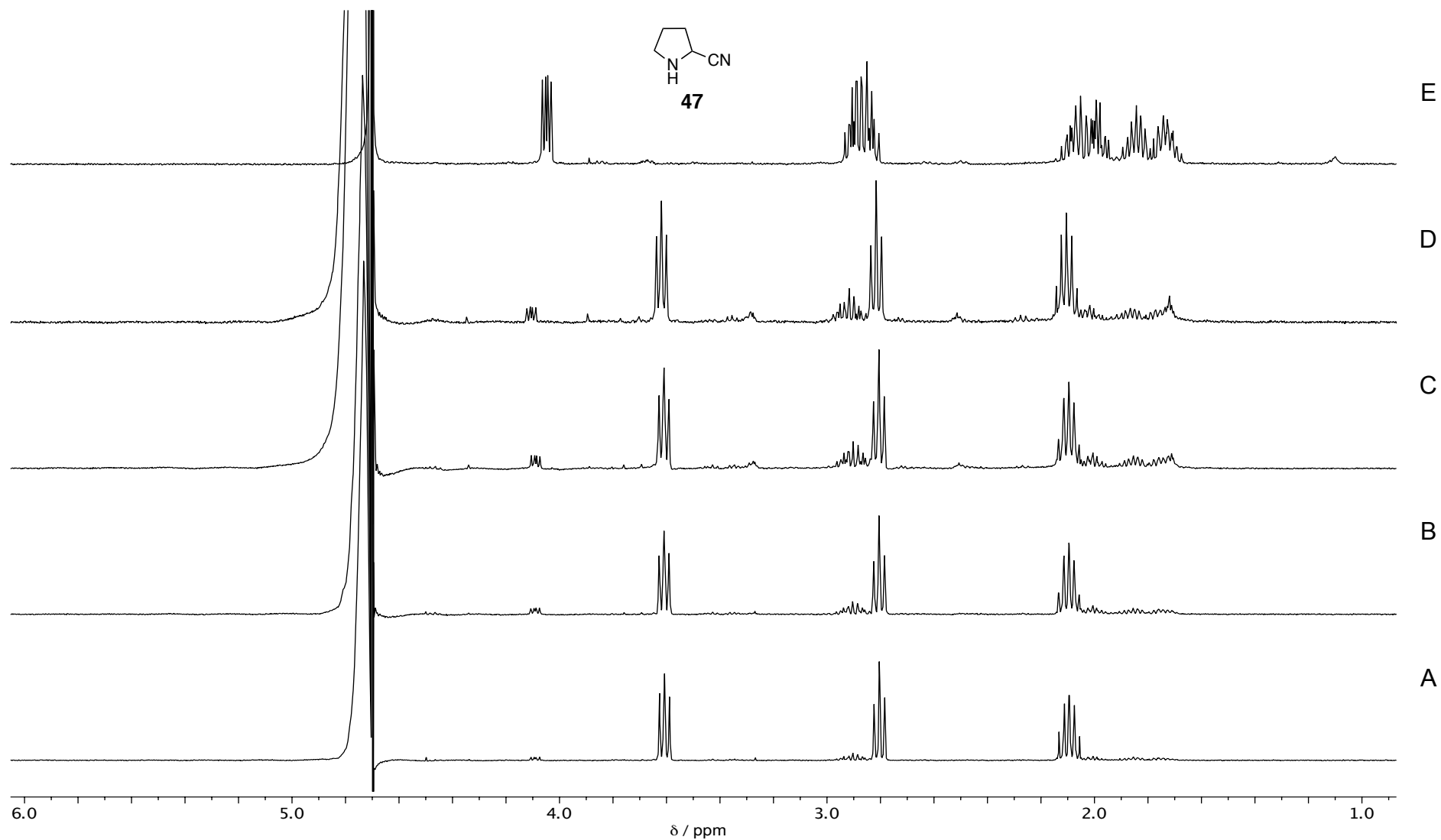
**Figure S20.** <sup>1</sup>H-NMR analysis (H<sub>2</sub>O/D<sub>2</sub>O, 9:1) of the photochemical conversion of α-hydroxythioamides **41** and **42** to the corresponding cyclic deoxygenated pyrrolidine-2-thione **44**. A – mixture of linear and cyclic α-hydroxythioamides **41** and **42**; B – reaction mixture after 3 h; C – reaction mixture after 5 h; D – reaction mixture after 7 h; E – reaction mixture after 12 h; F – commercially available pyrrolidine-2-thione **44**.

*Conversion of pyrrolidine-2-thione 44 to pyrrolidine-2-carbonitrile 47 by photoredox chemistry*



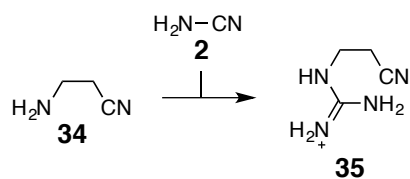
Pyrrolidine-2-thione **44** (3.0 mg, 0.03 mmol) and  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  (31 mg, 0.20 mmol) were dissolved in degassed  $\text{H}_2\text{O}/\text{D}_2\text{O}$  (6:1, 2.8 mL) and the solution was adjusted to pH 6.5 using degassed  $\text{NaOH}/\text{HCl}$ .  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  (43 mg, 0.18 mmol) and  $\text{KSCN}$  (9.0 mg, 0.09 mmol) were added and upon dissolution of the  $\text{NaSH}/\text{KSCN}$  the mixture was adjusted to pH 6.9 using degassed  $\text{NaOH}/\text{HCl}$ . The solution was then added to a quartz cuvette containing  $\text{CuCN}$  (ca. 0.5 mg), whereupon a black precipitate formed, and the cuvette was immediately sealed. The cuvette was placed in a Rayonet reactor and irradiated.  $\text{HCN}$  (1.0 M; 10  $\mu\text{L}$ , 0.01 mmol) at pH 7.2 was added periodically (1, 2, 4, 5.5 h) before a sample was taken for analysis in order to observe the reduction product as the  $\alpha$ -aminonitrile **47**. Samples of the reaction were taken at different time points (1, 2, 4, 5.5 h) and analysed by  $^1\text{H}$ -NMR spectroscopy (Figure S21). The yield of the conversion to **47** after 5.5 h was 32%.

$^1\text{H}$ -NMR (400 MHz,  $\text{H}_2\text{O}/\text{D}_2\text{O}$ , 9:1) *pyrrolidine-2-thione 44*:  $\delta$  3.61 (t,  $J = 7.4$  Hz, 2H), 2.81 (t,  $J = 8.0$  Hz, 2H), 2.11 (app p, 2H); *pyrrolidine-2-carbonitrile 47*:  $^1\text{H}$ -NMR (400 MHz,  $\text{H}_2\text{O}/\text{D}_2\text{O}$ , 9:1)  $\delta$  4.14 (dd,  $J = 8.1, 4.8$  Hz, 1H), 2.97 (m, 2H), 2.11 (m, 2H), 1.93 (m, 1H), 1.82 (m, 1H).



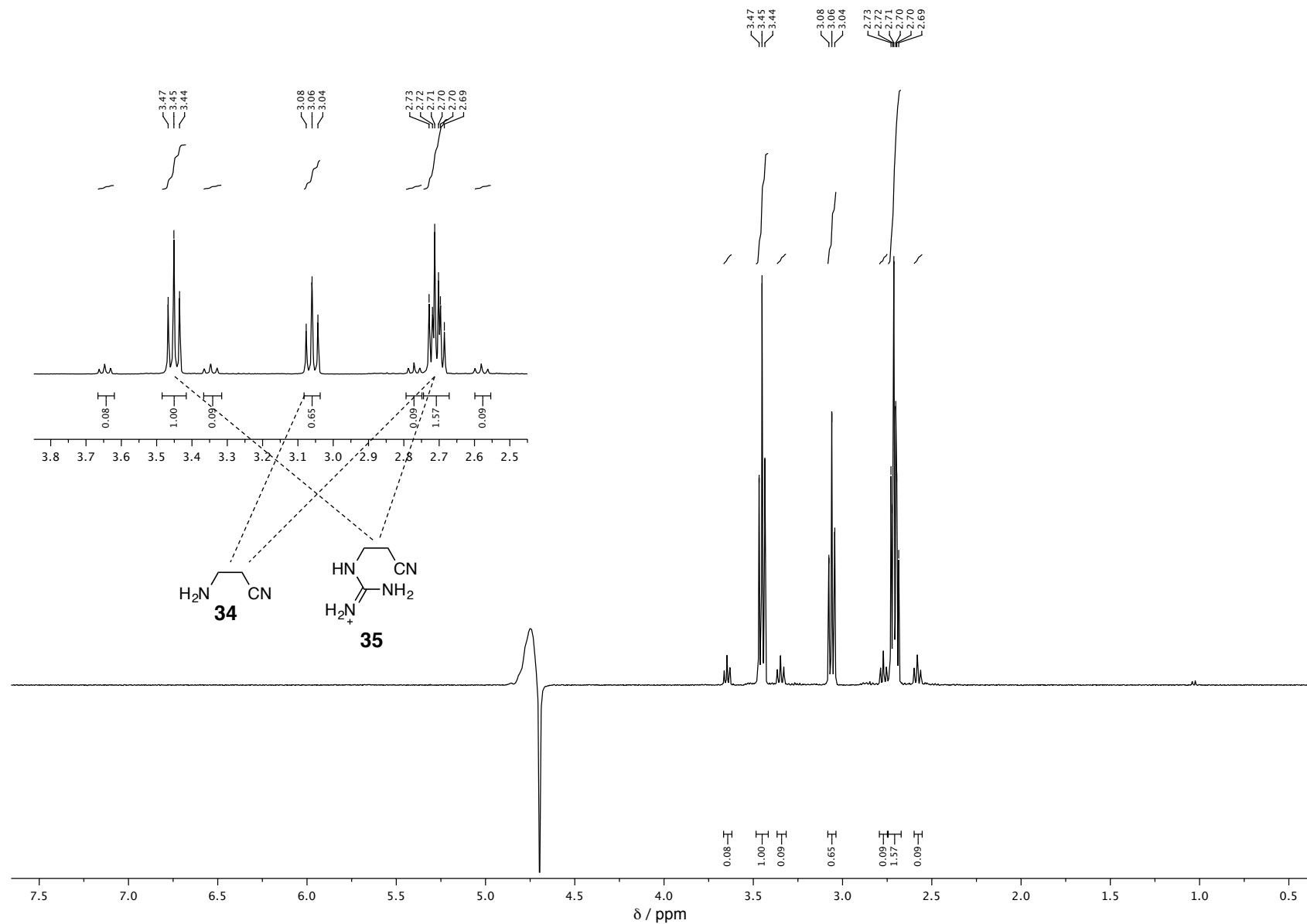
**Figure S21.** <sup>1</sup>H-NMR analysis (H<sub>2</sub>O/D<sub>2</sub>O, 9:1) of the photochemical conversion of pyrrolidine-2-thione **44** to α-aminonitrile **47**. A – reaction mixture after 1 h; B – reaction mixture after 2 h; C – reaction mixture after 4 h; D – reaction mixture after 5.5 h; E – synthetic standard of α-aminonitrile **47**.<sup>4</sup>

*Guanidinylation of aminopropionitrile 34 with cyanamide 2 and phosphate*



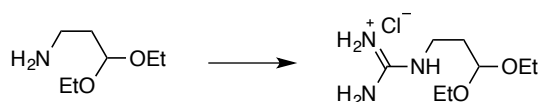
Aminopropionitrile **34** (73  $\mu$ L, 1.0 mmol), cyanamide **2** (84 mg, 2.0 mmol), and NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O (156 mg, 1.0 mmol) were dissolved in H<sub>2</sub>O/D<sub>2</sub>O (9:1, 1 mL). The pH of the solution was adjusted to 8.0 using NaOH/HCl and the reaction mixture stirred at 50 °C. Samples of the reaction were taken at different time points and analysed by <sup>1</sup>H-NMR spectroscopy. After 22h, the aminopropionitrile **34** had been converted to the corresponding guanidinylated product **35** (55%), with residual aminopropionitrile **34** (35%), and minor by-products (~10%) arising most probably from hydrolysis of the nitrile group (Figure S22).

<sup>1</sup>H-NMR (400 MHz, H<sub>2</sub>O/D<sub>2</sub>O, 9:1) *aminopropionitrile 34*:  $\delta$  3.06 (t,  $J$  = 6.6 Hz, 2H), 2.70 (t,  $J$  = 6.6 Hz, 2H); *amino((2-cyanoethyl)amino)methaniminium 35*:  $\delta$  3.45 (t,  $J$  = 6.5 Hz, 2H), 2.71 (t,  $J$  = 6.4 Hz, 2H).



**Figure S22.**  $^1\text{H-NMR}$  analysis ( $\text{H}_2\text{O}/\text{D}_2\text{O}$ , 9:1) of the guanidinylation of aminopropionitrile **34** to give **35**.

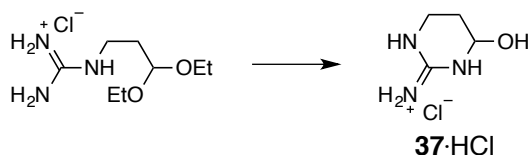
*Procedure for the synthesis of amino((3,3-diethoxypropyl)amino)methaniminium hydrochloride*



3,3-Diethoxypropan-1-amine (0.32 mL, 2.0 mmol) and triethylamine (0.70 mL, 4.0 mmol) were dissolved in MeOH/H<sub>2</sub>O (1:1, 8.0 mL), and *O*-methylisourea hydrochloride (0.44 g, 4.0 mmol) was added in one portion. The reaction mixture was stirred at room temperature for 15 h. The solvent was evaporated and the residue purified by column chromatography (EtOAc/CHCl<sub>3</sub>/MeOH; 1:1:0 to 1:1:0.1 to 1:1:0.2) to give the desired product (0.25 g, 66%) as a colourless gum (Figure S23).

*Amino((3,3-diethoxypropyl)amino)methaniminium hydrochloride*: <sup>1</sup>H-NMR (400 MHz, D<sub>2</sub>O) δ 4.63 (t, *J* = 5.7 Hz, 1H), 3.67 (dq, *J* = 9.7, 7.1 Hz, 2H), 3.53 (dq, *J* = 9.7, 7.1 Hz, 2H), 3.19 (t, *J* = 6.7 Hz, 2H), 1.84 (m, 2H), 1.11 (t, *J* = 7.1 Hz, 6H).

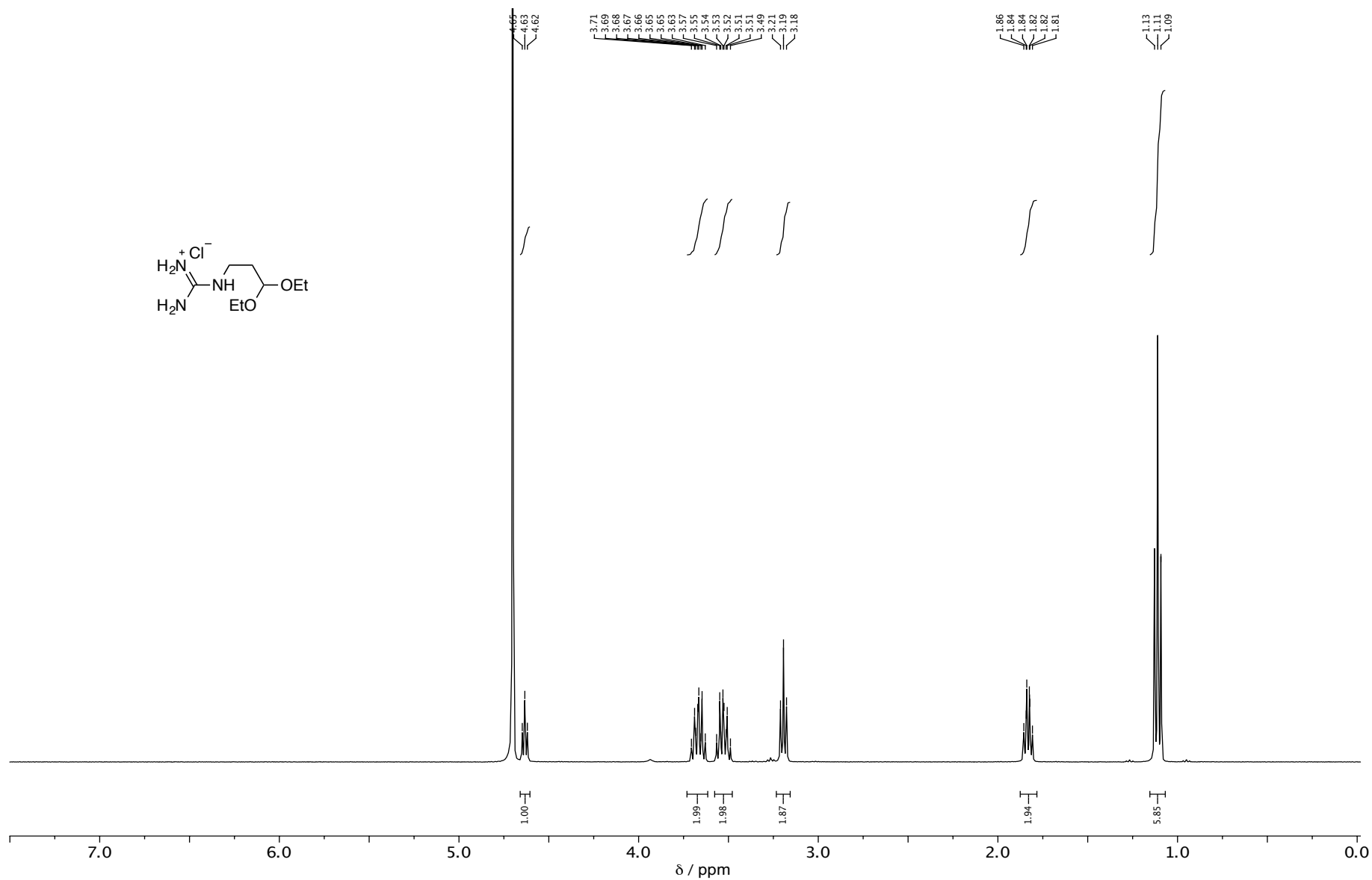
*Procedure for the synthesis of a standard sample of 4-hydroxytetrahydropyrimidin-2(1H)-iminium hydrochloride 37·HCl*



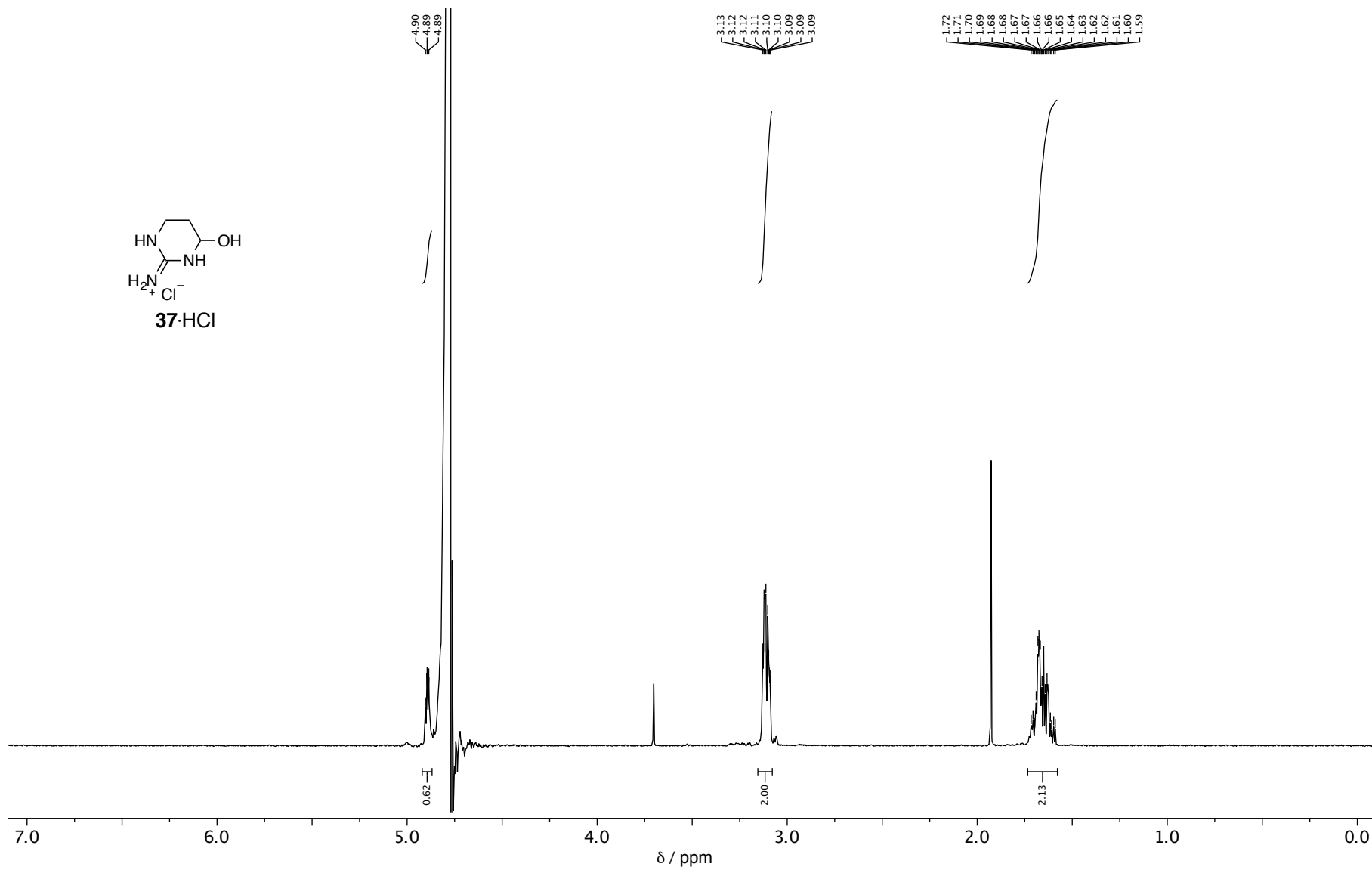
Amino((3,3-diethoxypropyl)amino)methaniminium hydrochloride (19 mg, 0.1 mmol) was treated with aqueous HCl (2.0 M; 0.8 mL, 1.6 mmol) and the reaction mixture stirred at room temperature. After 1 h, H<sub>2</sub>O (0.8 mL) was added and the mixture subjected to slow evaporation (45-50 mbar, 36 °C, 45 min) to remove the residual EtOH. The mixture was then analysed by <sup>1</sup>H-NMR spectroscopy to confirm clean formation of a standard sample of **37·HCl** (Figure S24).

*4-Hydroxytetrahydropyrimidin-2(1H)-iminium hydrochloride 37·HCl*: <sup>1</sup>H-NMR (400 MHz, H<sub>2</sub>O/D<sub>2</sub>O, 9:1) δ 4.89 (m, 1H), 3.10 (m, 2H), 1.66 (m, 2H).



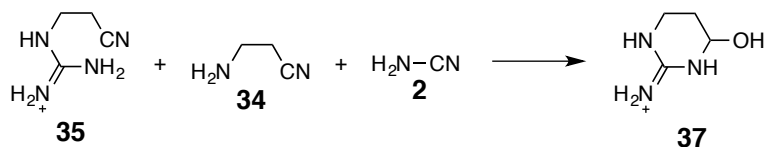


**Figure S23.** <sup>1</sup>H-NMR spectrum of amino((3,3-diethoxypropyl)amino)methaniminium hydrochloride.

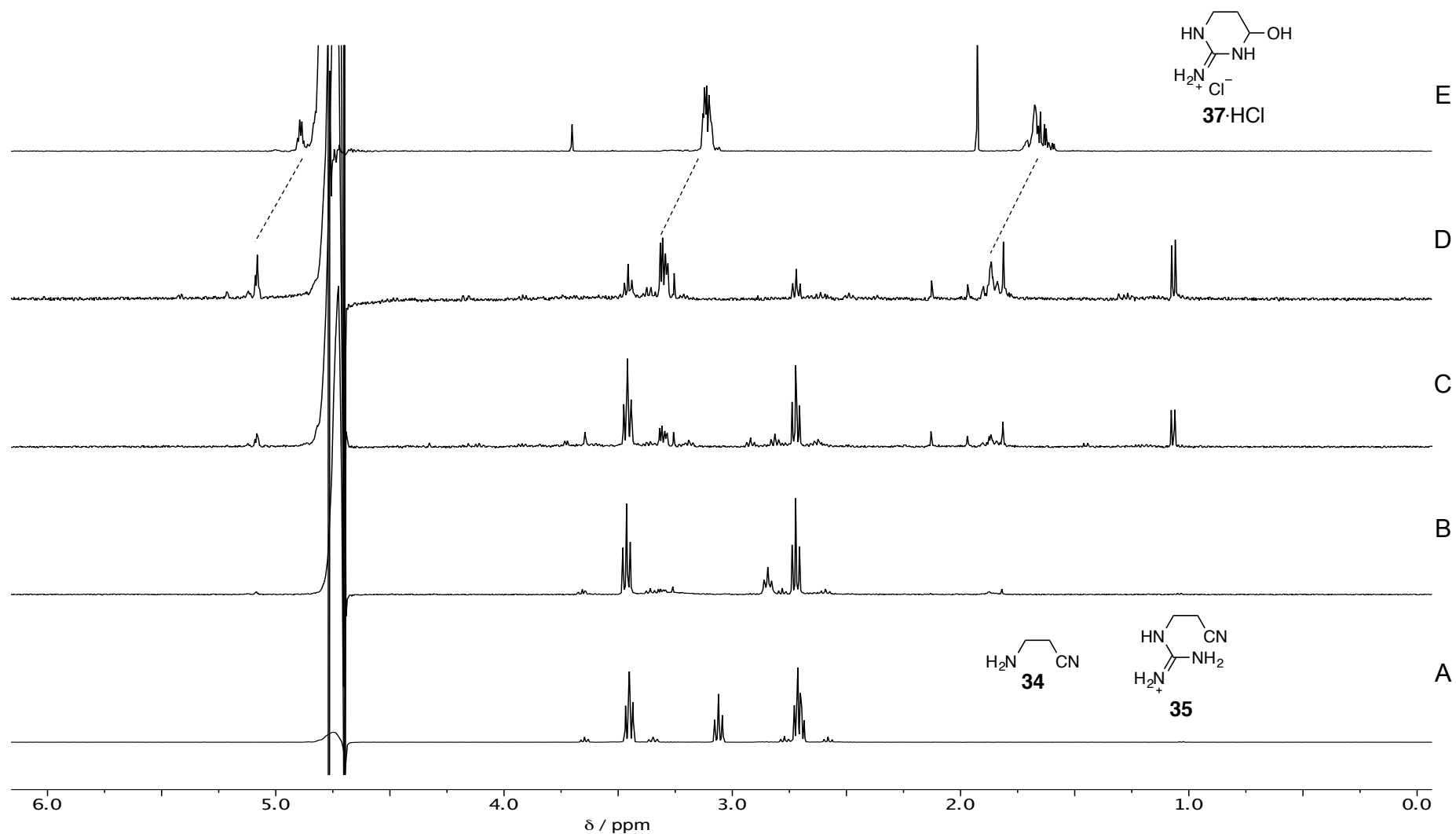


**Figure S24.** <sup>1</sup>H-NMR spectrum of 4-hydroxytetrahydropyrimidin-2(1H)-iminium hydrochloride **37**·HCl.

*Conversion of a mixture of aminopropionitrile 34 and guanidylated-aminopropionitrile 35 to 4-hydroxy-tetrahydropyrimidin-2(1H)-iminium 37·HCl by photoredox chemistry*

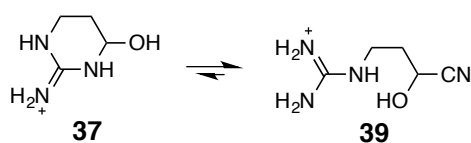


The previously generated mixture (see page S46) of **35** and **34** (1.0 M; 30  $\mu$ L, 0.03 mmol) and  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  (47 mg, 0.30 mmol) were dissolved in degassed  $\text{H}_2\text{O}/\text{D}_2\text{O}$  (6:1, 2.8 mL) and the solution was adjusted to pH 6.5 using degassed  $\text{NaOH}/\text{HCl}$ .  $\text{NaSH} \cdot x\text{H}_2\text{O}$  (21 mg, assumed 60%  $\text{NaSH}$ , 0.23 mmol) and  $\text{KSCN}$  (9.0 mg, 0.09 mmol) was added and upon dissolution of the  $\text{NaSH}/\text{KSCN}$  the mixture was adjusted to pH 6.9 using degassed  $\text{NaOH}/\text{HCl}$ . The solution was then added to a quartz cuvette containing  $\text{CuCN}$  (ca. 1.5 mg), whereupon a black precipitate formed, and the cuvette was immediately sealed. The cuvette was placed in a Rayonet reactor and irradiated. Samples of the reaction were taken at different time points (3, 15 h, 2 d) and analysed by  $^1\text{H}$ -NMR spectroscopy (Figure S25). The yield of the conversion to **37** after 2 d was 77%.



**Figure S25.**  $^1\text{H-NMR}$  analysis ( $\text{H}_2\text{O}/\text{D}_2\text{O}$ , 9:1) of the photochemical conversion of a mixture of aminopropionitrile **34** and guanidylated-aminopropionitrile **35** to 4-hydroxy-tetrahydropyrimidin-2(1H)-iminium **37**. A – mixture of aminopropionitrile **34** and guanidylated-aminopropionitrile **35** generated using cyanamide **2** and phosphate after 22 h (see page S46); B – reaction mixture after 3 h; C – reaction mixture after 15 h; D – reaction mixture after 2 d; E – synthetic standard of 4-hydroxy-tetrahydropyrimidin-2(1H)-iminium hydrochloride **37**·HCl.

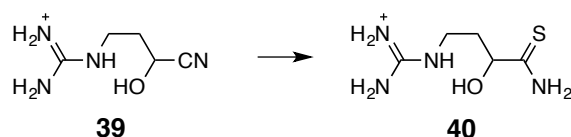
### Conversion of hemiaminal **37** to the corresponding cyanohydrin **39**



A mixture of an aqueous solution of hemiaminal **37** (0.067 M; 1.35 mL, 0.09 mmol), KCN (23 mg, 0.36 mmol), and D<sub>2</sub>O (0.15 mL) was adjusted to pH 7.1 and stirred at room temperature. After 20 h, further KCN (23 mg, 0.36 mmol) was added and the pH readjusted to 7.1 and stirring continued at room temperature. After 9 h, analysis by <sup>1</sup>H-NMR spectroscopy showed ~50% conversion to **39**, therefore further KCN (12 mg, 0.18 mmol) was added and the pH readjusted to 7.1 and stirring continued at room temperature. After 6 d, conversion to the cyanohydrin **39** was analysed by <sup>1</sup>H-NMR spectroscopy (Figure S26, A-C). The yield of the conversion to **39** after 6 d was 77%.

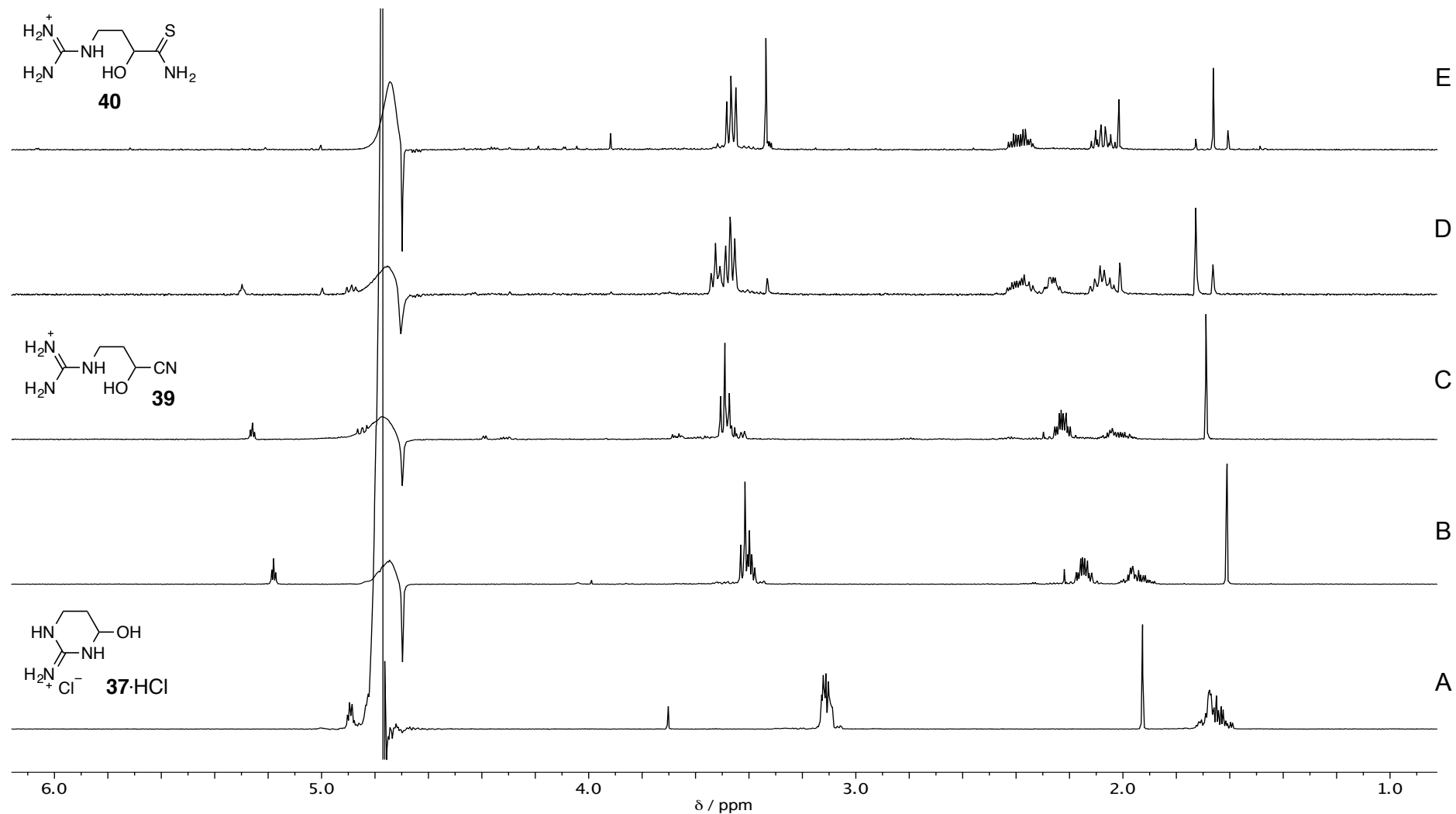
*Amino((3-cyano-3-hydroxypropyl)amino)methaniminium 39*: <sup>1</sup>H-NMR (400 MHz, H<sub>2</sub>O/D<sub>2</sub>O, 9:1) δ 3.49 (t, *J* = 6.6 Hz, 2H), 2.22 (m, 2H), other 1H not assigned due to HOD suppression of the signal.

### Conversion of cyanohydrin **39** to α-hydroxythioamide **40**



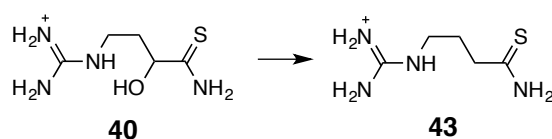
A mixture of a solution of cyanohydrin **39** (0.033M; 1.8 mL, 0.06 mmol) and NaSH.xH<sub>2</sub>O (28 mg, assumed 60% NaSH, 0.30 mmol) was adjusted to pH 8.2 and stirred at room temperature. After 22 h, analysis by <sup>1</sup>H-NMR spectroscopy showed some formation of **40** along with residual **39** and **37** (Figure S26-D; **40**:**39**:**37** = ~29:62:9), therefore further NaSH.xH<sub>2</sub>O (17 mg, assumed 60% NaSH, 0.18 mmol) and KCN (4 mg, 0.06 mmol) were added, the pH readjusted to 8.2 and stirring continued at room temperature. After 4 d, conversion to α-hydroxythioamide **40** was analysed by <sup>1</sup>H-NMR spectroscopy and shown to be complete (Figure S26-E). The yield of the conversion to **40** after 4 d was ~100%.

*Amino((4-amino-3-hydroxy-4-thioxobutyl)amino)methaniminium 40*: <sup>1</sup>H-NMR (400 MHz, H<sub>2</sub>O/D<sub>2</sub>O, 9:1) δ 3.47 (dd, *J* = 7.4, 6.2 Hz, 2H), 2.39 (dtd, *J* = 14.5, 7.4, 3.6 Hz, 1H), 2.07 (ddt, *J* = 14.6, 8.5, 6.2 Hz, 1H), other 1H not assigned due to HOD suppression of the signal.



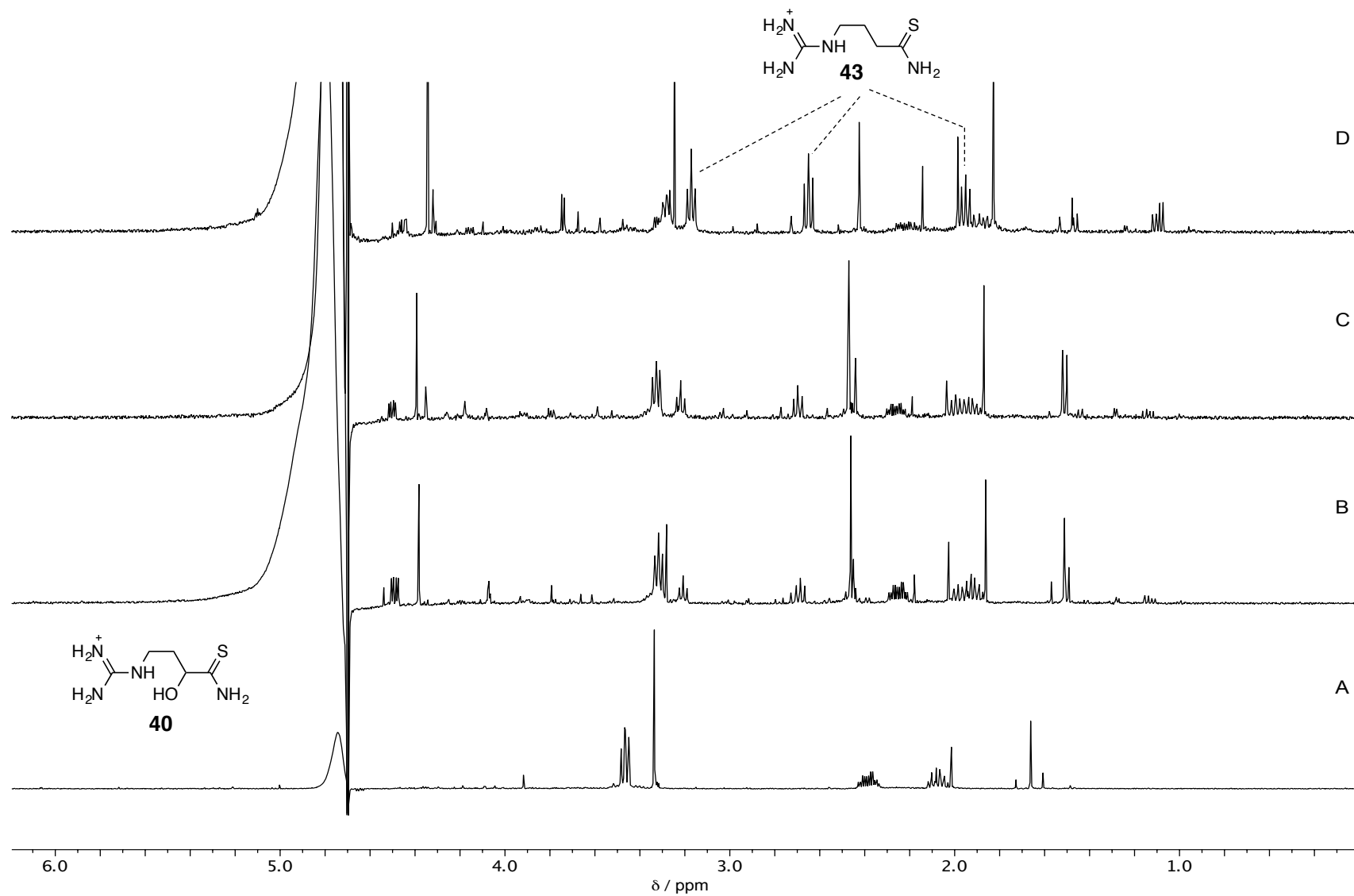
**Figure S26.** <sup>1</sup>H-NMR analysis (H<sub>2</sub>O/D<sub>2</sub>O, 9:1) of the conversion of 4-hydroxy-tetrahydropyrimidin-2(1H)-iminium hydrochloride **37·HCl** to  $\alpha$ -hydroxythioamide **40** by the way of cyanohydrin **39**. A – synthetic standard of 4-hydroxy-tetrahydropyrimidin-2(1H)-iminium hydrochloride **37·HCl**; B – reaction with HCN at pH 7.1 after 20 h; C – reaction mixture with HCN at pH 7.1 after 6 d giving predominantly the corresponding cyanohydrin **39**; D – reaction with NaSH at pH 8.2 after 22 h; E – reaction with NaSH at pH 8.2 after 4 d giving the corresponding  $\alpha$ -hydroxythioamide **40**.

Conversion of  $\alpha$ -hydroxythioamide **40** to thioamide **43** by photoredox chemistry



A solution of  $\alpha$ -hydroxythioamide **40** (0.033 M; 0.9 mL, 0.03 mmol) and  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  (47 mg, 0.30 mmol) were dissolved in degassed  $\text{H}_2\text{O}/\text{D}_2\text{O}$  (5:1, 2.4 mL) and the mixture was adjusted to pH 6.5 using degassed  $\text{NaOH}/\text{HCl}$ .  $\text{NaSH} \cdot x\text{H}_2\text{O}$  (18 mg, assumed 60%  $\text{NaSH}$ , 0.18 mmol) and  $\text{KSCN}$  (9.0 mg, 0.09 mmol) were added and upon dissolution of the  $\text{NaSH}/\text{KSCN}$  the mixture was adjusted to pH 6.9 using degassed  $\text{NaOH}/\text{HCl}$ . The solution was then added to a quartz cuvette containing  $\text{CuCN}$  (ca. 0.5 mg), whereupon a black precipitate formed, and the cuvette was immediately sealed. The cuvette was placed in a Rayonet reactor and irradiated. Samples of the reaction were taken at different time points (4, 8, 15 h) and analysed by  $^1\text{H}$ -NMR spectroscopy (Figure S27). The yield of the conversion to **43** after 15 h was 71%.

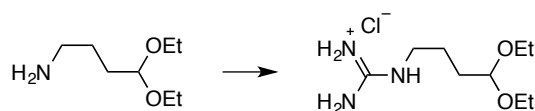
$^1\text{H}$ -NMR (400 MHz,  $\text{H}_2\text{O}/\text{D}_2\text{O}$ , 9:1) *amino((4-amino-3-hydroxy-4-thioxobutyl)amino)-methaniminium 40*:  $\delta$  3.47 (dd,  $J = 7.4, 6.2$  Hz, 2H), 2.39 (dtd,  $J = 14.5, 7.4, 3.6$  Hz, 1H), 2.07 (ddt,  $J = 14.6, 8.5, 6.2$  Hz, 1H), other 1H not assigned due to HOD suppression of the signal; *amino((4-amino-4-thioxobutyl)amino)methaniminium 43*:  $\delta$  3.17 (t,  $J = 6.9$  Hz, 2H), 2.65 (t,  $J = 7.4$  Hz, 2H), 1.96 (m, 2H).



**Figure S27.**  $^1\text{H-NMR}$  analysis ( $\text{H}_2\text{O}/\text{D}_2\text{O}$ , 9:1) of the photochemical conversion of  $\alpha$ -hydroxythioamide **40** to the corresponding deoxygenated thioamide **43**. A –  $\alpha$ -hydroxythioamide **40** produced from hemiaminal **37** (see Figure S26); B – reaction mixture after 4 h; C – reaction mixture after 8 h; D – reaction mixture after 15 h.



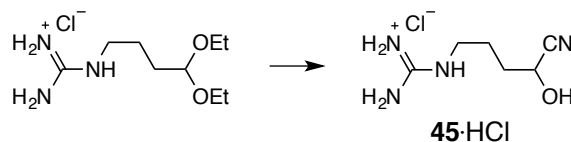
*Procedure for the synthesis of amino((4,4-diethoxybutyl)amino)methaniminium hydrochloride*



4,4-Diethoxybutan-1-amine (0.54 mL, 3.0 mmol) and triethylamine (0.42 mL, 3.0 mmol) were dissolved in MeOH (45 mL), and *O*-methylisourea hydrochloride (0.39 g, 3.5 mmol) was added in one portion. The reaction mixture was stirred at room temperature for 24 h. The solvent was evaporated and the residue purified by column chromatography (EtOAc/CHCl<sub>3</sub>/MeOH; 1:1:0 to 1:1:0.2) to give the desired product (0.41 g, 57%) as a colourless gum (Figure S28).

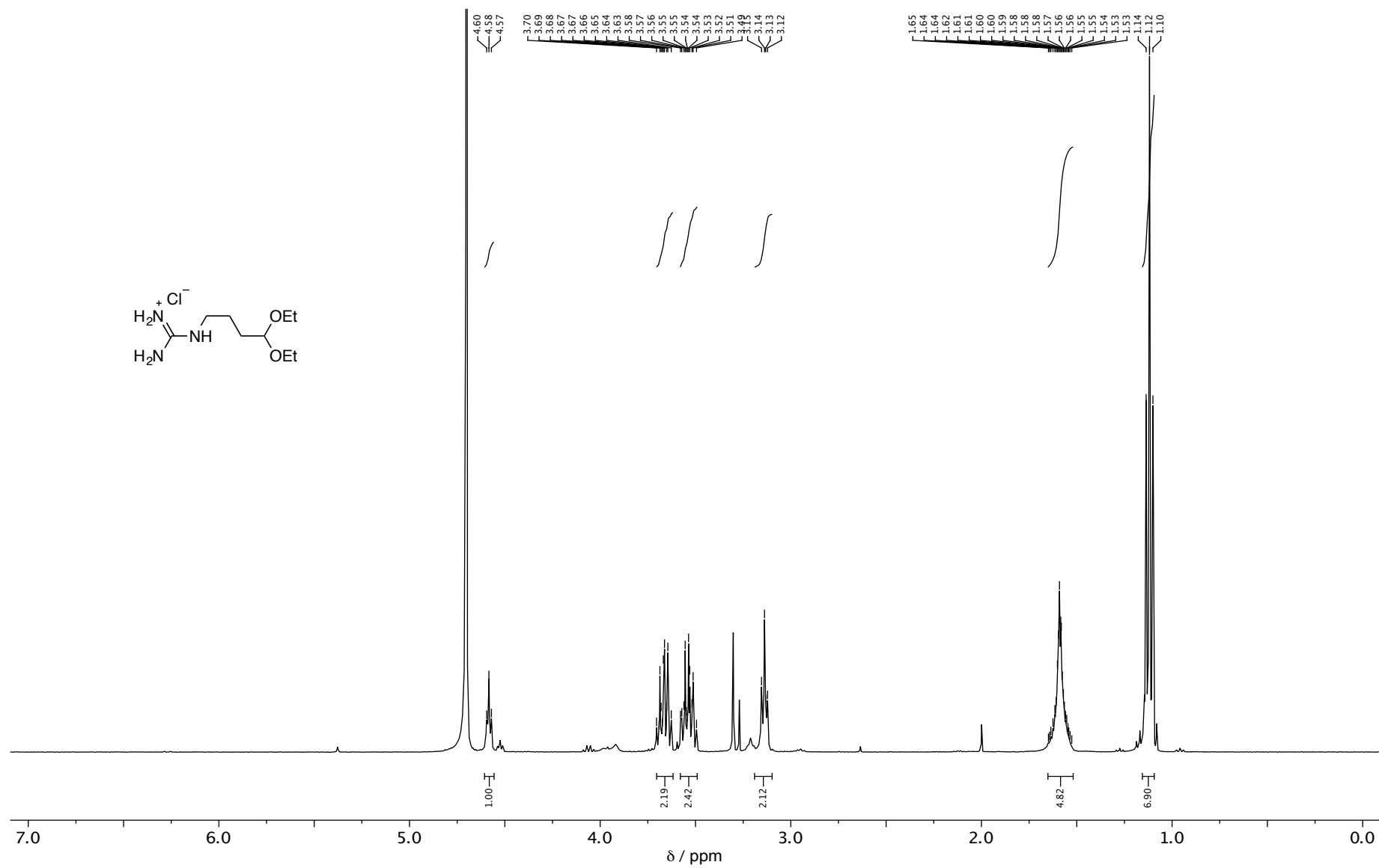
*Amino((4,4-diethoxybutyl)amino)methaniminium hydrochloride*: <sup>1</sup>H-NMR (400 MHz, D<sub>2</sub>O) δ 4.58 (t, *J* = 5.1 Hz, 1H), 3.67 (dq, *J* = 9.6, 7.1 Hz, 2H), 3.53 (dq, *J* = 9.5, 7.1 Hz, 2H), 3.14 (t, *J* = 6.3 Hz, 2H), 1.58 (m, 4H), 1.12 (t, *J* = 7.1 Hz, 6H).

*Procedure for the synthesis of a standard sample of cyanohydrin 45*

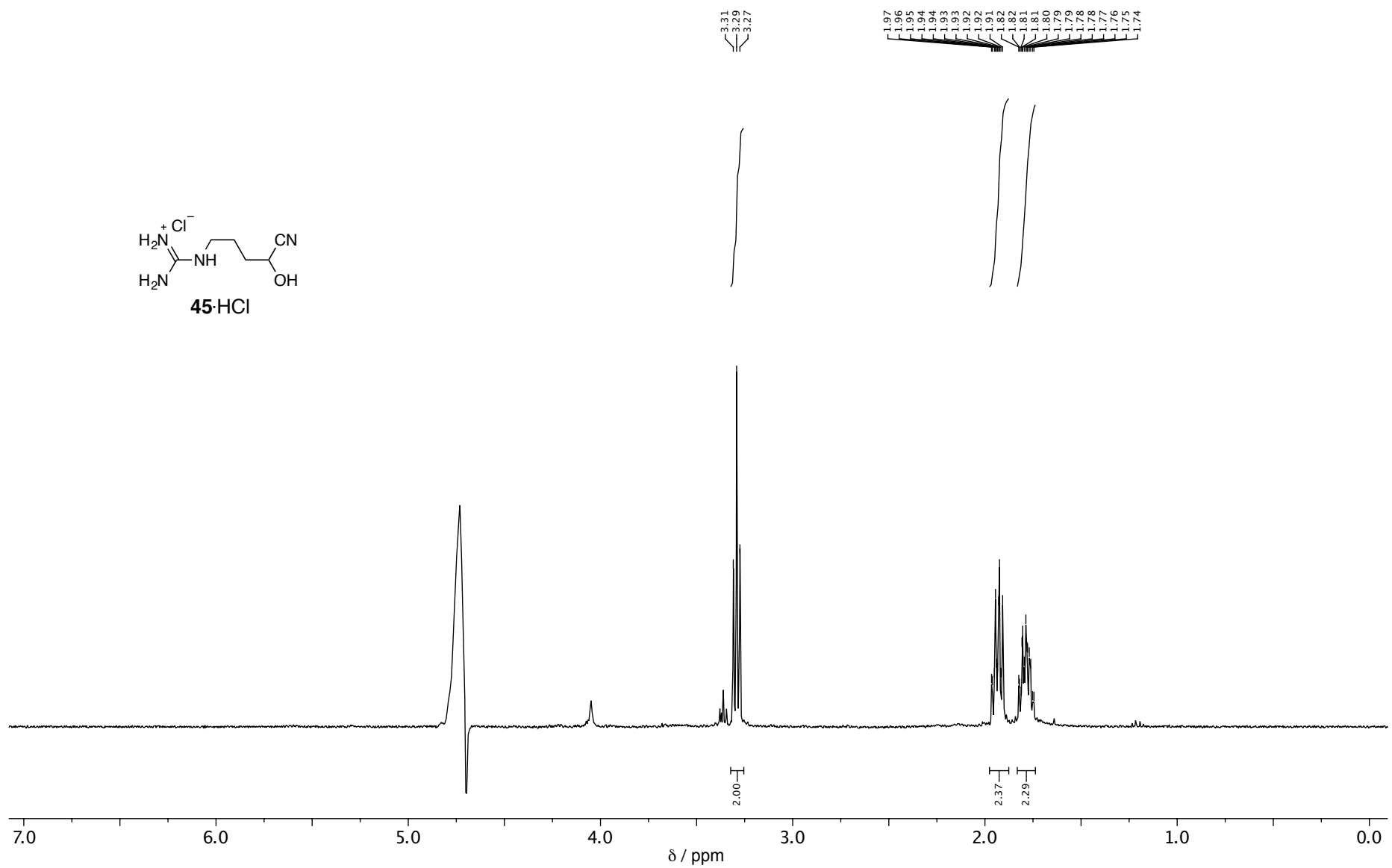


*Amino((4,4-diethoxybutyl)amino)methaniminium hydrochloride* (102 mg, 0.5 mmol) was treated with aqueous HCl (1.2 M; 2.5 mL, 3.0 mmol) and the reaction mixture stirred at room temperature. After 1 h, KCN (40 mg, 0.6 mmol) was added, the pH adjusted to 7.2, and the mixture stirred at room temperature. After 16 h, analysis by <sup>1</sup>H-NMR spectroscopy confirmed clean formation of a standard sample of *45·HCl* (Figure S29).

*Amino((4-cyano-4-hydroxybutyl)amino)methaniminium hydrochloride 45·HCl*: <sup>1</sup>H-NMR (400 MHz, H<sub>2</sub>O/D<sub>2</sub>O, 9:1) δ 3.29 (t, *J* = 6.9 Hz, 2H), 1.94 (m, 2H), 1.79 (m, 2H), other 1H not assigned due to HOD suppression of the signal.

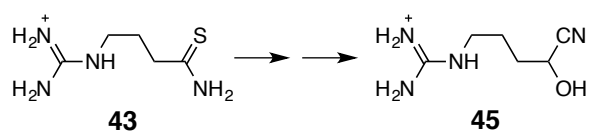


**Figure S28.**  $^1\text{H}$ -NMR spectrum of amino((4,4-diethoxybutyl)amino)methaniminium hydrochloride.



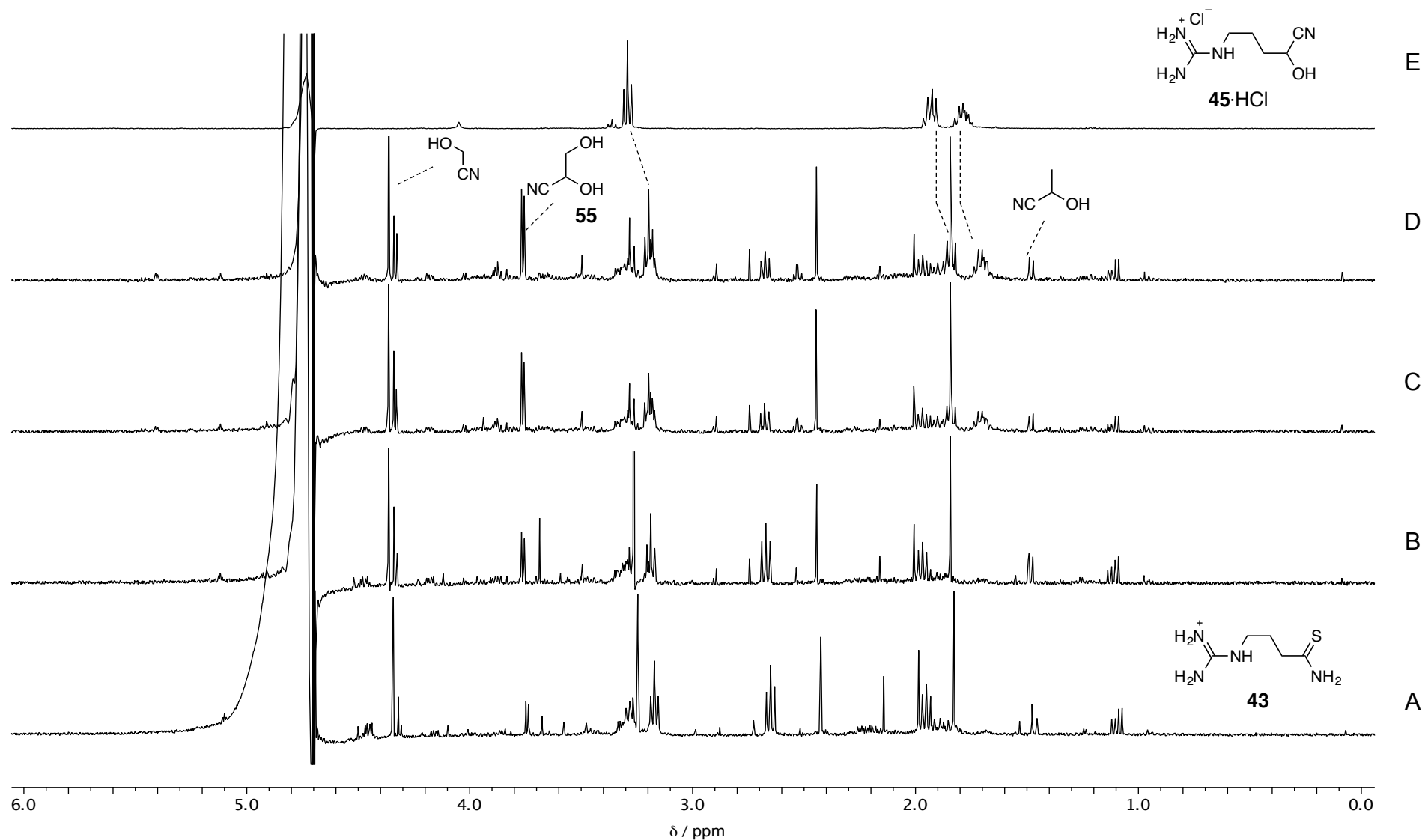
**Figure S29.** <sup>1</sup>H-NMR spectrum of amino((4-cyano-4-hydroxybutyl)amino)methaniminium hydrochloride **45·HCl**.

Conversion of thioamide **43** to cyanohydrin **45** by photoredox chemistry



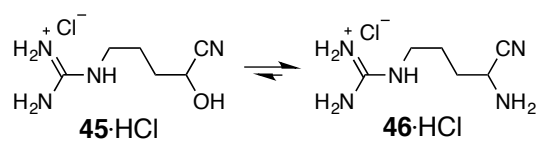
A solution of thioamide **43** (0.03 M; 1.0 mL, 0.03 mmol) and NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O (31 mg, 0.20 mmol) were dissolved in degassed H<sub>2</sub>O/D<sub>2</sub>O (6:1, 2.8 mL) and the mixture was adjusted to pH 6.5 using degassed NaOH/HCl. NaSH·xH<sub>2</sub>O (16 mg, assumed 60% NaSH, 0.16 mmol) and KSCN (7.0 mg, 0.07 mmol) were added and upon dissolution of the NaSH/KSCN the mixture was adjusted to pH 6.9 using degassed NaOH/HCl. The solution was then added to a quartz cuvette containing CuCN (ca. 0.5 mg), whereupon a black precipitate formed, and the cuvette was immediately sealed. The cuvette was placed in a Rayonet reactor and irradiated. HCN (1.0 M; 10 μL, 0.01 mmol) at pH 7.0 was added periodically (1.5, 6, 12 h) before a sample was taken for analysis in order to observe the reduction product as the desired cyanohydrin **45**. A sample of the reaction was taken at different time points (1.5, 6, 12 h) and analysed by <sup>1</sup>H-NMR spectroscopy (Figure S30). The yield of the conversion to **45** after 12 h was 76%.

<sup>1</sup>H-NMR (400 MHz, H<sub>2</sub>O/D<sub>2</sub>O, 9:1) *amino((4-amino-4-thioxobutyl)amino)methaniminium 43*: δ 3.17 (t, *J* = 6.9 Hz, 2H), 2.65 (t, *J* = 7.4 Hz, 2H), 1.95 (m, 2H); *amino((4-cyano-4-hydroxybutyl)amino)methaniminium 45*: δ 3.19 (t, *J* = 6.9 Hz, 2H), 1.84 (m, 2H), 1.70 (m, 2H), other 1H not assigned due to HOD suppression of the signal.



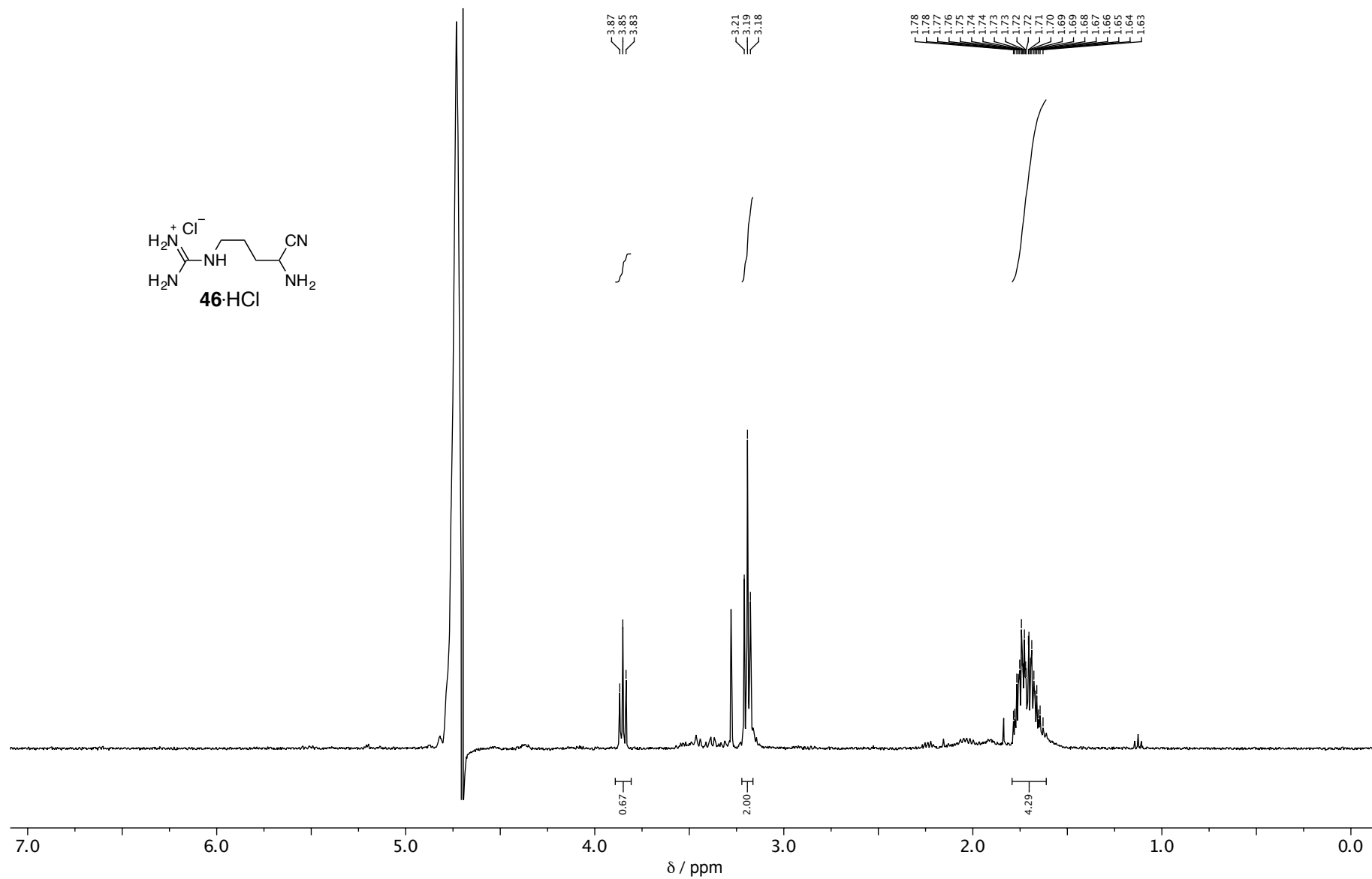
**Figure S30.** <sup>1</sup>H-NMR analysis (H<sub>2</sub>O/D<sub>2</sub>O, 9:1) of the photochemical conversion of thioamide **43** to cyanohydrin **45**. A – starting reaction mixture of thioamide **43**; B – reaction mixture after 1.5 h; C – reaction mixture after 6 h; D – reaction mixture after 12 h, also showing the presence of Gly, Ser **55**, and Ala cyanohydrins; E – synthetic standard of cyanohydrin hydrochloride **45·HCl**.

*Conversion of cyanohydrin 45 to  $\alpha$ -aminonitrile 46*



The cyanohydrin hydrochloride **45·HCl** (33 mg, 0.20 mmol) was dissolved H<sub>2</sub>O/D<sub>2</sub>O (4:1, 0.5 mL) and NH<sub>3</sub>/NH<sub>4</sub>Cl buffer (1.0 M; 0.50 mL, 0.50 mmol) was added. The solution was adjusted to pH 8.2 using NaOH/HCl and stirred at room temperature. After 5 d, analysis by <sup>1</sup>H-NMR spectroscopy showed 90% conversion to the  $\alpha$ -aminonitrile **46·HCl** (Figure S31).

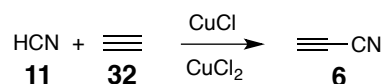
*Amino((4-amino-4-cyanobutyl)amino)methaniminium hydrochloride 46·HCl*: <sup>1</sup>H-NMR (400 MHz, H<sub>2</sub>O/D<sub>2</sub>O, 9:1)  $\delta$  3.85 (t,  $J$  = 6.6 Hz, 1H), 3.19 (t,  $J$  = 6.5 Hz, 2H), 1.72 (m, 4H).



**Figure S31.**  $^1\text{H-NMR}$  spectrum of  $\alpha$ -aminonitrile **46**·HCl generated from cyanohydrin hydrochloride **45**·HCl.

## 5. Oxidative coupling to form cyanoacetylene **6**

Formation of cyanoacetylene **6** from acetylene **32** and hydrogen cyanide **11**



Copper(I) chloride (0.69 g, 7.0 mmol) and potassium chloride (0.50 g, 6.7 mmol) were suspended in H<sub>2</sub>O (0.6 mL) and the resultant suspension heated at 70 °C for 1 h to generate the *Nieuwland* catalyst mixture, K[CuCl<sub>2</sub>]. The mixture was allowed to cool to room temperature. The resultant suspension of K[CuCl<sub>2</sub>] (~7.0 M, 71 μL, 0.5 mmol) was diluted with H<sub>2</sub>O (0.38 mL). Acetylene (generated from addition of CaC<sub>2</sub> to H<sub>2</sub>O) was bubbled into the mixture in short bursts over 1 min. After 1 h, CuCl<sub>2</sub> (67 mg, 0.5 mmol) in H<sub>2</sub>O (100 μL) followed by KCN (16 mg, 0.25 mmol) in H<sub>2</sub>O (50 μL) were added and stirring continued at room temperature. The reaction was monitored by <sup>1</sup>H-NMR spectroscopy by extraction of reaction aliquots with CDCl<sub>3</sub> (Figure S32/33-A). In order to observe cyanoacetylene **6**, further KCN (7 mg, 0.1 mmol) was added to the aliquot prior to extraction with CDCl<sub>3</sub>, to release **6**, which was presumably bound to copper (Figure S32/33-B). A further experiment was conducted where K<sup>13</sup>CN (7 mg, 0.1 mmol) was used initially and for the second addition (Figure S32/33-C,D), or only for the second addition in order to determine the yield with respect to the initial amount of KCN used (Figure S32/33-E,F).

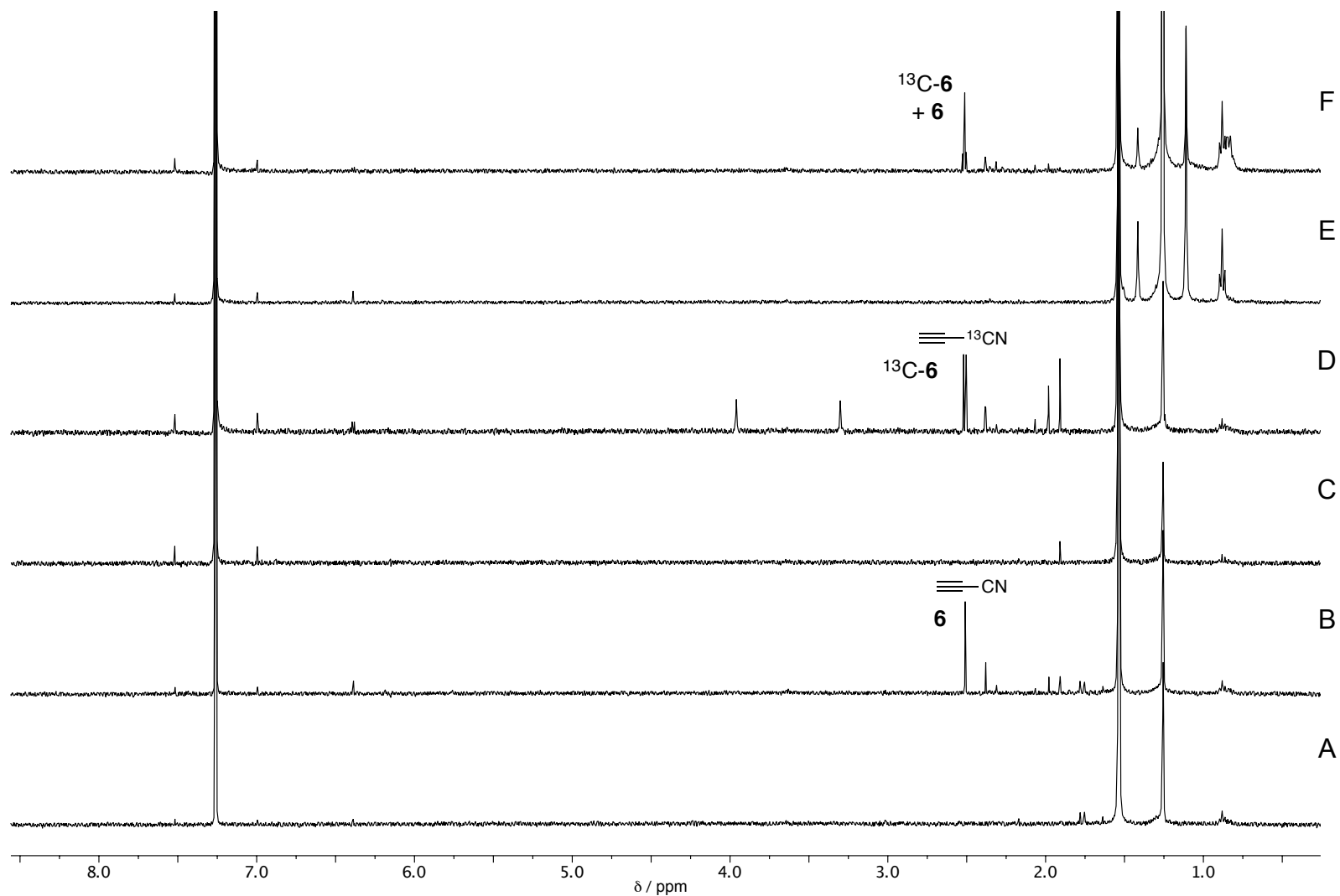
*KCN experiment* (Figure S32/33-A,B): The yield of cyanoacetylene **6** was estimated to be 36% after 3 h by relative integration of the <sup>1</sup>H-NMR signal after spiking with Me<sub>2</sub>SO<sub>2</sub> (0.2 M in CDCl<sub>3</sub>, 40 μL).

*Cyanoacetylene 6*: <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) δ 2.51 (s, 1H).

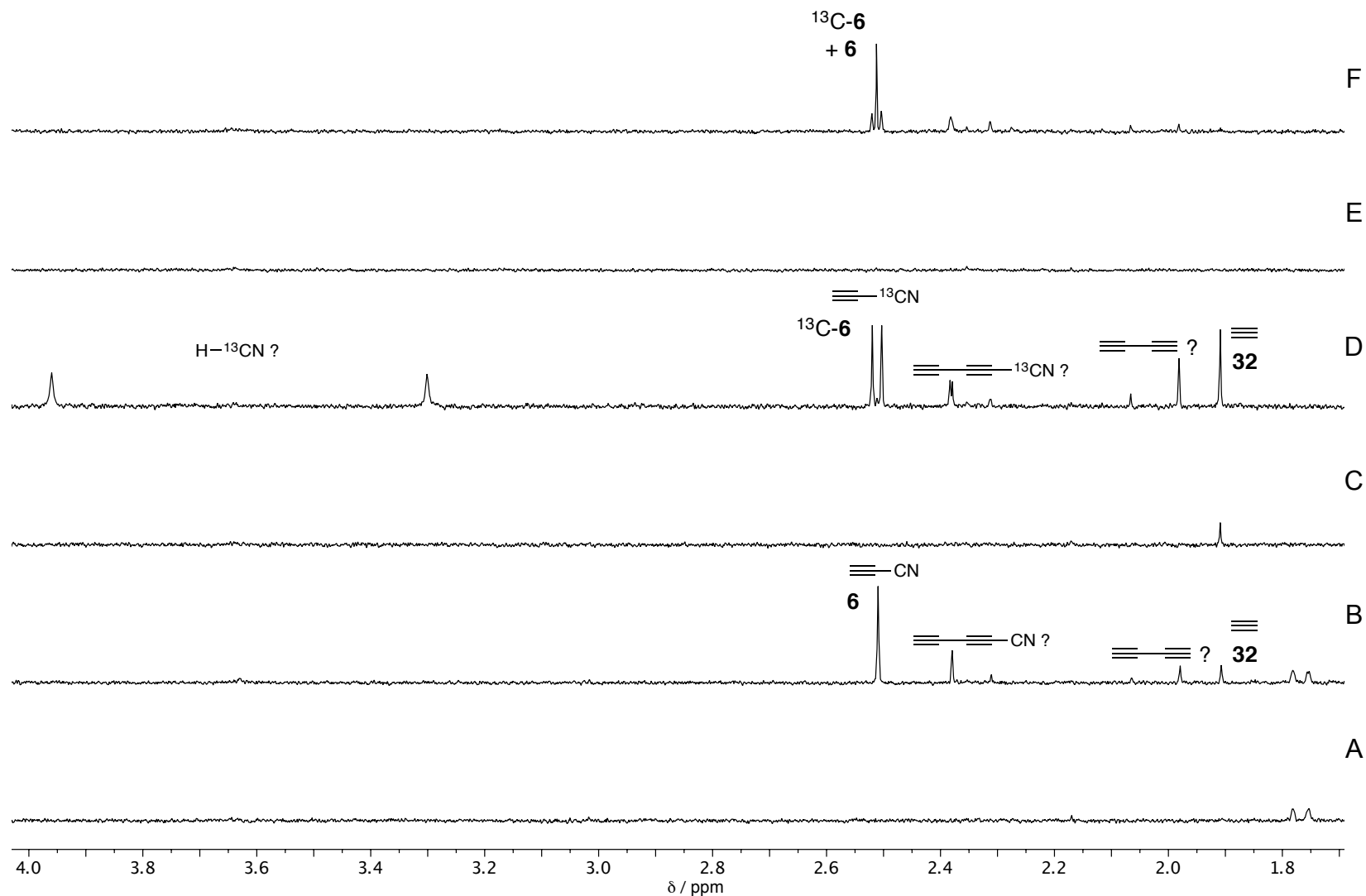
*KCN/K<sup>13</sup>CN experiment* (Figure S32/33-E,F): The yield of cyanoacetylene **6** from the initial addition of unlabelled KCN was estimated to be 26% after 3 h by relative integration of the <sup>1</sup>H-NMR signal after spiking with Me<sub>2</sub>SO<sub>2</sub> (0.2 M in CDCl<sub>3</sub>, 40 μL).

*Cyanoacetylene 6, using K<sup>13</sup>CN*: <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) δ 2.51 (d, *J* = 6.6 Hz, 1H).





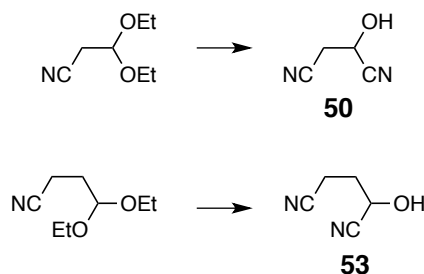
**Figure S32.**  $^1\text{H}$ -NMR analysis ( $\text{CDCl}_3$ ) of the formation of cyanoacetylene **6** from acetylene **32** and  $\text{HCN}/\text{H}^{13}\text{CN}$  **11**/ $^{13}\text{C}$ -**11**. A – KCN experiment: extract after 3 h; B – KCN experiment + KCN: extract after 3 h; C –  $\text{K}^{13}\text{CN}$  experiment: extract after 3 h; D –  $\text{K}^{13}\text{CN}$  experiment +  $\text{K}^{13}\text{CN}$ : extract after 3 h; E – KCN experiment: extract after 3 h; F – KCN experiment +  $\text{K}^{13}\text{CN}$ : extract after 3 h.



**Figure S33.**  $^1\text{H}$ -NMR analysis ( $\text{CDCl}_3$ ) of the formation of cyanoacetylene **6** from acetylene **32** and  $\text{HCN}/\text{H}^{13}\text{CN}$  **11**/ $^{13}\text{C}$ -**11** – expansion of spectra from Figure S32. Question marks indicate inferred assignments. A – KCN experiment: extract after 3 h; B – KCN experiment + KCN: extract after 3 h; C –  $\text{K}^{13}\text{CN}$  experiment: extract after 3 h; D –  $\text{K}^{13}\text{CN}$  experiment +  $\text{K}^{13}\text{CN}$ : extract after 3 h; E – KCN experiment: extract after 3 h; F – KCN experiment +  $\text{K}^{13}\text{CN}$ : extract after 3 h, showing both cyanoacetylene **6** and  $^{13}\text{C}$ -cyanoacetylene  $^{13}\text{C}$ -**6**.

## 6. Synthesis of Asn/Asp and Gln/Glu $\alpha$ -aminonitriles (49 & 54)

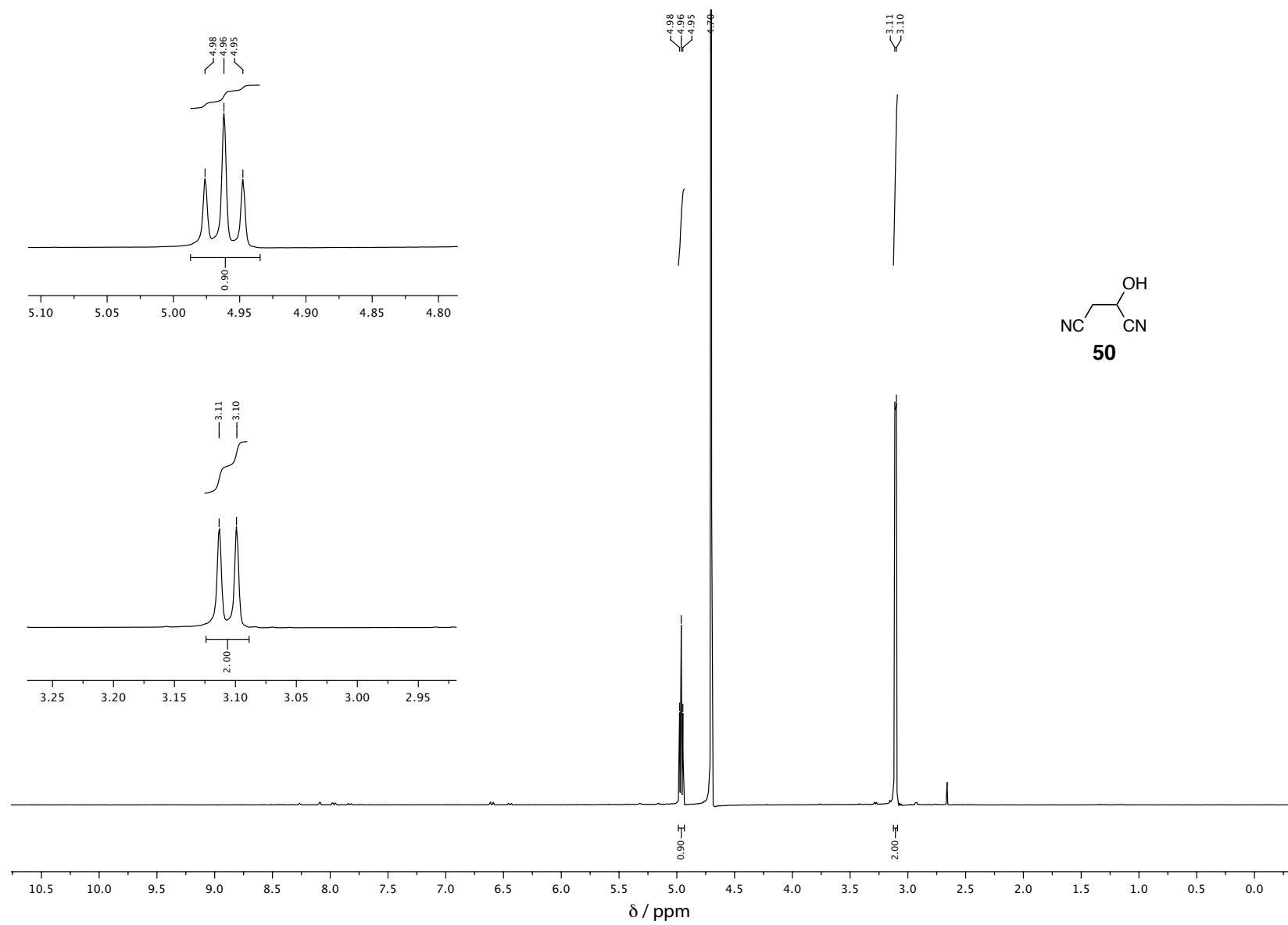
General procedure for the synthesis of standard samples of cyanohydrins **50** and **53**<sup>5</sup>



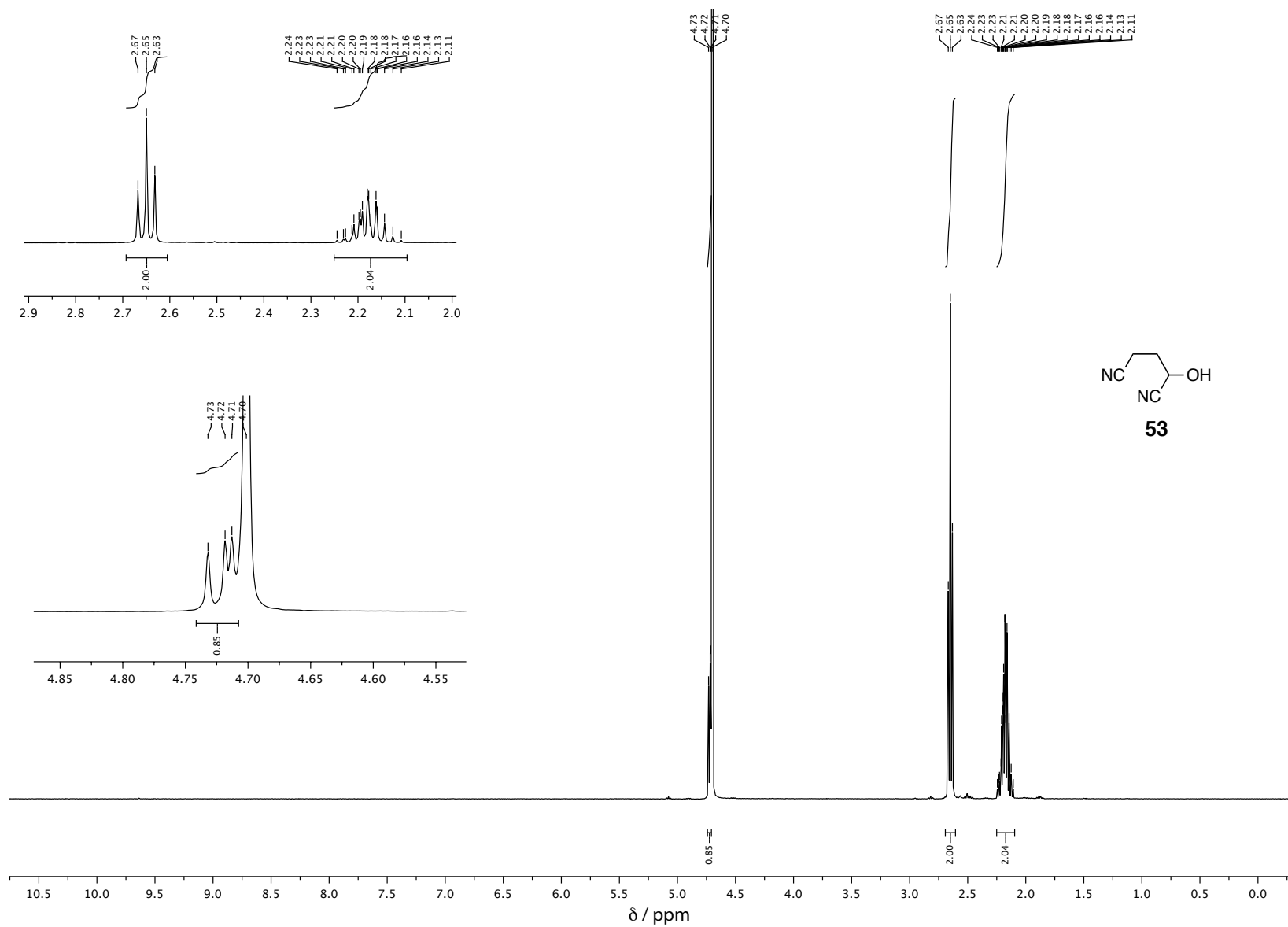
3,3-Diethoxypropionitrile (0.30 mL, 2.0 mmol) or 3-cyanopropionaldehyde diethyl acetal (0.34 mL, 2.0 mmol) was dissolved in H<sub>2</sub>O (3.2 mL) and concentrated HCl (10  $\mu$ L) was added. The reaction mixture was heated at 100 °C for 10 min and then allowed to cool to room temperature. An aliquot was taken at this point to obtain a reference sample of the aldehyde. The remaining mixture was diluted with ice-cold H<sub>2</sub>O (9.5 mL) and KCN (0.12 g, 1.9 mmol) was added. The solution was adjusted to pH 3 using 3.0 M HCl and stirred at room temperature for 6 h. The mixture was degassed for 2 h and the water was evaporated to leave a residual solid confirmed to be the desired cyanohydrin by <sup>1</sup>H-NMR spectroscopy.

*2-Hydroxysuccinonitrile* **50** (Figure S34): orange solid residue (0.16 g, 83%); <sup>1</sup>H-NMR (400 MHz, D<sub>2</sub>O)  $\delta$  4.96 (t,  $J$  = 5.7 Hz, 1H), 3.11 (d,  $J$  = 5.7 Hz, 2H).

*2-Hydroxypentanedinitrile* **53** (Figure S35): white solid residue (0.18 g, 87%); <sup>1</sup>H-NMR (400 MHz, D<sub>2</sub>O)  $\delta$  4.72 (dd,  $J$  = 7.6, 5.6 Hz, 1H), 2.65 (t,  $J$  = 7.2 Hz, 2H), 2.18 (m, 2H).

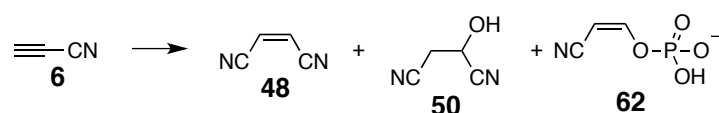


**Figure S34.** <sup>1</sup>H-NMR spectrum of the synthetic standard of Asn/Asp cyanohydrin **50**.



**Figure S35.** <sup>1</sup>H-NMR spectrum of the synthetic standard of Gln/Glu cyanohydrin **53**.

*Formation of a mixture of maleonitrile 48, cyanohydrin 50 and cyanovinylphosphate 62*



*Procedure 1:*  $\text{CuC}_3\text{N}$  (10 mg), KCN (24 mg, 0.37 mmol) and  $\text{NH}_4\text{H}_2\text{PO}_4$  (32 mg, 0.27 mmol) were mixed in  $\text{H}_2\text{O}$  (0.8 mL). The reaction mixture was rapidly adjusted to pH 7.4 using 1.0 M NaOH/HCl, sealed and stirred at room temperature.

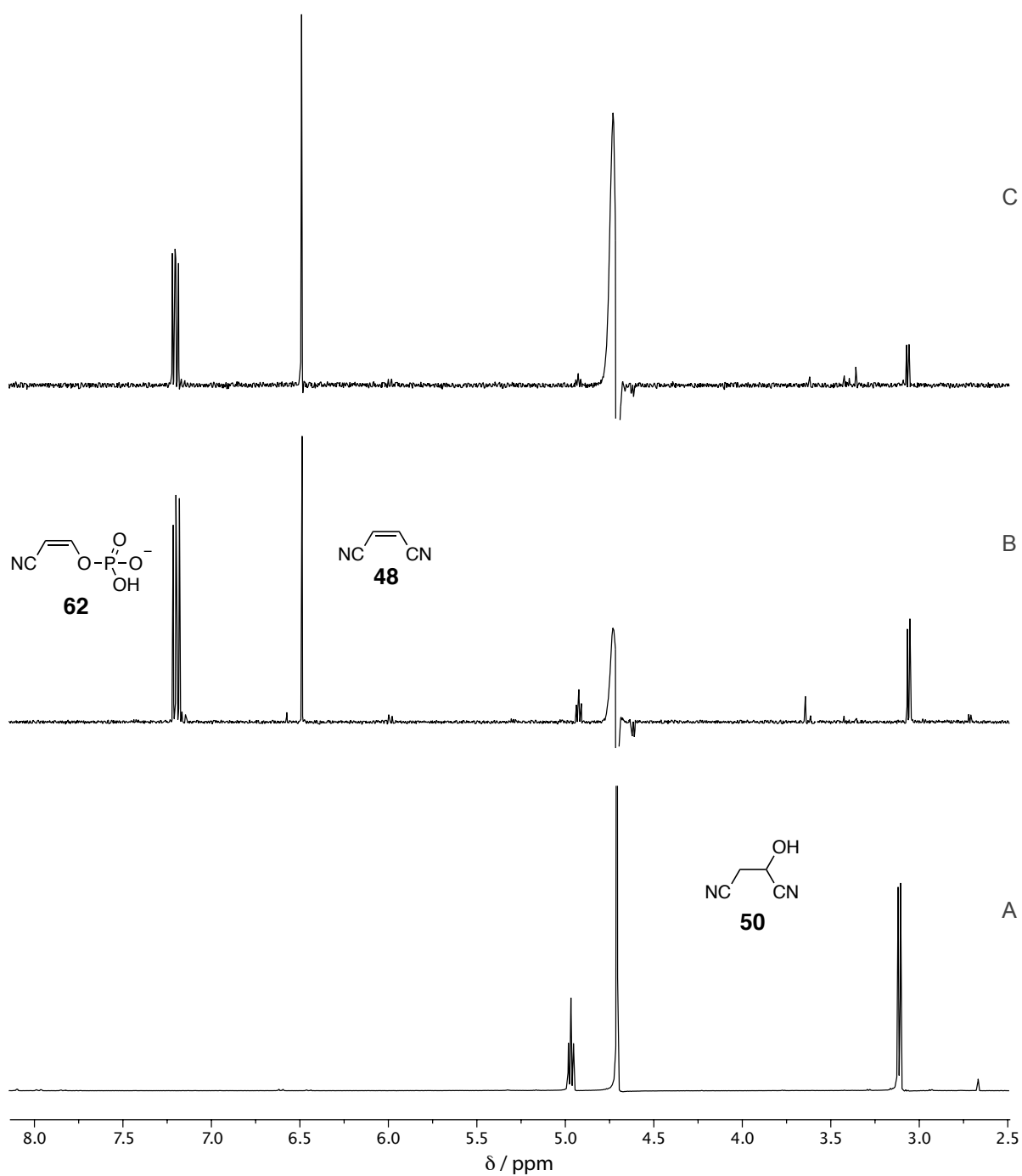
*Procedure 2:*  $\text{CuC}_3\text{N}$ -copper(I) phosphate (10 mg), KCN (24 mg, 0.37 mmol) and  $\text{NH}_4\text{H}_2\text{PO}_4$  (24 mg, 0.19 mmol) were mixed in  $\text{H}_2\text{O}$  (0.8 mL). The reaction mixture was rapidly adjusted to pH 7.4 using 1.0 M NaOH/HCl, sealed and stirred at room temperature.

After 4 d, both reaction mixtures were analysed by  $^1\text{H}$ -NMR spectroscopy (Figure S36).

*Procedure 1:* maleonitrile **48** (12%), cyanohydrin **50** (10%), cyanovinylphosphate **62** (78%).

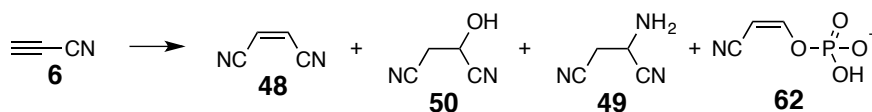
*Procedure 2:* maleonitrile **48** (27%), cyanohydrin **50** (6%), cyanovinylphosphate **62** (67%).

$^1\text{H}$ -NMR (400 MHz,  $\text{H}_2\text{O}/\text{D}_2\text{O}$ , 9:1) *maleonitrile 48*:  $\delta$  6.48 (s, 2H); *2-hydroxysuccinonitrile 50*:  $\delta$  4.92 (t,  $J = 5.6$  Hz, 1H), 3.05 (d,  $J = 5.8$  Hz, 2H); *cyanovinylphosphate 62*:  $\delta$  7.19 (dd,  $J = 7.7, 6.3$  Hz, 1H), other 1H not assigned due to HOD suppression of the signal.



**Figure S36.**  $^1\text{H-NMR}$  analysis of the formation of a mixture of maleonitrile **48**, cyanohydrin **50** and cyanovinylphosphate **62**. A ( $\text{D}_2\text{O}$ ) – synthetic standard of cyanohydrin **50**; B ( $\text{H}_2\text{O}/\text{D}_2\text{O}$ , 9:1) – signals from procedure 1 showing formation of maleonitrile (12%), Asn/Asp cyanohydrin (10%), cyanovinylphosphate (78%); C ( $\text{H}_2\text{O}/\text{D}_2\text{O}$ , 9:1) – signals from procedure 2 showing formation of maleonitrile (27%), Asn/Asp cyanohydrin (6%) and cyanovinylphosphate (67%).

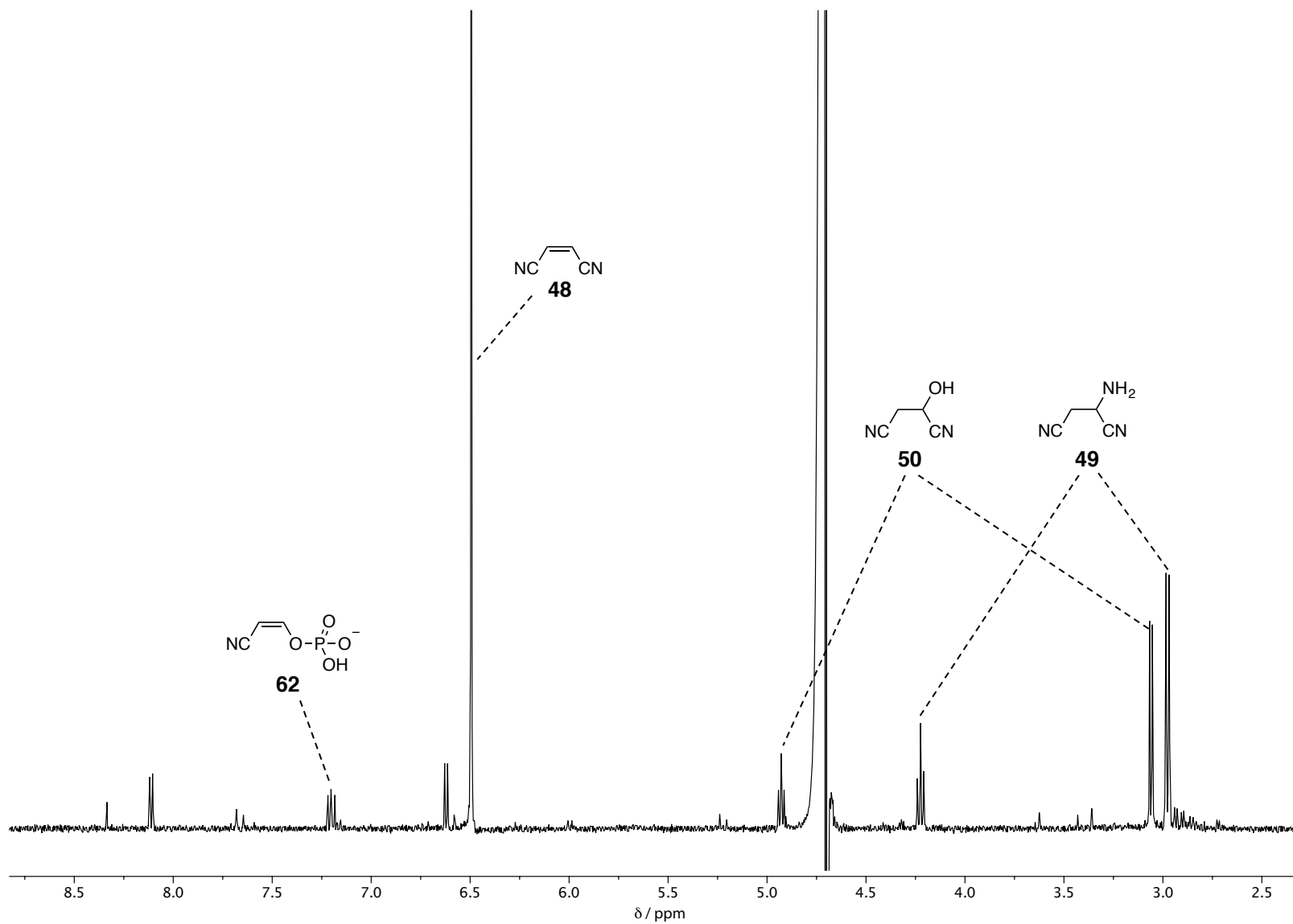
Formation of a mixture of maleonitrile **48**, cyanohydrin **50**,  $\alpha$ -aminonitrile **49** and cyanovinylphosphate **62**



$\text{CuC}_3\text{N}$ -copper(I) phosphate (11 mg), KCN (26 mg, 0.40 mmol),  $\text{NH}_4\text{H}_2\text{PO}_4$  (23 mg, 0.20 mmol) and  $\text{NH}_3/\text{NH}_4\text{Cl}$  buffer (1.0 M; 0.30 mL, 0.30 mmol) were mixed in  $\text{H}_2\text{O}$  (0.8 mL). The reaction mixture was rapidly adjusted to pH 8.5 using  $\text{NaOH}/\text{HCl}$ , sealed and stirred at room temperature. After 18 h, the reaction mixture was analysed by  $^1\text{H-NMR}$  spectroscopy (Figure S37). The yields of conversion after 18 h: maleonitrile **48** (50%), cyanohydrin **50** (16%),  $\alpha$ -aminonitrile **49** (25%), cyanovinylphosphate **62** (9%).

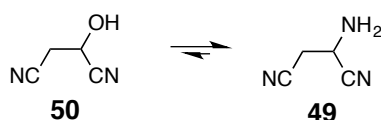
$^1\text{H-NMR}$  (400 MHz,  $\text{H}_2\text{O}/\text{D}_2\text{O}$ , 9:1) maleonitrile **48**:  $\delta$  6.49 (s, 2H); 2-hydroxysuccinonitrile **50**:  $\delta$  4.93 (t,  $J = 5.6$  Hz, 1H), 3.06 (d,  $J = 5.7$  Hz, 2H); 2-aminosuccinonitrile **49**:  $\delta$  4.22 (t,  $J = 6.4$  Hz, 1H), 2.98 (d,  $J = 6.2$  Hz, 2H); cyanovinylphosphate **62**:  $\delta$  7.20 (dd,  $J = 7.8, 6.2$  Hz, 1H), other 1H not assigned due to HOD suppression of the signal.





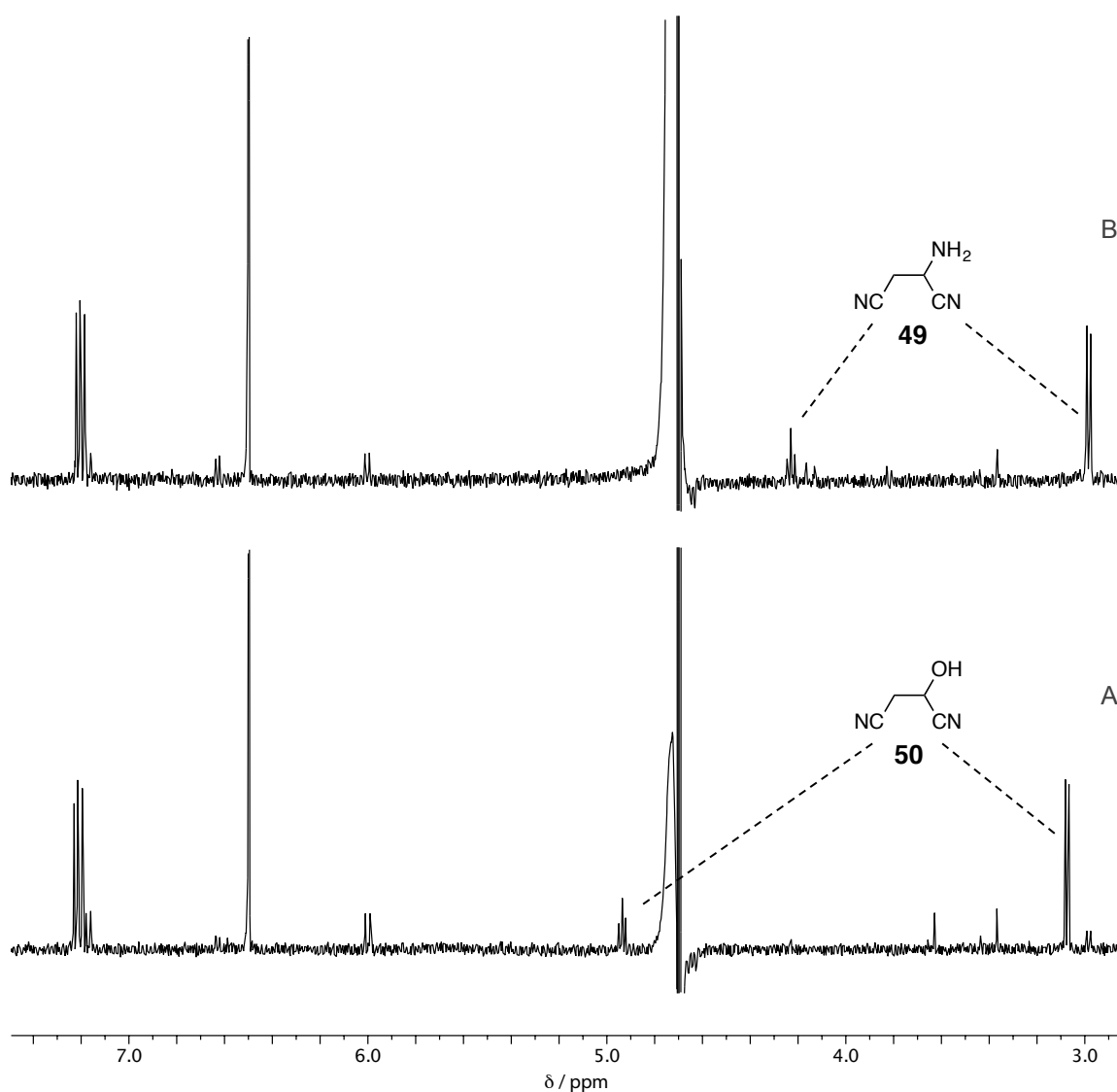
**Figure S37.** <sup>1</sup>H-NMR spectra from the formation of a mixture of maleonitrile **48**, cyanohydrin **50**, α-aminonitrile **49** and cyanovinylphosphate **62**.

Conversion of cyanohydrin **50** to  $\alpha$ -aminonitrile **49**



A solution formed following procedure 2 (see page S70) described previously was adjusted from pH 7.4 to 9.0 using NaOH/HCl and analysed by  $^1\text{H-NMR}$  spectroscopy (Figure S38).

$^1\text{H-NMR}$  (400 MHz,  $\text{H}_2\text{O}/\text{D}_2\text{O}$ , 9:1) 2-hydroxysuccinonitrile **50**:  $\delta$  4.93 (t,  $J = 5.6$  Hz, 1H), 3.06 (d,  $J = 5.7$  Hz, 2H); 2-aminosuccinonitrile **49**:  $\delta$  4.23 (t,  $J = 6.4$  Hz, 1H), 2.98 (d,  $J = 6.3$  Hz, 2H).



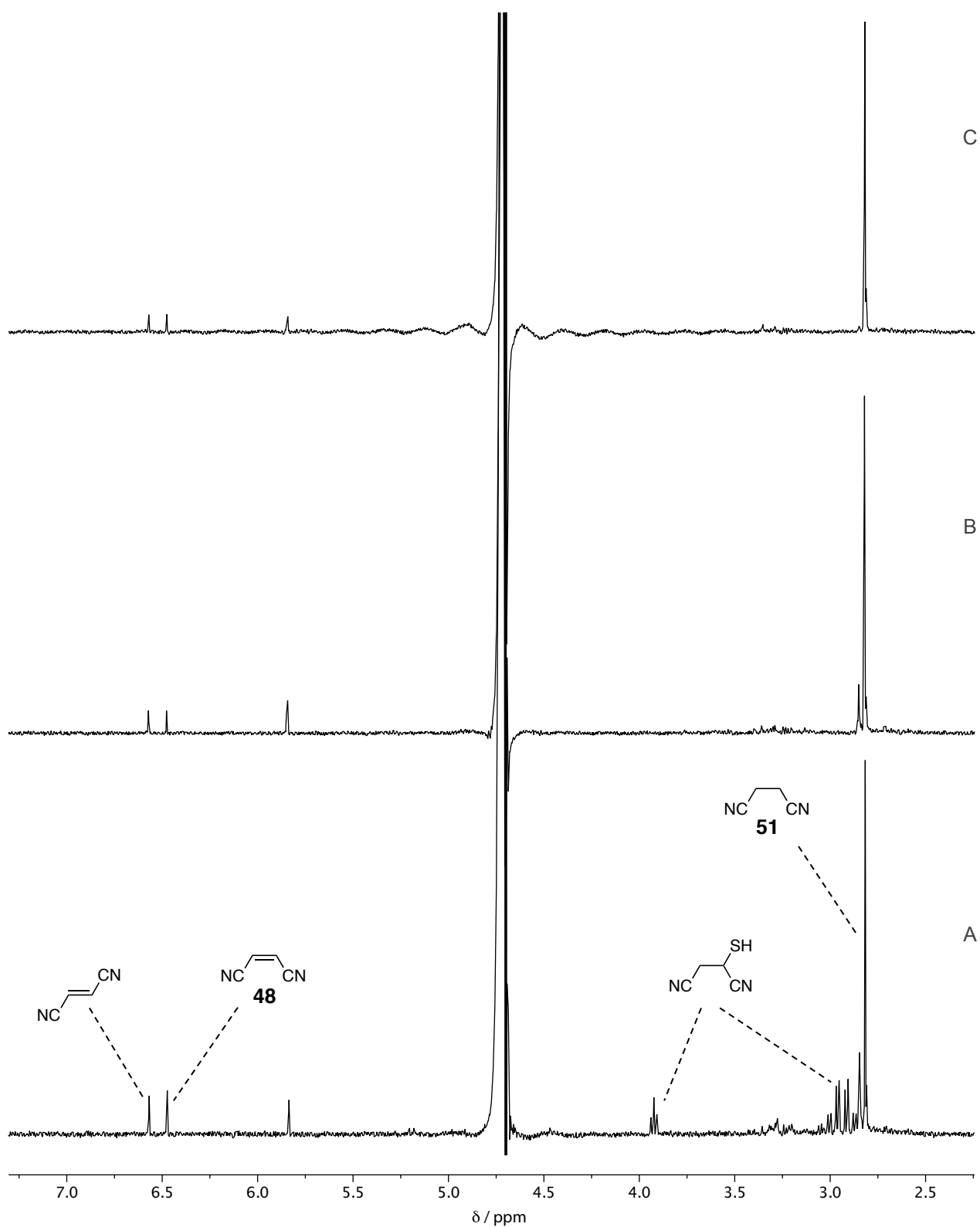
**Figure S38.**  $^1\text{H-NMR}$  spectra ( $\text{H}_2\text{O}/\text{D}_2\text{O}$ , 9:1) showing the conversion of cyanohydrin **50** to the  $\alpha$ -aminonitrile **49**. A – reaction mixture at pH 7.4 showing signals for the cyanohydrin **50**; B – reaction mixture at pH 9.0 showing signals for the  $\alpha$ -aminonitrile **49**.

*Conversion of fumaronitrile to succinonitrile **51** by photoredox chemistry*



Fumaronitrile (2.5 mg, 0.03 mmol) and NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O (16 mg, 0.10 mmol) were dissolved in degassed H<sub>2</sub>O/D<sub>2</sub>O (6:1, 2.8 mL) and the solution was adjusted to pH 6.5 using degassed NaOH/HCl. NaSH·xH<sub>2</sub>O (8 mg, assumed 60% NaSH, 0.09 mmol) was added and upon dissolution of the NaSH the mixture was adjusted to pH 6.9 using degassed NaOH/HCl. The solution was then added to a quartz cuvette containing CuCN (*ca.* 0.5 mg), whereupon a black precipitate formed, and the cuvette was immediately sealed. The cuvette was placed in a Rayonet reactor and irradiated. Samples of the reaction were taken at different time points (1, 4, 7 h) and analysed by <sup>1</sup>H-NMR spectroscopy (Figure S39). The yield of the conversion to **51** after 7 h was 90%.

<sup>1</sup>H-NMR (400 MHz, H<sub>2</sub>O/D<sub>2</sub>O, 9:1) *fumaronitrile*: δ 6.57 (s, 2H); *maleonitrile 48*: δ 6.47 (s, 2H); *2-mercaptosuccinonitrile*: δ 3.92 (t, *J* = 6.2 Hz, 1H), 2.98 (dd, *J* = 16.9, 5.9 Hz, 1H), 2.90 (dd, *J* = 16.9, 6.3 Hz, 1H); *succinonitrile 51*: δ 2.82 (s, 4H).



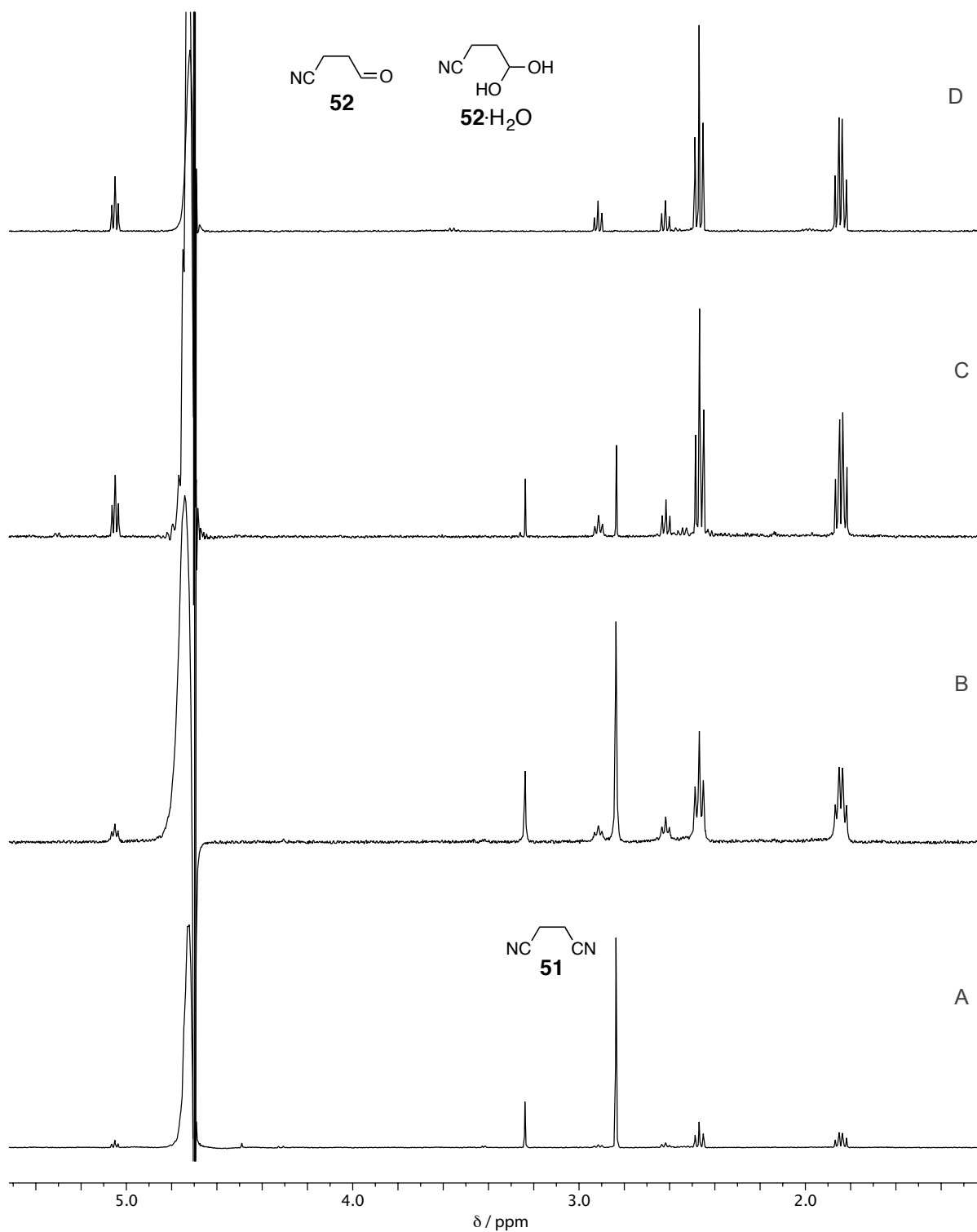
**Figure S39.** <sup>1</sup>H-NMR analysis (H<sub>2</sub>O/D<sub>2</sub>O, 9:1) of the photochemical conversion of fumaronitrile to succinonitrile **51**. A – reaction mixture after 1 h showing significant reduction to succinonitrile, as well as photo-equilibration with maleonitrile **48**, and the addition of HS<sup>-</sup> to give 2-mercaptosuccinonitrile; B – reaction mixture after 4 h; C – reaction mixture after 7 h.

Conversion of succinonitrile **51** to 3-cyanopropionaldehyde **52**



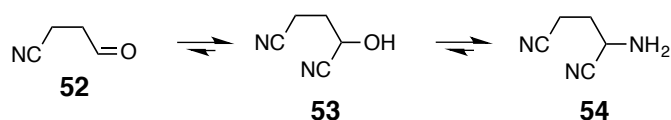
Succinonitrile **51** (2.5 mg, 0.03 mmol) and  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  (16 mg, 0.10 mmol) were dissolved in degassed  $\text{H}_2\text{O}/\text{D}_2\text{O}$  (6:1, 2.8 mL) and the solution was adjusted to pH 6.4 using degassed  $\text{NaOH}/\text{HCl}$ .  $\text{NaSH} \cdot x\text{H}_2\text{O}$  (16 mg, assumed 60%  $\text{NaSH}$ , 0.18 mmol) was added and upon dissolution of the  $\text{NaSH}$  the mixture was adjusted to pH 6.9 using degassed  $\text{NaOH}/\text{HCl}$ . The solution was then added to a quartz cuvette containing  $\text{CuCN}$  (*ca.* 0.5 mg), whereupon a black precipitate formed, and the cuvette was immediately sealed. The cuvette was placed in a Rayonet reactor and irradiated. A sample of the reaction was taken at different time points (2, 4, 7 h) and analysed by  $^1\text{H}$ -NMR spectroscopy (Figure S40). The yield of the conversion to **52**· $\text{H}_2\text{O}$  after 7 h was 89%.

$^1\text{H}$ -NMR (400 MHz,  $\text{H}_2\text{O}/\text{D}_2\text{O}$ , 9:1) *succinonitrile* **51**:  $\delta$  2.84 (s, 4H); *3-cyanopropionaldehyde hydrate* **52**· $\text{H}_2\text{O}$ :  $\delta$  5.05 (t,  $J = 5.6$  Hz, 1H), 2.47 (t,  $J = 7.2$  Hz, 2H), 1.84 (td,  $J = 7.1, 5.6$  Hz, 2H). *3-cyanopropionaldehyde* **52**:  $\delta$  9.60 (s, 1H), 2.91 (t,  $J = 6.7$  Hz, 2H), 2.62 (t,  $J = 6.7$  Hz, 2H).



**Figure S40.** <sup>1</sup>H-NMR analysis (H<sub>2</sub>O/D<sub>2</sub>O, 9:1) of the photochemical conversion of succinonitrile **51** to 3-cyanopropionaldehyde **52** and hydrate **52**·H<sub>2</sub>O. A – reaction mixture after 2 h; B – reaction mixture after 4 h; C – reaction mixture after 7 h; D – synthetic standard of 3-cyanopropionaldehyde **52** (12%) and hydrate **52**·H<sub>2</sub>O (88%).

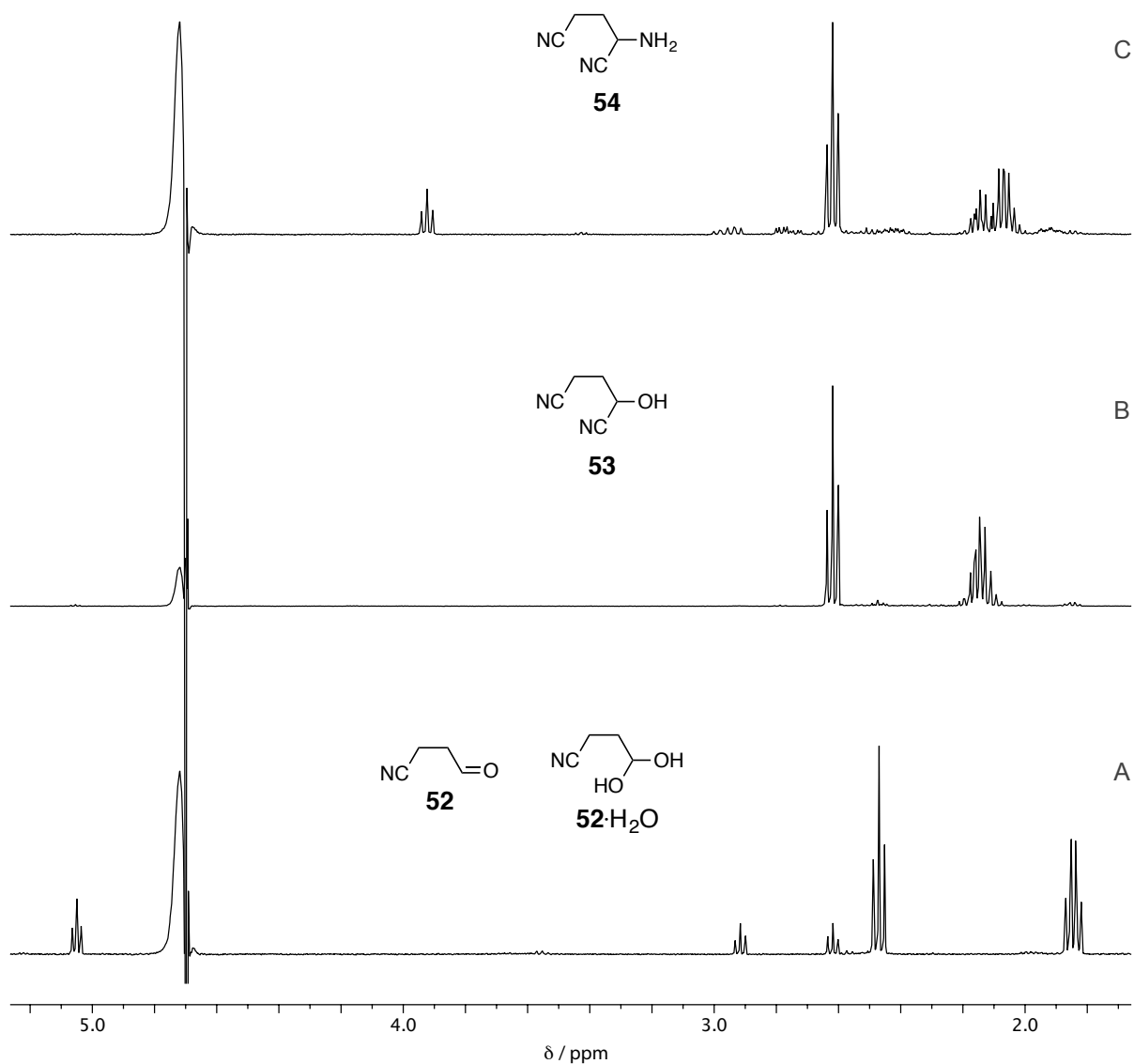
Conversion of 3-cyanopropionaldehyde **52** to cyanohydrin **53** to  $\alpha$ -aminonitrile **54**



3-Cyanopropionaldehyde **52** in H<sub>2</sub>O (2.0 M; 0.10 mL, 0.20 mmol) and NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O (31 mg, 0.20 mmol) were dissolved in H<sub>2</sub>O/D<sub>2</sub>O (8:1, 1.8 mL) and the solution was adjusted to pH 7.0 using NaOH/HCl. KCN (20 mg, 0.30 mmol) in H<sub>2</sub>O (0.1 mL) at pH 7.0 was then added and conversion to the cyanohydrin **53** was analysed by <sup>1</sup>H-NMR spectroscopy (Figure S41-B). The yield of the conversion to **53** after 20 h was ~100%.

The synthetic standard of cyanohydrin **53** (11 mg, 0.10 mmol) was dissolved H<sub>2</sub>O (0.6 mL) and NH<sub>3</sub>/NH<sub>4</sub>Cl buffer (1.0 M; 0.20 mL, 0.20 mmol) was added. The solution was adjusted to pH 9.0 using NaOH/HCl, sealed, stirred at room temperature and analysed by <sup>1</sup>H-NMR spectroscopy (Figure S41-C). The yield of the conversion to **54** after 3 h was 70%.

<sup>1</sup>H-NMR (400 MHz, H<sub>2</sub>O/D<sub>2</sub>O, 9:1) 3-cyanopropionaldehyde hydrate **52**·H<sub>2</sub>O:  $\delta$  5.05 (t,  $J$  = 5.6 Hz, 1H), 2.47 (t,  $J$  = 7.2 Hz, 2H), 1.84 (td,  $J$  = 7.1, 5.6 Hz, 2H); 3-cyanopropionaldehyde **52**:  $\delta$  9.60 (s, 1H), 2.91 (t,  $J$  = 6.7 Hz, 2H), 2.62 (t,  $J$  = 6.7 Hz, 2H); 2-hydroxypentanedinitrile **53**:  $\delta$  2.62 (t,  $J$  = 7.2 Hz, 2H), 2.15 (m, 2H), other 1H not assigned due to HOD suppression of the signal; 2-aminopentanedinitrile **54**:  $\delta$  3.92 (t,  $J$  = 7.2 Hz, 1H), 2.62 (t,  $J$  = 7.1 Hz, 2H), 2.07 (m, 2H).



**Figure S41.**  $^1\text{H-NMR}$  spectra ( $\text{H}_2\text{O}/\text{D}_2\text{O}$ , 9:1) from the conversion of cyanohydrin **53** to the  $\alpha$ -aminonitrile **54**. A – synthetic standard of 3-cyanopropionaldehyde **52** and hydrate **52·H<sub>2</sub>O**. B – formation of cyanohydrin **53** by addition of HCN at pH 7.0; C – formation of the  $\alpha$ -aminonitrile **54** by addition of  $\text{NH}_3$  at pH 9.0.



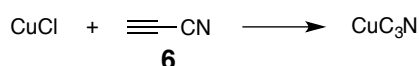
**Table S2. Yields for that part of the reaction network shown in Figure 1b and c.**

Conversion	No. steps	Yield /%	Conversion	No. steps	Yield /%
<b>33 → 34 + 61</b>	1	83 17	<b>38 → 41 + 42</b>	1	30 60
<b>34 → 35 + 34</b>	1	55 35	<b>41 + → 44 42</b>	1	78
<b>34 → 37</b>	2	77	<b>38 → 44</b>	2	70
<b>34 → 36</b>	1	45	<b>44 → 47</b>	2	32
<b>37 → 39</b>	1	77	<b>45 → 46</b>	1	90
<b>37 → 40</b>	2	~100	<b>6 → 48 + 49 + 50 + 62</b>	1 (pH 8.5)	50 25 16 9
<b>37 → 43</b>	3	~70	<b>6 → 48 + 49 + 50 + 62</b>	1 (pH 7.4)	27 0 10 67
<b>37 → 45</b>	5	~50	<b>48 → 51</b>	1	90
<b>36 → 38</b>	1	~100	<b>51 → 52</b>	1	89
<b>40 → 43</b>	1	~71	<b>52 → 53</b>	1	~100
<b>43 → 45</b>	1	~76	<b>52 → 54</b>	2	~70

## 7. Investigation of CuC<sub>3</sub>N

We decided to investigate an alternative scenario involving the absorption of atmospheric cyanoacetylene **6** onto copper(I), which would provide a store for **6** as solid CuC<sub>3</sub>N. Sanchez, Ferris and Orgel described a spark discharge reaction of a methane-nitrogen mixture that resulted in the production of cyanoacetylene.<sup>6</sup> Therefore, we explored the precipitation of cyanoacetylene **6** as CuC<sub>3</sub>N and subsequent reaction with *arabino-/ribo*-aminooxazoline, in the presence of hydrogen cyanide **11**, to form the *arabino-/ribo*-anhydronucleoside. Furthermore, due to the requirement of phosphate in the formation of the anhydronucleoside **7**, we also investigated the selective release of phosphate, by addition of hydrogen cyanide **11**, from a CuC<sub>3</sub>N–copper(I) phosphate co-precipitate. Finally, we explored the simultaneous sequestering of hydrogen cyanide **11** and cyanoacetylene **6** in a iron(II)/copper(I) mixture in order to confirm the relative affinity of **11** and **6** to iron(II) and copper(I), respectively.

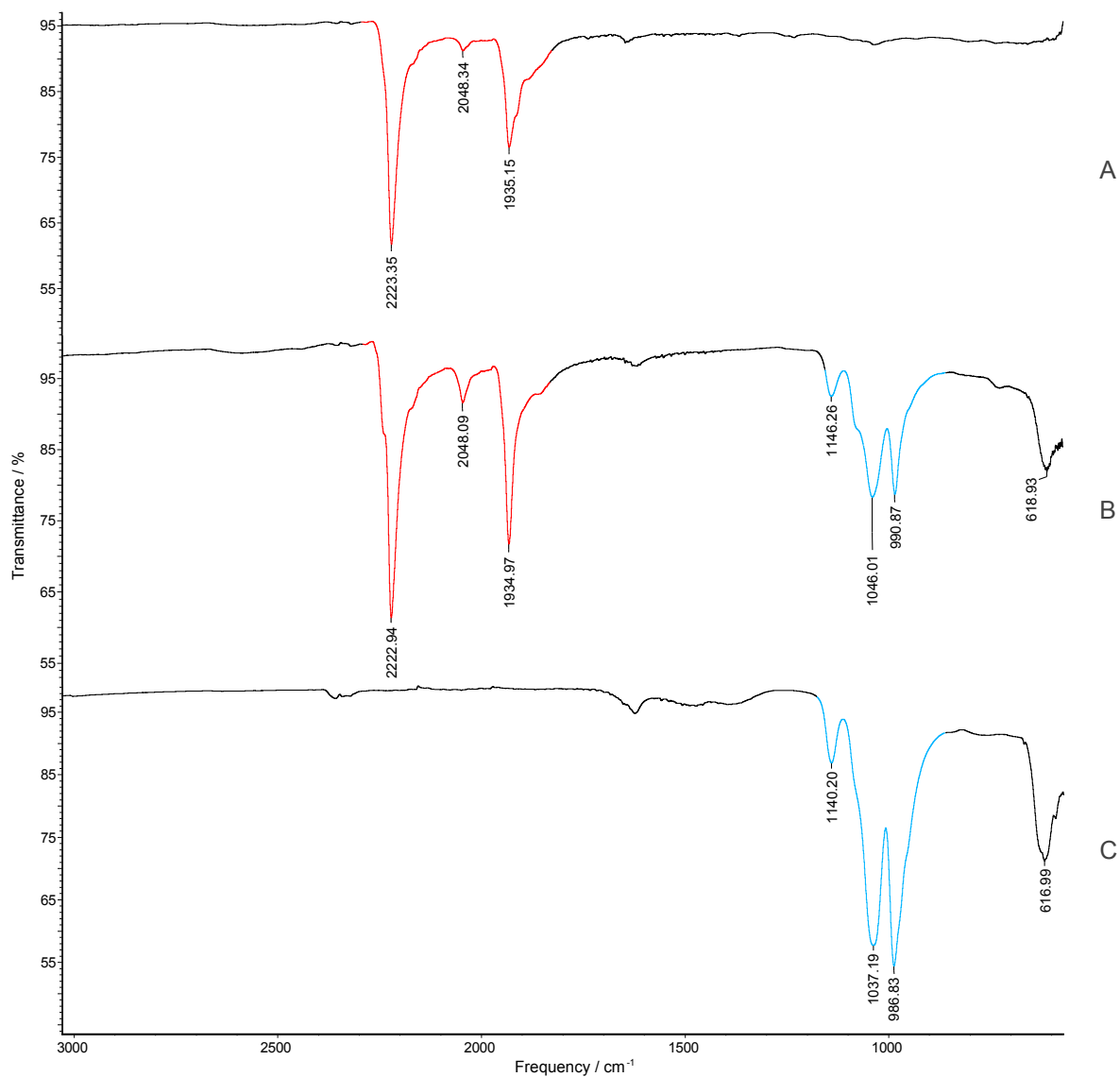
### *General procedure for CuC<sub>3</sub>N formation*



Copper(I) chloride (0.10 g, 1.0 mmol) and NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O (0.16 g, 1.0 mmol) or Na<sub>2</sub>HPO<sub>4</sub> (0.14 g, 1.0 mmol) were mixed in H<sub>2</sub>O (5.0 mL). Cyanoacetylene **6** (1.0 M in H<sub>2</sub>O; 1.0 mL, 1.0 mmol) was added rapidly to the resultant suspension, the vessel sealed immediately and the reaction mixture stirred for 4 h, during which the precipitate changed from a white/yellow co-precipitate to a yellow-green precipitate. The mixture was filtered under suction and the resulting solid was dried under vacuum over P<sub>2</sub>O<sub>5</sub> overnight.

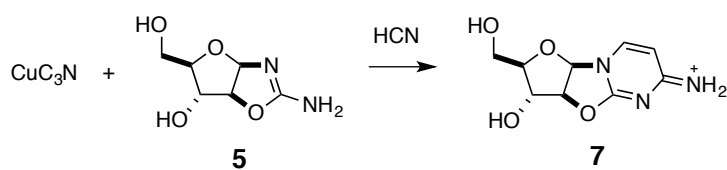
NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O gave a dark yellow solid (77 mg): IR (neat) 2223, 2048, 1935 cm<sup>-1</sup> (Figure S42-A); Analysis % calcd for C<sub>3</sub>CuN: C, 31.72; N, 12.33; % Found (1<sup>st</sup> analyst, LMEAS): C, 17.57; H, <0.1; N, 6.72. % Found (2<sup>nd</sup> analyst, UCMD): C, 17.27; H, 0.26; N, 6.23, Cl, 18.37; indicated formation of a CuC<sub>3</sub>N compound with residual copper(I) chloride still present.

Na<sub>2</sub>HPO<sub>4</sub> gave a yellow-green solid (95 mg): IR (neat) 2223, 2048, 1935, 1146, 1046, 991 cm<sup>-1</sup> (Figure S42-B); Analysis % calcd for C<sub>3</sub>CuN: C, 31.72; N, 12.33; % Found (1<sup>st</sup> analyst, LMEAS): C, 9.12; H, 0.34; N, 3.81. % Found (2<sup>nd</sup> analyst, UCMD): C, 9.90; H, 0.21; N, 3.03; P, 1.25; indicated formation of a CuC<sub>3</sub>N–copper(I) phosphate mixture.



**Figure S42.** IR spectra of solids from the  $\text{Cu}_3\text{N}$  formation. A – product isolated using  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ , highlighting  $\text{Cu}_3\text{N}$  signals in red; B – product isolated using  $\text{Na}_2\text{HPO}_4$  highlighting  $\text{Cu}_3\text{N}$  signals in red and copper(I) phosphate signals in blue; C – copper(I) phosphate prepared from copper(I) chloride and  $\text{Na}_2\text{HPO}_4$  in  $\text{H}_2\text{O}$ .

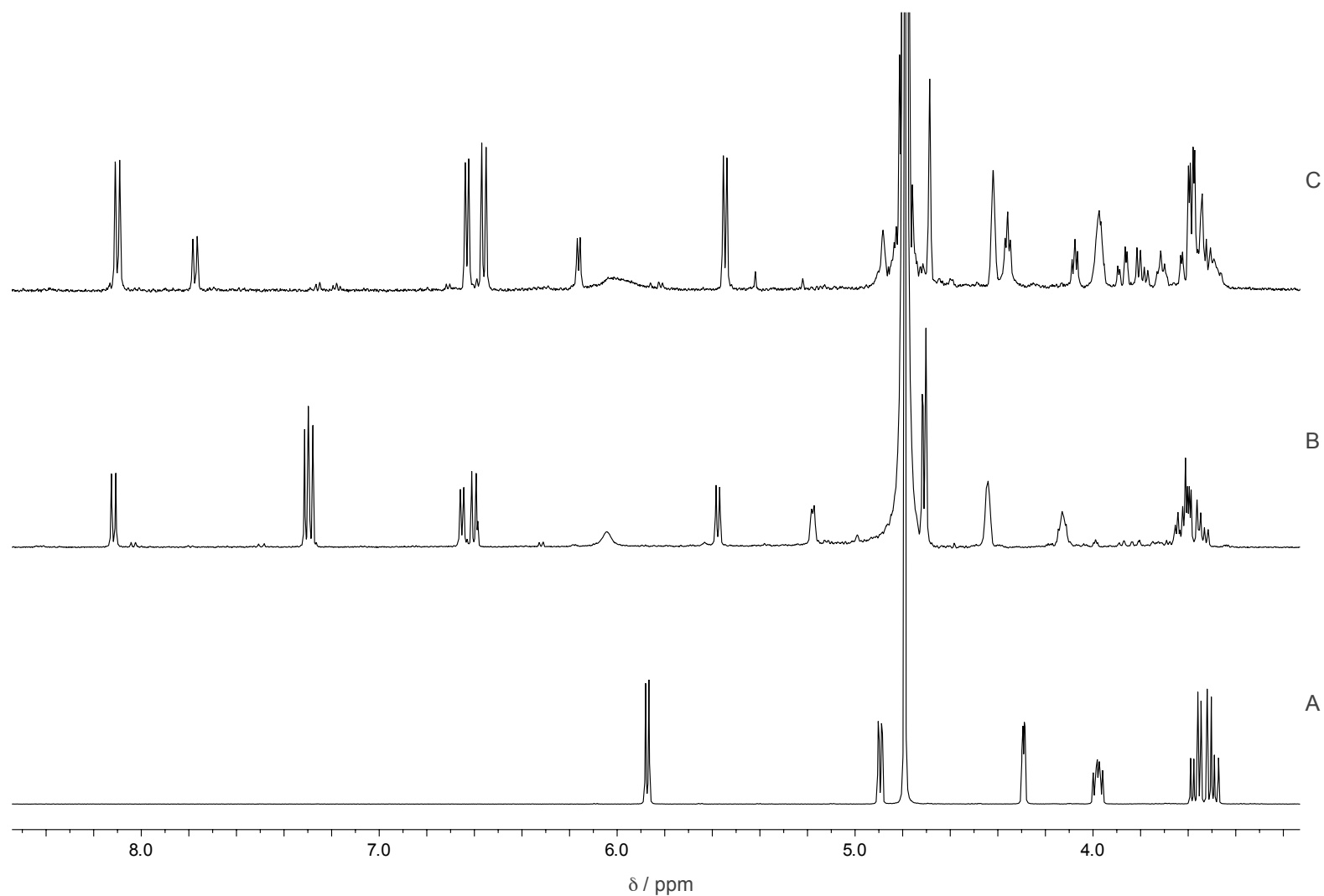
General procedure for anhydronucleoside formation



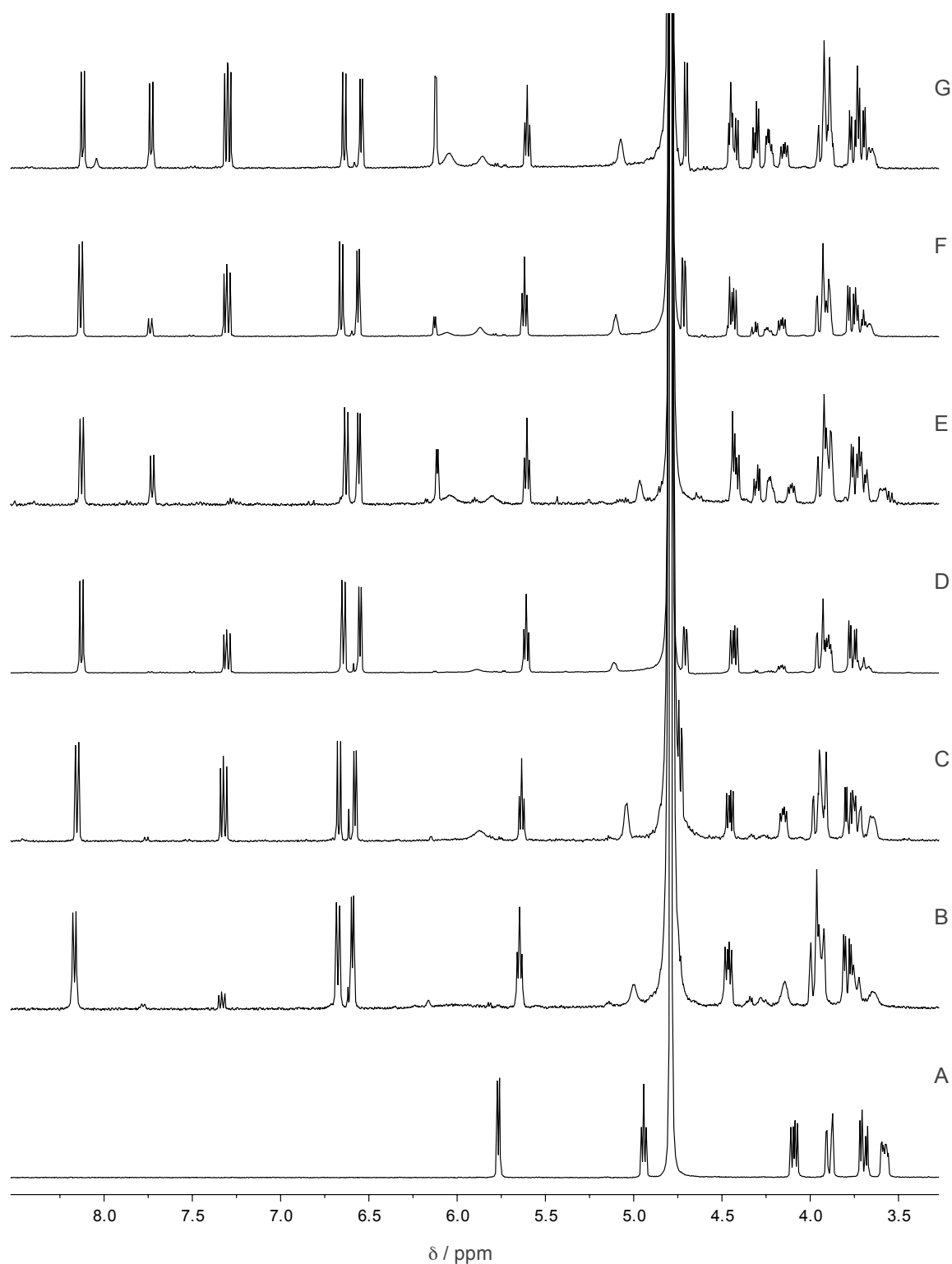
$\text{CuC}_3\text{N}$ –copper(I) phosphate (34 mg, ~0.15 mmol of each assuming a 1:1 composition), *arabino-ribo*-aminooxazoline (5.2 mg, 0.03 mmol), KCN (39 mg, 0.60 mmol), and  $\text{NaH}_2\text{PO}_4$  (variable amounts) were mixed in  $\text{H}_2\text{O}$  (0.6 mL) and the pH adjusted to 6.5 using aqueous HCl/NaOH. The vessel was immediately sealed and the contents stirred for 18 h. The solvent was evaporated and the grey residue suspended in  $\text{D}_2\text{O}$ . Following centrifugation the solution was examined by  $^1\text{H}$ -NMR analysis ( $\text{H}_2\text{O}/\text{D}_2\text{O}$ , 9:1).

*Arabino*-:  $^1\text{H}$ -NMR analysis showed conversion from the aminooxazoline **5** to the anhydronucleoside **7** (Figure S43). The yield of anhydronucleoside in the mixture was estimated to be 38% after 18 h by relative integration of  $^1\text{H}$ -NMR signals after spiking with pentaerythritol (0.1 M in  $\text{H}_2\text{O}$ ; 20  $\mu\text{L}$ ).

*Ribo*-:  $^1\text{H}$ -NMR analysis showed conversion from the aminooxazoline to the anhydronucleoside (Figure S44). The yield of anhydronucleoside in the mixture was estimated to be 40% after 18 h by relative integration of  $^1\text{H}$ -NMR signals after spiking with pentaerythritol (0.1 M in  $\text{H}_2\text{O}$ ; 20  $\mu\text{L}$ ).



**Figure S43.** <sup>1</sup>H-NMR analysis (D<sub>2</sub>O) of *arabino*-anhydronucleoside formation. A – *arabino*-aminooxazoline **5**; B – reaction mixture after 18 h, showing formation of *arabino*-anhydronucleoside **7** and cyanovinylphosphate **62**; C – reaction mixture without any additional phosphate present after 18 h, showing formation of *arabino*-anhydronucleoside **7** and the corresponding hydrolysed product, *arabino*-cytidine.

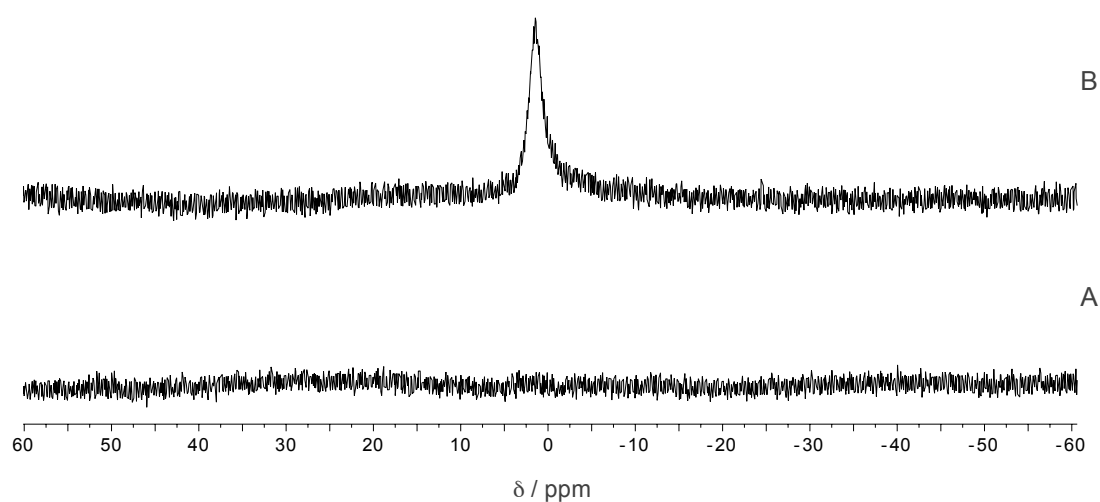


**Figure S44.** <sup>1</sup>H-NMR analysis (D<sub>2</sub>O) of *ribo*-anhydronucleoside formation. A – *ribo*-aminooxazoline; B – reaction mixture after 18 h using CuC<sub>3</sub>N, showing formation of *ribo*-anhydronucleoside and small amounts of cyanovinylphosphate **62**; C – reaction mixture after 18 h using CuC<sub>3</sub>N–copper(I) phosphate mixture; D – as C spiked with *ribo*-anhydronucleoside; E – reaction mixture without any phosphate present after 18 h, showing formation of *ribo*-anhydronucleoside and the corresponding hydrolysed product α-cytidine; F – as C after 7 d, also showing some hydrolysis; G – as F spiked with α-cytidine.

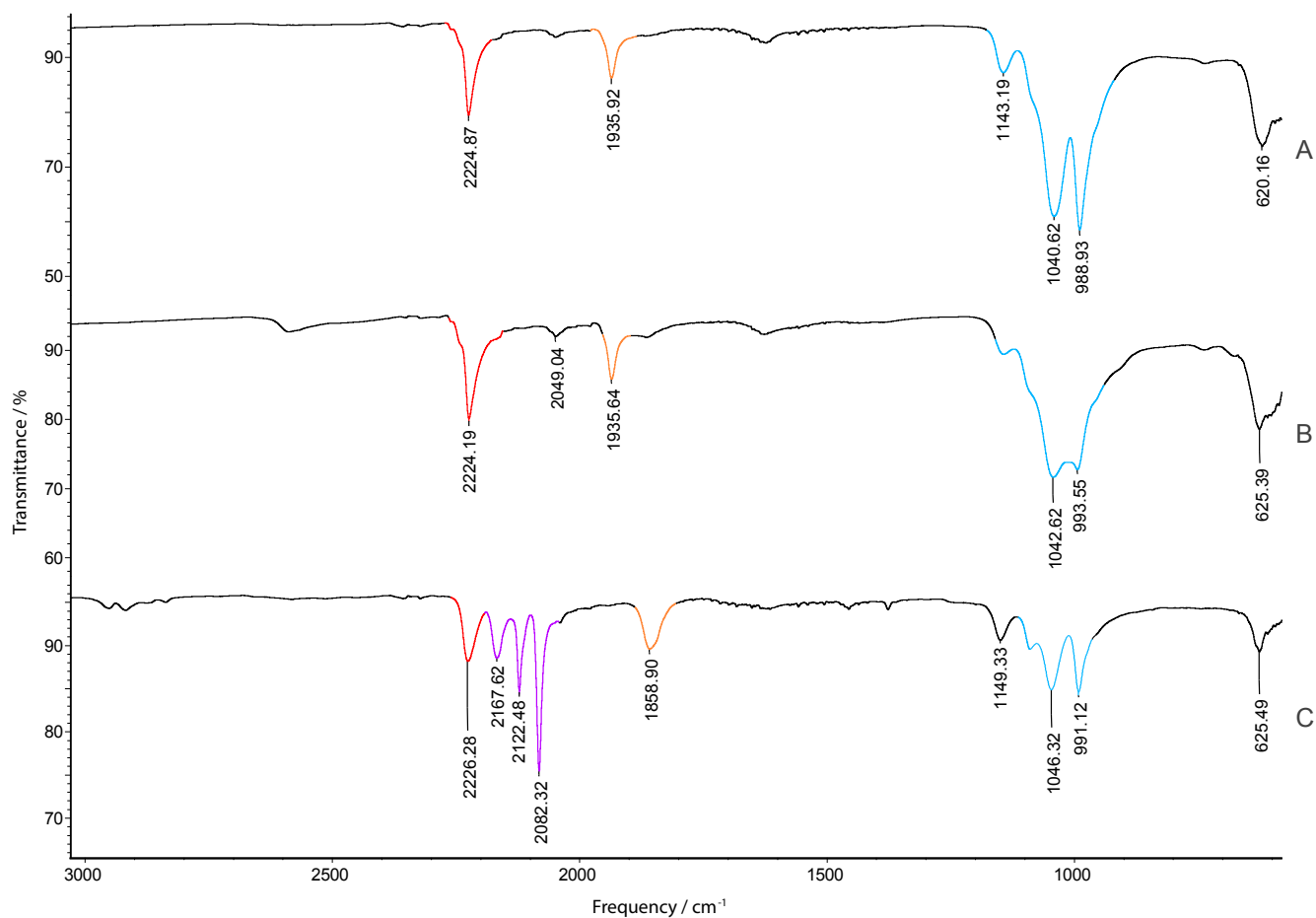
*Procedure for the selective phosphate release from CuC<sub>3</sub>N–copper(I) phosphate mixture*

CuC<sub>3</sub>N–copper(I) phosphate (11 mg, assume 0.05 mmol of each) was stirred in D<sub>2</sub>O for 2 h, centrifuged and the resultant solution and residual precipitate were analysed by <sup>31</sup>P-NMR and IR spectroscopies, respectively.

CuC<sub>3</sub>N–copper(I) phosphate (11 mg, assume 0.05 mmol of each) was stirred in KCN (0.2 M in D<sub>2</sub>O; pD 6.5; 0.5 mL, 0.10 mmol) for 2 h, centrifuged and the resultant solution and residual precipitate were analysed by <sup>31</sup>P-NMR and IR spectroscopies, respectively.



**Figure S45.** <sup>31</sup>P-NMR analysis (D<sub>2</sub>O) from investigation of selective phosphate release. A – resultant solution without the addition of HCN, showing no <sup>31</sup>P signals; B – resultant solution with the addition of HCN, showing the release of phosphate into the solution.

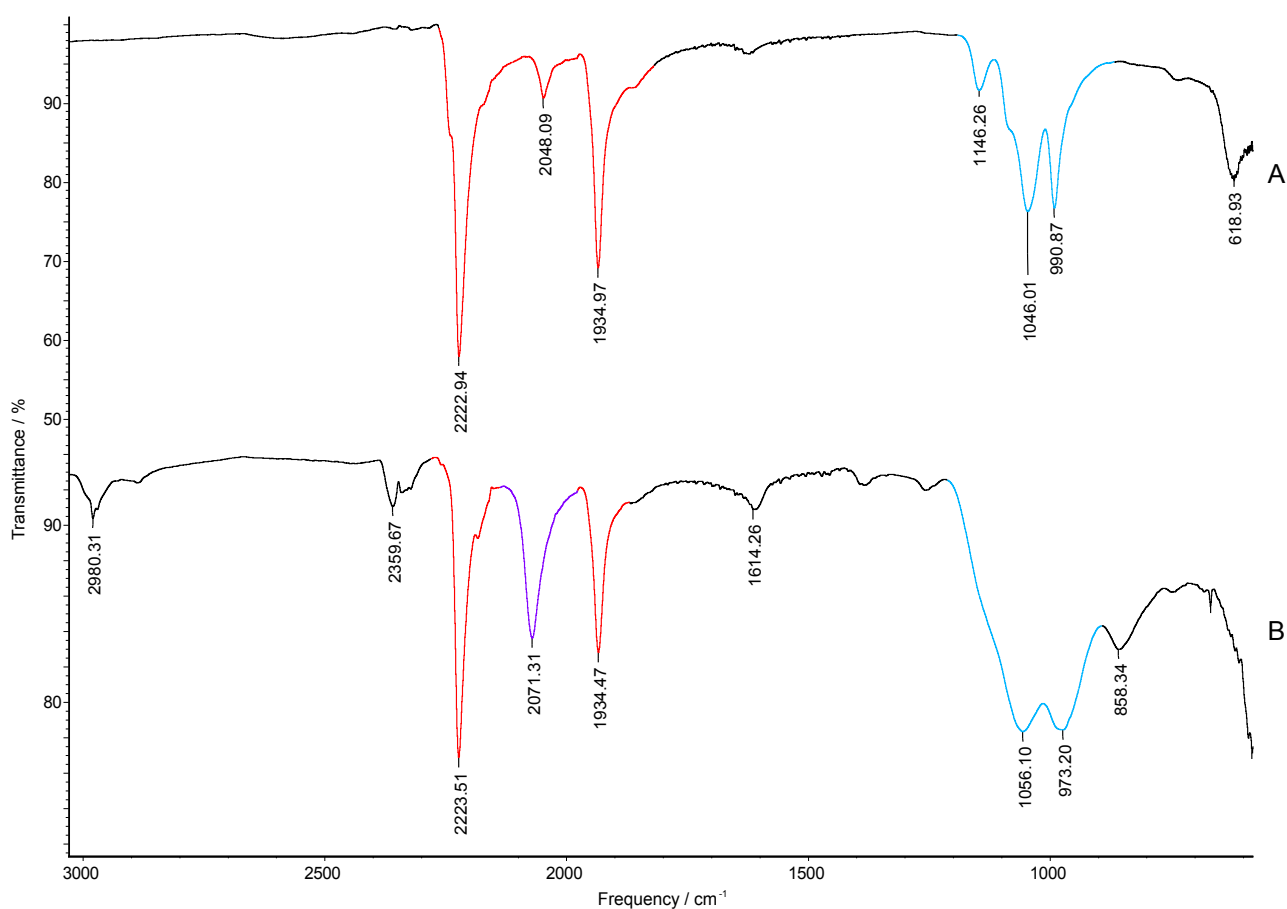


**Figure S46.** IR spectra from investigation of selective phosphate release from the  $\text{CuC}_3\text{N}$ –copper(I) phosphate mixture. A – starting  $\text{CuC}_3\text{N}$ –copper(I) phosphate mixture; B – residual precipitate without the addition of HCN, showing  $\text{CuC}_3\text{N}$ –copper(I) phosphate mixture signals (as in A); C – residual precipitate with the addition of HCN, suggesting reduction in amount of phosphate (blue signals), a similar signal for  $\text{C}\equiv\text{N}$  (red), a shift in the signal for  $\text{C}\equiv\text{C}$  (orange), and new signals in the  $\text{C}\equiv\text{N}$  region (purple) indicative of some  $\text{CuCN}$  and a possible  $[(\text{NC})_n\text{CuC}_3\text{N}]^{\text{p-}}$  type species prior to release of the cyanoacetylene **6**.



*Procedure for simultaneous sequestering of hydrogen cyanide 11 and cyanoacetylene 6*

Copper(I) chloride (25 mg, 0.25 mmol), iron(II) chloride tetrahydrate (50 mg, 0.25 mmol) and  $\text{Na}_2\text{HPO}_4$  (0.25 g, 1.75 mmol) were mixed in degassed  $\text{H}_2\text{O}$  (1 mL). Cyanoacetylene **6** (1.0 M in  $\text{H}_2\text{O}$ ; 0.50 mL, 0.50 mmol) was added rapidly to the resultant suspension, and within 3 min, KCN (1.0 M in  $\text{H}_2\text{O}$ ; 1.5 mL, 1.5 mmol) was added and stirring continued. After 1 h, a yellow-green/blue-brown co-precipitate had formed which was separated by filtration from the pale yellow supernatant. The precipitate was dried under vacuum over  $\text{P}_2\text{O}_5$  for 2 h and analysed by IR spectroscopy.



**Figure S47.** IR spectra from investigation of simultaneous sequestering of hydrogen cyanide **11** and cyanoacetylene **6**. A – standard sample of  $\text{Cu}_3\text{N}$ -copper(I) phosphate mixture; B – precipitate isolated after 1 h showing  $\text{Cu}_3\text{N}$  signals (red), copper(I) phosphate signals (blue), and a copper(I) ferrocyanide signal (purple).<sup>7</sup>

## 8. Notes and references

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- <sup>2</sup> McLaughlin, M., Mohareb, R. M. & Rapoport, H. An efficient procedure for the preparation of 4-substituted 5-aminoimidazoles. *J. Org. Chem.* **68**, 50 (2003).
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- <sup>4</sup> For procedure see: Bahde, R. J. & Rychnovsky, S. D. Cyclization via carbolithiation of  $\alpha$ -amino alkyllithium reagents. *Org. Lett.* **10**, 4017 (2008).
- <sup>5</sup> Procedure modified from: Hoshino, T.-I., Yamamoto, Y., Hasegawa, T., Takahashi, S. & Sawada, S. Facile synthesis of  $^{13}\text{C}$ -2-butenedinitrile and regiospecifically labeled  $^{13}\text{C}$ ,  $^{15}\text{N}$ -pyridoxines. *Biosci. Biotech. Biochem.* **58**, 1939 (1994).
- <sup>6</sup> Sanchez, R. A., Ferris, J. P. & Orgel, L. E. Cyanoacetylene in prebiotic synthesis. *Science* **154**, 784 (1966).
- <sup>7</sup> Garcia, S. C. & Domingues, P. H. Mössbauer study of the valency effects in copper ferrocyanides. *Rev. Bras. Fis.* **17**, 31, (1987).