

Supplementary Information (SI)

Non-enzymatic coupling of protometabolic reactions with a prebiotic redox cofactor

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1. Materials and general methods

General experimental conditions. Solvents, reagents and authentic samples (standards) were obtained from commercial sources and used without further purification. Aqueous solutions were prepared using Milli-Q® water. The commercial α -ketoacids used were: sodium pyruvate (**2a**), α -ketoglutaric acid disodium salt dihydrate (**2b**) and glyoxylic acid monohydrate (**2c**); pH adjustment to 8-9 (depending on the reaction) shifted them to their deprotonated forms, as depicted in the schemes in the main text. The commercial cofactors used, NAD⁺ and NADH, were: β -nicotinamide adenine dinucleotide monosodium salt and β -nicotinamide adenine dinucleotide disodium salt hydrate reduced form, respectively. All the reactions that required inert nitrogen atmosphere were performed with a Schlenk line and Schlenk glassware.

NMR spectroscopy for reaction monitoring. NMR spectra were recorded in H₂O:D₂O (9:1) solutions at 298 K using one of the next spectrometers, depending on availability:

- Bruker Avance III-HD Nanobay 300 MHz spectrometer
- Bruker Avance Neo 500 MHz spectrometer

The ¹H-NMR water signal was suppressed using noesygppr1d pulse, acquiring 128 scans for each sample with a relaxation delay of 2 seconds. To prepare samples for analysis, 450 μ L of the reaction mixture were taken and mixed with 50 μ L of a solution of TMSP-*d*₄ 3 mM in D₂O, reaching the ratio H₂O:D₂O (9:1). All chemical shifts are referenced to the standard TMSP-*d*₄: 0.00 (s, 9H) ppm.

NMR spectroscopy for characterization of pyridinium and dihydropyridine derivatives. NMR spectra were recorded in DMSO-*d*₆ or in D₂O using one of the next spectrometers, depending on availability:

- Bruker Avance II 300 MHz.
- Bruker Avance Neo 500 MHz spectrometer.

HRMS spectrometry. High resolution mass spectra were recorded on a Bruker timsTOF Pro 2 (UHPLC, ESI/APCI-IM-QTOF). For each molecule, the ionization source used is indicated: Electrospray ionization (ESI) or Atmospheric Pressure Chemical Ionization (APCI).

GC-MS spectrometry. GC analyses were performed on a 7820A gas chromatographer coupled to an Agilent Technologies 5977A mass selective detector equipped with an achiral capillary column HP-5 (30 m, 0.25 mm inner diameter, 25 μ m thickness), which operated at 1.372 V using He as the carrier gas, supplied at a constant flow rate of 1.0 mL min⁻¹. The analysis was carried out on a 1 μ L injection volume (split 20:1). The injection port temperature was 250 °C, and the column oven temperature program was: 100 °C for 2 min, then increased to 275 °C with a 20 °C min⁻¹ ramp, followed by a 5-min hold (total running time 15.75 min).

pH measurements. pH was determined with a Mettler Toledo SevenCompact pH meter equipped with a InLab Routine Pro sensor.

2. Experimental procedures

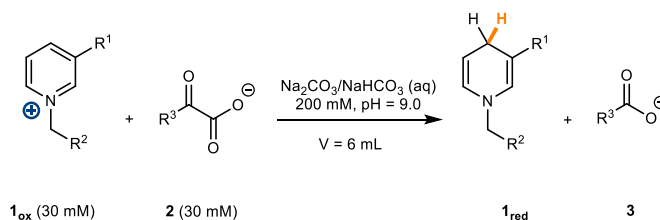
Preparation of bicarbonate and ammonia solutions

The solution $\text{NH}_3/\text{NH}_4\text{Cl}$ (600 mM, pH = 8.0) was prepared as follows: Two different solutions of NH_3 (600 mM) and NH_4Cl (600 mM) were prepared, mixed in an appropriate ratio (approximately 35 mL of NH_4Cl for each mL of NH_3) and its pH was measured. If necessary, more NH_3 (600 mM) or NH_4Cl (600 mM) were added to increase or decrease the pH until achieving pH 8.0.

Similarly, for preparing $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ (200 mM, pH = 9.0), solutions of Na_2CO_3 (200 mM) and NaHCO_3 (200 mM) were mixed in an appropriate ratio (approximately 70 mL of NaHCO_3 and 10 mL of Na_2CO_3) and the pH was adjusted, if necessary, with more Na_2CO_3 (200 mM) or NaHCO_3 (200 mM), to 9.0.

Same procedure was applied for preparing $\text{NH}_3/\text{NH}_4\text{Cl}$, $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ or $\text{HNa}_2\text{PO}_4/\text{H}_2\text{NaPO}_4$ solutions with other concentrations or at other pH. More information in our previous work.¹

General procedure for oxidative decarboxylation of α -ketoacids with pyridiniums

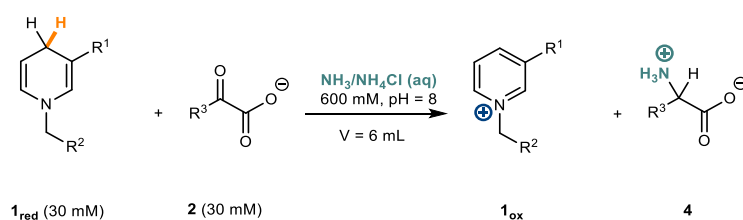


Two different stock solutions of the pyridinium (1_{ox}, 60 mM) and α -ketoacid (2, 60 mM) derivatives were prepared in aqueous $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ (200 mM, pH = 9.0). The pH of each solution was measured again and, if necessary, adjusted to pH = 9.0 using aqueous HCl (1 M) or NaOH (1 M).^{*} 4 mL of the pyridinium 1_{ox} solution[†] was transferred to a Schlenk tube. Separately, 3 mL of α -ketoacid 2 solution was transferred to another Schlenk tube equipped with a magnetic stirring bar. Both Schlenk tubes were sealed with septa and three freeze-pump-thaw cycles were carried out, backfilling with nitrogen. With a needle purged 3 times with nitrogen, 3 mL of the pyridinium 1_{ox} solution was transferred to the Schlenk tube containing the 3 mL of α -ketoacid 2 solution, resulting in a final volume of 6 mL and a concentration of 30 mM for each reagent. This procedure ensured that both reagents were under oxygen-free conditions before being mixed. The reaction mixture was stirred at 25 °C for up to 168 h. Control experiments, containing only pyridinium 1_{ox} or α -ketoacid 2, were performed using the same procedure.

^{*} For most pyridiniums, dihydropyridines and α -ketoacids (among them, 1_{a_ox}, 1_{a_red} and 2_a), dissolution did not alter the pH, and this step was therefore skipped in replicate experiments.

[†] The excess volume of this solution allows for more accurate withdrawal of 3 mL later.

General procedure for reductive amination of α -ketoacids with dihydropyridines



Two different stock solutions of the dihydropyridine (**1_{red}**, 60 mM)[‡] and α -ketoacid (**2**, 60 mM) derivatives were prepared in aqueous $\text{NH}_3/\text{NH}_4\text{Cl}$ (600 mM, pH = 8.0). The pH of each solution was measured again and, if necessary, adjusted to pH = 8.0 using aqueous HCl (1 M) or NaOH (1 M).^{*} 4 mL of α -ketoacid **2** solution[†] was transferred to a Schlenk tube. Separately, 3 mL of dihydropyridine **1_{red}** solution was transferred to another Schlenk tube equipped with a magnetic stirring bar. Both Schlenk tubes were sealed with septa and three freeze-pump-thaw cycles were carried out, backfilling with nitrogen. With a needle purged 3 times with nitrogen, 3 mL of the α -ketoacid **2** solution was transferred to the Schlenk tube containing the 3 mL of **1_{red}** solution, resulting in a final volume of 6 mL and a concentration of 30 mM for each reagent. This procedure ensured that both reagents were under oxygen-free conditions before being mixed. The reaction mixture was stirred at 25 °C for up to 168 h. Control experiments, containing only dihydropyridine **1_{red}** or α -ketoacid **2**, were performed using the same procedure.

[‡] Most dihydropyridines **1_{red}** dissolved slowly in water and solid traces initially remained undissolved. If a transfer was necessary, careful handling was required to avoid material loss. For this reason, the α -ketoacid **2** solution was always added to the dihydropyridine **1_{red}** solution. Within the first hours of reaction, the dihydropyridine fully dissolved.

3. Procedure for quantification by ^1H -NMR

To prepare samples for analysis, an aliquot of **450 μL of the reaction mixture** was taken and combined with **50 μL of a 3 mM TMSP- d_4** solution in D_2O , achieving an $\text{H}_2\text{O}:\text{D}_2\text{O}$ ratio of 9:1. The solution was then transferred to an NMR tube. If the spectrometer was not available in that moment, the tubes were frozen and subsequently thawed before analysis. ^1H -NMR spectra were recorded using water suppression with nuseygppr1d pulse sequence (see parameters in section 1, Materials and general methods). The resulting spectra were processed using MestReNova (version 14.3.2-32681) software with the following protocol:

1. All chemical shifts were referenced to the internal standard TMSP- d_4 : 0.00 (s, 9H) ppm.
2. Phase correction was applied only when the automatic phasing was not optimal.
3. Baseline correction was necessary for most spectra. It was performed using the “Multipoint Baseline Correction” tool in MestReNova, which has been reported to be suitable for quantitative analysis,² with the “Smooth Segments” algorithm.

Baseline correction was found to be essential to avoid overestimation of integral values and, consequently, product yields.

Depending on the compound that is analysed, the quantification was performed by two different methods:

- Quantification by direct integral comparison.
- Quantification based on calibration lines.

Quantification by direct comparison of integrals

The signal of TMSP- d_4 at 0.00 (s, 9H) ppm was integrated and set to a reference value of 9.00. One signal of the analyte (which do not overlap with others) was integrated. The concentration of the analyte in mM (mmol/L) in the reaction mixture was calculated using the following equation:

$$\text{Analyte (mM)} = \left(\frac{\text{Analyte signal integral} \times \text{mmol of TMSP}}{\text{Analyte signal number of protons}} \right) \times \frac{1}{\text{mL of taken aliquot}} \quad (\text{S1})$$

Where:

- **mmol of TMSP** is the mmol amount of TMSP- d_4 present in the NMR sample, which in all cases is approx. $3 \text{ mmol/L} \cdot 0.00005 \text{ L} = \mathbf{0.00015 \text{ mmol}}$.
The term enclosed in parentheses in Equation S1 gives the mmol amount of analyte present in the NMR sample. This is valid only when the integral of the signal of TMSP- d_4 has been normalized to 9.00, corresponding to its nine equivalent protons.
- **mL of taken aliquot** is **0.00045 L** in all cases. Thus, the result corresponds to the concentration of the analyte in the taken aliquot and, by extension, in the original reaction mixture at the moment the aliquot was taken.

This procedure assumes that the analyte protons and the TMSP- d_4 protons produce exactly the same area in ^1H -NMR.

Quantification based on calibration lines

Preparation of calibration samples:

The stock solution of TMSP- d_4 3 mM was made with pure D_2O as solvent.

For all other cases, stock solutions and dilutions of the analytes were made using the corresponding ammonia or bicarbonate solutions (NH_3/NH_4Cl 600 mM at pH 8, $Na_2CO_3/NaHCO_3$ 200 mM at pH 9, etc.). To simplify, these solutions will be named generically *basic mixture* in this section.

Preparation of stock solution: The desired amount of the analyte (typically 0.6 mmol) was firstly dissolved in half the required volume of *basic mixture*. The pH was measured and, if needed, adjusted with aqueous HCl (1 M) or NaOH (1 M) to reach the desired pH. It was transferred to a volumetric flask and more *basic mixture* was added to complete the desired final volume (typically 20 mL, reaching a stock solution of 30 mM).

Preparation of dilutions and NMR tubes: Solutions of 2 mL of the analyte were made diluting the stock solution of the analyte with *basic mixture*. To prepare the samples for measurements in NMR, 450 μL of the solution were taken and mixed with 50 μL of a solution of TMSP- d_4 3 mM in D_2O , reaching the ratio $H_2O:D_2O$ (9:1).

The signal of TMSP- d_4 at 0.00 (s, 9H) ppm was integrated and set to a reference value of 9.00. The integral value of the analyte is measured after this adjustment. The correlation line was obtained from the least-squares fitting (intercept = 0).

See calibration lines in next page. For all of them, a theoretical calibration line, calculated point by point using Equation S1, has been included to visualize the deviation of the experimental line with respect to the quantification by direct comparison of integrals.

Other considerations in the quantification

The time past between the start of the reaction and the acquisition of the first NMR spectrum (*ca.* 1 hour) is an estimated value that may vary slightly between experiments, depending on the position and timing within the NMR autosampler carousel. As a result, the 1H -NMR yield values (%) reported at the 1-hour time point may carry some degree of uncertainty.

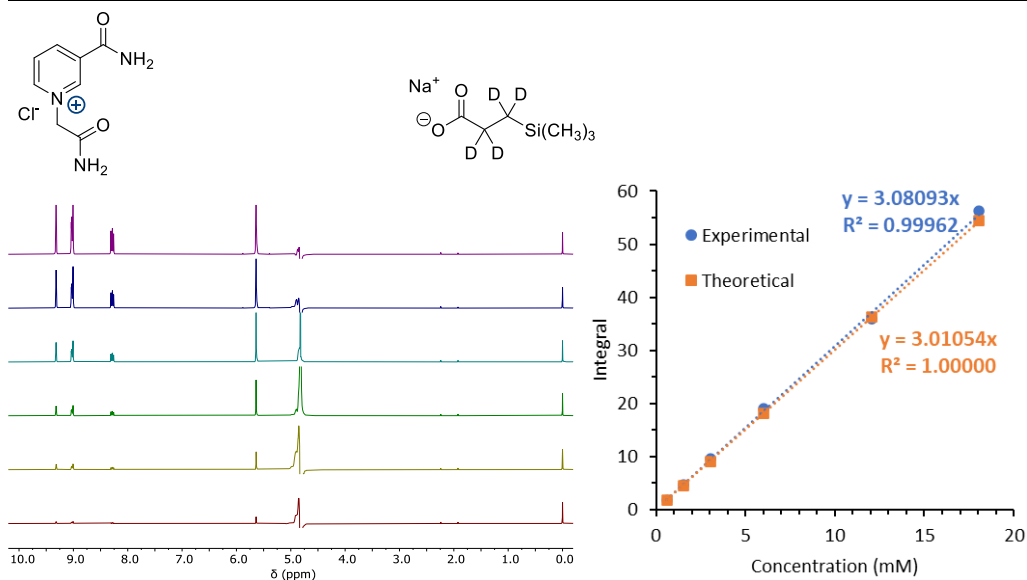
Sections 5 and 6 present the reaction spectra with assigned signals for the identified reagents and products. The signal used for quantification is indicated. When two signals are marked, the one highlighted with an arrow corresponds to the quantified signal.

Calibration lines

As an example, calibration line of pyridinium **1a_{ox}** in NH₃/NH₄Cl (600 mM, pH = 8) is shown:

Stock solution	Weighted mass (mg)	Mmol (g/mol)	V (mL)	Conc. (mM)	V (mL) aliquot	n (mmol) in aliquot	Integrated signal (ppm)
1a_{ox}	129.9	215.637	20	30.12	0.450	See each case	9.32 (s, 1H)
TMSP- <i>d</i> ₄	10.3	172.27	10	2.98	0.050	0.000149	0.00 (s, 9H) ppm

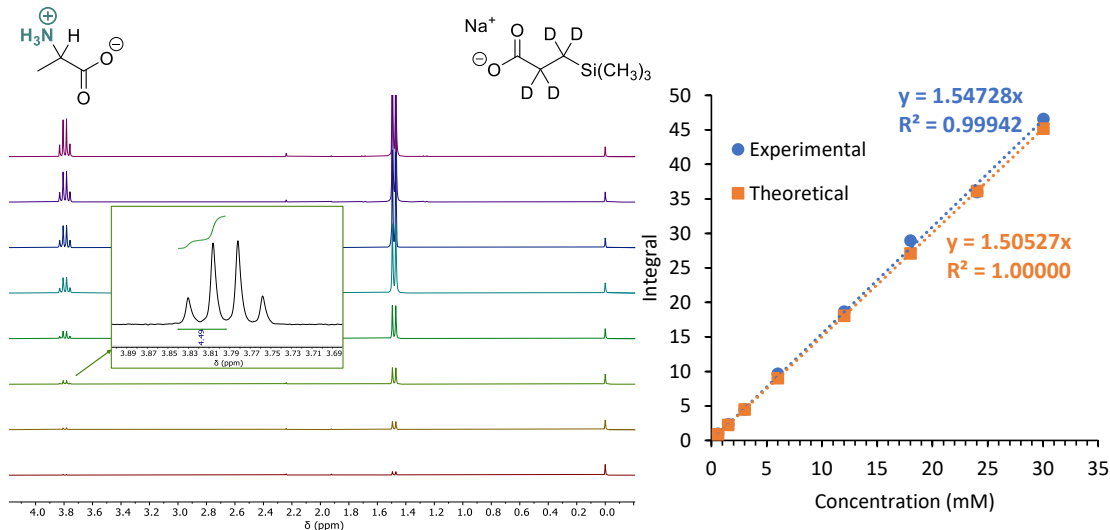
Dilution	Concentration (mM)	n (mmol) in aliquot	Measured integral of analyte	Theoretical integral of analyte
02:98	0.60	0.00027	1.82	1.81
05:95	1.51	0.00068	4.69	4.52
10:90	3.01	0.00135	9.69	9.04
20:80	6.02	0.00270	18.96	18.08
40:60	12.05	0.00540	36.00	36.16
60:40	18.07	0.00811	56.22	54.24



In the case of alanine (**4a**) in $\text{NH}_3/\text{NH}_4\text{Cl}$ (600 mM, pH = 8), we found the following issue during quantification in the reductive amination reactions using **1a_{red}**-**1g_{red}** as reductants: in the studied signal, 3.79 (q, $J = 7.2$ Hz, 1 H) the rightmost peak of the quartet slightly overlaps with impurities (see below, Figure S33). Therefore, we performed the quantification using only the left half part of the signal, integrating the two leftmost peaks of the quartet. The resulting calibration line closely matches the theoretical one for an effective number of protons of 0.5 H, confirming the reliability of this approach for quantifying alanine.

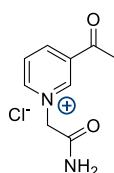
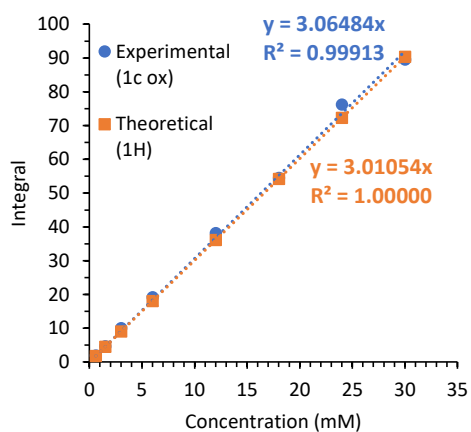
Stock solution	Weighted mass (mg)	Mmol (g/mol)	V (mL)	Conc. (mM)	V (mL) aliquot	n (mmol) in aliquot	Integrated signal (ppm)
4a	53.5	89.09	20	30.03	0.450	See each case	left half of 3.79 (q, $J = 7.2$ Hz, 0.5 H)
TMSP- d_4	10.3	172.27	10	2.98	0.050	0.000149	0.00 (s, 9H) ppm

Dilution	Concentration (mM)	n (mmol) in aliquot	Measured integral of analyte	Theoretical integral of analyte
02:98	0.60	0.00027	0.98	0.90
05:95	1.50	0.00068	2.34	2.26
10:90	3.00	0.00135	4.49	4.52
20:80	6.01	0.00270	9.70	9.04
40:60	12.01	0.00540	18.68	18.08
60:40	18.02	0.00811	28.99	27.12
80:20	24.02	0.01081	36.02	36.16
100:0	30.03	0.01351	46.60	45.20



Note: With these calibration spectra, if the entire signal is integrated, the resulting calibration line is $y = 3.11197x$ ($R^2 = 0.99950$). This was applied in the reaction where NADH was used as the reductant, as no overlapping issues were observed and the full alanine quartet could be integrated.

Other calibration lines:

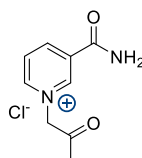
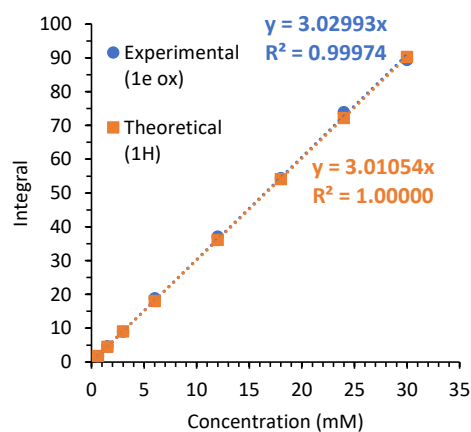


1c_{ox} (NH₃/NH₄Cl, 600 mM, pH = 8.0)

Integrated signal = 9.42 ppm

$y = 3.06484x$

$R^2 = 0.99913$

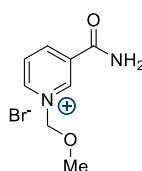
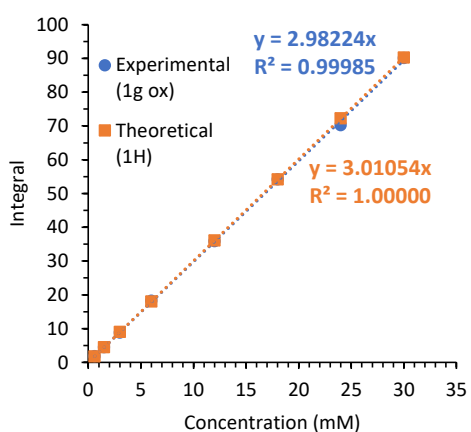


1e_{ox} (NH₃/NH₄Cl, 600 mM, pH = 8.0)

Integrated signal = 9.16 ppm

$y = 3.02993x$

$R^2 = 0.99973$

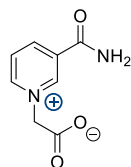
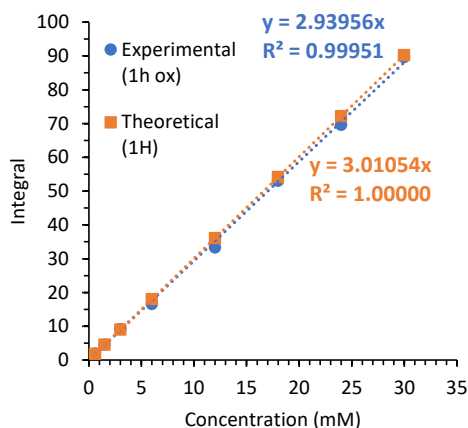


1g_{ox} (NH₃/NH₄Cl, 600 mM, pH = 8.0)

Integrated signal = 9.43 ppm

$y = 2.98224x$

$R^2 = 0.99985$

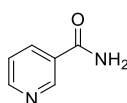
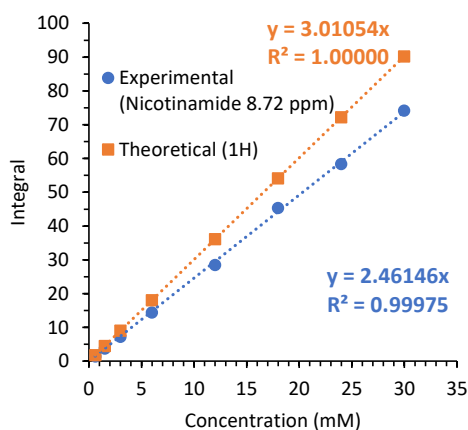


1h_{ox} (NH₃/NH₄Cl, 600 mM, pH = 8.0)

Integrated signal = 9.26 ppm

$y = 2.93956x$

$R^2 = 0.99951$

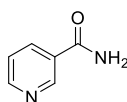
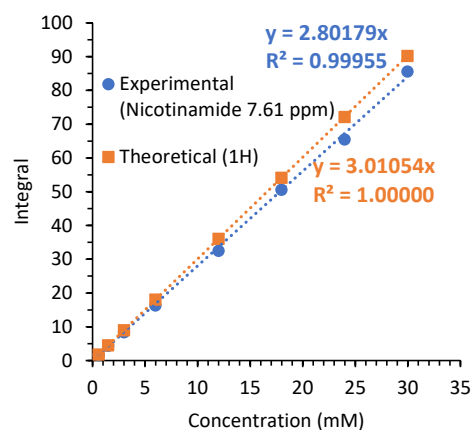


Nicotinamide (NH₃/NH₄Cl, 600 mM, pH = 8.0)

Integrated signal: 8.72 ppm

$y = 2.46146x$

$R^2 = 0.99975$



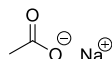
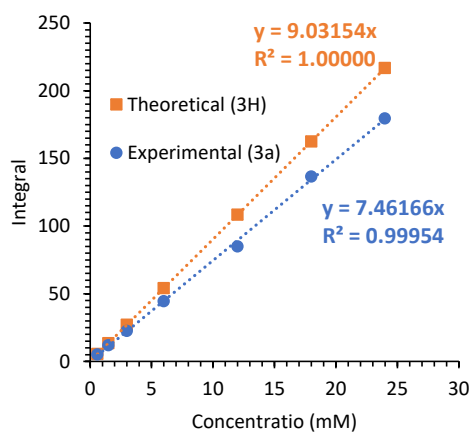
Nicotinamide (NH₃/NH₄Cl, 600 mM, pH = 8.0)

Integrated signal: 7.61 ppm

$y = 2.80179x$

$R^2 = 0.99955$

In reactions where nicotinamide was detected, it could be quantified using the signal at 8.72 ppm or the signal at 7.61 ppm, depending on which of them was in a cleaner part of the spectrum without overlapping with other signals.

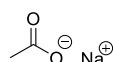
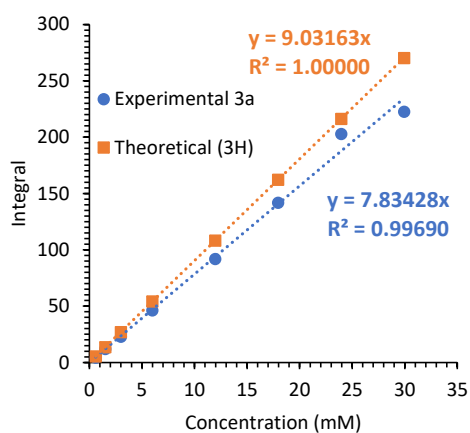


Acetate **3a** ($\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ 200 mM, pH = 9.0)

Integrated signal: 1.92 ppm

$y = 7.46166 x$

$R^2 = 0.99954$

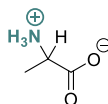
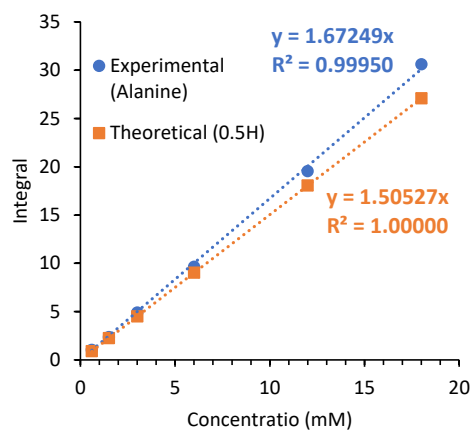


Acetate **3a** ($\text{NH}_3/\text{NH}_4\text{Cl}$, 600 mM, pH = 8.5)

Integrated signal: 1.92 ppm

$y = 7.83428 x$

$R^2 = 0.99690$

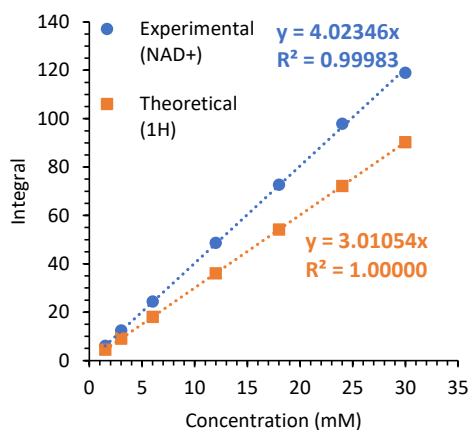


Alanine **4a** ($\text{NH}_3/\text{NH}_4\text{Cl}$, 600 mM, pH = 8.5)

Integrated left half part of the signal: 3.78 ppm

$y = 1.67249$

$R^2 = 0.99950$

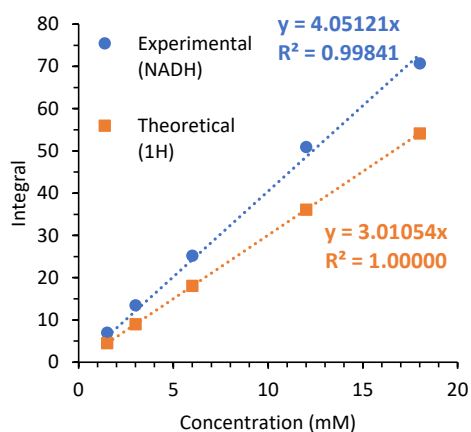


NAD⁺ (NH₃/NH₄Cl, 200 mM, pH = 8.0)

Integrated signal: 9.34 ppm

$$y = 4.02346x$$

$$R^2 = 0.99983$$



NADH (NH₃/NH₄Cl, 200 mM, pH = 8.0)

Integrated signal: 6.94 ppm

$$y = 4.05121x$$

$$R^2 = 0.99841$$

We observed that variation in aqueous buffer composition, concentration, pH (within the range of 8-9) do not significantly affect the slope or correlation of the calibration lines.

Analytes that are not in this list were quantified using equation S1.

4. Control reactions of α -ketoacids

Pyruvate (**2a**)

When pyruvate is dissolved in aqueous solution (pH 7-9), several species can be identified: pyruvate (**2a**), pyruvate hydrate (**2a.II**) and the self-condensation form parapyruvate (**2a.III**) (Figure S1). In the presence of $\text{NH}_3/\text{NH}_4\text{Cl}$, an additional form can be detected, **2a.IV**, likely corresponding to a self-condensation adduct of pyruvate with its imine form. These forms are always present in the spectra and should be taken into account when analysing pyruvate reactions with other reagents. Further spectroscopic details are available in the previous publication.¹

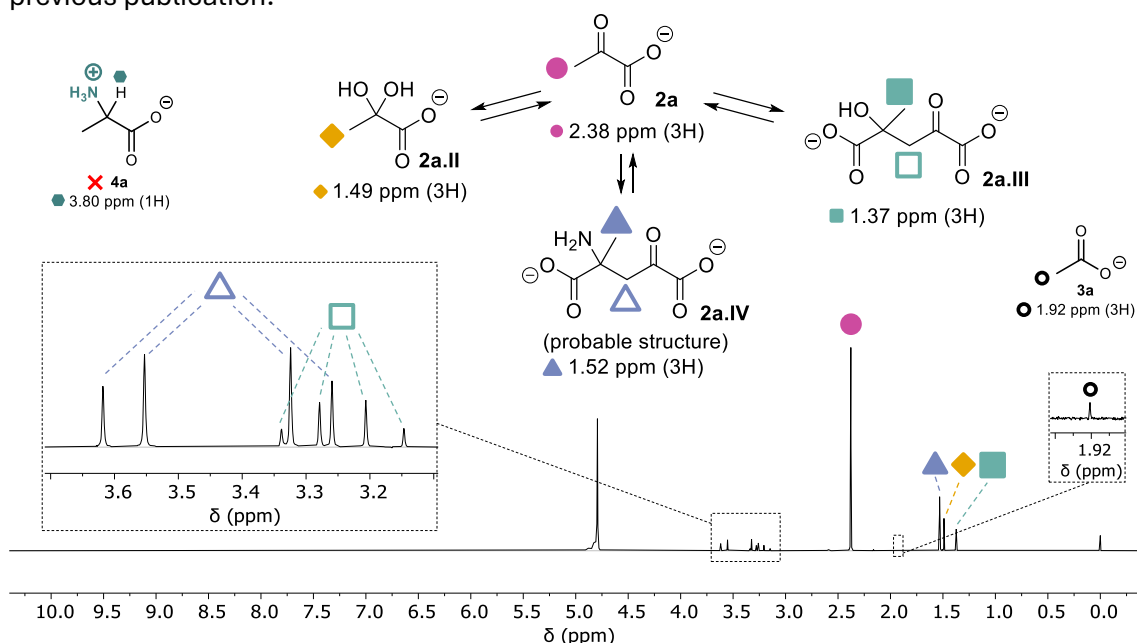


Figure S1. ^1H -NMR (300 MHz, $\text{H}_2\text{O}:\text{D}_2\text{O}$ 9:1, noesygppr1d) spectra of pyruvate (**2a**, 30 mM) in aqueous $\text{NH}_3/\text{NH}_4\text{Cl}$ solution (600 mM) at pH 8, after 72 h. Structure and assignment of the different forms detected in the spectrum.

The equilibria between these species are fast and highly reversible, and their relative distribution are sensitive to small variations in measurement conditions. Table S1 reports the molar ratio of the different equilibrating species (including the background formation of acetate **3a**) observed in one representative replicate of the control reaction of pyruvate (**2a**, 30 mM) for each aqueous buffer over time.

In media without ammonia (Figure S2A), the hydrate (**2a.II**) almost maintains the same molar ratio in all measures. In contrast, parapyruvate (**2a.III**) ratio fluctuates, especially at pH 9, confirming that this equilibrium is highly reversible in these conditions.^s In any case, inset shows that pH 9 favours parapyruvate compared to pH 7 or 8.^{**} Since parapyruvate

^s As indicated in section 3 of this document, NMR aliquots are frozen and thawed depending on NMR equipment availability and the temperature at which each sample is measured can vary slightly from tube to tube, being this one of the possible causes of the equilibrium shift.

^{**} Due to the aforementioned fluctuation, parapyruvate (**2a.III**) formation exhibited some variability over time in replicates of this control, however, a consistent trend was always observed across all experiments: parapyruvate (**2a.III**) levels were significantly higher at pH 9 than at pH 7 or 8.

formation involves necessarily condensation with the enol form of pyruvate, this observation might provide indirect evidence that enol tautomer is more accessible at pH 9.

In $\text{NH}_3/\text{NH}_4\text{Cl}$ solutions (Figure S2B), species **2a.IV** reaches a molar ratio of 19 % while parapyruvate (**2a.III**) reaches up to 7 % even at pH 8, indicating that these auto-condensation products are more readily formed in ammoniacal media due to the more accessible enamine and imine forms.^{††}

Table S1. Ratio **2a** : **2a.II** : **2a.III** : **2a.IV** : **3a** (normalized to 100, considering stoichiometry relationships) in the controls of pyruvate (**2a**, 30 mM) in different conditions. The absolute acetate (**3a**) yield (%) has also been presented.

Conditions	Time (h)	Pyruvate (2a)	Hydrate (2a.II)	Parapyruv. (2a.III)	(2a.IV)	Acetate (3a)	Acetate (3a) yield (%)
NaH ₂ PO ₄ / Na ₂ HPO ₄ (200 mM, pH = 7.0)	1	86.9	11.6	0.7	0.00	0.04	0.04
	24	87.0	11.4	0.8	0.00	0.03	0.03
	48	86.4	11.8	0.9	0.00	0.03	0.03
	72	86.6	11.8	0.8	0.00	0.03	0.03
	168	86.9	11.4	0.9	0.00	0.02	0.01
NaH ₂ PO ₄ / Na ₂ HPO ₄ (200 mM, pH = 8.0)	1	86.6	11.7	0.8	0.00	0.06	0.05
	24	87.0	10.8	1.1	0.00	0.04	0.03
	48	86.7	11.3	1.0	0.00	0.04	0.03
	72	86.5	11.6	0.9	0.00	0.06	0.06
	168	85.4	11.2	1.6	0.00	0.18	0.16
NaHCO ₃ / Na ₂ CO ₃ (200 mM, pH = 9.0)	1	78.3	8.5	6.6	0.00	0.03	0.02
	24	87.6	9.4	1.5	0.00	0.06	0.06
	48	86.0	9.8	2.1	0.00	0.04	0.04
	72	85.1	9.5	2.7	0.00	0.02	0.02
	168	78.9	8.4	6.3	0.00	0.02	0.02
NH ₄ Cl/ NH ₃ (600 mM, pH = 8.0)	1	85.4	11.4	1.0	0.5	0.06	0.04
	24	68.6	9.3	3.2	7.9	0.06	0.06
	48	57.1	8.0	4.8	12.7	0.08	0.08
	72	50.6	7.0	5.6	15.5	0.09	0.10
	168	41.0	5.6	7.5	19.1	0.35	0.35
NH ₄ Cl/ NH ₃ (600 mM, pH = 8.5)	1	86.4	9.9	1.4	0.4	0.06	0.05
	24	51.9	6.9	5.6	14.9	0.07	0.08
	48	40.0	6.0	7.7	19.2	0.18	0.20
	72	37.6	6.0	8.5	19.5	0.30	0.30
	168	35.1	4.9	9.3	20.0	1.40	1.20

When analysing reactions, given that pyruvate quantification requires monitoring three or four interconverting species and that their relative proportion may vary with small changes in conditions, we therefore focused our quantitative analysis on products formation, which, as shown below, displays high reproducibility and low associated error.

^{††} Across different replicates, the relative ratio of **2a.IV** also showed some fluctuation. For instance, in one replicate its molar ratio decreased between 24 and 48 hours and increased again at 72 h, consistent with a rapid and highly reversible equilibrium that is sensitive to small variations in measurements conditions.

Importantly, the background formation of **alanine was never observed**. Besides, **pyruvate background formation is low and reproducible** (see discussion below, in Figure S5, Figure S7 and Figure S55).

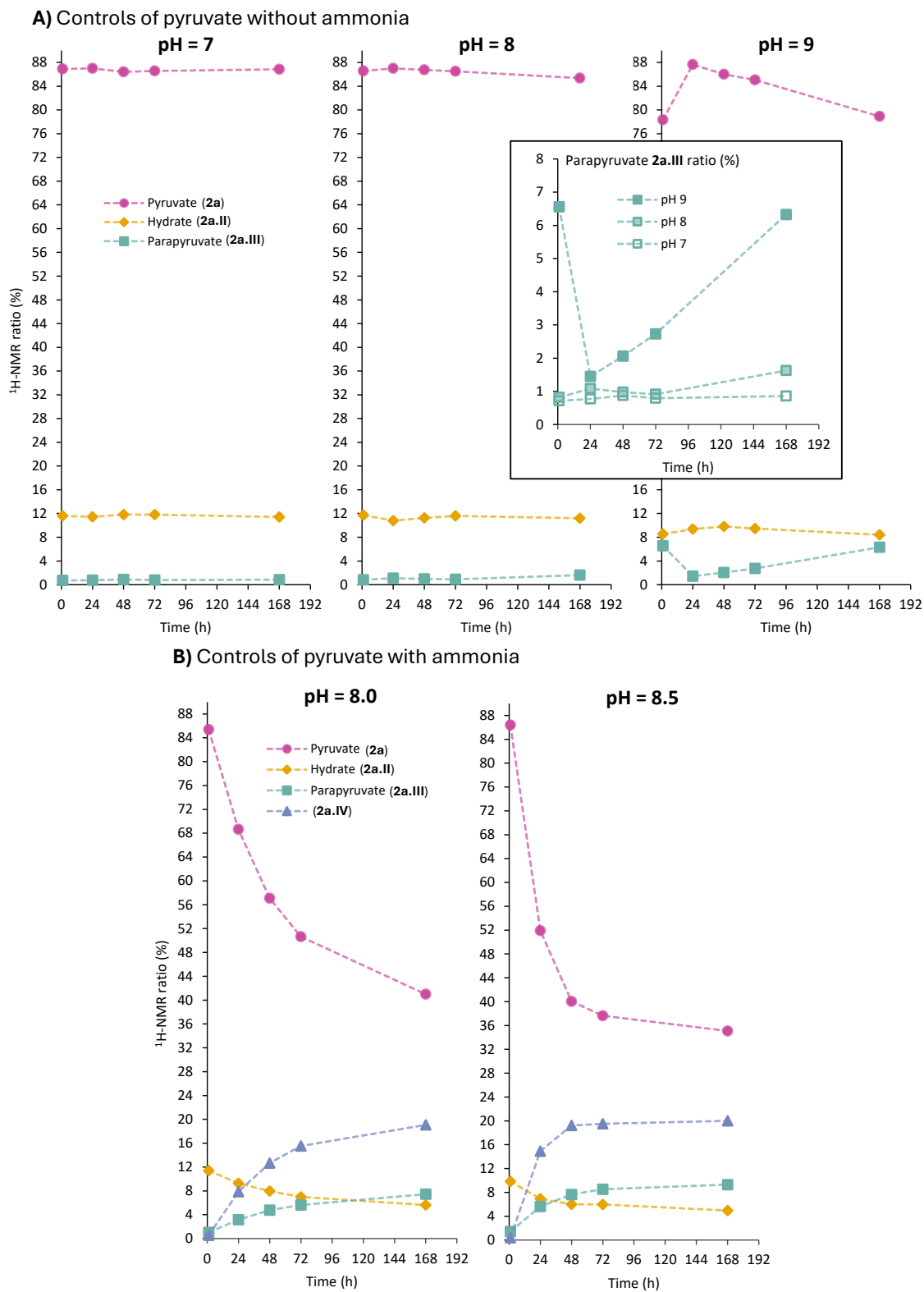


Figure S2. Plots of ^1H -NMR monitoring of molar ratios of the different species in equilibria in control reactions of pyruvate (**2a**, 30 mM) in the following media. **A)** pH 7.0 ($\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$, 200 mM), pH 8.0 ($\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$, 200 mM) and pH 9.0 ($\text{H}_2\text{CO}_3/\text{Na}_2\text{CO}_3$, 200 mM). **B)** pH 8.0 ($\text{NH}_4\text{Cl}/\text{NH}_3$, 600 mM) and pH 8.5 ($\text{NH}_4\text{Cl}/\text{NH}_3$, 600 mM). All numerical data are presented in Table S1.

α -Ketoglutarate (**2b**)

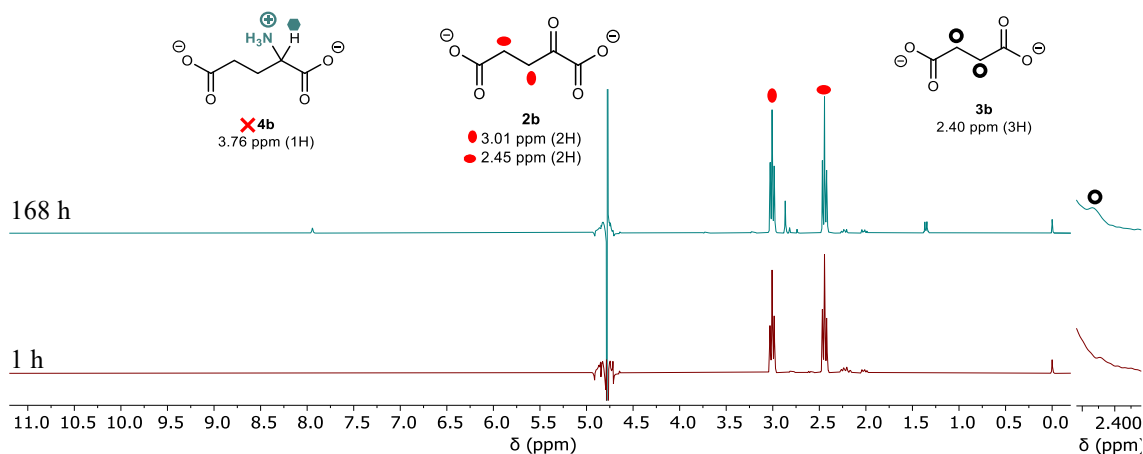


Figure S3. ^1H -NMR (300 MHz, $\text{H}_2\text{O}:\text{D}_2\text{O}$ 9:1, noesygppr1d) spectra of α -ketoglutarate (**2b**, 30 mM) in aqueous $\text{NH}_3/\text{NH}_4\text{Cl}$ solution (600 mM) at pH 8, after 1 h and 168 hours, showing that the compound is quite stable in this medium. α -Ketoglutarate is in equilibrium with other minor forms. **No traces of glutamate (**4b**) were observed.**

Table S2. ^1H -NMR yield of succinate (**3b**) in the control reaction of α -ketoglutarate (**2b**, 30 mM) in aqueous $\text{NH}_3/\text{NH}_4\text{Cl}$ solution (600 mM) at pH 8. Comparison with the reductive amination of **2b** using **1a_{red}** (Figure S37) leads to the conclusion that succinate arises predominantly from the action of the *in situ* generated **1a_{ox}**, with a very minor contribution from background process.

Time (h)	3b	4b
1	traces	0.0
24	traces	0.0
48	traces	0.0
72	0.6	0.0
168	0.7	0.0

Control of α -ketoglutarate in $\text{NaHCO}_3/\text{Na}_2\text{CO}_3$ (200 mM) at pH = 9 also showed <1% yield of succinate after 168 h, with no detectable glutamate formation.

Glyoxylate (2c)

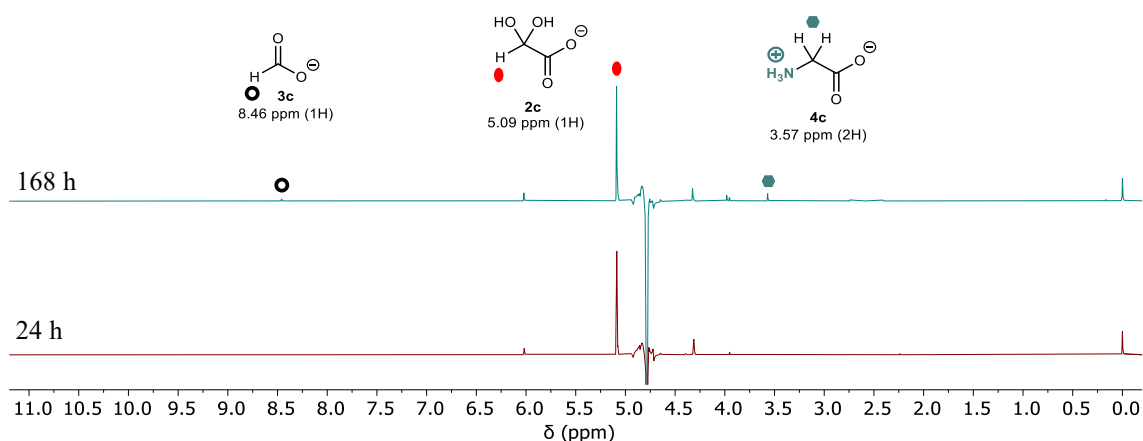


Figure S4. ^1H -NMR (300 MHz, $\text{H}_2\text{O}:\text{D}_2\text{O}$ 9:1, noesygppr1d) spectra of glyoxylate (**2c**, 30 mM) in aqueous $\text{NH}_3/\text{NH}_4\text{Cl}$ solution (600 mM) at pH 8, after 24 h and 168 hours, showing that the compound is quite stable in this medium. The compound is predominantly present as its hydrate and it is in equilibrium with other minor forms.

Table S3. ^1H -NMR yield of formate (**3c**) and glycine (**4c**) in the control reaction of glyoxylate (**2c**, 30 mM) in aqueous $\text{NH}_3/\text{NH}_4\text{Cl}$ solution (600 mM) at pH 8.0. Comparison with the reductive amination of glyoxylate (**2c**) using **1a_{red}** (Figure S39) confirms that glycine (**4c**) and formate (**3c**) formations arise predominantly from the action of **1a_{red}** and *in situ* generated **1a_{ox}**, with a minor contribution from background formation in both compounds.

Time (h)	3c	4c
24	0.2	0.1
48	0.3	0.2
72	0.3	0.4
168	0.6	1.4

5. Optimization of reaction conditions

Optimization of oxidative decarboxylation

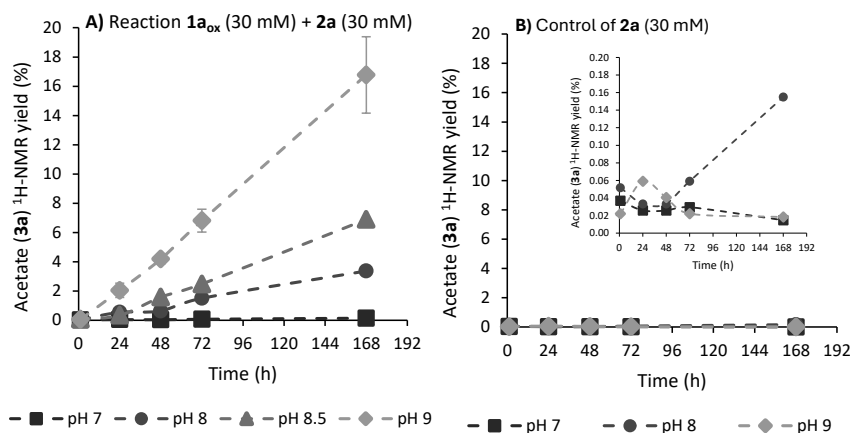


Figure S5. Optimization and control experiments of oxidative decarboxylation by pyridinium **1a_{ox}**. **A)** Monitoring of acetate (**3a**) formation in the reaction of pyridinium (**1a_{ox}**, 30 mM) and pyruvate (**2a**, 30 mM) in Na₂CO₃/NaHCO₃ (200 mM) at pH 9.0 or in Na₂HPO₄/NaH₂PO₄ (200 mM) at pH 8.5, 8.0 or 7.0. (Data in Source Data file) **B)** Monitoring of acetate (**3a**) formation in the control of pyruvate (**2a**, 30 mM) in Na₂CO₃/NaHCO₃ (200 mM) at pH 9.0 or in Na₂HPO₄/NaH₂PO₄ (200 mM) at pH 8.0 or 7.0 (inset: expanded yield axis) (data from Table S1). Errors bars represent $\pm$ S.D.

The oxidative decarboxylation was almost null at pH 7 and took place sluggishly at pH 8. Dihydropyridine **1a_{red}** was never observed at these pH values, indicating that its degradation was always faster than its formation. At pH 8.5, the formation of acetate increased and the dihydropyridine **1a_{red}** could be observed at the 24 h time point, but not at any other stage of the reaction. In contrast, considerable amount of acetate was observed at pH 9, conditions that also enabled to identify the dihydropyridine presence through the course of the reaction, as indicated in the main text.

Control experiments with pyruvate alone in the corresponding buffer showed only traces of acetate (below 0.16 % yield). The evolution of these values over time was erratic, likely because they fall close to the detection limit. Therefore, in reactions in the presence of **1a_{ox}**, acetate (**3a**) formation arises predominantly from the action of the cofactor analogue, while the background formation is negligible.

Optimization of reductive amination

After monitoring the reaction at pH 8.0 with different NH₃/NH₄Cl concentration (200 mM, 600 mM and 1 M, Figure S6A), concentration of 600 mM was selected as a compromise between observing good yields and maintaining plausible prebiotic conditions.

Small amounts of glycnamide are generated during the reaction, as a consequence of the cofactor rupture. Due to the proximity of its pK_a (8.2)³ with the working pH, its NMR chemical shift is sensitive to small pH variations (Figure S6B). At pH 7.0, the glycnamide signal overlaps with that from alanine, avoiding accurate quantification of the amino acid (although a qualitative increase in alanine could still be observed over the week). The pH was optimized by comparing the formation of pyridinium **1a_{ox}**. Given the similarity of results

(Figure S6C), pH 8 was selected as the working pH, in analogy with our previous study on reductive amination using NADH,¹ which allowed us to quantify the alanine formation.

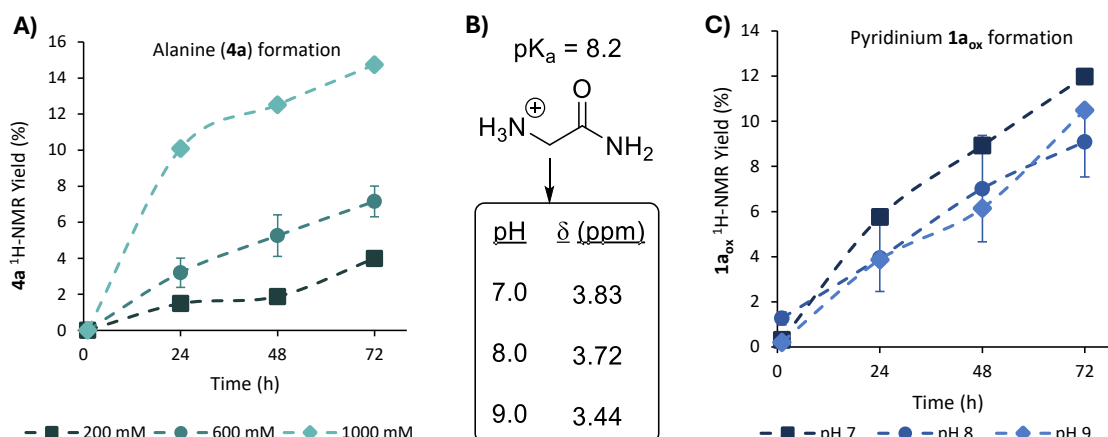


Figure S6. Monitoring of the reaction between dihydropyridine (**1a_{red}**, 30 mM) and pyruvate (**2a**, 30 mM) in $\text{NH}_3/\text{NH}_4\text{Cl}$. **A)** Monitoring of alanine (**4a**) formation at different concentrations of $\text{NH}_3/\text{NH}_4\text{Cl}$ (pH = 8). **B)** Chemical shifts of methylenic protons of glycnamide at different pH. **C)** Monitoring of pyridinium **1a_{ox}** formation at different pH in $\text{NH}_3/\text{NH}_4\text{Cl}$ (600 mM). Plots data in Source Data file. Errors bars represent $\pm$ S.D.

Therefore, $\text{NH}_3/\text{NH}_4\text{Cl}$ conditions of 600 mM at pH = 8.0 were selected, as they enhance alanine (**4a**) formation and enabled its quantification, while knowing that the reaction also proceeded at 200 mM of $\text{NH}_3/\text{NH}_4\text{Cl}$ or at pH = 7.0, expanding the range of plausible conditions under which this chemistry could have occurred in prebiotic scenarios.

Importantly, a control of pyruvate in $\text{NH}_3/\text{NH}_4\text{Cl}$ (600 mM, pH = 8.0) was performed, showing no detectable alanine background formation (see previous section). As discussed in the main text, acetate (**3a**) is produced in the reaction of **1a_{red}** with pyruvate (**2a**) in $\text{NH}_3/\text{NH}_4\text{Cl}$ as a consequence of the *in situ* generation of **1a_{ox}** (Figure S7A). Comparison with the pyruvate control confirms that acetate formation arises predominantly from the action of *in situ* generated **1a_{ox}**, with a minor contribution from background process (Figure S7B).

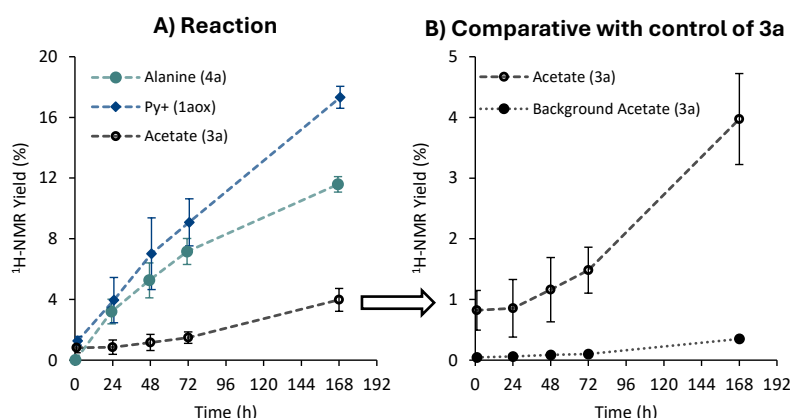


Figure S7. Reaction of **1a_{red}** (30 mM) and **2a** (30 mM) in aq. $\text{NH}_3/\text{NH}_4\text{Cl}$ (600 mM) at pH 8. **A)** Plots of ^1H -NMR monitoring of alanine (**4a**), pyridinium **1a_{ox}** and acetate (**3a**) formation (data from Table S26, details in Figure S34). **B)** Comparative of the acetate formed in previous reaction with the background acetate (**3a**) formation in the control reaction of pyruvate (**2a**, 30 mM) in $\text{NH}_4\text{Cl}/\text{NH}_3$ (600 mM, pH = 8.0) (Data from Table S1). Errors bars represent $\pm$ S.D.

6. Results and ^1H -NMR spectra of oxidative decarboxylation

Procedure for integration and quantification in oxidative decarboxylation

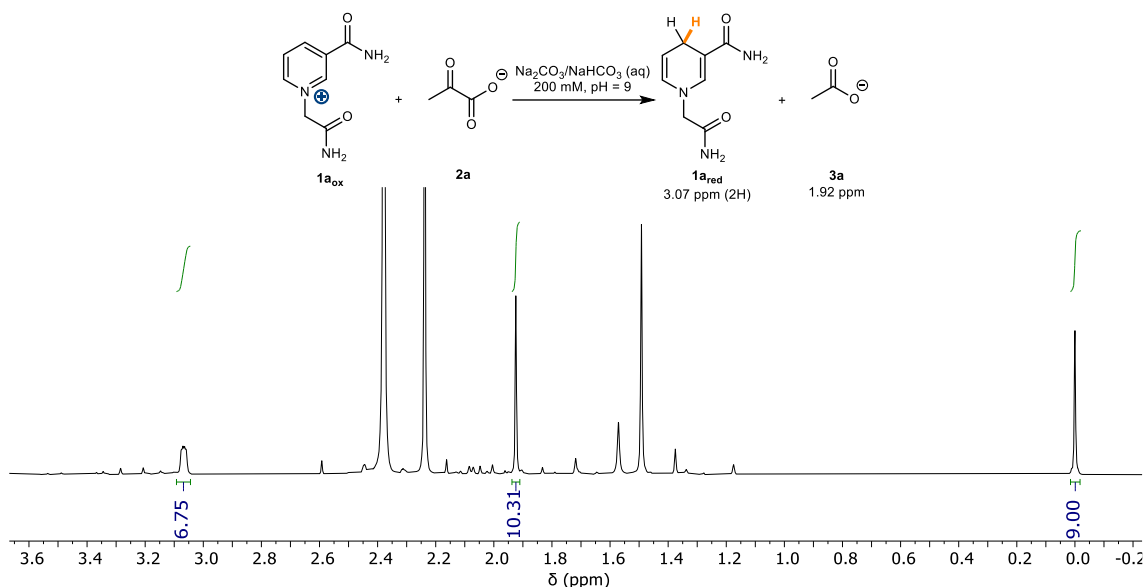


Figure S8. ^1H -NMR (300 MHz, $\text{H}_2\text{O}:\text{D}_2\text{O}$ 9:1, noesygppr1d) spectrum of the reaction between pyridinium (**1a_{ox}**, 30 mM) and pyruvate (**2a**, 30 mM) in aqueous $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ solution (200 mM) at pH 9. Example of integration at 48 h of reaction.

See section 3 for calibration lines and preparation of samples for NMR analysis.

Initial concentrations: Pyridinium [**1a_{ox}**]₀ = 30 mM Pyruvate [**2a**]₀ = 30 mM

Once the integral of TMSP- d_4 [0.00 (s, 9H)] has been set to 9.00, the integral of dihydropyridine **1a_{red}** [3.07 (dd, J = 3.7, 1.8 Hz, 2H)] and the integral of acetate **3a** [1.92 (s, 3H)] are measured. Concentration (mM) of acetate **3a** is obtained with the calibration line and concentration (mM) of dihydropyridine **1a_{red}** is obtained with equation S1 (section 3). The yields are calculated for every replicate, then the average and its standard deviation are obtained. This protocol has been used for other signals and the other reactions.

$$\text{Integral } \mathbf{3a} = 7.4617 \cdot [\mathbf{3a}] \quad \text{Yield} = [\text{Product}]/[\text{Reagent}]_0 \cdot 100$$

Table S4. Integral values and calculated ^1H -NMR Yields of products **1a_{red}** and **3a**.

Time (h)	Integral dihydropy. 1a_{red}			Yield (%) dihydropy. 1a_{red}			Average Yield 1a_{red} (%)
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	
1	0.00	0.00	1.04	0.0	0.0	0.6	0.2 ±0.3
24	5.89	6.04	3.92	3.3	3.3	2.2	2.9 ±0.5
48	4.58	6.75	5.08	2.5	3.7	2.8	3.0 ±0.5
72	4.90	3.75	7.73	2.7	2.1	4.3	3.0 ±0.9
168	1.99	0.62	1.52	1.1	0.3	0.8	0.8 ±0.3

Time (h)	Integral acetate 3a			Yield (%) acetate 3a			Average Yield 3a (%)
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	
1	0.00	0.00	0.39	0.0	0.0	0.2	0.1 ±0.1
24	3.70	3.89	6.12	1.7	1.7	2.7	2.0 ±0.5
48	8.50	10.31	9.31	3.8	4.6	4.2	4.2 ±0.3
72	12.96	17.21	15.56	5.8	7.7	7.0	6.8 ±0.8
168	29.78	38.90	43.94	13.3	17.4	19.6	16.8 ±2.6

Spectra related to the reaction of pyridinium **1a_{ox}** with pyruvate (**2a**)

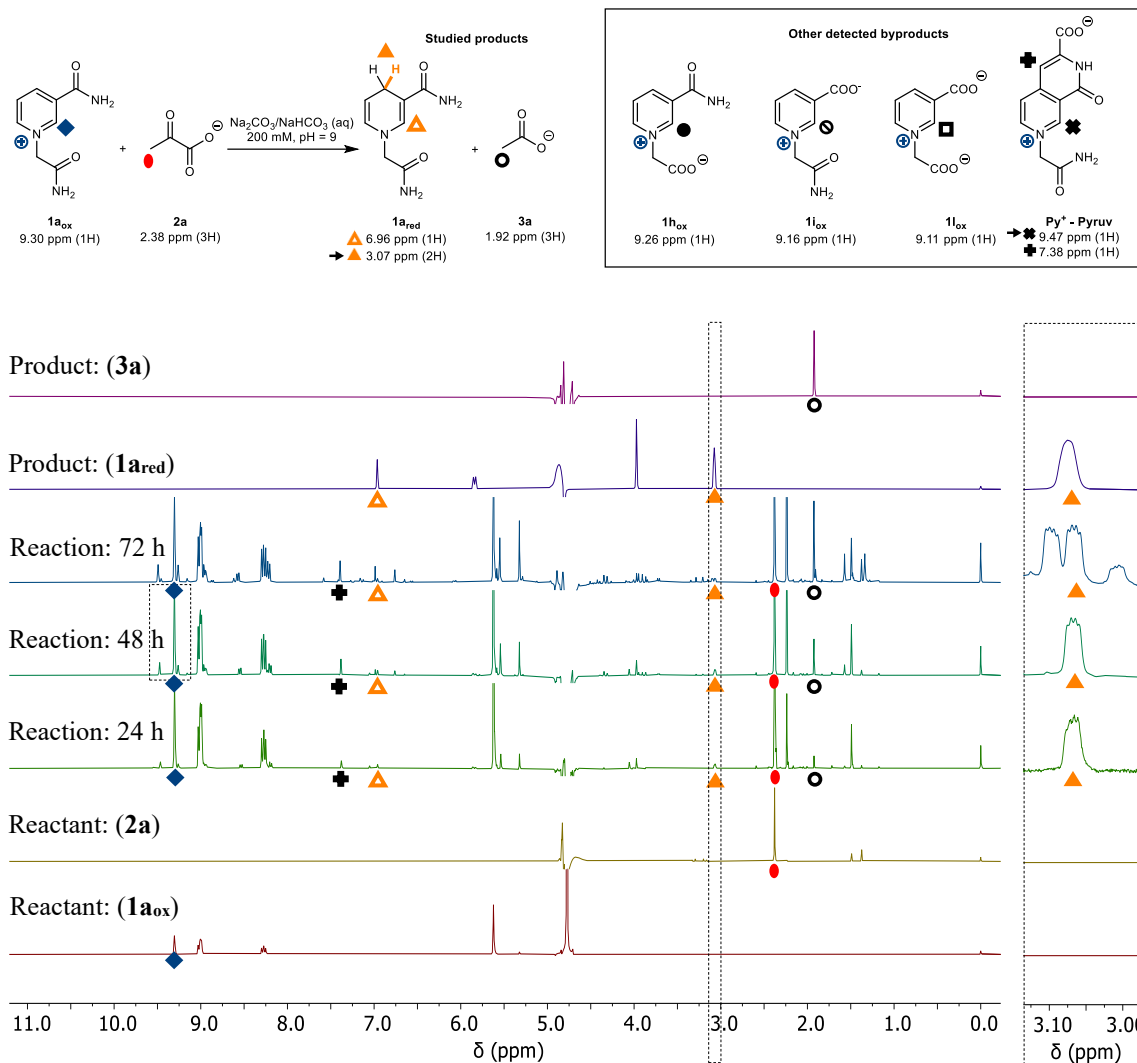
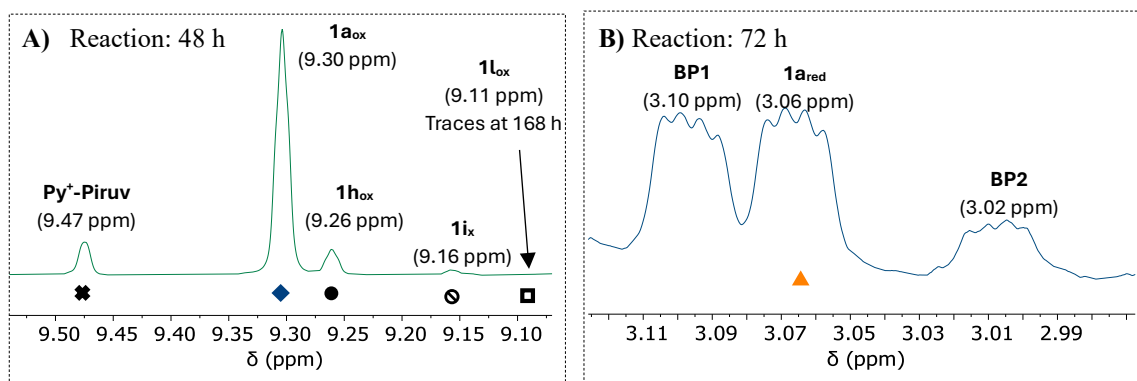


Figure S9. ¹H-NMR (300 MHz, H₂O:D₂O 9:1, noesygppr1d) spectra of the reaction between pyridinium (**1a_{ox}**, 30 mM) and pyruvate (**2a**, 30 mM) in aqueous Na₂CO₃/NaHCO₃ solution (200 mM) at pH 9, after 24 h, 48 h and 72 h of reaction (middle spectra). Spectra of pure reactants (below) and pure products (top) in the same bicarbonate solution are given for comparison.



A) Expanded view of the 9.60–9.05 ppm region of the spectrum at 48 h, showing the signals used to quantify **1a_{ox}** and the pyridinium byproducts. **B)** Expanded view of the 3.13–2.97 ppm region of the spectrum at 72 h, showing the signals used to quantify **1a_{red}** and the signals of byproducts **BP1** and **BP2**.

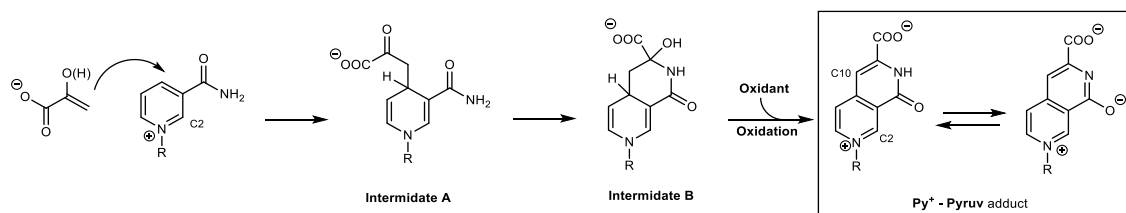


Figure S10. Formation **Py⁺-Pyruv** adduct.

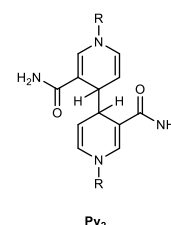
According to A. Gafni, there is a secondary process in which the pyruvate (in the form of enol or enolate) is added to the pyridinium,⁴ forming **Intermediate A**, followed by an intramolecular cyclization to give **Intermediate B** (Figure S10). Finally, it is oxidized to the **Py⁺-Pyruv** adduct.

J. Everse *et al.* described the adduct between NAD^+ and pyruvate (**2a**).⁵ The most characteristic changes in $^1\text{H-NMR}$, according to their data,^{††} were a downfield shift of the C2-H signal from 9.44 ppm (in **NAD⁺**) to 9.63 ppm (in the **NAD⁺-Pyruv** adduct) and the appearance of the C10-H signal at 7.23 ppm.^{§§} This adduct exhibits an acid-base equilibrium with a pK_a of 9.6. In addition, L. Sebastianelli *et al.* also described this type of adduct by HR-MS and isotopic labelling.⁶

Similarly, in the present work, during the reaction between pyruvate (**2a**) and pyridinium **1a_{ox}**, we observed the formation of a byproduct whose more recognisable signals are at 9.47 ppm and 7.38 ppm. These signals were assigned the **Py⁺-Pyruv** adduct, specifically to C2-H (which has experienced a down-field shift from 9.30 ppm in **1a_{ox}**) and C10-H, respectively. These signals were absent in the controls of **1a_{ox}**, **1a_{red}** or pyruvate (**2a**), where these compounds were not mixed, further supporting their assignment to **Py⁺-Pyruv**. Since the pK_a (expected to be close to 9.6, as in the **NAD⁺-Pyruv** case) is similar to the working pH (9.0), very small variations of pH during the reaction slightly shift the product signals.

The oxidant of the oxidation step (from **Intermediate B** to **Py⁺-Pyruv**, Figure S10) could be another molecule of pyridinium **1a_{ox}**, rendering dihydropyridinium **1a_{red}**. Consequently, the total amount of **1a_{red}** observed would originate from two process: the oxidative decarboxylation of pyruvate and the formation of **Py⁺-Pyruv**. This would explain why, in some reactions involving other pyridiniums **1_{ox}**, we occasionally observed a greater amount of dihydropyridine **1_{red}** than acetate **3a**.

In the spectra shown in Figure S9B (which corresponds to one of the three replicates of the reaction of pyruvate and **1a_{ox}**), there can be seen two signals centred at 3.10 ppm (**BP1**) and 3.02 ppm (**BP2**) at 72 hours. These signals have a very erratic behaviour: in other replicates they appeared at different times, so we could not quantify them. In analogy with the spectrochemical data of NAD_2 in this region of the $^1\text{H-NMR}$ spectra,⁷ one of those signals could tentatively belong to Py_2 (**1a-1a**), although additional evidence would be needed for definitive confirmation.



^{††} Differences in spectrometer frequency, pH, concentration and other conditions cause the signals that we obtain for these compounds (Figure S31) to differ slightly.

^{§§} Data extracted from Figure 7 of reference 5, transforming the values given in Hz into values in ppm (220 MHz spectrometer).

Table S5. Yields (%)^a of the quantified compounds in the reaction of **1a_{ox}** (30 mM) and **2a** (30 mM) in aqueous Na₂CO₃/NaHCO₃ solution (200 mM) at pH 9.

	Reagents ^b	Studied products			Other detected byproducts			
Time (h)	1a_{ox}	1a_{red}	3a	1h_{ox}	1i_{ox}	1l_{ox}	Py⁺ - Pyruv	
1	103.7 ±3.4	0.2 ±0.3	0.1 ±0.1	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	
24	83.5 ±4.5	2.9 ±0.5	2.0 ±0.5	7.2 ±3.6	1.5 ±0.6	0.2 ±0.3	5.6 ±1.9	
48	64.4 ±4.3	3.0 ±0.5	4.2 ±0.3	10.2 ±3.9	2.0 ±0.6	0.3 ±0.4	7.4 ±2.5	
72	54.7 ±9.6	3.0 ±0.9	6.8 ±0.8	13.5 ±3.5	2.5 ±0.8	0.4 ±0.5	8.2 ±2.7	
168	36.9 ±7.0	0.8 ±0.3	16.8 ±2.6	22.2 ±5.5	4.0 ±0.9	1.2 ±0.7	12.2 ±4.0	

^aAverage yield (%) and standard deviation (±%) of three replicates. ^bPercentage of remaining reagent.

Table S6. Yields (%) of the quantified compounds in the control of pyridinium **1a_{ox}** (30 mM) in aq. Na₂CO₃/NaHCO₃ (200 mM) at pH 9.

Time (h)	1a_{ox} ^a	1a_{red}	3a	1h_{ox}	1i_{ox}	1l_{ox}	Py⁺ - Pyruv
1	99.8	0.0	0.0	0.0	0.0	0.0	0.0
24	93.5	0.0	0.0	4.8	1.0	0.0	0.0
48	86.5	0.0	0.0	10.1	1.5	0.0	0.0
72	82.3	0.0	0.0	14.1	2.5	0.0	0.0
168	67.8	0.0	0.0	28.1	4.7	1.0	0.0

^aPercentage of remaining reagent.

Table S7. Yields (%) of the quantified compounds in the control of pyridinium **1a_{red}** (30 mM) in aq. Na₂CO₃/NaHCO₃ (200 mM) at pH 9.

Time (h)	1a_{ox}	3a	1h_{ox}	1i_{ox}	1l_{ox}	Py⁺ - Pyruv
1	0.1	0.0	0.0	0.0	0.0	0.0
24	0.3	0.0	0.1	0.0	0.0	0.0
48	0.5	0.0	0.3	0.0	0.0	0.0
72	0.6	0.0	0.4	0.0	0.0	0.0
168	1.5	0.0	1.2	0.1	0.0	0.0

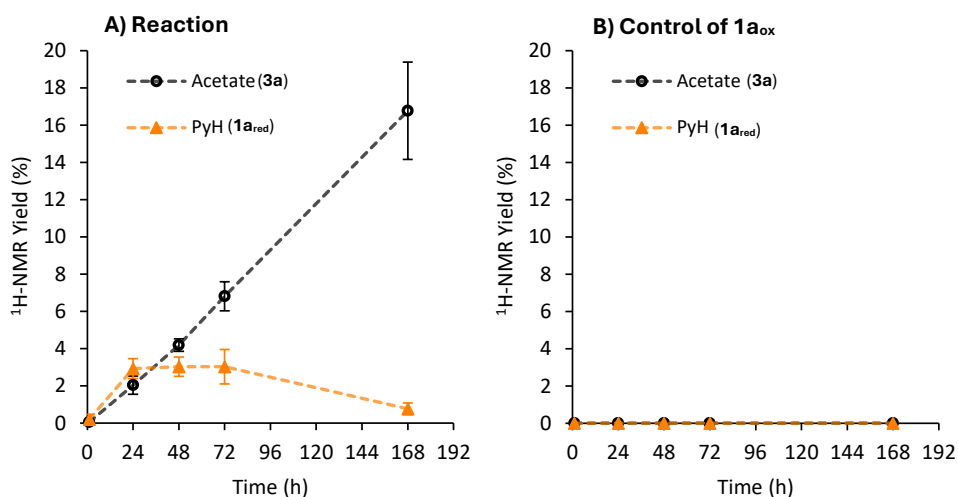


Figure S11. ¹H-NMR monitoring of acetate (**3a**) and dihydropyridine **1a_{red}** formation in: **A)** Reaction of **1a_{ox}** (30 mM) and **2a** (30 mM). **B)** Control of pyridinium **1a_{ox}** (30 mM). Both in aq. Na₂CO₃/NaHCO₃ (200 mM) at pH 9. Plotted values from the preceding tables.

Spectra related to the reaction of pyridinium **1a_{ox}** with α -ketoglutarate (**2b**)

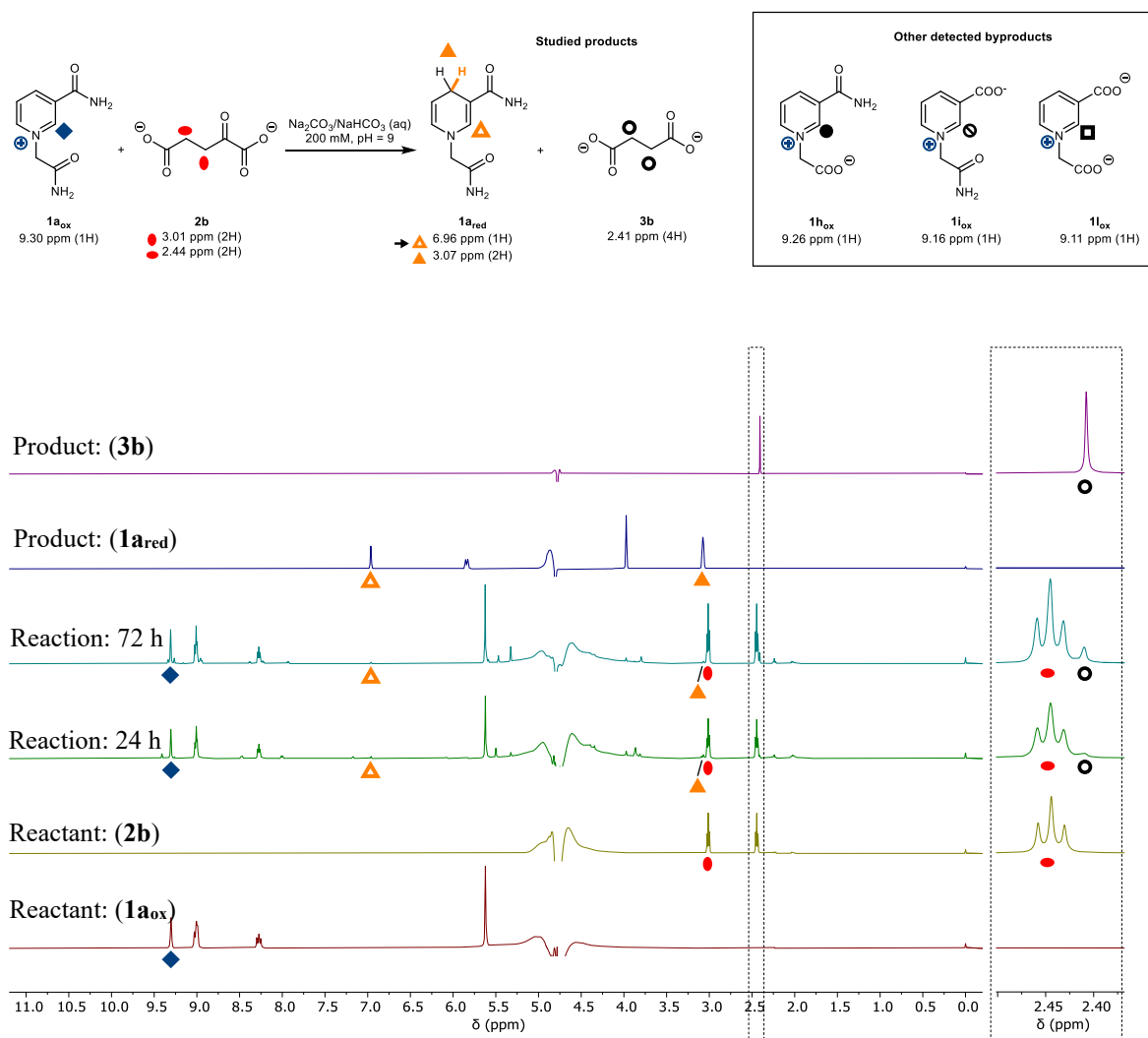


Figure S12. ^1H -NMR (300 MHz, $\text{H}_2\text{O}:\text{D}_2\text{O}$ 9:1, noesygppr1d) spectra of the reaction between pyridinium (**1a_{ox}**, 30 mM) and α -ketoglutarate (**2b**, 30 mM) in aqueous $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ solution (200 mM) at pH 9, after 24 h and 72 h of reaction (middle spectra). Spectra of pure reactants (below) and pure products (top) in the same bicarbonate solution are given for comparison.

See Figure S9A for the chemical shifts of **1h_{ox}**, **1i_{ox}** and **1l_{ox}** in the spectra.

Table S8. Yields (%) of the quantified compounds in the reaction of **1a_{ox}** (30 mM) and **2b** (30 mM) in aqueous Na₂CO₃/NaHCO₃ solution (200 mM) at pH 9.

Time (h)	Studied products		Other detected byproducts		
	1a_{red}	3b	1h_{ox}	1i_{ox}	1l_{ox}
1	0.0	0.0	1.6	0.2	0.0
24	5.0	1.7	4.5	0.7	0.4
48	6.8	3.9	12.6	2.0	0.6
72	3.1	4.0	14.0	2.4	0.6
168	0.9	11.3	33.7	4.9	1.5

See Table S6 for the control reaction of **1a_{ox}**.

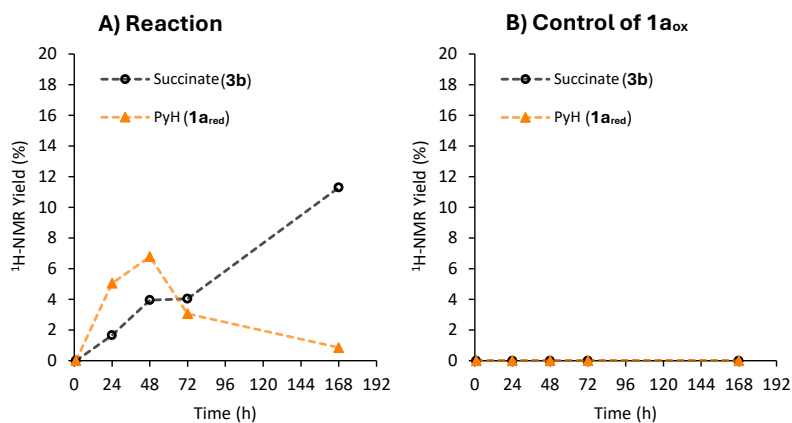


Figure S13. ¹H-NMR monitoring of succinate (**3b**) and dihydropyridine **1a_{red}** formation in: **A)** Reaction of **1a_{ox}** (30 mM) and **2b** (30 mM). **B)** Control of pyridinium **1a_{ox}** (30 mM). Both in aq. Na₂CO₃/NaHCO₃ (200 mM) at pH 9. Plotted values from the preceding tables.

Spectra related to the reaction of pyridinium **1b_{ox}** with pyruvate (**2a**)

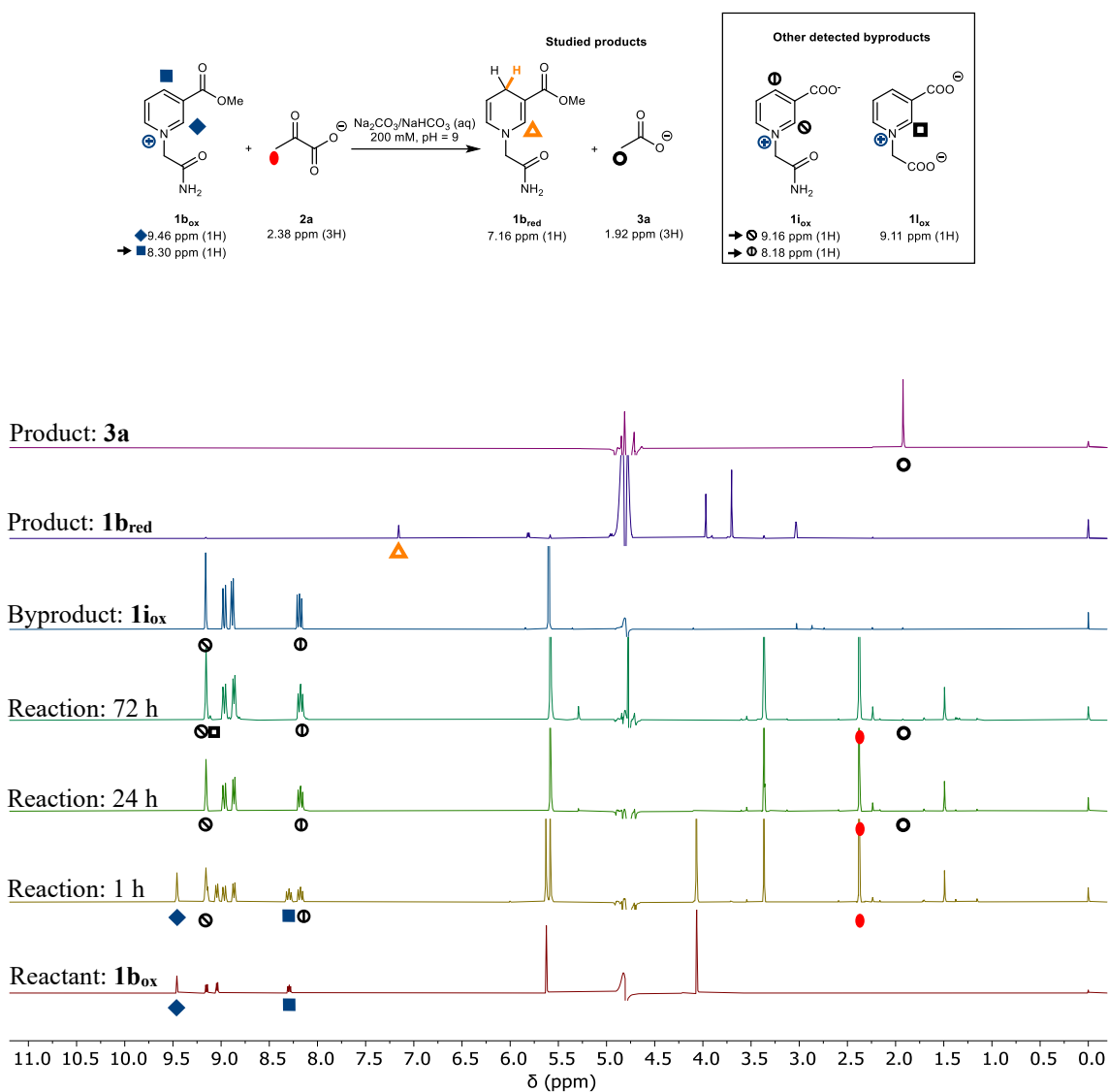


Figure S14. ¹H-NMR (300 MHz, H₂O:D₂O 9:1, noesygppr1d) spectra of the reaction between pyridinium (**1b_{ox}**, 30 mM) and pyruvate (**2a**, 30 mM) in aqueous Na₂CO₃/NaHCO₃ solution (200 mM) at pH 9, after 1 h, 24 h and 72 h of reaction (middle spectra). Spectra of pure products and byproducts in the same bicarbonate solution are given for comparison (spectrum of pure reactant **1b_{ox}** in pure H₂O:D₂O 9:1).

(For the quantification of **1i_{ox}**, the signal at 8.18 ppm is used at 1 hour reaction time and the signal at 9.16 ppm is used for all other time points).

Table S9. Yields (%) of the quantified compounds in the reaction of **1b_{ox}** (30 mM) and **2a** (30 mM) in aqueous Na₂CO₃/NaHCO₃ solution (200 mM) at pH 9.

	Reagents ^a	Studied products		Other detected byproducts	
Time (h)	1b_{ox}	1b_{red}	3a	1i_{ox}	1l_{ox}
1	47.0	0.0	traces	49.8	0.0
24	0.0	0.0	0.2	97.0	1.6
48	0.0	0.0	0.3	93.8	3.7
72	0.0	0.0	0.4	93.0	6.9
168	0.0	0.0	0.4	84.6	15.7

^aPercentage of remaining reagent.

From the ¹H-NMR spectra and the table, it can be inferred that there is a total hydrolysis of **1b_{ox}** (3-COOMe) into **1i_{ox}** (3-COO⁻) within the first hours of reaction. Pyridinium **1i_{ox}** is a weak oxidant which cannot perform the oxidative decarboxylation. No dihydropyridine is detected. The small amount of acetate **3a** formed during the initial hours of the reaction is due to the action of **1b_{ox}**, which quickly disappears (the slight increase of acetate remains within the quantification error range).

Table S10. Yields (%) of the quantified compounds in the control of pyridinium **1b_{ox}** (30 mM) in aq. Na₂CO₃/NaHCO₃ (200 mM) at pH 9.

	Reagents	Studied products		Other detected byproducts	
Time (h)	1b_{ox} ^a	1b_{red}	3a	1i_{ox}	1l_{ox}
1	43.1	0.0	0.0	57.0	0.0
24	0.0	0.0	0.0	98.8	1.1
48	0.0	0.0	0.0	98.0	2.6
168	0.0	0.0	0.0	86.8	12.1

^aPercentage of remaining reagent.

Byproduct **1i_{ox}** also appears rapidly in the control reaction. Therefore, the hydrolysis of **1b_{ox}** into **1i_{ox}** is due to the medium at pH 9.

Spectra related to the reaction of pyridinium **1c_{ox}** with pyruvate (**2a**)

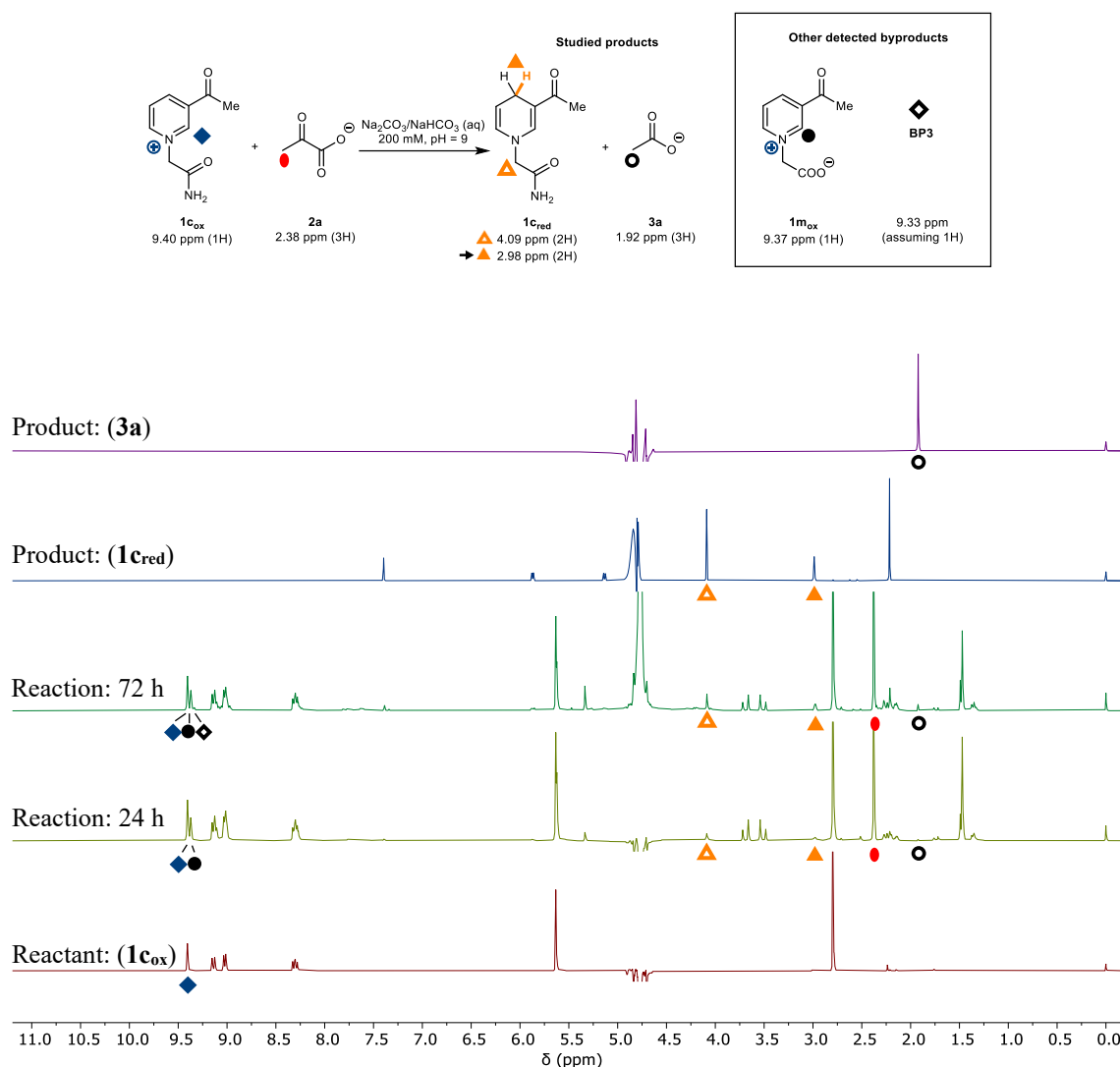


Figure S15. ^1H -NMR (300 MHz, $\text{H}_2\text{O}:\text{D}_2\text{O}$ 9:1, noesygppr1d) spectra of the reaction between pyridinium (**1c_{ox}**, 30 mM) and pyruvate (**2a**, 30 mM) in aqueous $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ solution (200 mM) at pH 9, after 24 h and 72 h of reaction (middle spectra). Spectra of pure reactants (below) and pure products (top) in the same bicarbonate solution are given for comparison.

Signal at 9.37 ppm was assigned to **1m_{ox}**, resulting from the hydrolysis of the $-\text{CONH}_2$ group in **1c_{ox}** to $-\text{COO}^-$, as it exhibits the same upfield shift observed between the signals of **1a_{ox}** and **1h_{ox}** (see Figure S9).

Signal at 9.33 ppm (assigned to unidentified byproduct **BP3**) only appears in the presence of pyruvate.

Table S11. Yields (%)^a of the quantified compounds in the reaction of **1c_{ox}** (30 mM) and **2a** (30 mM) in aqueous Na₂CO₃/NaHCO₃ solution (200 mM) at pH 9.

	Reagents ^b	Studied products			Other detected byproducts	
Time (h)	1c_{ox}	1c_{red}	3a	1m_{ox}	BP3	
1	82.4 ±7.1	0.8 ±0.2	0.1 ±0.0	13.8 ±2.2	0.0 ±0.0	
24	45.9 ±2.6	3.7 ±0.4	0.4 ±0.0	28.0 ±1.4	2.7 ±0.2	
48	28.2 ±10.9	6.2 ±0.9	1.0 ±0.3	25.7 ±2.3	6.1 ±1.7	
72	23.8 ±10.2	5.8 ±0.6	1.4 ±0.1	22.0 ±2.5	7.3 ±2.2	
168	21.7 ±6.0	5.9 ±1.3	4.8 ±0.3	23.2 ±1.5	7.2 ±1.2	

^aAverage yield (%) and standard deviation (±%) of three replicates. ^bPercentage of remaining reagent.

Table S12. Yields (%) of the quantified compounds in the control of pyridinium **1c_{ox}** (30 mM) in aq. Na₂CO₃/NaHCO₃ (200 mM) at pH 9.

	Reagents ^a	Studied products		Other detected byproducts	
Time (h)	1c_{ox}	1c_{red}	3a	1m_{ox}	BP3
1	96.0	0.0	0.0	0.0	0.0
24	75.8	2.7	0.0	4.7	0.0
48	68.2	4.6	0.2	10.2	0.0
72	49.8	4.1	0.7	12.2	0.0
168	24.8	1.0	4.1	13.0	0.0

^aPercentage of remaining reagent.

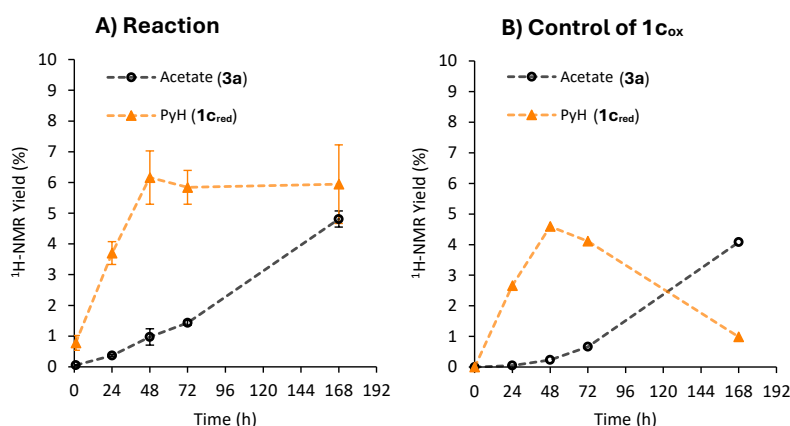


Figure S16. ¹H-NMR monitoring of acetate (**3a**) and dihydropyridine **1c_{red}** formation in: **A)** Reaction of **1c_{ox}** (30 mM) and **2a** (30 mM). **B)** Control of pyridinium **1c_{ox}** (30 mM). Both in aq. Na₂CO₃/NaHCO₃ (200 mM) at pH 9. Plotted values from the preceding tables.

In the control of pyridinium **1c_{ox}**, formation of acetate (**3a**) and dihydropyridine (**1c_{red}**) is observed. A plausible explanation is some type of disproportionation reaction in which the acyl group (-COMe) of one molecule of **1c_{ox}** is released and oxidised to acetate (**3a**) while another molecule of **1c_{ox}** is reduced to **1c_{red}**.

Therefore, acetate (**3a**) and dihydropyridine (**1c_{red}**) formed in the main reaction come from two process: the aforementioned disproportionation of **1c_{ox}** and the proper oxidative decarboxylation of **2a** by **1c_{ox}**.

Spectra related to the reaction of pyridinium **1d_{ox}** with pyruvate (**2a**)

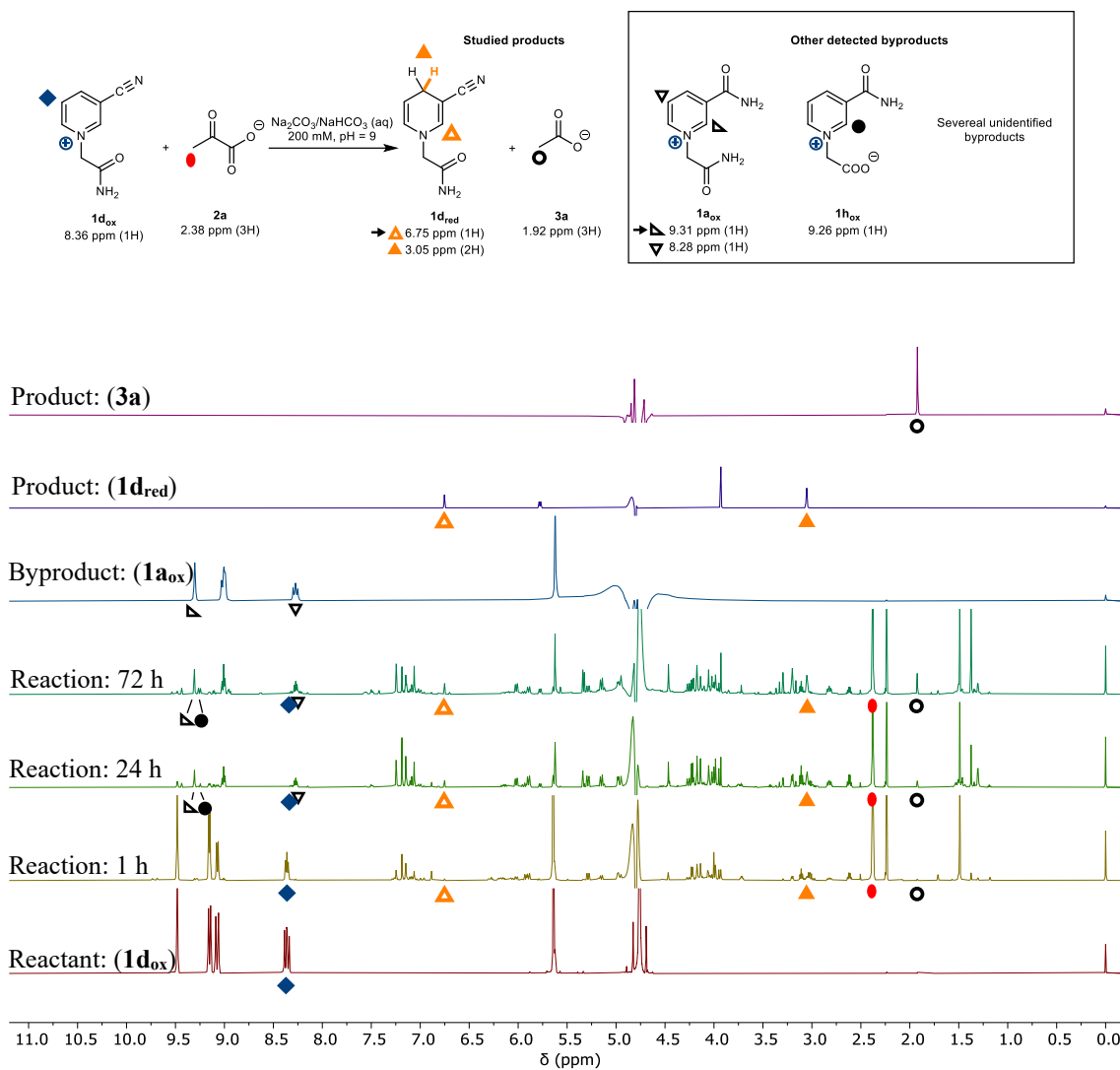


Figure S17. ^1H -NMR (300 MHz, $\text{H}_2\text{O}:\text{D}_2\text{O}$ 9:1, noesygppr1d) spectra of the reaction between pyridinium (**1d_{ox}**, 30 mM) and pyruvate (**2a**, 30 mM) in aqueous $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ solution (200 mM) at pH 9, after 1 h, 24 h and 72 h of reaction (middle spectra). Spectra of pure reactants (below) and pure products and by products (top) in the same bicarbonate solution are given for comparison (spectrum of pure reactant **1d_{ox}** in pure D_2O)

Table S13. Yields (%)^a of the quantified compounds in the reaction of **1d_{ox}** (30 mM) and **2a** (30 mM) in in aqueous Na₂CO₃/NaHCO₃ solution (200 mM) at pH 9.

Time (h)	Reagents ^b		Studied products		Other detected byproducts	
	1d_{ox}		1d_{red}	3a	1a_{ox}	1h_{ox}
1	12.4 ±9.0		0.5 ±0.1	0.1 ±0.0	1.1 ±0.4	0.0 ±0.0
24	1.7 ±0.3		2.7 ±0.6	0.7 ±0.2	8.5 ±1.9	1.4 ±0.8
48	1.5 ±0.3		3.7 ±0.6	1.5 ±0.3	11.0 ±2.4	2.6 ±0.9
72	1.0 ±0.7		4.3 ±0.5	2.4 ±0.6	9.1 ±2.0	5.1 ±2.1
168	0.5 ±0.6		4.4 ±0.5	4.0 ±0.7	9.0 ±2.4	8.3 ±2.3

^aAverage yield (%) and standard deviation (±%) of three replicates. ^bPercentage of remaining reagent.

Table S14. Yields (%) of the quantified compounds in the control of pyridinium **1d_{ox}** (30 mM) in aq. Na₂CO₃/NaHCO₃ (200 mM) at pH 9.

Time (h)	1d_{ox} ^a	1d_{red}	3a	1a_{ox}	1h_{ox}
1	6.6	0.4	0.0	1.0	0.0
24	2.5	2.3	0.0	7.8	1.2
48	1.5	2.6	0.0	11.6	3.9
72	0.0	2.6	0.0	11.7	6.4
168	0.0	1.6	0.0	8.0	8.6

^aPercentage of remaining reagent.

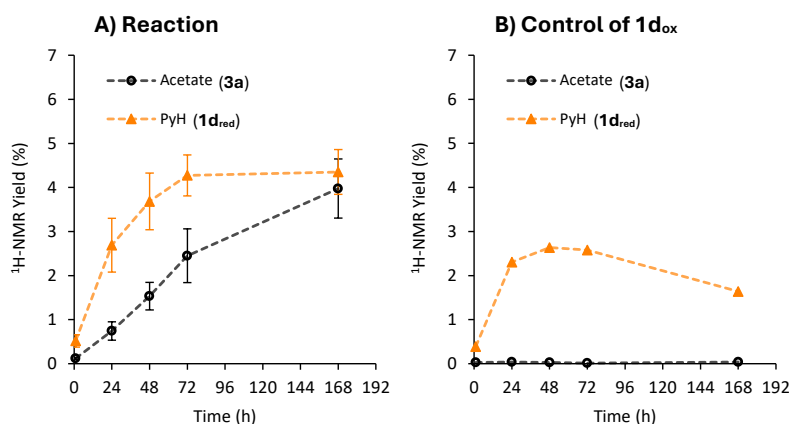


Figure S18. ¹H-NMR monitoring of acetate (**3a**) and dihydropyridine **1d_{red}** formation in: **A)** Reaction of **1d_{ox}** (30 mM) and **2a** (30 mM). **B)** Control of pyridinium **1d_{ox}** (30 mM). Both in aq. Na₂CO₃/NaHCO₃ (200 mM) at pH 9. Plotted values from the preceding tables.

Spectra and tables show that pyridinium **1d_{ox}** is especially labile in the buffer Na₂CO₃/NaHCO₃ (200 mM) at pH 9, forming rapidly other byproducts that could not be identified for the most part. One of these byproducts is **1a_{ox}** (due to hydrolysis of 3-CN into 3-CONH₂).

Therefore, two possible oxidants could be involved in the oxidation of pyruvate (**2a**) to acetate (**3a**): pyridinium **1d_{ox}** and pyridinium **1a_{ox}**. However, only dihydropyridine **1d_{red}** is detected. According to DFT calculations presented in Figure 3B (in the main text) pyridinium **1d_{ox}** has a greater thermodynamic tendency to be reduced into **1d_{red}** than **1a_{ox}** has to be converted into **1a_{red}**. Hence, a hypothetical explanation for the absence of **1a_{red}** is a rapid hydride transfer from the initially formed **1a_{red}** to **1d_{ox}**, regenerating **1a_{ox}** and producing additional **1d_{red}**.

In any case, the control reaction shows a significant amount of **1d_{red}**, indicating that this dihydropyridine is also formed through other secondary processes.

Spectra related to the reaction of pyridinium **1e_{ox}** with pyruvate (**2a**)

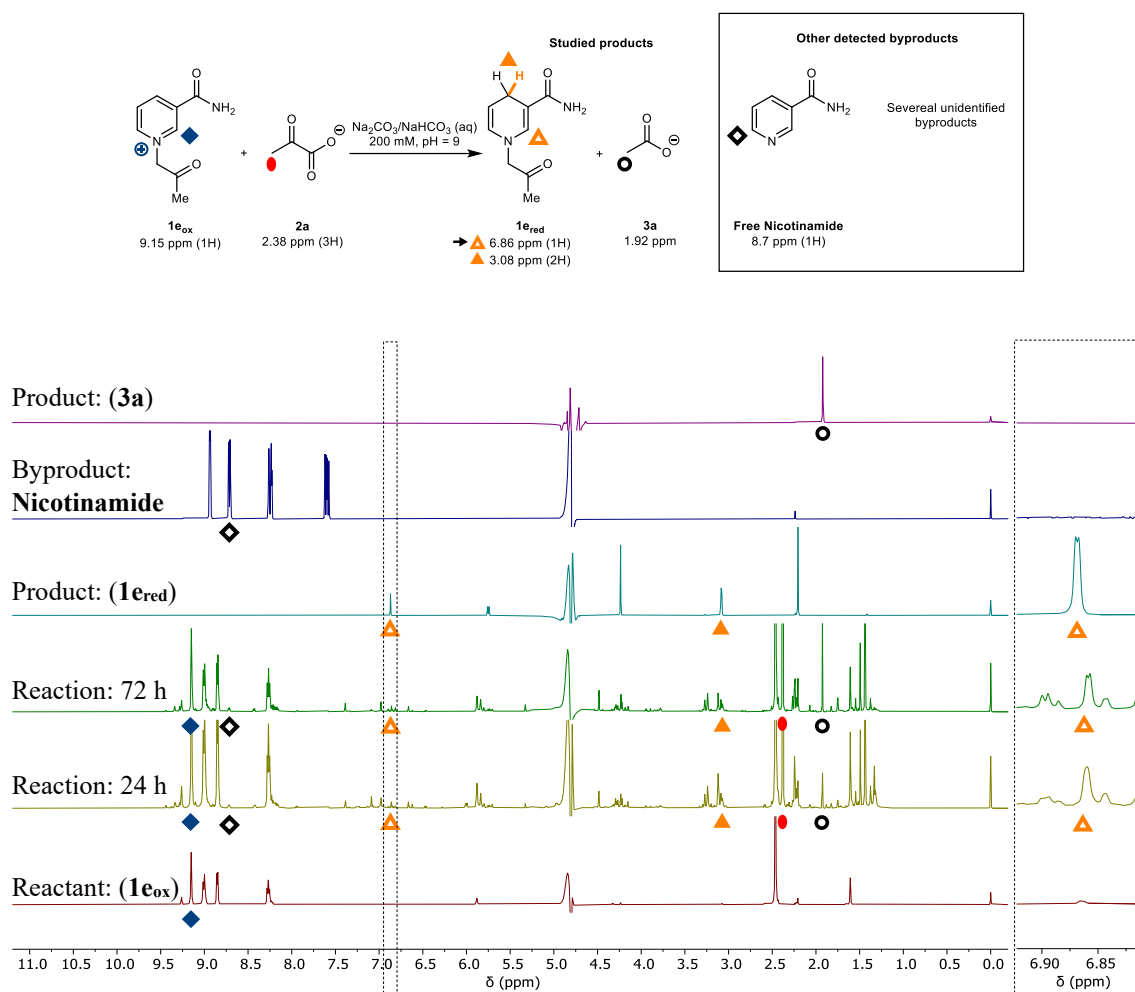


Figure S19. ^1H -NMR (300 MHz, $\text{H}_2\text{O}:\text{D}_2\text{O}$ 9:1, noesygppr1d) spectra of the reaction between pyridinium (**1e_{ox}**, 30 mM) and pyruvate (**2a**, 30 mM) in aqueous $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ solution (200 mM) at pH 9, after 24h and 72 h of reaction (middle spectra). Spectra of pure reactants (below) and pure products and byproducts (top) in the same bicarbonate solution are given for comparison (spectrum of pure nicotinamide in $\text{NH}_3/\text{NH}_4\text{Cl}$ solution).

Table S15. Yields (%)^a of the quantified compounds the in reaction of **1e_{ox}** (30 mM) and **2a** (30 mM) in in aqueous Na₂CO₃/NaHCO₃ solution (200 mM) at pH 9.

	Reagents ^b	Studied products		Other detected byproducts
Time (h)	1e_{ox}	1e_{red}	3a	Free Nicotinamide
1	84.4 ±7.9	1.8 ±0.4	0.3 ±0.1	0.4 ±0.1
24	80.8 ±6.1	2.2 ±0.2	2.5 ±0.2	2.7 ±0.1
48	61.5 ±11.2	2.3 ±0.3	5.2 ±0.2	4.0 ±0.3
72	59.0 ±12.7	2.0 ±0.6	7.9 ±1.1	5.4 ±0.7
168	50.5 ±4.3	0.8 ±0.3	17.4 ±2.3	9.2 ±1.2

^aAverage yield (%) and standard deviation (±%) of three replicates. ^bPercentage of remaining reagent.

Table S16. Yields (%) of the quantified compounds in the control of pyridinium **1e_{ox}** (30 mM) in aq. Na₂CO₃/NaHCO₃ (200 mM) at pH 9.

Time (h)	1e_{ox} ^a	1e_{red}	3a	Nicotinamide
1	97.6	1.1	0.1	0.7
24	92.5	1.7	2.0	3.1
48	54.1	2.7	4.0	5.9
72	63.7	2.0	4.9	8.6
168	46.2	1.0	8.7	16.8

^aPercentage of remaining reagent.

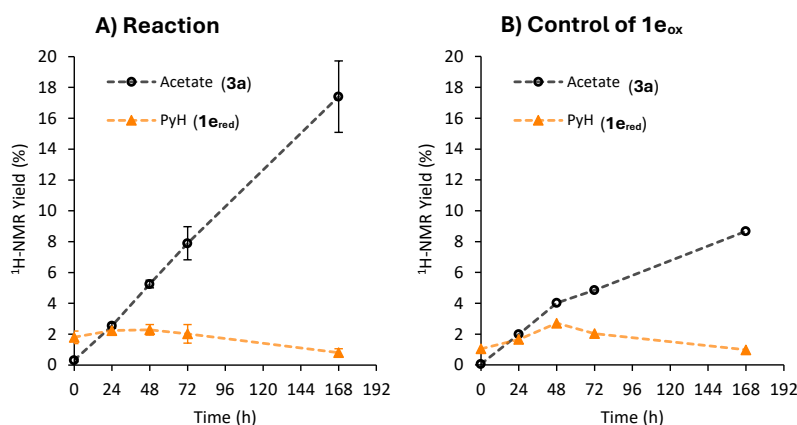


Figure S20. ¹H-NMR monitoring of acetate (**3a**) and dihydropyridine **1e_{red}** formation in: **A**) Reaction of **1e_{ox}** (30 mM) and **2a** (30 mM). **B**) Control of pyridinium **1e_{ox}** (30 mM). Both in aq. Na₂CO₃/NaHCO₃ (200 mM) at pH 9. Plotted values from the preceding tables.

As in the case of pyridinium **1c_{ox}**, the control of pyridinium **1e_{ox}** shows formation of acetate (**3a**) and dihydropyridine (**1e_{red}**). A plausible explanation is some type of disproportionation reaction in which the acyl group (-COMe) of one molecule of **1e_{ox}** is released and oxidised to acetate (**3a**) while another molecule of **1e_{ox}** is reduced to **1e_{red}**.

Therefore, acetate (**3a**) and dihydropyridine (**1e_{red}**) formed in the main reaction come from two process: the aforementioned disproportionation of **1e_{ox}** and the proper oxidative decarboxylation of **2a** by **1e_{ox}**.

Among the byproducts of this reaction is nicotinamide (without any substitution at the *N*-position). This is further evidence that the -CH₂COMe substituent undergoes other secondary processes that release nicotinamide, in contrast to the cases where the substituent was -CH₂CONH₂ (**1a_{ox}**-**1d_{ox}**), where no free pyridines were observed.

Spectra related to the reaction of pyridinium **1f_{ox}** with pyruvate (**2a**)

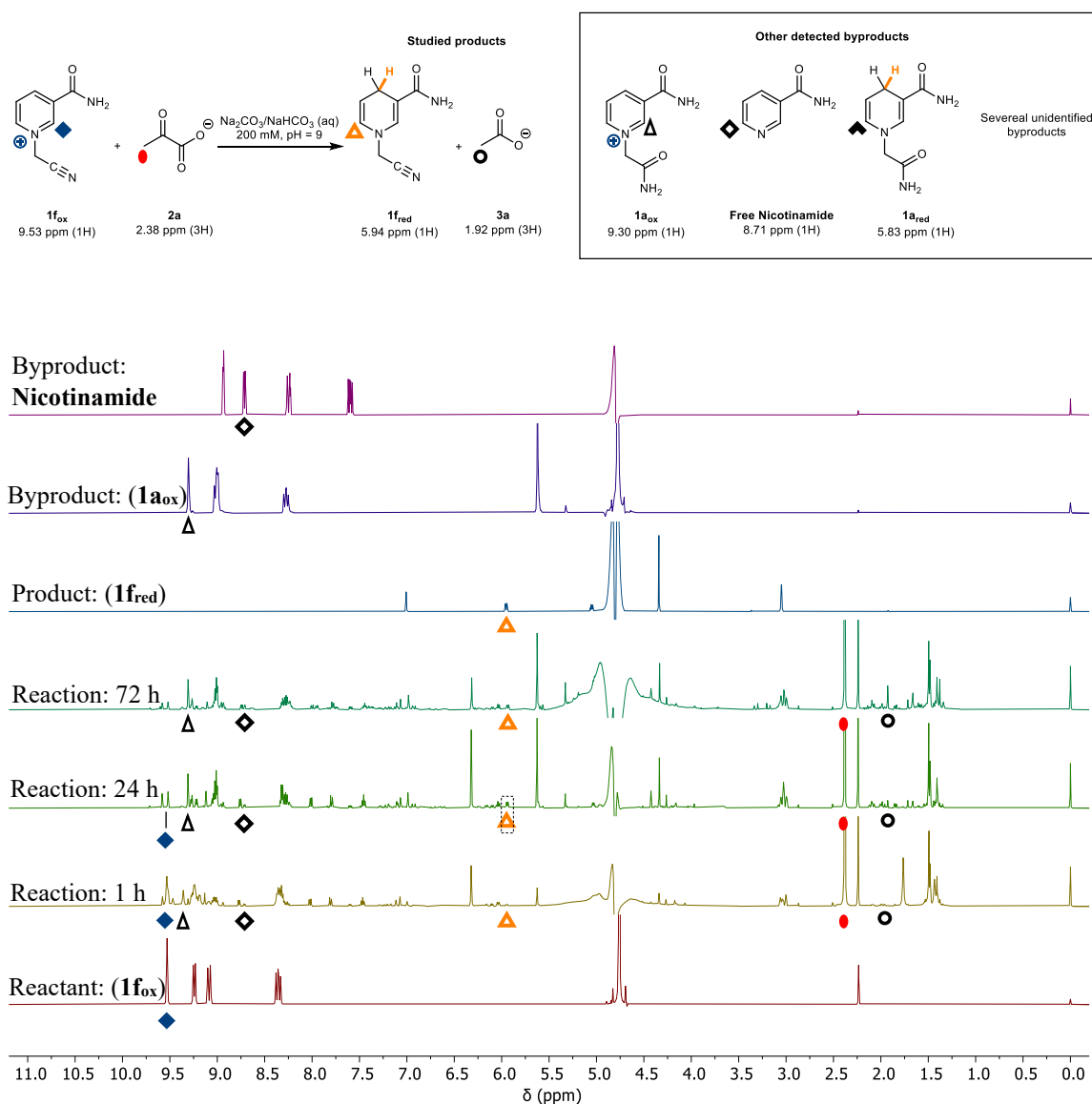
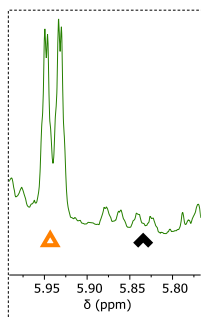


Figure S21. ¹H-NMR (300 MHz, H₂O:D₂O 9:1, noesygppr1d) spectra of the reaction between pyridinium (**1f_{ox}**, 30 mM) and pyruvate (**2a**, 30 mM) in aqueous Na₂CO₃/NaHCO₃ solution (200 mM) at pH 9, after 24h and 72 h of reaction (middle spectra). Spectra of pure reactants (below) and pure products and byproducts (top) in the same bicarbonate solution are given for comparison (spectrum of pure reactant **1f_{ox}** in pure D₂O; spectrum of pure nicotinamide in NH₃/NH₄Cl solution).



Expanded view of the 6.00–5.75 ppm region of the spectrum at 24 h, showing the signals used to quantify product **1f_{red}** and the byproduct **1a_{red}**.

Table S17. Yields (%)^a of the quantified compounds in the reaction of **1f_{ox}** (30 mM) and **2a** (30 mM) in aqueous Na₂CO₃/NaHCO₃ solution (200 mM) at pH 9.

Time (h)	Reagents ^b	Studied products		Other detected byproducts		
	1f_{ox}	1f_{red}	3a	1a_{ox}	Nicotinamide	1a_{red}
1	23.9 ±1.3	1.9 ±0.7	0.0 ±0.1	10.3 ±4.5	2.6 ±0.2	0.0 ±0.0
24	0.7 ±0.5	6.7 ±0.0	0.8 ±0.2	14.9 ±0.4	3.5 ±0.2	0.3 ±0.0
48	0.0 ±0.0	5.6 ±0.8	1.5 ±0.2	14.1 ±1.3	3.8 ±0.3	0.3 ±0.1
72	0.0 ±0.0	5.9 ±0.6	2.5 ±0.4	14.7 ±0.2	4.1 ±0.1	0.4 ±0.0
168	0.0 ±0.0	4.2 ±0.3	5.9 ±0.9	12.7 ±0.6	4.2 ±0.1	0.6 ±0.1

^aAverage yield (%) and standard deviation (±%) of three replicates. ^bPercentage of remaining reagent.

Table S18. Yields (%) of the quantified compounds in the control of pyridinium **1f_{ox}** (30 mM) in aq. Na₂CO₃/NaHCO₃ (200 mM) at pH 9.

Time (h)	1f_{ox} ^a	1f_{red}	3a	1a_{ox}	Nicotinamide	1a_{red}
1	24.2	7.2	0.0	4.9	2.3	0.0
24	1.2	12.5	0.0	15.0	3.0	0.4
48	0.0	10.4	0.0	15.9	3.0	0.4
72	0.0	10.0	0.0	16.2	3.2	0.5
168	0.0	7.5	0.0	16.6	4.2	0.4

^aPercentage of remaining reagent.

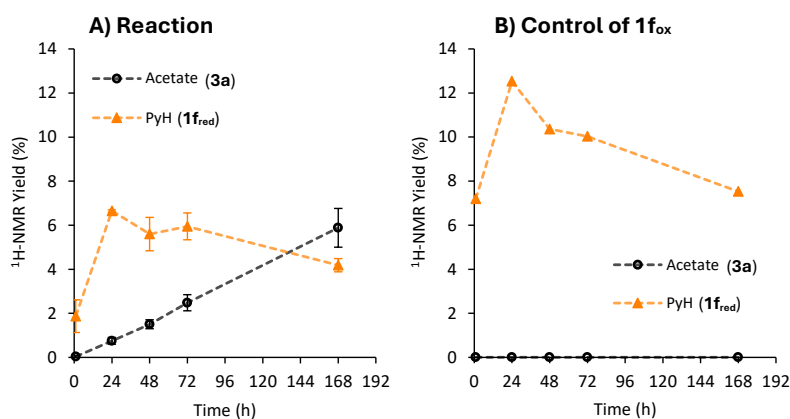


Figure S22. ¹H-NMR monitoring of acetate (**3a**) and dihydropyridine **1f_{red}** formation in: **A)** Reaction of **1f_{ox}** (30 mM) and **2a** (30 mM). **B)** Control of pyridinium **1f_{ox}** (30 mM). Both in aq. Na₂CO₃/NaHCO₃ (200 mM) at pH 9. Plotted values from the preceding tables.

Similar to the case of **1d_{ox}**, pyridinium **1f_{ox}** in the carbonate solution at pH 9 rapidly decomposes into various byproducts, including pyridinium **1a_{ox}**. Consequently, the acetate (**3a**) formed through oxidative decarboxylation of pyruvate (**2a**) can be attributed to the sum of the actions of oxidants **1f_{ox}** and **1a_{ox}**.

Among the byproducts of this reaction is nicotinamide (without any substitution at the *N*-position). This is further evidence that the -CH₂CN substituent undergoes other secondary processes that release nicotinamide, in contrast to the cases where the substituent was -CH₂CONH₂ (**1a_{ox}**-**1d_{ox}**), where no free pyridines were observed.

The control reaction shows a significant amount of **1f_{red}**, indicating that this dihydropyridine is also formed through other secondary processes.

Spectra related to the reaction of pyridinium **1g_{ox}** with pyruvate (**2a**)

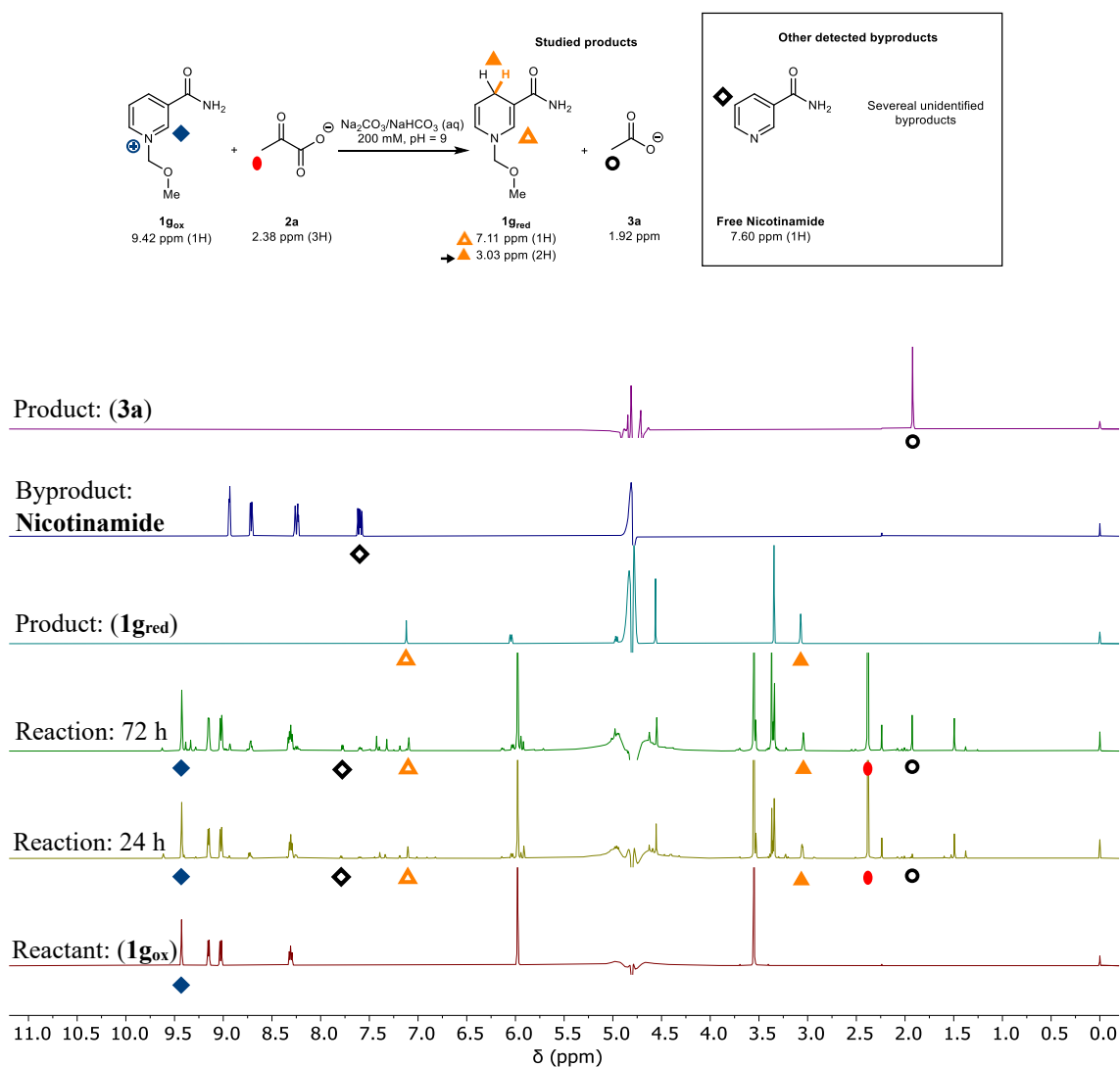


Figure S23. ^1H -NMR (300 MHz, $\text{H}_2\text{O}:\text{D}_2\text{O}$ 9:1, noesygppr1d) spectra of the reaction between pyridinium (**1g_{ox}**, 30 mM) and pyruvate (**2a**, 30 mM) in aqueous $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ solution (200 mM) at pH 9, after 24h and 72 h of reaction (middle spectra). Spectra of pure reactants (below) and pure products and byproducts (top) in the same bicarbonate solution are given for comparison (spectrum of pure nicotinamide in $\text{NH}_3/\text{NH}_4\text{Cl}$ solution).

The signal at 9.62 ppm (not quantified) is absent in the control of **1g_{ox}** without **2a**, suggesting that it likely belongs to the adduct formed between pyruvate (**2a**) and pyridinium **1g_{ox}**.

Methanol is formed in the course of the reaction.

Table S19. Yields (%)^a of the quantified compounds in the reaction of **1g_{ox}** (30 mM) and **2a** (30 mM) in aqueous Na₂CO₃/NaHCO₃ solution (200 mM) at pH 9.

	Reagents ^b	Studied products		Other detected byproducts
Time	1g_{ox}	1g_{red}	3a	Free Nicotinamide
1	97.2 ±4.3	5.4 ±3.6	0.1 ±0.0	2.1 ±0.1
24	62.0 ±2.7	12.2 ±1.0	0.9 ±0.1	3.2 ±0.1
48	54.3 ±0.3	13.5 ±2.3	2.6 ±0.5	5.3 ±0.8
72	57.5 ±3.2	13.3 ±1.0	5.7 ±0.9	7.7 ±0.4
168	42.5 ±1.3	7.2 ±0.8	13.6 ±1.8	12.2 ±1.4

^aAverage yield (%) and standard deviation (±%) of three replicates. ^bPercentage of remaining reagent.

Table S20. Yields (%) of the quantified compounds in the control of pyridinium **1g_{ox}** (30 mM) in aq. Na₂CO₃/NaHCO₃ (200 mM) at pH 9.

Time (h)	1g_{ox} ^a	1g_{red}	3a	Nicotinamide
1	95.7	0.0	0.0	2.1
24	97.5	0.0	0.0	3.3
48	96.0	0.0	0.0	4.4
72	86.6	0.0	0.0	5.3
168	77.6	0.0	0.0	7.2

^aPercentage of remaining reagent.

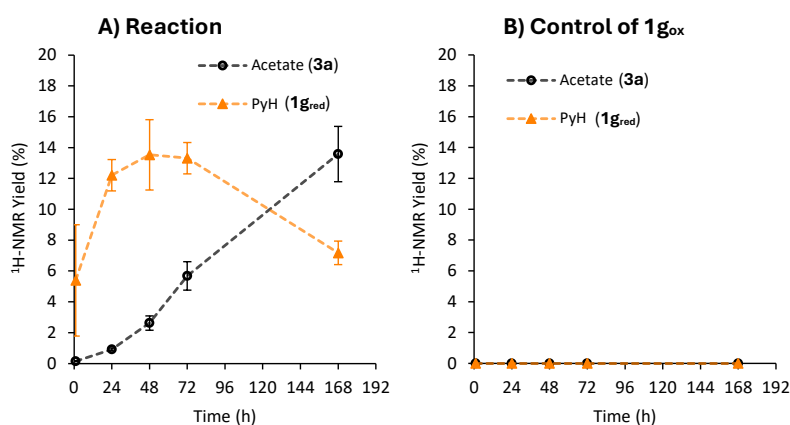


Figure S24. ¹H-NMR monitoring of acetate (**3a**) and dihydropyridine **1g_{red}** formation in: **A)** Reaction of **1g_{ox}** (30 mM) and **2a** (30 mM). **B)** Control of pyridinium **1g_{ox}** (30 mM). Both in aq. Na₂CO₃/NaHCO₃ (200 mM) at pH 9. Plotted values from the preceding tables.

Among the byproducts of this reaction (and in the control of **1g_{ox}**) is nicotinamide (without any substitution at the *N*-position). This is further evidence that the -OMe substituent undergoes other secondary processes that release nicotinamide, in contrast to the cases where the substituent was -CH₂CONH₂ (**1a_{ox}**-**1d_{ox}**), where no free pyridines were observed.

Spectra related to the reaction of pyridinium **1h_{ox}** with pyruvate (**2a**)

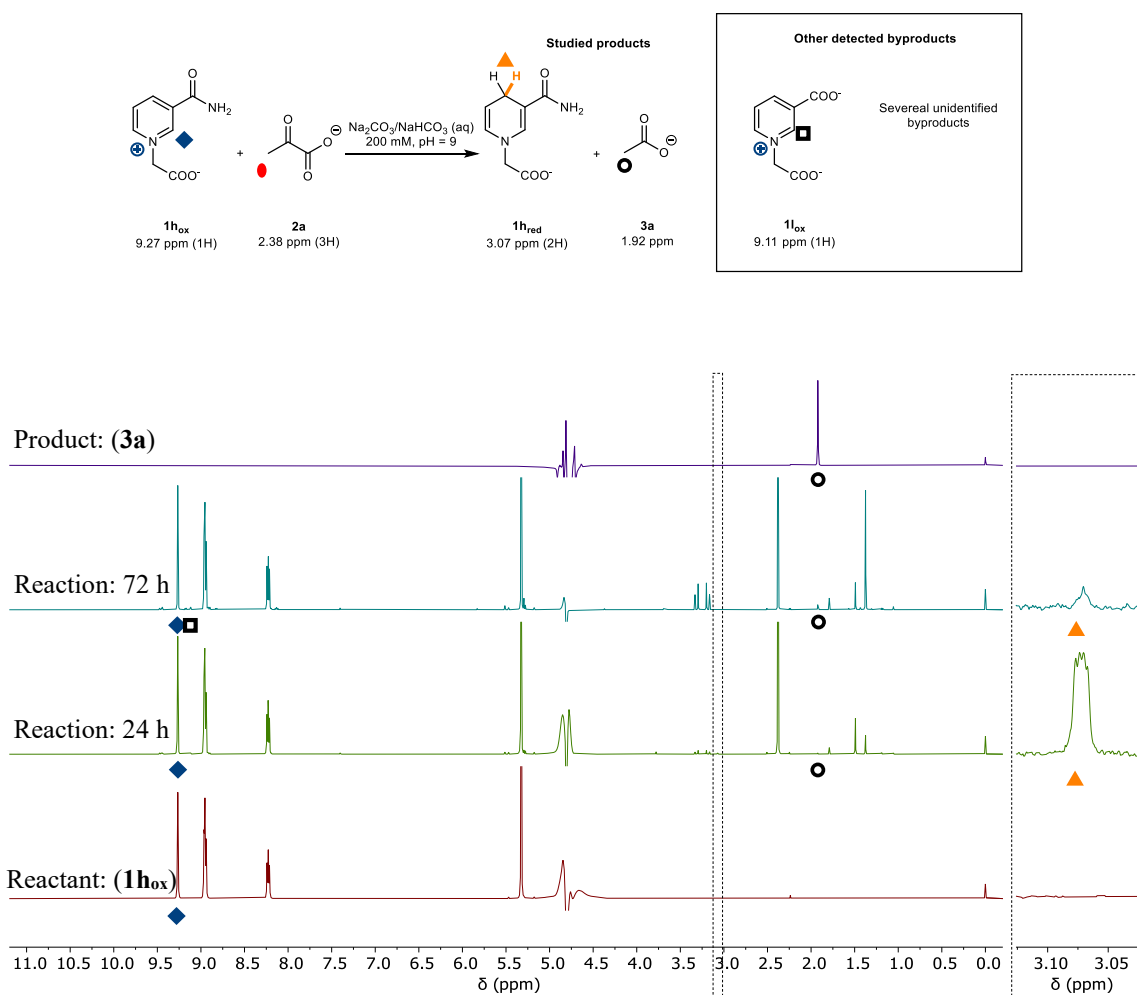


Figure S25. ^1H -NMR (300 MHz, $\text{H}_2\text{O}:\text{D}_2\text{O}$ 9:1, noesygppr1d) spectra of the reaction between pyridinium (**1h_{ox}**, 30 mM) and pyruvate (**2a**, 30 mM) in aqueous $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ solution (200 mM) at pH 9, after 24h and 72 h of reaction (middle spectra). Spectra of pure reactants (below) and pure products and byproducts (top) in the same bicarbonate solution are given for comparison.

Table S21. Yields (%)^a of the quantified compounds in the reaction of **1h_{ox}** (30 mM) and **2a** (30 mM) in aqueous Na₂CO₃/NaHCO₃ solution (200 mM) at pH 9.

Time	Studied products		Other detected byproducts
	1h_{red}	3a	1l_{ox}
1	0.1 ±0.2	0.1 ±0.0	0.6 ±0.0
24	0.6 ±0.3	0.3 ±0.0	1.6 ±0.4
48	0.6 ±0.1	1.0 ±0.3	2.7 ±0.2
72	0.2 ±0.1	1.3 ±0.3	3.0 ±0.3
168	0.4 ±0.3	3.7 ±0.5	5.5 ±1.2

^aAverage yield (%) and standard deviation (±%) of three replicates.

Table S22. Yields (%) of the quantified compounds in the control of pyridinium **1h_{ox}** (30 mM) in aq. Na₂CO₃/NaHCO₃ (200 mM) at pH 9.

Time (h)	1h_{red}	3a	1l_{ox}
1	0.0	0.0	0.7
24	0.0	0.0	1.5
48	0.0	0.0	2.1
72	0.0	0.0	3.2
168	0.0	0.0	5.2

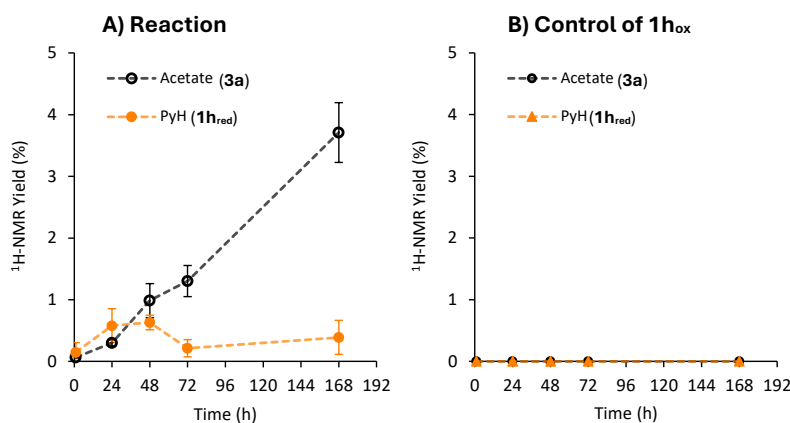


Figure S26. ¹H-NMR monitoring of acetate (**3a**) and dihydropyridine **1h_{red}** formation in: **A)** Reaction of **1h_{ox}** (30 mM) and **2a** (30 mM). **B)** Control of pyridinium **1h_{ox}** (30 mM). Both in aq. Na₂CO₃/NaHCO₃ (200 mM) at pH 9.

Pyridinium **1h_{ox}** promoted a slow conversion of pyruvate into acetate (**3a**) but dihydropyridine **1h_{red}** was detected in a scarce yield. Although the carboxylate group (COO⁻) is not directly attached to the aromatic ring, the negative charge that provides to the molecule decrease its oxidative power.

It is possible that in the other reactions where **1h_{ox}** is formed as a byproduct, it contributes marginally to the oxidative decarboxylation. However, we have not detected dihydropyridine **1h_{red}** in any of those cases.

Although dihydropyridine **1h_{red}** was detected *in situ* in this reaction, attempts to synthesise and isolate it were unsuccessful.

Spectra related to the reaction of pyridinium **1i_{ox}** with pyruvate (**2a**)

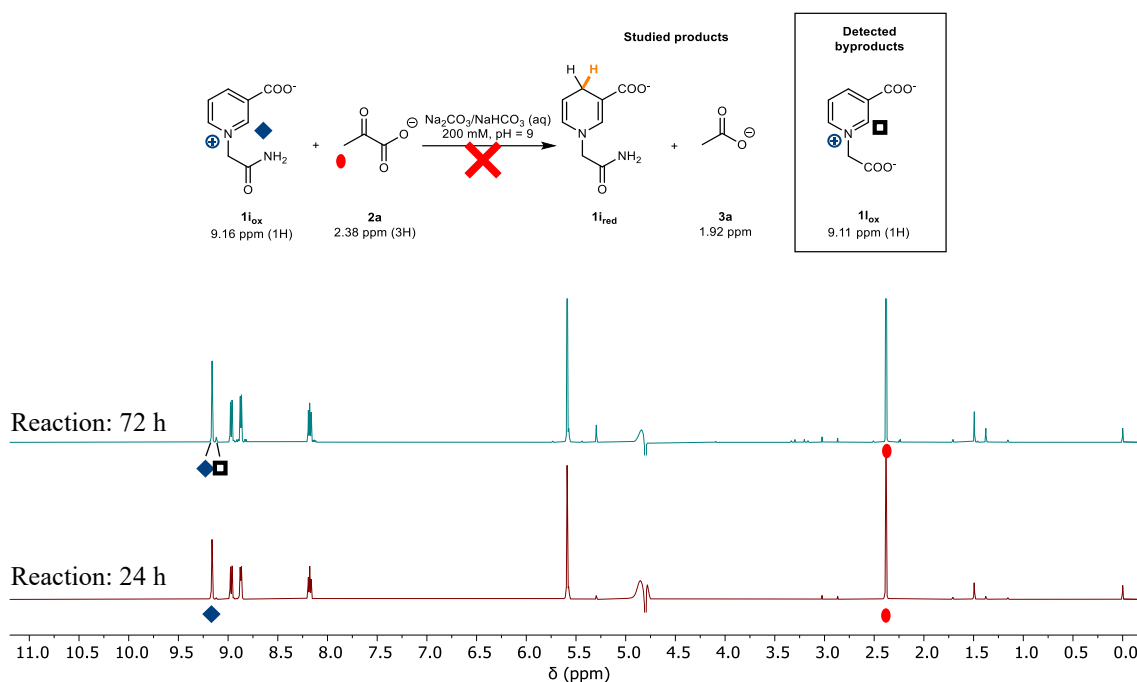


Figure S27. ^1H -NMR (300 MHz, $\text{H}_2\text{O}:\text{D}_2\text{O}$ 9:1, noesygppr1d) spectra of the reaction between pyridinium (**1i_{ox}**, 30 mM) and pyruvate (**2a**, 30 mM) in aqueous $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ solution (200 mM) at pH 9, after 24h and 72 h of reaction. Neither dihydropyridine **1i_{red}** nor acetate (**3a**) were observed at any time.

Spectra related to the reaction of pyridinium **1j_{ox}** with pyruvate (**2a**)

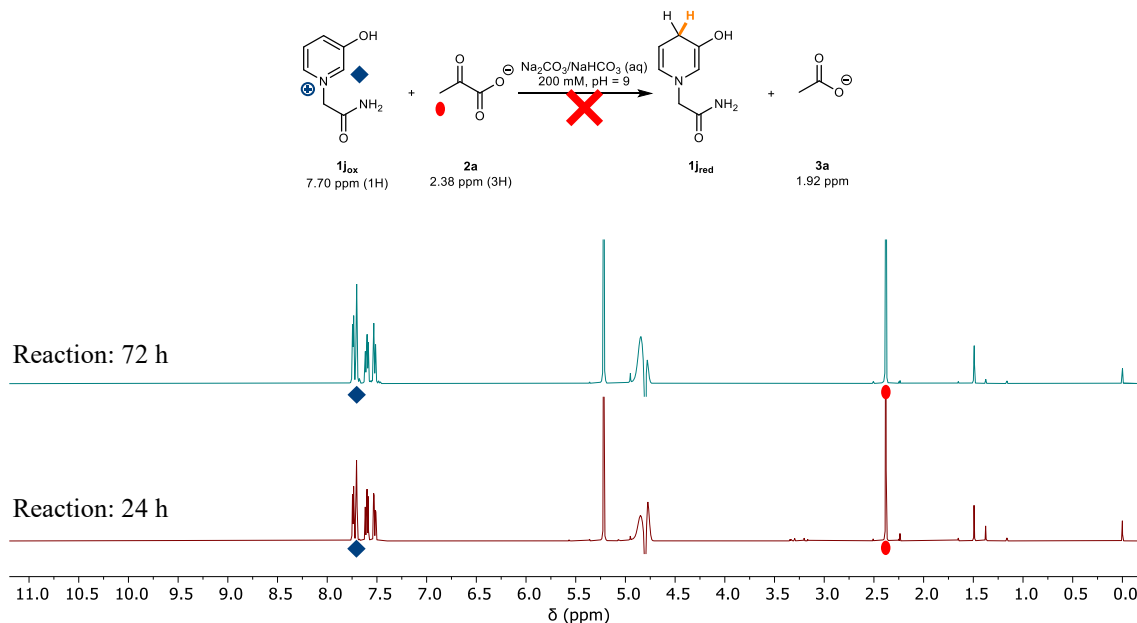


Figure S28. ^1H -NMR (300 MHz, $\text{H}_2\text{O}:\text{D}_2\text{O}$ 9:1, noesygppr1d) spectra of the reaction between pyridinium (**1j_{ox}**, 30 mM) and pyruvate (**2a**, 30 mM) in aqueous $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ solution (200 mM) at pH 9, after 24h and 72 h of reaction. Neither dihydropyridine **1j_{red}** nor acetate (**3a**) were observed at any time.

Spectra related to the reaction of pyridinium **1k_{ox}** with pyruvate (**2a**)

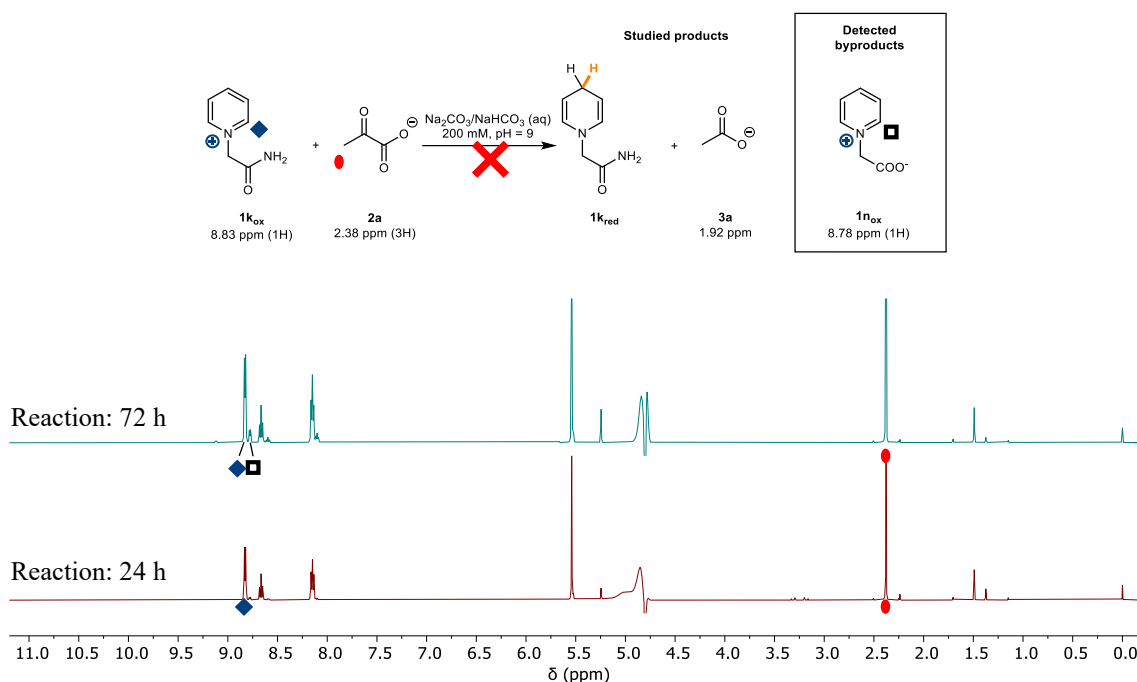


Figure S29. ^1H -NMR (300 MHz, $\text{H}_2\text{O}:\text{D}_2\text{O}$ 9:1, noesygppr1d) spectra of the reaction between pyridinium (**1k_{ox}**, 30 mM) and pyruvate (**2a**, 30 mM) in aqueous $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ solution (200 mM) at pH 9, after 24h and 72 h of reaction. Neither dihydropyridine **1k_{red}** nor acetate (**3a**) were observed at any time.

Spectra related to the reaction of free nicotinamide with pyruvate (**2a**)

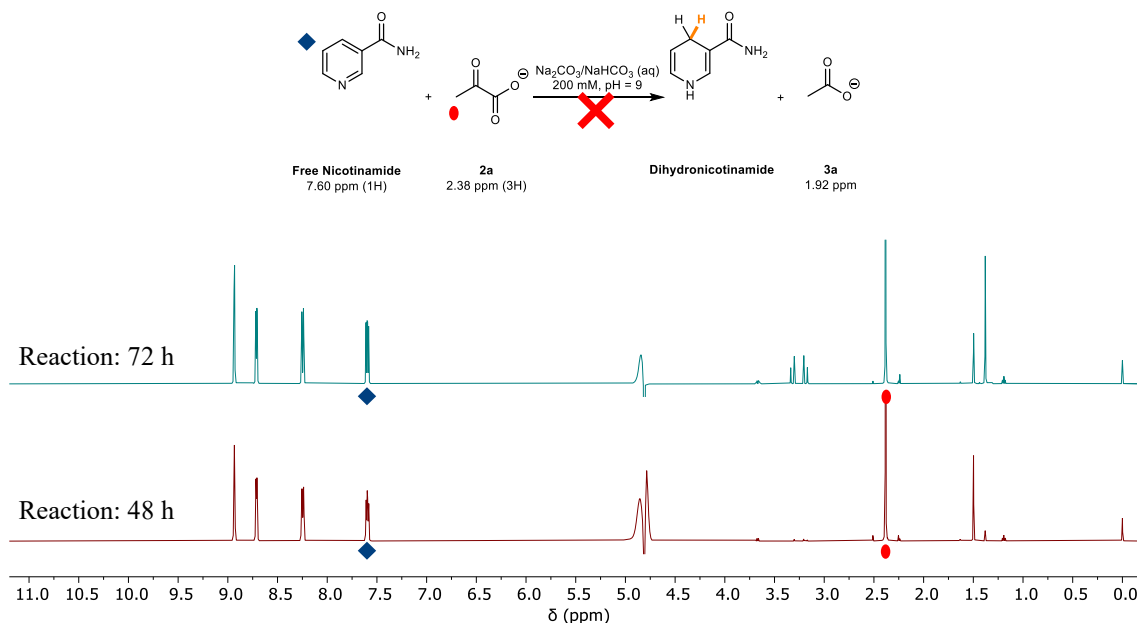


Figure S30. ^1H -NMR (300 MHz, $\text{H}_2\text{O}:\text{D}_2\text{O}$ 9:1, noesygppr1d) spectra of the reaction between **free nicotinamide** (30 mM) and pyruvate (**2a**, 30 mM) in aqueous $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ solution (200 mM) at pH 9, after 24h and 72 h of reaction. **Dihydronicotinamide** was not detected and acetate (**3a**) was seen at a negligible amount (< 0.08 % yield).

Spectra related to the reaction of NAD⁺ with pyruvate (2a)

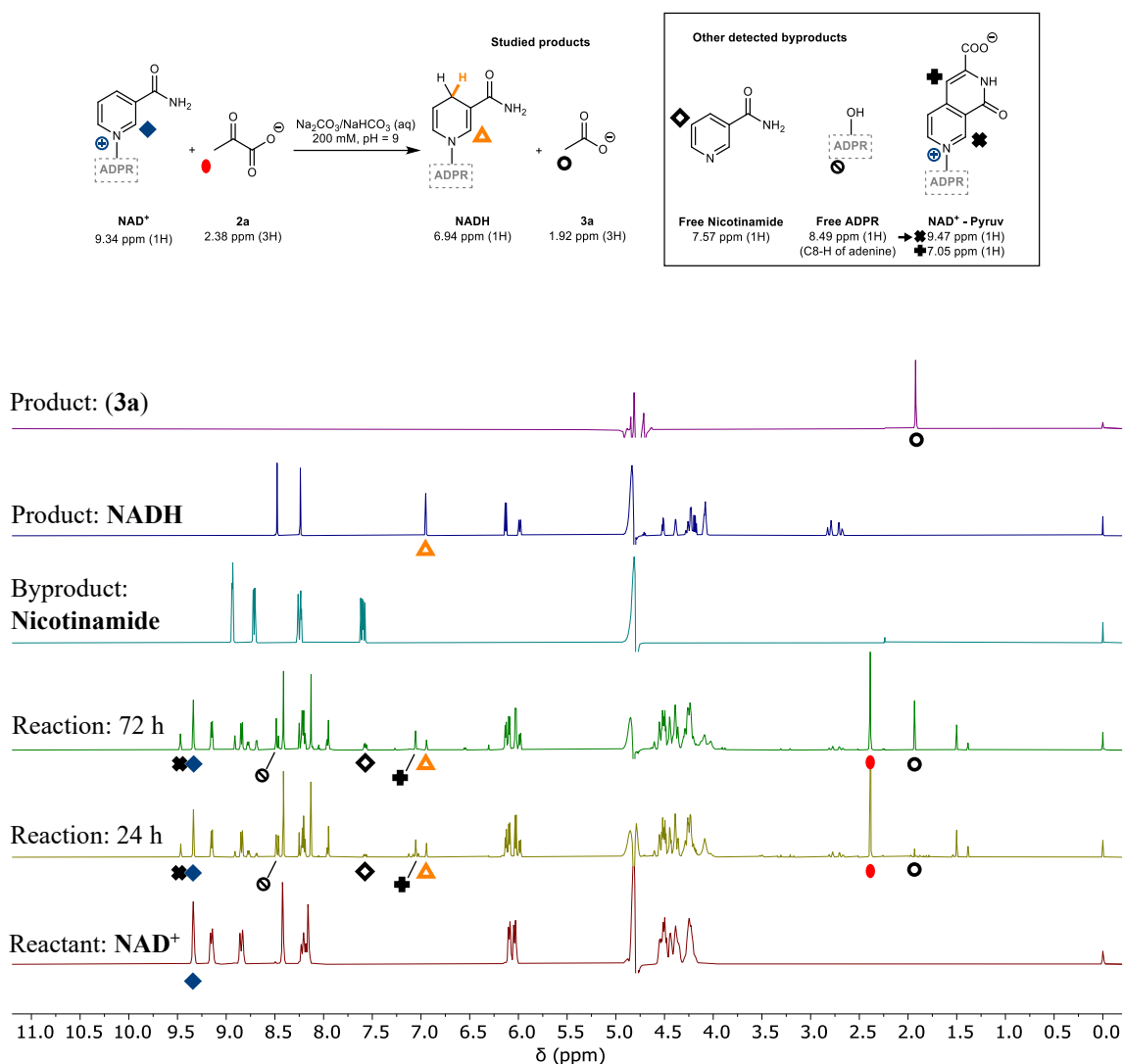


Figure S31. ¹H-NMR (300 MHz, H₂O:D₂O 9:1, noesygppr1d) spectra of the reaction between NAD⁺ (30 mM) and pyruvate (2a, 30 mM) in aqueous Na₂CO₃/NaHCO₃ solution (200 mM) at pH 9, after 24 h and 72 h of reaction (middle spectra). Spectra of pure reactants (below) and pure products and byproducts (top) in the same bicarbonate solution are given for comparison (spectrum of pure nicotinamide in NH₃/NH₄Cl solution).

Table S23. Yields (%)^a of the quantified compounds in the reaction of **NAD**⁺ (30 mM) and **2a** (30 mM) in aqueous Na₂CO₃/NaHCO₃ solution (200 mM) at pH 9.

	Reagents	Studied products		Other detected byproducts		
Time (h)	NAD ⁺	NADH	3a	Nicotinamide	ADPR	NAD-Pyruv
1	83.6 ±3.0	1.5 ±0.4	0.1 ±0.0	2.1 ±0.1	5.8 ±0.8	2.0 ±0.4
24	46.9 ±3.2	12.2 ±0.4	2.0 ±0.7	8.9 ±0.4	24.5 ±0.9	17.4 ±1.2
48	50.0 ±0.8	7.3 ±0.5	5.1 ±1.9	12.9 ±0.6	22.4 ±0.3	14.2 ±1.1
72	39.5 ±3.0	7.0 ±1.2	9.0 ±1.8	15.8 ±0.7	22.4 ±1.5	17.2 ±1.2
168	24.5 ±1.5	4.3 ±1.2	19.5 ±4.9	24.3 ±1.7	38.9 ±1.1	12.9 ±3.7

^aAverage yield (%) and standard deviation (±%) of three replicates. ^bPercentage of remaining reagent.

Table S24. Yields (%) of the quantified compounds in the control of **NAD**⁺ (30 mM) in aq. Na₂CO₃/NaHCO₃ (200 mM) at pH 9.

Time (h)	NAD ⁺ ^a	NADH	3a	Nicotinamide	ADPR	NAD-Pyruv
1	88.5	0.0	0.0	3.1	3.2	0.0
24	62.9	0.7	0.1	21.5	23.1	0.0
48	49.4	1.7	0.1	35.6	35.6	0.0
72	39.1	2.1	0.2	46.2	41.2	0.0
168	23.3	1.1	0.9	58.2	48.0	0.0

^aPercentage of remaining reagent.

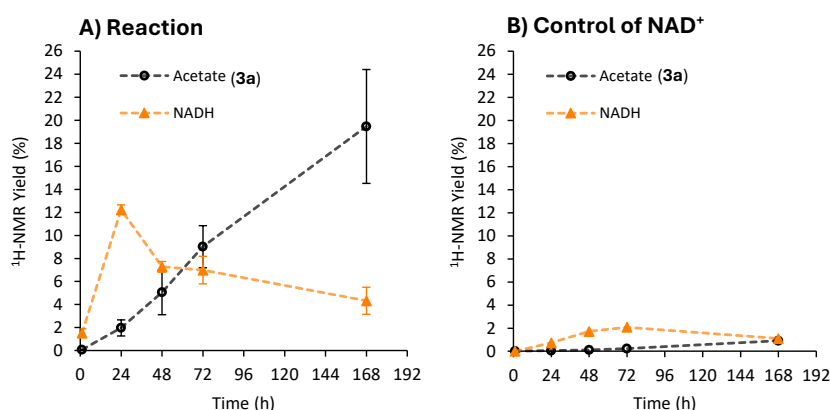


Figure S32. ¹H-NMR monitoring of acetate (**3a**) and **NADH** formation in: A) Reaction of **NAD**⁺ (30 mM) and **2a** (30 mM). B) Control of **NAD**⁺ (15 mM). Both in aq. Na₂CO₃/NaHCO₃ (200 mM) at pH 9.

The effect described in Figure S10 is clearly observed in this case. **NADH** is formed through two processes: the oxidative decarboxylation of pyruvate **2a** to acetate **3a** and the formation of the **NAD-Pyruv** adduct. During the first 24 hours, the second process predominates over the first one. From 24 hours onwards, NADH decomposition is faster than its generation by the combined contribution of both processes.

Note: As discussed in our previous work,¹ the quantification of **NAD**⁺ byproducts, such as **ADPR**, involves certain limitations, including signal overlap issues. Accordingly, the results for **ADPR** may bear slightly higher uncertainty compared to other measurements presented in this study.

7. Results and ^1H -NMR spectra of reductive amination

Procedure for integration and quantification in the reductive amination

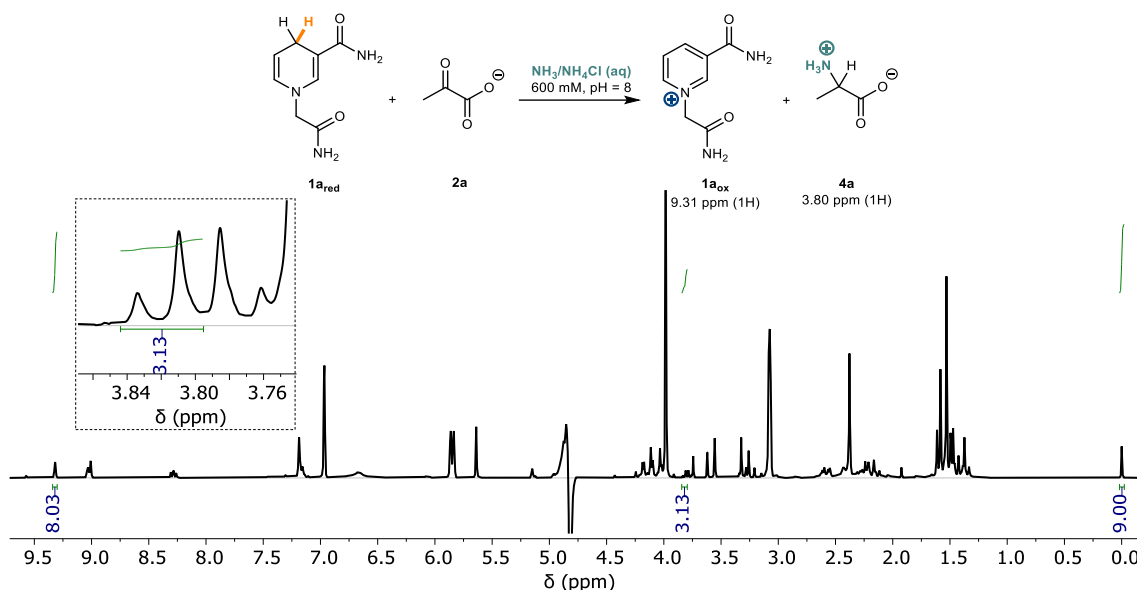


Figure S33. ^1H -NMR (300 MHz, $\text{H}_2\text{O}:\text{D}_2\text{O}$ 9:1, noesygppr1d) spectrum of the reaction between dihydropyridine (**1ared**, 30 mM) and pyruvate (**2a**, 30 mM) in aqueous $\text{NH}_3/\text{NH}_4\text{Cl}$ solution (600 mM) at pH 8. Example of integration at 48 h of reaction.

See section 3 for calibration lines and preparation of samples for NMR analysis.

Initial concentrations: Dihydropyridine [**1ared**]₀ = 30 mM Pyruvate [**2a**]₀ = 30 mM

Once the integral of TMS- d_4 [0.00 (s, 9H)] has been set to 9.00, the integral of pyridinium **1aox** [9.32 (s, 1H)] and the left half integral of alanine **4a** [3.80 (q, J = 7.2 Hz, 1H)] are taken and the concentrations (mM) are obtained with the calibration lines:

$$\text{Integral } \mathbf{1aox} = 3.0809 \cdot [\mathbf{1aox}] \quad \text{Half left integral } \mathbf{4a} = 1.5473 \cdot [\mathbf{4a}] \quad \text{Yield} = [\text{P}]/[\text{R}]_0 \cdot 100$$

The yields are calculated for every replicate, then the average and its standard deviation are obtained. This protocol has been used for other signals and the other reactions.

Table S25. Integral values and calculated ^1H -NMR yields of products **1aox** and **4a**.

Time (h)	Integral pyridinium 1aox				Yield (%) pyridinium 1aox				Average Yield 3a (%)
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 1	Rep 2	Rep 3	Rep 4	
1	1.39	0.77	1.1	1.44	1.5	0.8	1.2	1.6	1.3 ± 0.3
24	5.04	1.77	4.15	-	5.5	1.9	4.5	-	4.0 ± 1.5
48	8.03	3.4	8.02	-	8.7	3.7	8.7	-	7.0 ± 2.4
72	9.77	6.52	9.81	7.5	10.6	7.1	10.6	8.1	9.1 ± 1.5
168	15.07	16.33	15.77	16.87	16.3	17.7	17.1	18.3	17.3 ± 0.7

Time (h)	Hald left integral acetate 4a				Yield (%) alanine 4a				Average Yield 4a (%)
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 1	Rep 2	Rep 3	Rep 4	
1	0	0	0	0	0.0	0.0	0.0	0.0	0.0 ± 0.0
24	2.01	1.14	1.31	-	4.3	2.5	2.8	-	3.2 ± 0.8
48	3.13	1.82	2.37	-	6.7	3.9	5.1	-	5.3 ± 1.2
72	3.91	3.12	3.42	2.84	8.4	6.7	7.4	6.1	7.2 ± 0.9
168	5.14	5.55	5.15	5.67	11.1	12.0	11.1	12.2	11.6 ± 0.5

Spectra related to the reaction of dihydropyridine **1a_{red}** with pyruvate (**2a**)

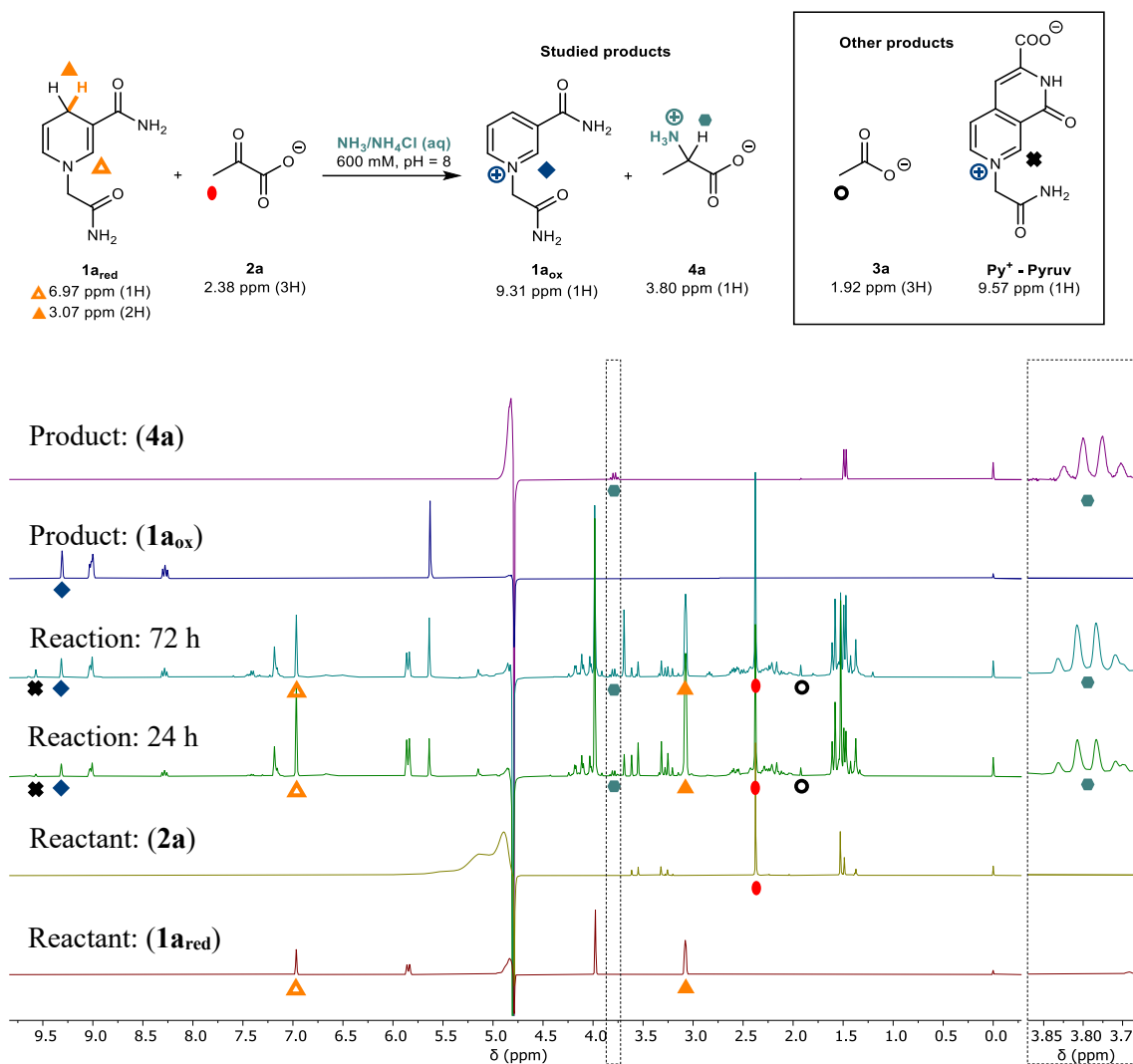


Figure S34. ^1H -NMR (300 MHz, $\text{H}_2\text{O}:\text{D}_2\text{O}$ 9:1, noesygppr1d) spectra of the reaction between dihydropyridine (**1a_{red}**, 30 mM) and pyruvate (**2a**, 30 mM) in aqueous $\text{NH}_3/\text{NH}_4\text{Cl}$ solution (600 mM) at pH 8, after 24 h and 72 h of reaction (middle spectra). Spectra of pure reactants (below) and pure products (top) in $\text{NH}_3/\text{NH}_4\text{Cl}$ solution are given for comparison.

The signal at 9.57 ppm only appears in the presence of pyruvate and it is absent in the control of **1a_{red}** in $\text{NH}_3/\text{NH}_4\text{Cl}$ solution (600 mM) at pH 8. It is assigned to the adduct **Py⁺-Pyruv** (since its pK_a is close to the working pH, the variation from pH 9 in previous experiments to the present pH 8 can cause a slight shift in the NMR signals).

Table S26. Yields (%)^a of the quantified compounds in the reaction of **1a_{red}** (30 mM) and **2a** (30 mM) in aqueous NH₃/NH₄Cl solution (600 mM) at pH 8.

Time (h)	Studied products		Other products	
	1a_{ox}	4a	3a	Py⁺-Pyruv
1	1.3 ±0.3	0.0 ±0.0	0.8 ±0.3	0.0 ±0.0
24	4.0 ±1.5	3.2 ±0.8	0.9 ±0.5	0.2 ±0.1
48	7.0 ±2.4	5.3 ±1.2	1.2 ±0.5	0.7 ±0.3
72	9.1 ±1.5	7.2 ±0.9	1.5 ±0.4	1.4 ±0.4
168	17.3 ±0.7	11.6 ±0.5	4.0 ±0.7	4.3 ±0.8

^aAverage yield (%) and standard deviation (±%) of three replicates.

Table S27. Yields (%) of the quantified compounds in the control of **1a_{red}** (30 mM) in aqueous NH₃/NH₄Cl solution (600 mM) at pH 9.

Time (h)	Studied products		Other detected byproducts	
	1a_{ox}	4a	3a	Py⁺-Pyruv
1	0.2	0.0	0.0	0.0
24	1.4	0.0	0.0	0.0
48	1.6	0.0	0.0	0.0
72	2.1	0.0	0.0	0.0
168	3.4	0.0	0.0	0.0

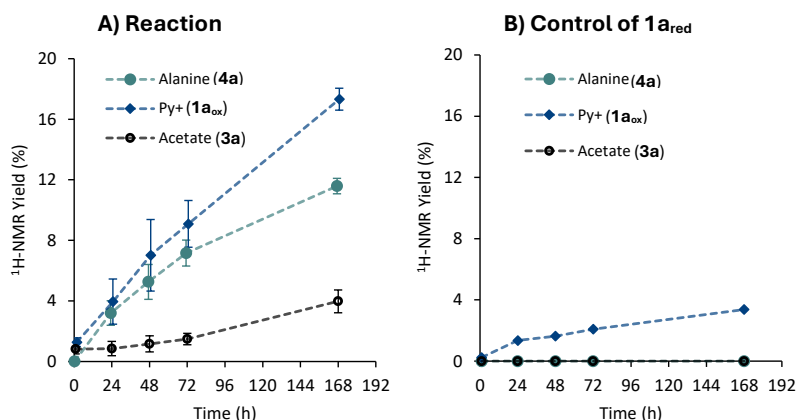


Figure S35. Plots of ¹H-NMR monitoring of alanine (**4a**), pyridinium **1a_{ox}** and acetate (**3a**) formation in: **A)** Reaction of **1a_{red}** (30 mM) and **2a** (30 mM). **B)** Control of dihydropyridine **1a_{red}** (30 mM). Both in aq. NH₃/NH₄Cl (600 mM) at pH 8. See control reaction of pyruvate (**3a**) in these conditions in Figure S7.

During the reaction, small amounts of glycynamide (probably from decomposition of **1a_{red}**) were formed. It could not be quantified since it overlaps (3.72 ppm) with the right half of the alanine signal.

Spectra related to the reaction of dihydropyridine **1a_{red}** with α -ketoglutarate (**2b**)

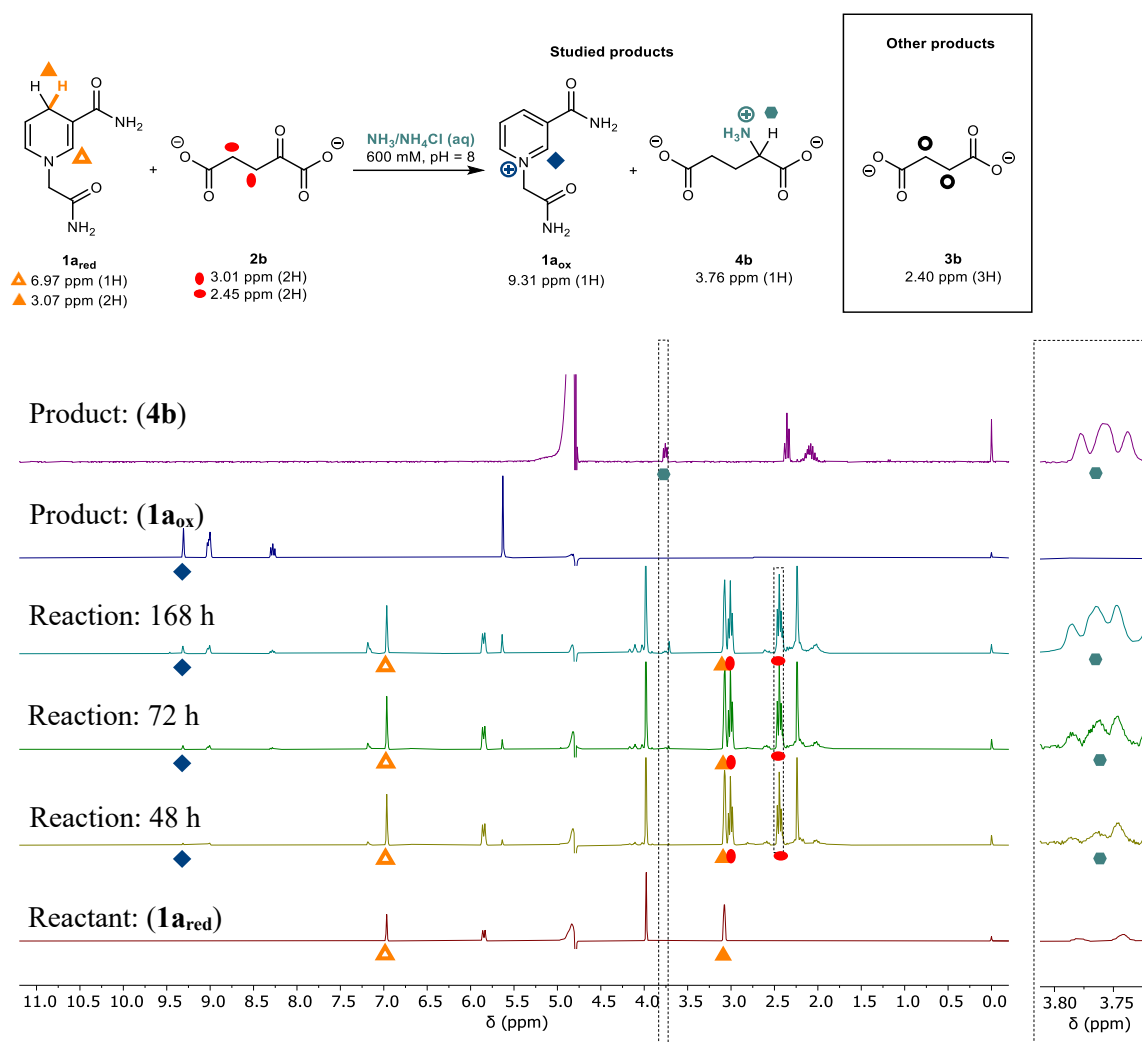


Figure S36. ¹H-NMR (300 MHz, H₂O:D₂O 9:1, noesygppr1d) spectra of the reaction between dihydropyridine (**1a_{red}**, 30 mM) and pyruvate (**2a**, 30 mM) in aqueous $\text{NH}_3/\text{NH}_4\text{Cl}$ solution (600 mM) at pH 8, after 24 h, 72 h and 168 h of reaction (middle spectra). Spectra of pure reactants (below) and pure products (top) in $\text{NH}_3/\text{NH}_4\text{Cl}$ solution are given for comparison.

After 72 hours, a small signal at 9.47 ppm begins to appear, which could belong to the corresponding Py^+ - α -ketoglutarate adduct.

Small amounts of glycnamide were also observed in this reaction.

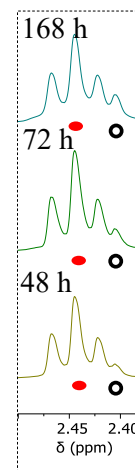


Table S28. Yields (%) of the quantified compounds in the reaction of **1a_{red}** (30 mM) and **2b** (30 mM) in in aqueous NH₃/NH₄Cl solution (600 mM) at pH 8.

Time (h)	Studied products		Other products	
	1a_{ox}	4b	3b	Py⁺-Pyruv
1	1.0	0.0	1.5	0.0
24	2.2	1.6	2.6	0.0
48	2.8	2.2	2.8	0.0
72	4.7	3.6	4.6	0.3
168	11.0	6.5	7.1	1.8

See Table S27 for the control reaction of **1a_{red}**.

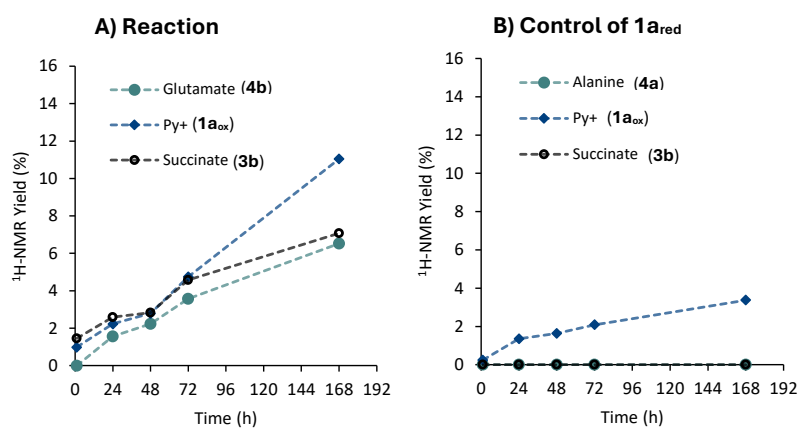


Figure S37. Plots of ¹H-NMR monitoring of glutamate (**4b**), pyridinium **1a_{ox}** and succinate (**3b**) formation in: **A)** Reaction of **1a_{red}** (30 mM) and **2b** (30 mM). **B)** Control of dihydropyridine **1a_{red}** (30 mM). Both in aq. NH₃/NH₄Cl (600 mM) at pH 8.

Spectra related to the reaction of dihydropyridine **1a_{red}** with glyoxylate (**2c**)

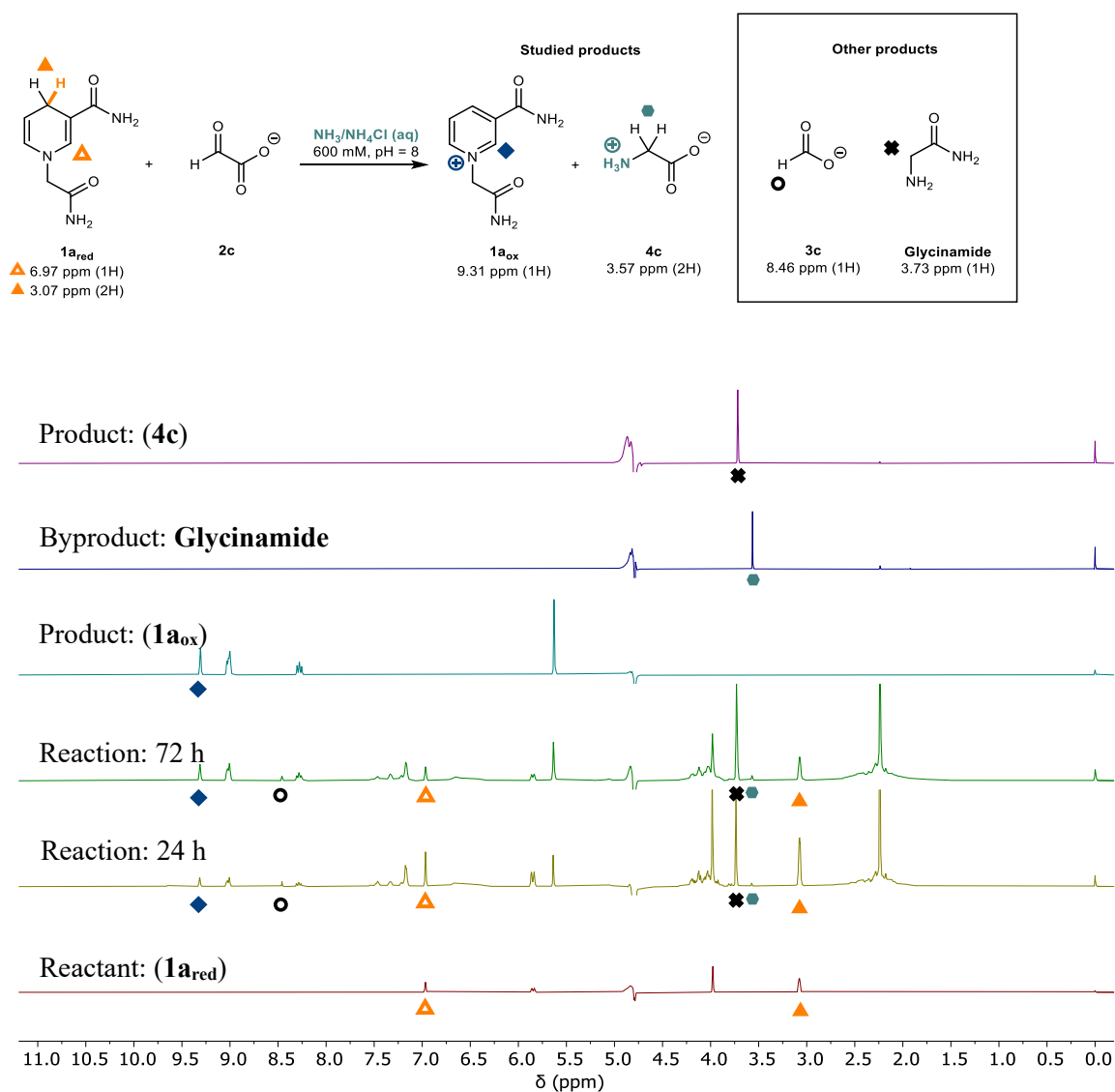


Figure S38. ¹H-NMR (300 MHz, H₂O:D₂O 9:1, noesygppr1d) spectra of the reaction between dihydropyridine (**1a_{red}**, 30 mM) and glyoxylate (**2c**, 30 mM) in aqueous NH₃/NH₄Cl solution (600 mM) at pH 8, after 24 h and 72 h of reaction (middle spectra). Spectra of pure reactants (below) and pure products (top) in NH₃/NH₄Cl solution are given for comparison.

Table S29. Yields (%) of the quantified compounds in the reaction of **1a_{red}** (30 mM) and **2c** (30 mM) in aqueous NH₃/NH₄Cl solution (600 mM) at pH 8.

Time (h)	Studied products		Other products	
	1a_{ox}	4c	3c	Glycinamide
1	1.0	0.3	0.4	6.8
48	14.2	2.6	3.4	37.9
72	17.9	2.6	3.8	44.7
168	28.3	7.2	6.6	70.9

See Table S27 for the control reaction of **1a_{red}**.

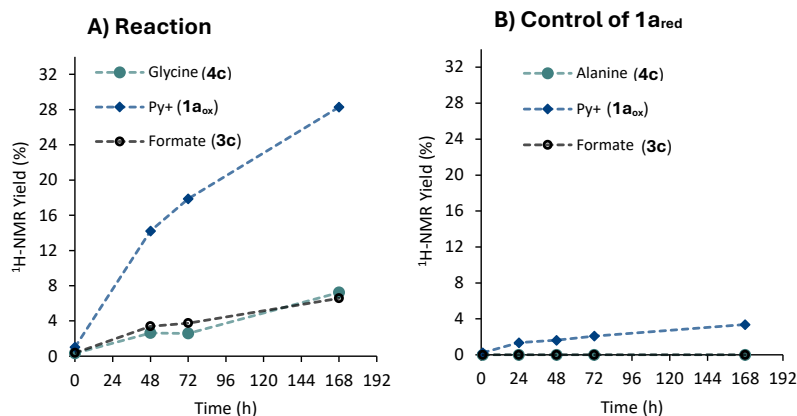


Figure S39. Plots of ¹H-NMR monitoring of glycine (**4c**), pyridinium **1a_{ox}** and formate (**3c**) formation in: **A)** Reaction of **1a_{red}** (30 mM) and **2c** (30 mM). **B)** Control of dihydropyridine **1a_{red}** (30 mM). Both in aq. NH₃/NH₄Cl (600 mM) at pH 8.

The reaction is particularly impure. As previously reported in our study on NADH,¹ glyoxylate can promote degradation of the nicotinamide ring. As a result, the byproduct glycinamide was obtained in significantly higher amounts compared to the reaction with the other α-ketoacids, **2a** and **2b**.

The high yield of pyridinium **1a_{ox}** relative to glycine (**4c**) suggests that its excess could arise from additional side reactions triggered by glyoxylate.

Spectra related to the reaction of dihydropyridine **1b_{red}** with pyruvate (**2a**)

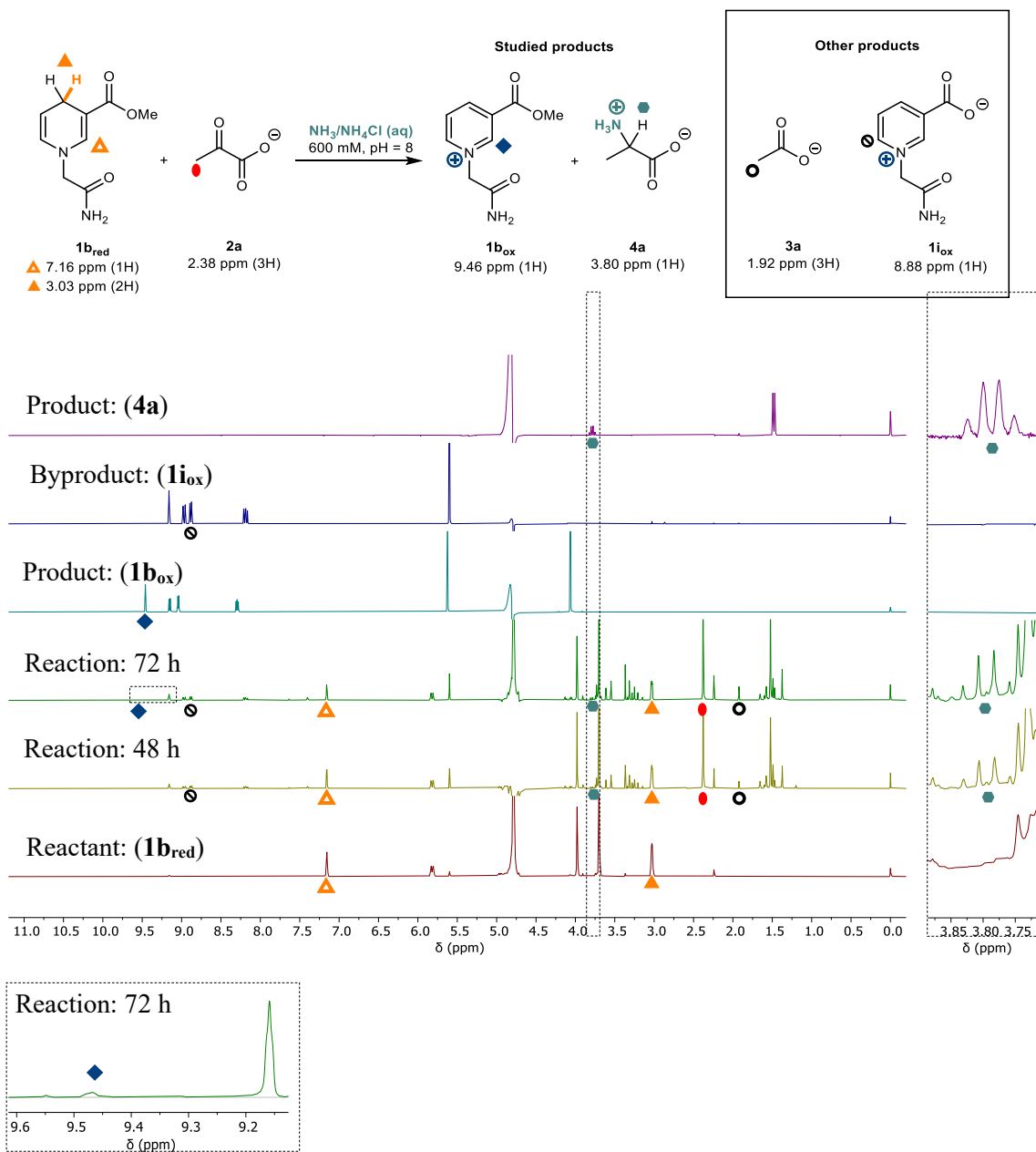


Figure S40. ^1H -NMR (300 MHz, $\text{H}_2\text{O}:\text{D}_2\text{O}$ 9:1, noesygppr1d) spectra of the reaction between dihydropyridine (**1b_{red}**, 30 mM) and pyruvate (**2a**, 30 mM) in aqueous $\text{NH}_3/\text{NH}_4\text{Cl}$ solution (600 mM) at pH 8, after 48 h and 72 h of reaction (middle spectra). Spectra of pure reactants (below) and pure products (top) and byproducts in $\text{NH}_3/\text{NH}_4\text{Cl}$ solution are given for comparison (spectrum of pure **1b_{ox}** in $\text{H}_2\text{O}:\text{D}_2\text{O}$ 9:1; spectrum of pure **1i_{ox}** in $\text{NH}_3/\text{NH}_4\text{Cl}$ solution).

Table S30. Yields (%)^a of the quantified compounds in the reaction of **1b_{red}** (30 mM) and **2a** (30 mM) in aqueous NH₃/NH₄Cl solution (600 mM) at pH 8.

Time (h)	Studied products		Other products	
	1b_{ox}	4a	3a	1i_{ox}
1	1.6 ±0.1	0.0 ±0.0	0.3 ±0.1	1.4 ±1.1
24	1.1 ±0.5	1.9 ±0.2	0.5 ±0.2	4.8 ±0.5
48	0.9 ±0.1	3.4 ±0.3	1.0 ±0.4	6.8 ±0.3
72	1.2 ±0.4	5.3 ±0.7	1.7 ±0.9	9.8 ±0.7
168	1.5 ±0.8	10.3 ±2.5	5.4 ±2.9	20.9 ±5.2

^aAverage yield (%) and standard deviation (±%) of three replicates.

Table S31. Yields (%) of the quantified compounds in the control of **1b_{red}** (30 mM) in aqueous NH₃/NH₄Cl solution (600 mM) at pH 9.

Time (h)	Studied products		Other detected byproducts	
	1b_{ox}	4a	3a	1i_{ox}
1	traces ^b	0.0	traces ^b	0.8
24	traces ^b	0.0	traces ^b	3.5
48	traces ^b	0.0	traces ^b	5.5
72	traces ^b	0.0	traces ^b	6.3
168	traces ^b	0.0	traces ^b	10.7

^aPercentage of remaining reagent. ^bYield <0.6 % in all cases

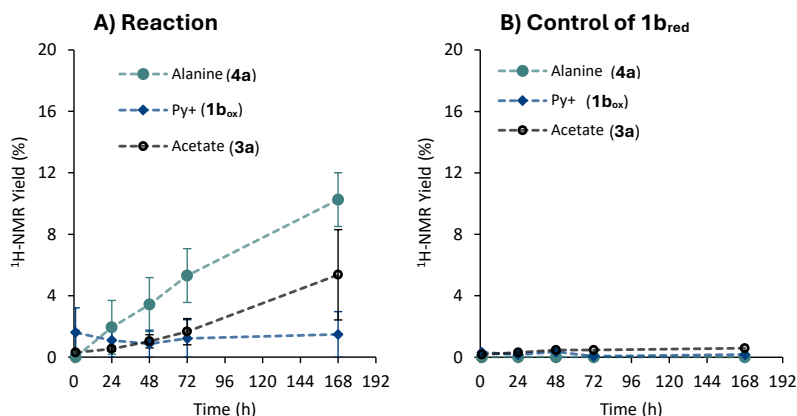


Figure S41. Plots of ¹H-NMR monitoring of alanine (**4a**), pyridinium **1b_{ox}** and acetate (**3a**) formation in: **A)** Reaction of **1b_{red}** (30 mM) and **2a** (30 mM). **B)** Control of dihydropyridine **1b_{red}** (30 mM). Both in aq. NH₃/NH₄Cl (600 mM) at pH 8.

Dihydropyridine **1b_{red}** is capable to efficiently carry out the reductive amination. However, as discussed above, the product pyridinium **1b_{ox}**, due to its positive charge, is much more susceptible to basic hydrolysis, and is rapidly converted mostly into **1i_{ox}**, which has already been shown not to have oxidative power. Consequently, although the reductive amination initially proceeds well, the dihydropyridine cannot be regenerated, rendering the **1b_{ox}/1b_{red}** pair as a poor NAD⁺/NADH cofactor analogue.

Spectra related to the reaction of dihydropyridine **1c_{red}** with pyruvate (**2a**)

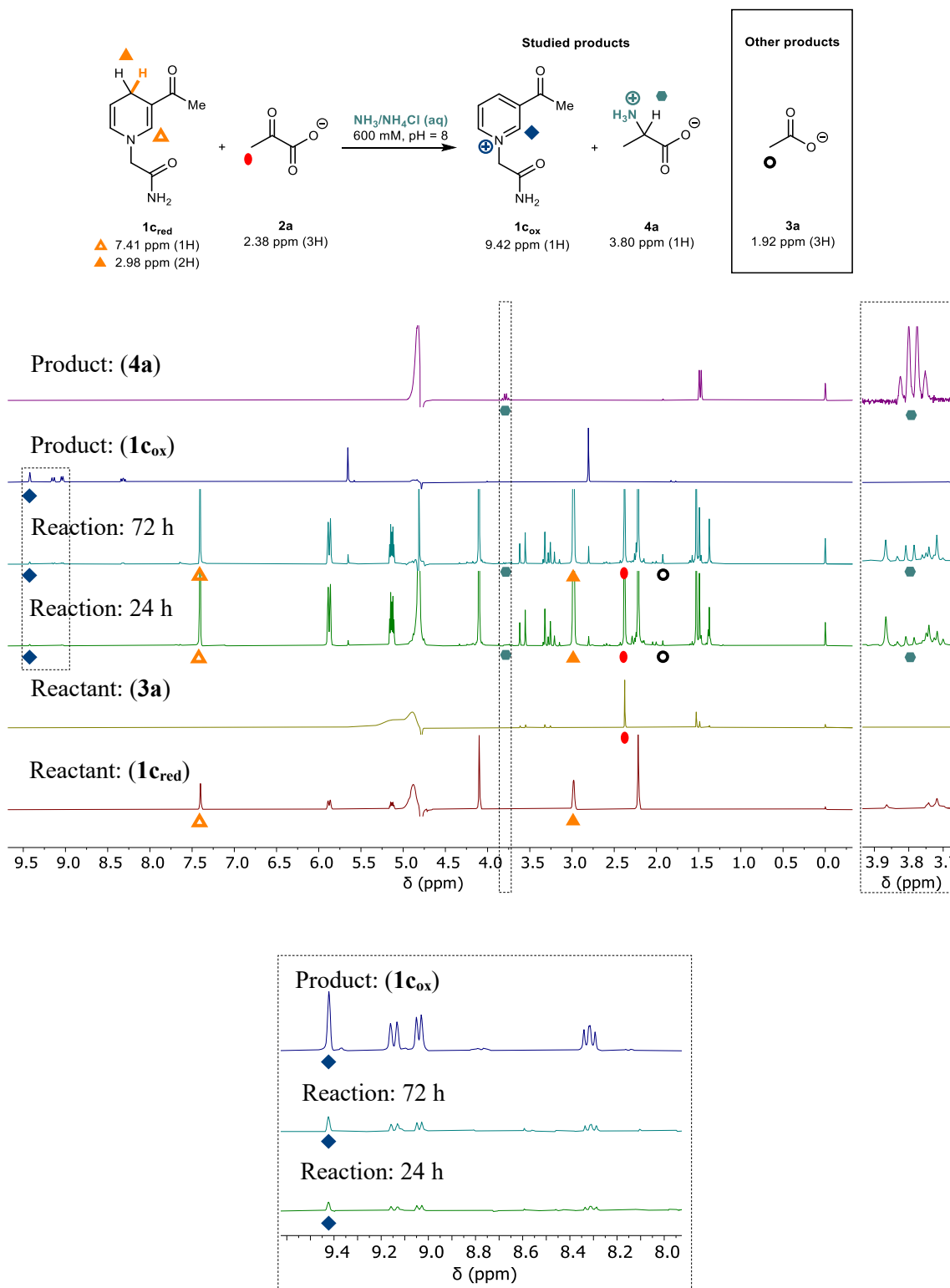


Figure S42. ^1H -NMR (300 MHz, $\text{H}_2\text{O}:\text{D}_2\text{O}$ 9:1, noesygppr1d) spectra of the reaction between dihydropyridine (**1c_{red}**, 30 mM) and pyruvate (**2a**, 30 mM) in aqueous $\text{NH}_3/\text{NH}_4\text{Cl}$ solution (600 mM) at pH 8, after 24 h and 72 h of reaction (middle spectra). Spectra of pure reactants (below) and pure products (top) in $\text{NH}_3/\text{NH}_4\text{Cl}$ solution are given for comparison.

Table S32. Yields (%)^a of the quantified compounds in the reaction of **1c_{red}** (30 mM) and **2a** (30 mM) in in aqueous NH₃/NH₄⁺ solution (600 mM) at pH 8.

Time (h)	Studied products		Other products	
	1c_{ox}	4a	3a	
1	0.4 ±0.1	0.0 ±0.0	0.4 ±0.1	
24	1.2 ±0.2	1.4 ±0.2	0.7 ±0.2	
48	1.4 ±0.3	1.5 ±0.4	0.9 ±0.2	
72	2.1 ±0.5	2.0 ±0.6	1.3 ±0.4	
168	4.5 ±1.9	3.1 ±0.7	3.8 ±1.7	

^a Average yield (%) and standard deviation (±%) of four replicates.

Table S33. Yields (%) of the quantified compounds in the control of **1c_{red}** (30 mM) in aqueous NH₃/NH₄⁺ solution (600 mM) at pH 8.

Time (h)	Studied products		Other detected byproducts
	1c_{ox}	4a	
1	0.3	0.0	0.1
24	0.8	0.0	0.1
48	0.9	0.0	0.2
72	1.0	0.0	0.2
168	0.8	0.0	1.1

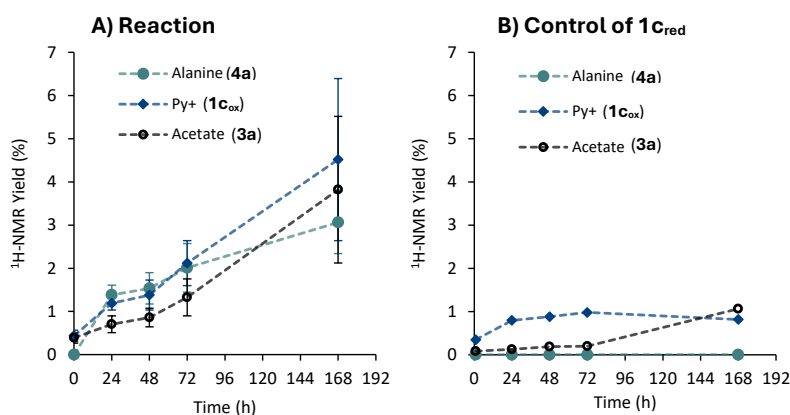


Figure S43. Plots of ¹H-NMR monitoring of alanine (**4a**), pyridinium **1c_{ox}** and acetate (**3a**) formation in: **A)** Reaction of **1c_{red}** (30 mM) and **2a** (30 mM). **B)** Control of dihydropyridine **1c_{red}** (30 mM). Both in aq. NH₃/NH₄Cl (600 mM) at pH 8.

As seen in these spectra and tables, as well as in previous Table S11, the pair **1c_{ox}**/**1c_{red}** can promote oxidative decarboxylation and reductive amination, respectively, generating **1c_{red}** in the former and **1c_{ox}** in the latter. Therefore, this pair behaves as a reversible electron shuttle and can act as an analogue of NAD⁺/NADH. However, it is less efficient than the pair **1a_{ox}**/**1a_{red}**, due to its lower yields in both reactions and its degradation problems.

Spectra related to the reaction of dihydropyridine **1d_{red}** with pyruvate (**2a**)

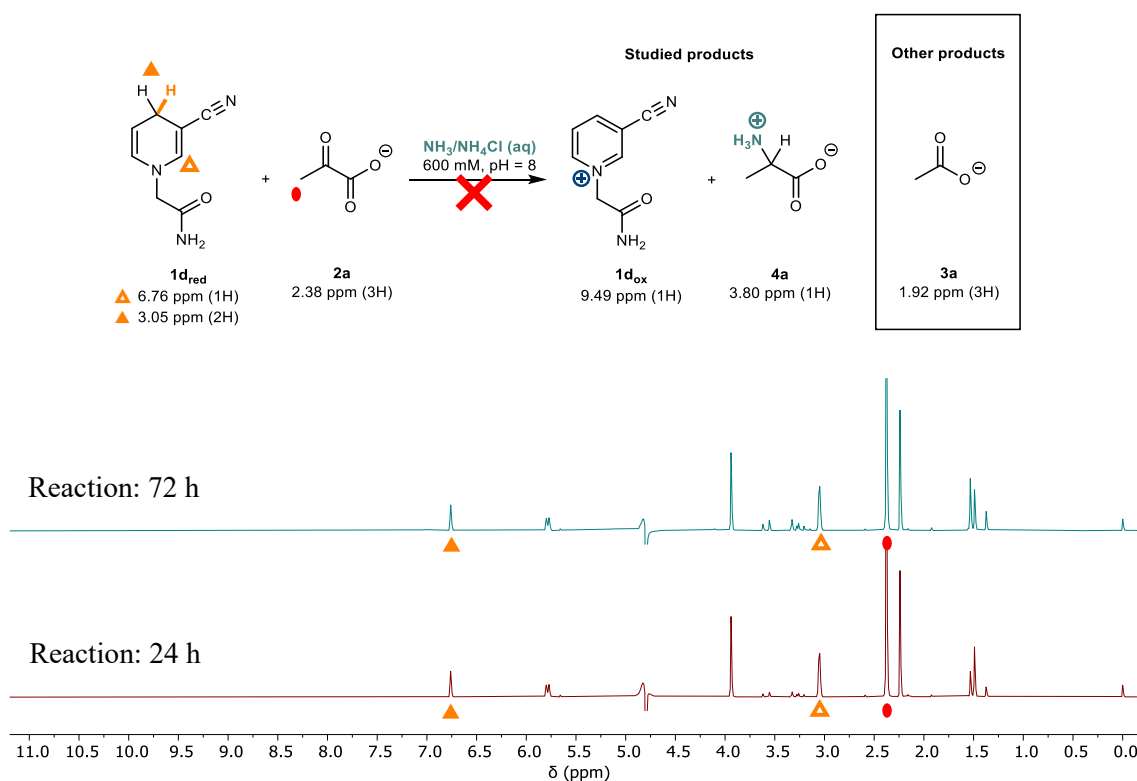


Figure S44. ¹H-NMR (300 MHz, H₂O:D₂O 9:1, noesygppr1d) spectra of the reaction between dihydropyridine (**1d_{red}**, 30 mM) and pyruvate (**2a**, 30 mM) in aqueous NH₃/NH₄Cl solution (600 mM) at pH 8, after 24 h and 72 h of reaction. Neither pyridinium **1d_{ox}** nor alanine (**4a**) were observed at any time. Traces of acetate (**3a**) were detected.

Although **1d_{ox}** can perform the oxidative decarboxylation and generate **1d_{red}**, this dihydropyridine does not show tendency to transfer the hydride, being a bad electron shuttle.

Consequently, the pair **1d_{ox}**/**1d_{red}** cannot function as a suitable analogue of the NAD⁺/NADH cofactor system. However, its hypothetical prebiotic existence would evolve, by hydrolysis, to the pair **1a_{ox}**/**1a_{red}**, a much better analogue (based on observations in Table S13 and Table S14).

Spectra related to the reaction of dihydropyridine **1e_{red}** with pyruvate (**2a**)

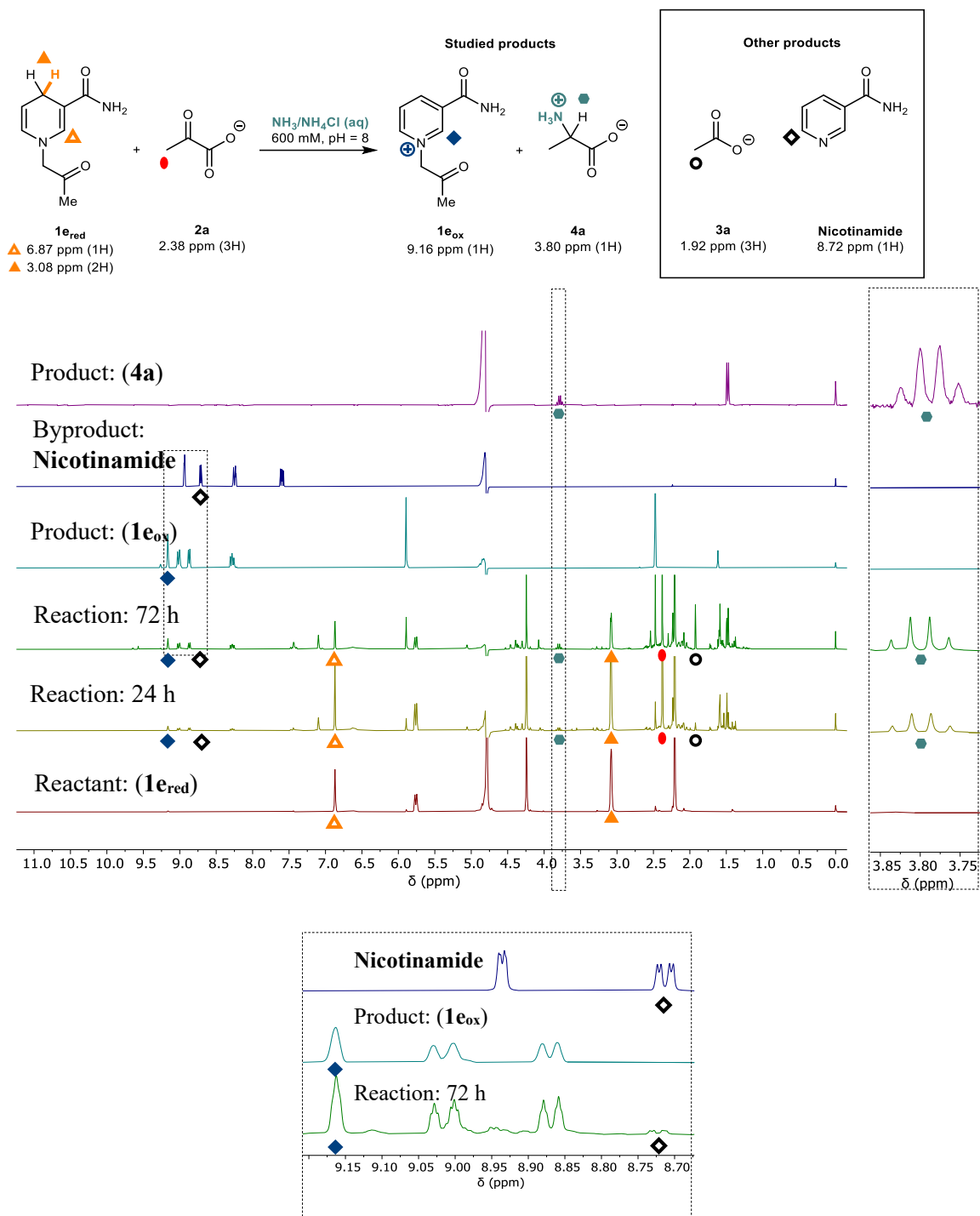


Figure S45. ¹H-NMR (300 MHz, H₂O:D₂O 9:1, noesygppr1d) spectra of the reaction between dihydropyridine (**1e_{red}**, 30 mM) and pyruvate (**2a**, 30 mM) in aqueous NH₃/NH₄Cl solution (600 mM) at pH 8, after 24 h and 72 h of reaction (middle spectra). Spectra of pure reactants (below) and pure products and byproducts (top) in NH₃/NH₄Cl solution are given for comparison.

Table S34. Yields (%)^a of the quantified compounds in the reaction of **1e_{red}** (30 mM) and **2a** (30 mM) in in aqueous NH₃/NH₄Cl solution (600 mM) at pH 8.

Time (h)	Studied products		Other products	
	1e_{ox}	4a	3a	Nicotinamide
1	2.1 ±0.7	1.4 ±0.6	0.8 ±0.2	0.3 ±0.2
24	5.3 ±0.5	5.3 ±0.3	1.4 ±0.3	0.6 ±0.1
48	8.0 ±1.3	8.2 ±0.7	2.8 ±1.0	1.2 ±0.2
72	11.7 ±2.9	9.2 ±1.1	5.4 ±2.7	2.3 ±0.4
168	19.6 ±5.4	11.4 ±1.6	11.1 ±7.0	4.9 ±0.5

^aAverage yield (%) and standard deviation (±%) of three replicates.

Table S35. Yields (%) of the quantified compounds in the control of **1e_{red}** (30 mM) in aqueous NH₃/NH₄Cl solution (600 mM) at pH 8.

Time (h)	Studied products		Other products	
	1e_{ox}	4a	3a	Nicotinamide
1	0.9	0.0	0.3	0.4
24	3.1	0.0	0.6	0.4
48	4.4	0.0	0.8	0.6
72	5.6	0.0	1.3	0.7
168	9.5	0.0	2.4	1.4

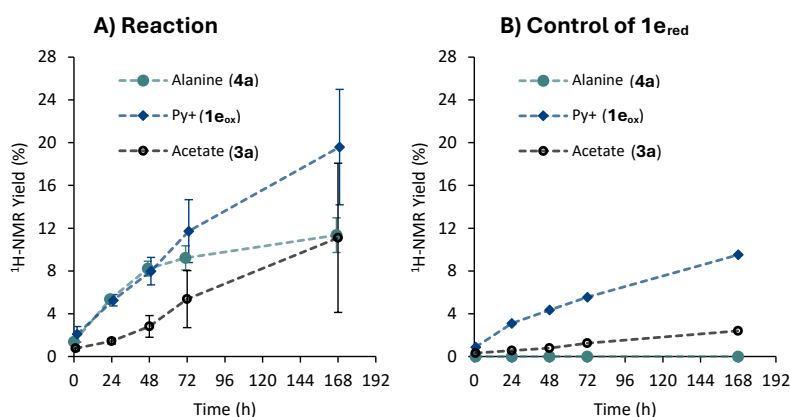


Figure S46. Plots of ¹H-NMR monitoring of alanine (**4a**), pyridinium **1e_{ox}** and acetate (**3a**) formation in: **A)** Reaction of **1e_{red}** (30 mM) and **2a** (30 mM). **B)** Control of dihydropyridine **1e_{red}** (30 mM). Both in aq. NH₃/NH₄Cl (600 mM) at pH 8.

Considerable amounts of pyridinium **1e_{ox}** and acetate **3a** are formed in the control, indicating that, in addition to reductive amination and oxidative decarboxylation, there are other secondary processes that contribute to the formation of these compounds in the main reaction.

As seen in these spectra and tables, as well as in previous Table S15, the pair **1e_{ox}**/**1e_{red}** can promote oxidative decarboxylation and reductive amination, respectively, generating **1e_{red}** in the former and **1e_{ox}** in the latter. Therefore, this pair behaves as a reversible electron shuttle and can act as an analogue of NAD⁺/NADH. However, as discussed on this page and in Figure S20, it presents more degradation problems than the pair **1a_{ox}**/**1a_{red}**.

Spectra related to the reaction of dihydropyridine **1f_{red}** with pyruvate (**2a**)

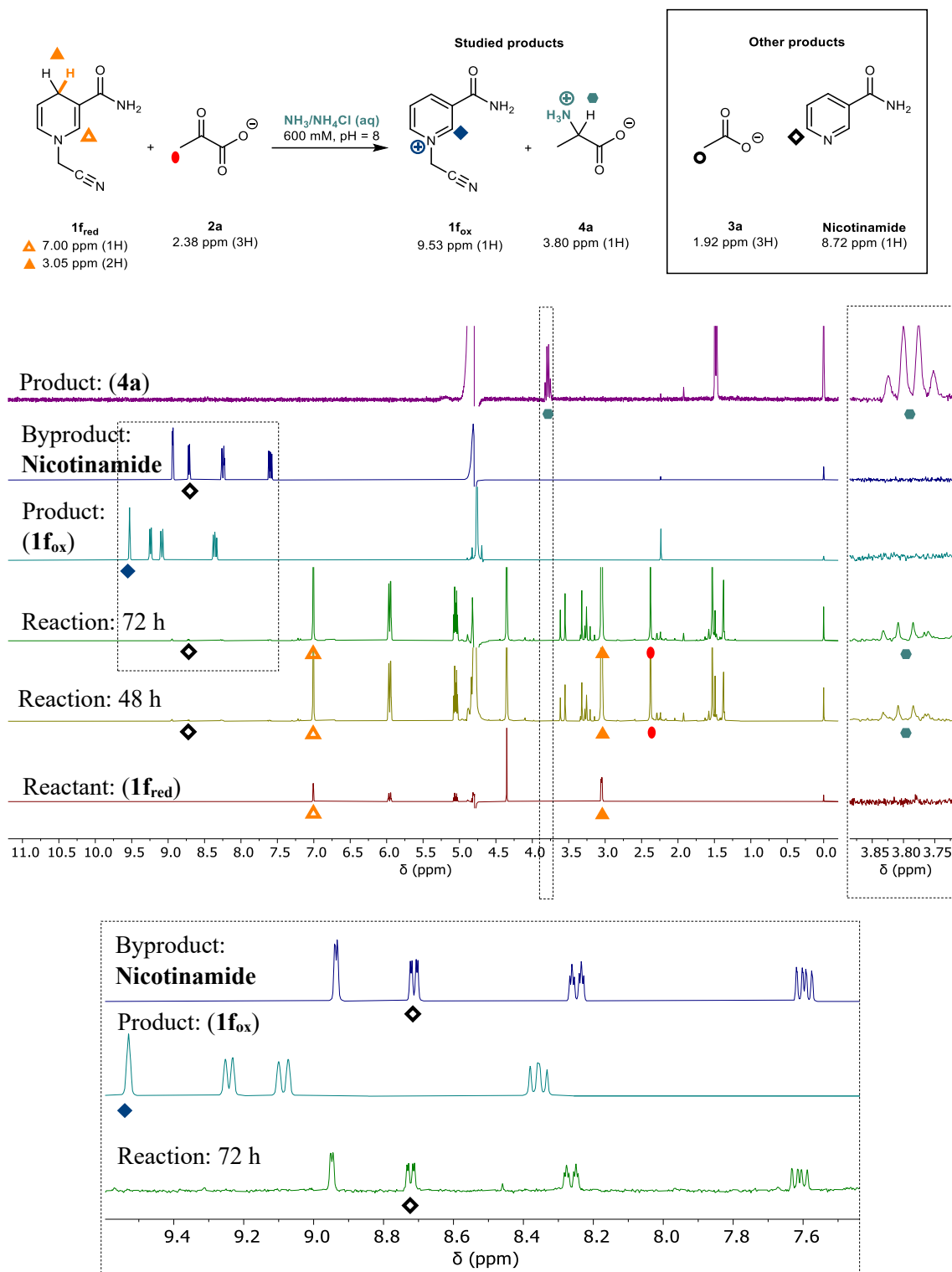


Figure S47. ^1H -NMR (300 MHz, $\text{H}_2\text{O}:\text{D}_2\text{O}$ 9:1, noesygppr1d) spectra of the reaction between dihydropyridine (**1f_{red}**, 30 mM) and pyruvate (**2a**, 30 mM) in aqueous $\text{NH}_3/\text{NH}_4\text{Cl}$ solution (600 mM) at pH 8, after 48 h and 72 h of reaction (middle spectra). Spectra of pure reactants (below) and pure products and byproducts (top) in $\text{NH}_3/\text{NH}_4\text{Cl}$ are given for comparison (spectrum of pure product **1f_{ox}** in pure D_2O).

Table S36. Yields (%) of the quantified compounds in the reaction of **1f_{red}** (30 mM) and **2a** (30 mM) in aqueous NH₃/NH₄Cl solution (600 mM) at pH 8.

Time	Studied products		Other products	
	1f_{ox}	4a	3a	Nicotinamide
1	0.0	0.0	0.3	1.5
24	0.0	0.0	0.4	1.6
48	0.0	0.6	0.7	1.8
72	0.0	0.6	0.7	1.9
168	0.0	1.1	1.2	2.7

Table S37. Yields (%) of the quantified compounds in the control of **1f_{red}** (30 mM) in aqueous NH₃/NH₄Cl solution (600 mM) at pH 8.

Time (h)	Studied products		Other products	
	1f_{ox}	4a	3a	Nicotinamide
1	0.0	0.0	0.1	1.4
24	0.3	0.0	0.0	1.4
48	0.2	0.0	0.1	1.5
72	0.1	0.0	0.1	1.6
168	0.3	0.0	0.1	1.8

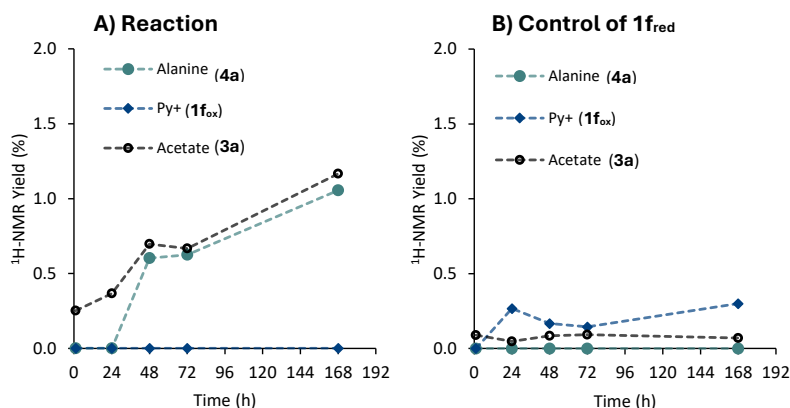


Figure S48. Plots of ¹H-NMR monitoring of alanine (**4a**), pyridinium **1f_{ox}** and acetate (**3a**) formation in: **A**) Reaction of **1f_{red}** (30 mM) and **2a** (30 mM). **B**) Control of dihydropyridine **1f_{red}** (30 mM). Both in aq. NH₃/NH₄Cl (600 mM) at pH 8.

Although the strong electron withdrawing group nitrile is not directly attached to the ring, it exerts influence in the redox active centre, since dihydropyridine **1f_{red}** barely produces alanine through reductive amination, not been detectable the product **1f_{ox}**.

Therefore, due to then null regeneration of the oxidized form, the pair **1f_{ox}**/**1f_{red}** would not behave as analogue of NAD⁺/NADH cofactor pair. However, its hypothetical prebiotic existence would evolve, by hydrolysis, to the pair **1a_{ox}**/**1a_{red}**, a much better analogue (based on observations in Table S17 and Table S18).

Spectra related to the reaction of dihydropyridine **1g_{red}** with pyruvate (**2a**)

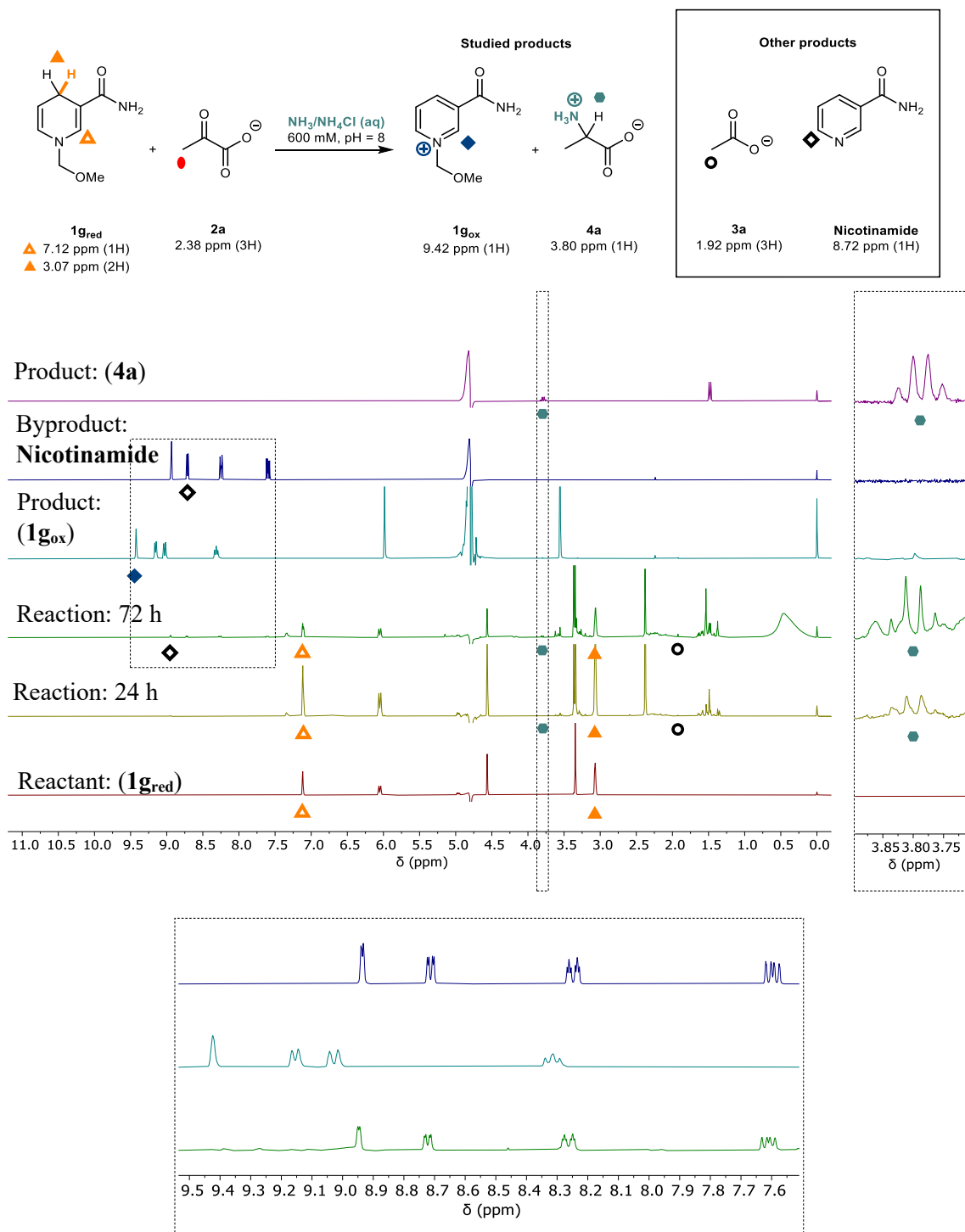


Figure S49. ^1H -NMR (300 MHz, $\text{H}_2\text{O}:\text{D}_2\text{O}$ 9:1, noesygppr1d) spectra of the reaction between dihydropyridine (**1g_{red}**, 30 mM) and pyruvate (**2a**, 30 mM) in aqueous $\text{NH}_3/\text{NH}_4\text{Cl}$ solution (600 mM) at pH 8, after 24 h and 72 h of reaction (middle spectra). Spectra of pure reactants (below) and pure products and byproducts (top) in $\text{NH}_3/\text{NH}_4\text{Cl}$ solution are given for comparison.

Table S38. Yields (%)^a of the quantified compounds in the reaction of **1g_{red}** (30 mM) and **2a** (30 mM) in in aqueous NH₃/NH₄Cl solution (600 mM) at pH 8.

Time (h)	Studied products		Other products	
	1g_{ox}	4a	3a	Nicotinamide
1	traces ^a	0.0	0.2	0.0
24	traces ^a	2.6	0.5	1.5
48	traces ^a	4.7	0.8	3.1
72	traces ^a	6.4	1.3	5.3
168	0.0	9.0	3.5	9.3

^aSmall and unquantifiable signal

Table S39. Yields (%) of the quantified compounds in the control of **1g_{red}** (30 mM) in aqueous NH₃/NH₄Cl solution (600 mM) at pH 8.

Time (h)	Studied products		Other products	
	1g_{ox}	4a	3a	Nicotinamide
1	traces ^a	0.0	traces ^a	0.0
24	0.0	0.0	traces ^a	1.2
48	0.0	0.0	traces ^a	2.1
72	0.0	0.0	traces ^a	3.6
168	0.0	0.0	traces ^a	5.3

^aSmall and unquantifiable signal

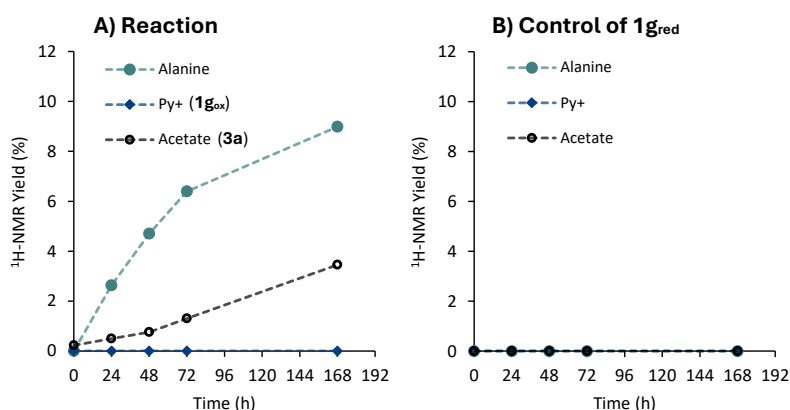


Figure S50. Plots of ¹H-NMR monitoring of alanine (**4a**), pyridinium **1g_{ox}** and acetate (**3a**) formation in: **A)** Reaction of **1g_{red}** (30 mM) and **2a** (30 mM). **B)** Control of dihydropyridine **1g_{red}** (30 mM). Both in aq. NH₃/NH₄Cl (600 mM) at pH 8.

Although alanine is produced through reductive amination, pyridinium **1g_{ox}** is not observed, probably due to a fast decomposition. Nicotinamide and methanol are observed as byproducts.

In view of these tables and Table S19, the pair **1g_{ox}/1g_{red}** gives very good results in the formation of acetate and alanine in the oxidative decarboxylation and reductive amination, respectively, but in the last process the pyridinium **1g_{ox}** is not regenerated. This lack of reversibility makes the pair **1g_{ox}/1g_{red}** not a suitable NAD⁺/NADH analogue.

Spectra related to the reaction of NADH with pyruvate (2a)

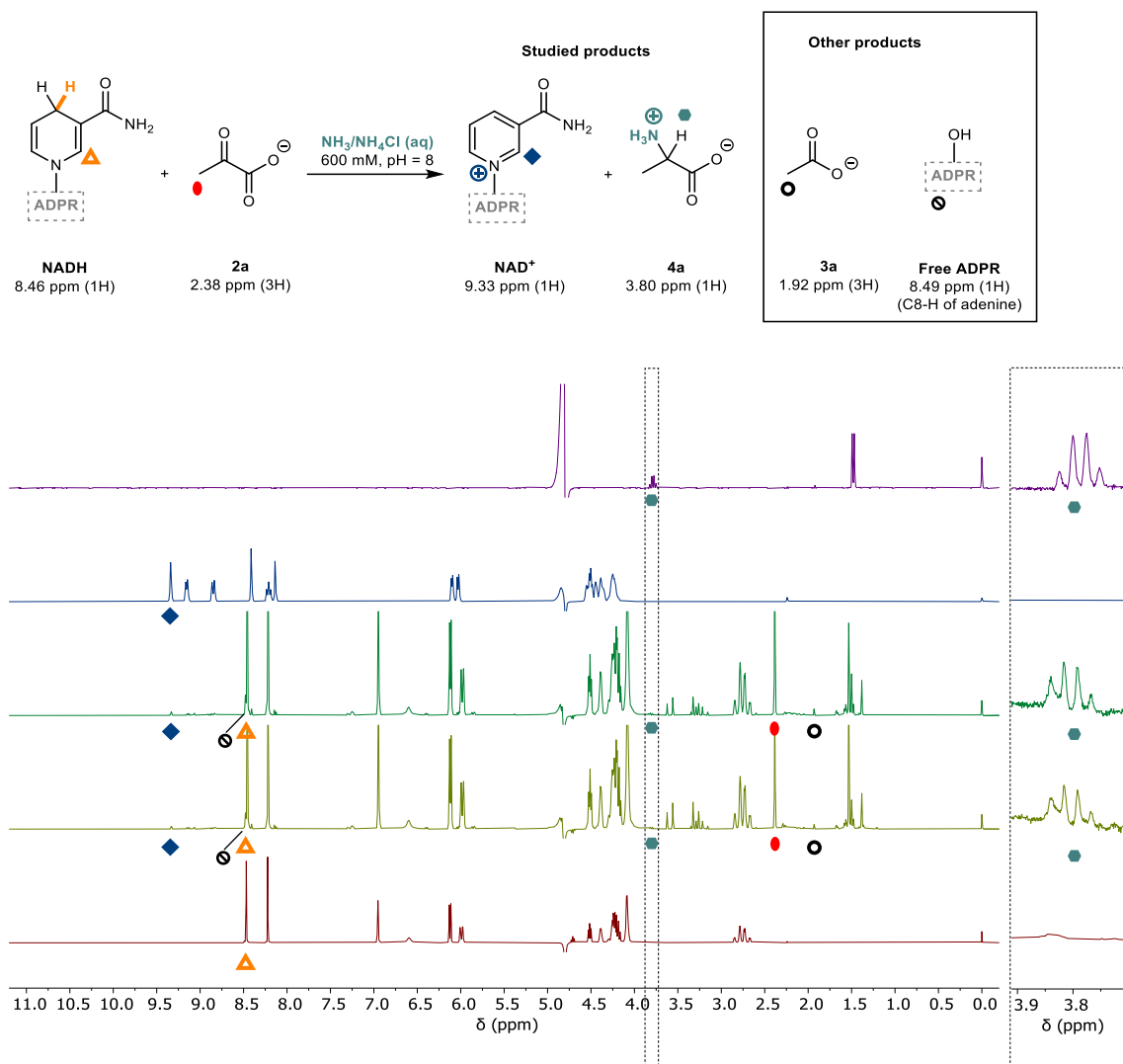


Figure S51. ^1H -NMR (300 MHz, $\text{H}_2\text{O}:\text{D}_2\text{O}$ 9:1, noesygppr1d) spectra of the reaction between **NADH** (30 mM) and pyruvate (**2a**, 30 mM) in aqueous $\text{NH}_3/\text{NH}_4\text{Cl}$ solution (600 mM) at pH 8, after 24 h and 72 h of reaction (middle spectra). Spectra of pure reactants (below) and pure products and byproducts (top) in $\text{NH}_3/\text{NH}_4\text{Cl}$ solution are given for comparison.

Table S40. Yields (%)^a of the quantified compounds in the reaction of **NADH** (30 mM) and **2a** (30 mM) in aqueous $\text{NH}_3/\text{NH}_4\text{Cl}$ solution (600 mM) at pH 8.

Time (h)	Studied products		Other products	
	NAD⁺	4a	3a	ADPR
1	1.1	0.0	0.3	8.7
48	2.6	6.5	1.2	17.3
72	3.3	8.3	1.7	22.9
168	4.4	11.8	4.7	30.7

Traces of **NAD⁺-Pyruv** were detected at 168h

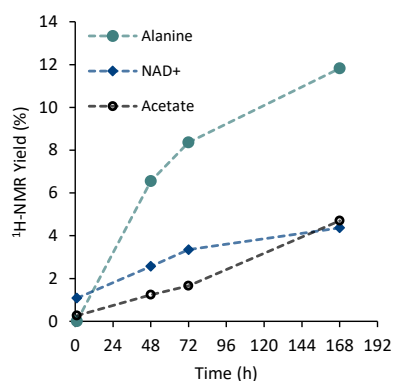


Figure S52. Plots of ^1H -NMR monitoring of alanine (**4a**), **NAD⁺** and acetate (**3a**) formation in: **A**) Reaction of **NADH** (30 mM) and **2a** (30 mM) in aq. $\text{NH}_3/\text{NH}_4\text{Cl}$ (600 mM) at pH 8.

We have re-examined the NMR data from our previous work¹ about the reductive amination of pyruvate by NADH and observed the appearance of acetate (**3a**) in amounts comparable to those detected in the reaction promoted by **1a_{red}**, indicating the *in situ* oxidative activity of the emerging **NAD⁺**.

See this article for more details about this reaction.

Spectra related to the reaction of Hantzsch ester with pyruvate (2a)

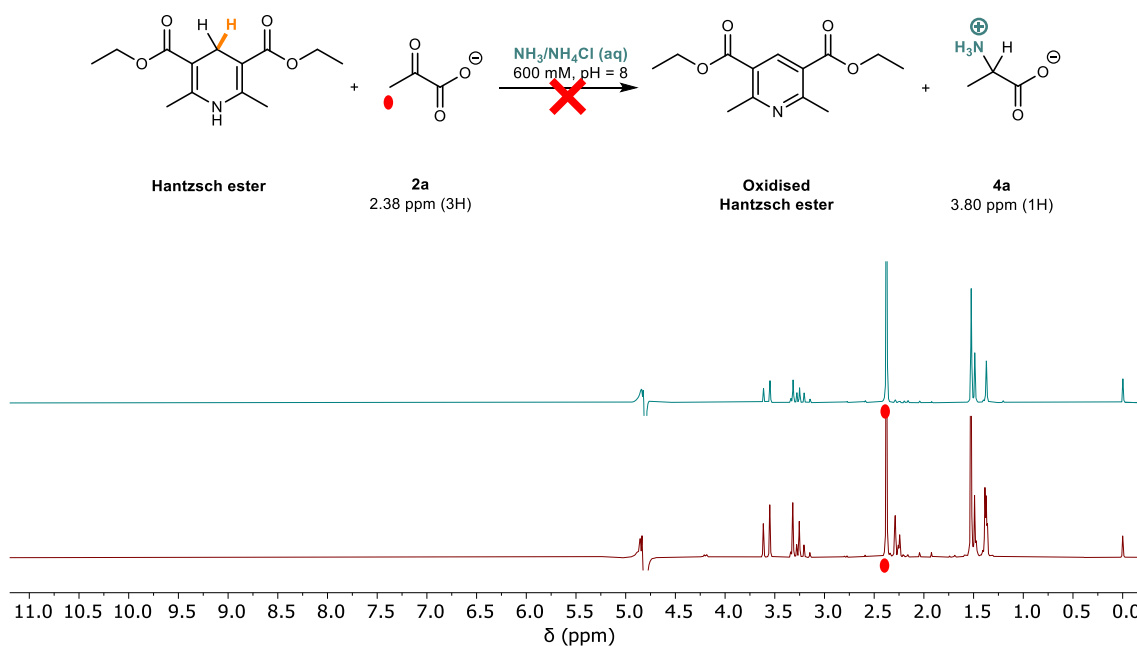


Figure S53. ^1H -NMR (300 MHz, $\text{H}_2\text{O}:\text{D}_2\text{O}$ 9:1, noesygppr1d) spectra of the reaction between Hantzsch ester (30 mM) and pyruvate (**2a**, 30 mM) in aqueous $\text{NH}_3/\text{NH}_4\text{Cl}$ solution (600 mM) at pH 8, after 24 h and 72 h of reaction. No alanine (**4a**) is observed at any reaction time. The reagent Hantzsch ester is completely insoluble in water, reason why it is invisible in the spectrum performed in $\text{H}_2\text{O}:\text{D}_2\text{O}$. Small amounts of acetate **3a** (<1 % yield at 168 h) are observed.

8. Comparison of results and other spectroscopic information

Comparison of results with the different pyridiniums 1_{ox} and dihydropyridines 1_{red}

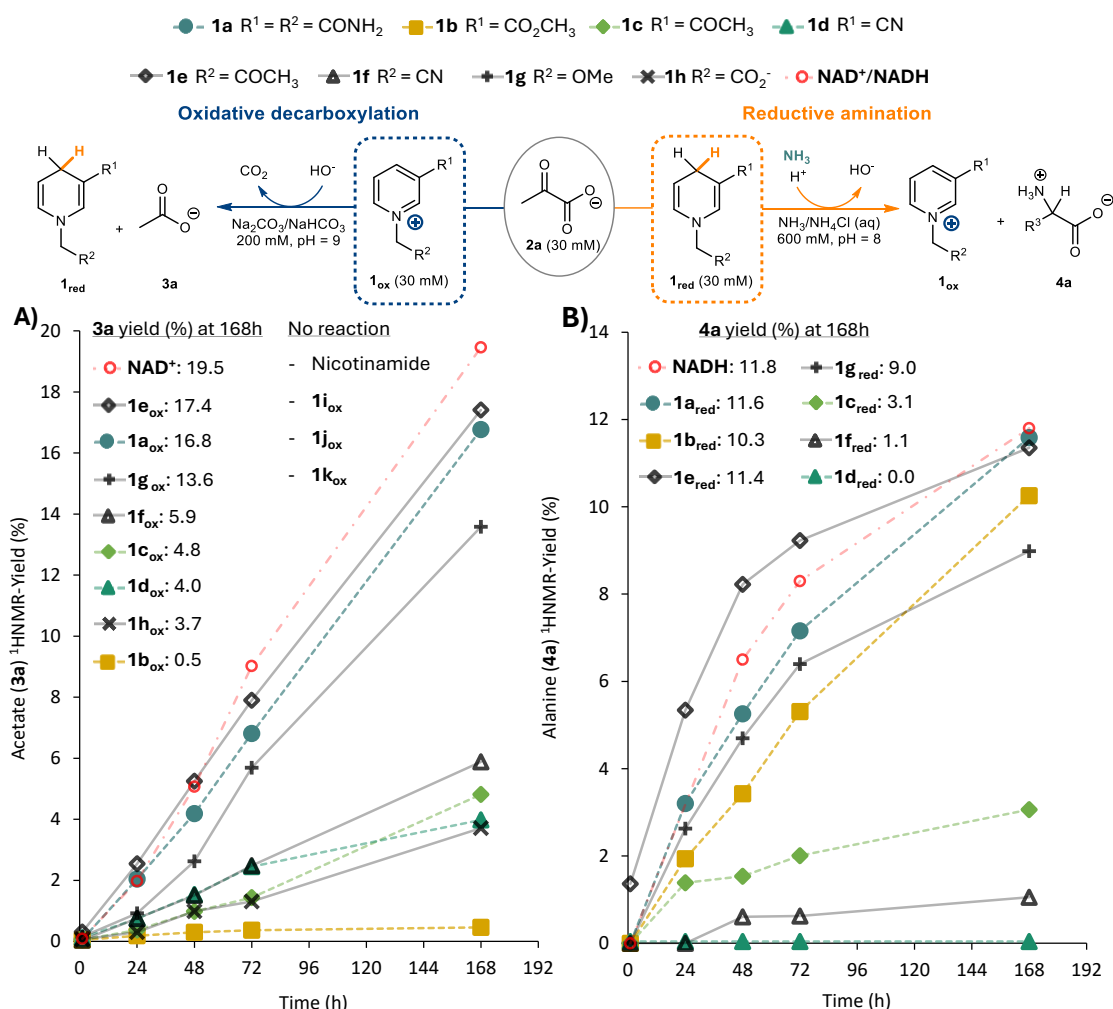


Figure S54. Comparison of results with the different pyridiniums 1_{ox} and dihydropyridines 1_{red} . **A)** 1H -NMR monitoring of acetate ($3a$) formation using different pyridiniums 1_{ox} as reagents in the oxidative decarboxylation. **B)** 1H -NMR monitoring of alanine ($4a$) formation using different dihydropyridines 1_{red} as reagents in the reductive amination. For a clear analysis, results for reagents $1a$ - $1d$ (varying substitution at R^1 , $R^2 = CONH_2$) are presented with coloured dashed lines and the rest of them with grey solid lines. The yield of the products $3a$ or $4a$ at 168 hours is given for each case and the legend is ordered according to this data. Reaction conditions are the same that those depicted in the scheme of Table 1 in the main text. All data are compiled from the tables presented in section 6 and section 7 of this document (Table S5–Table S40).

It is important to remark that in Figure S54A, in the reaction promoted by pyridiniums $1c_{ox}$ and $1e_{ox}$, the acetate ($3a$) 1H -NMR yield (%) comes from the sum of two processes: 1) the oxidative decarboxylation of $2a$ mediated by the pyridinium derivative and 2) the pyridinium decomposition as described in section 6 of this document. In the rest of the cases, the acetate ($3a$) 1H -NMR yield (%) arises almost entirely from the oxidative decarboxylation.

Direct reduction of pyruvate into lactic acid

In all spectra of the reductive amination of pyruvate (**2a**) with the different dihydropyridines (**1_{red}**) in $\text{NH}_4\text{Cl}/\text{NH}_3$ solution (600 mM, pH 8.0), the possible formation of lactate was examined by monitoring its characteristic signal at 4.12 ppm (q, $J = 6.9$ Hz, 1H).¹ Although, in these reactions, this region of the spectrum is often noisy, the signal was never detected, suggesting that the lactate was not formed. Therefore, under these non-enzymatic conditions, dihydropyridines are capable to transfer a hydride to the iminium ion, forming the amino acid alanine (**4a**), but not directly to pyruvate itself. According to the literature, such direct reduction to lactate requires catalysis, for instance by metal ions at lower values of pH.⁸



Other spectroscopic information

Spectroscopic data of NAD^+ , NADH , ADPR, free nicotinamide, pyruvate (**2a**), α -ketoglutarate (**2b**) and glyoxylate (**2c**), in $\text{H}_2\text{O}:\text{D}_2\text{O}$ (9:1) with water signal suppression can be found in our previous work.¹

The ^1H -NMR signal of the byproduct pyridinium **1_{ox}** ($\text{R}^1 = \text{R}^2 = \text{COO}^-$) was identified after synthesising the pyridinium with a reported procedure⁹ and analysing its ^1H -NMR spectrum.

9. Coupled reaction

Table S41. ¹H-NMR yields (%) of acetate (**3a**) and alanine (**4a**) in the reaction of pyruvate (**1a**, 30 mM) with different proportions of pyridinium **1a_{ox}** and dihydropyridine **1a_{red}** (30 mM total concentration), in a process that combines oxidative decarboxylation and reductive amination in aqueous NH₃/NH₄Cl solution (600 mM, pH = 8.5). Yields of **1a_{red}** and **1a_{ox}** have been given in the cases where they were not initially present and were formed exclusively as products. In all cases, average yields (%) and standard deviations (±%) are calculated from three replicates.

Initial - 1a_{ox} (30 mM) : 1a_{red} (0 mM) 1:0			
Time (h)	Acetate (3a)	Alanine (4a)	1a_{red}
1	0.4 ±0.1	0.0 ±0.0	0.5 ±0.1
24	1.0 ±0.1	0.0 ±0.0	1.4 ±0.4
48	2.1 ±0.2	0.1 ±0.1	1.5 ±0.4
72	2.9 ±0.3	0.4 ±0.1	1.1 ±0.3
168	8.0 ±0.4	0.6 ±0.1	0.7 ±0.2

Initial - 1a_{ox} (22.5 mM) : 1a_{red} (7.5 mM) 75:25		
Time (h)	Acetate (3a)	Alanine (4a)
1	0.5 ±0.0	0.3 ±0.2
24	0.6 ±0.2	1.4 ±0.2
48	1.2 ±0.1	1.4 ±0.1
72	2.0 ±0.3	1.6 ±0.3
168	4.7 ±0.5	2.8 ±0.1

Initial - 1a_{ox} (15 mM) : 1a_{red} (15 mM) 50:50		
Time (h)	Acetate (3a)	Alanine (4a)
1	0.5 ±0.2	0.4 ±0.3
24	0.9 ±0.1	2.7 ±0.2
48	1.7 ±0.5	3.3 ±0.2
72	2.4 ±0.6	3.6 ±0.4
168	6.5 ±0.6	4.6 ±0.7

Initial - 1a_{ox} (7.5 mM) : 1a_{red} (22.5 mM) 25:75		
Time (h)	Acetate (3a)	Alanine (4a)
1	0.6 ±0.1	0.0 ±0.0
24	1.1 ±0.0	3.8 ±0.5
48	2.1 ±0.2	5.4 ±0.5
72	2.8 ±0.4	6.3 ±0.4
168	6.3 ±1.2	7.9 ±0.8

Initial - 1a_{ox} (30 mM) : 1a_{red} (0 mM) 1:0			
Time (h)	Acetate (3a)	Alanine (4a)	1a_{ox}
1	0.7 ±0.1	0.0 ±0.0	0.9 ±0.1
24	1.3 ±0.3	4.2 ±0.3	6.3 ±0.6
48	1.5 ±0.1	6.1 ±0.1	9.1 ±0.5
72	1.8 ±0.2	6.7 ±0.2	10.3 ±0.4
168	3.1 ±1.3	7.5 ±0.8	12.4 ±0.9

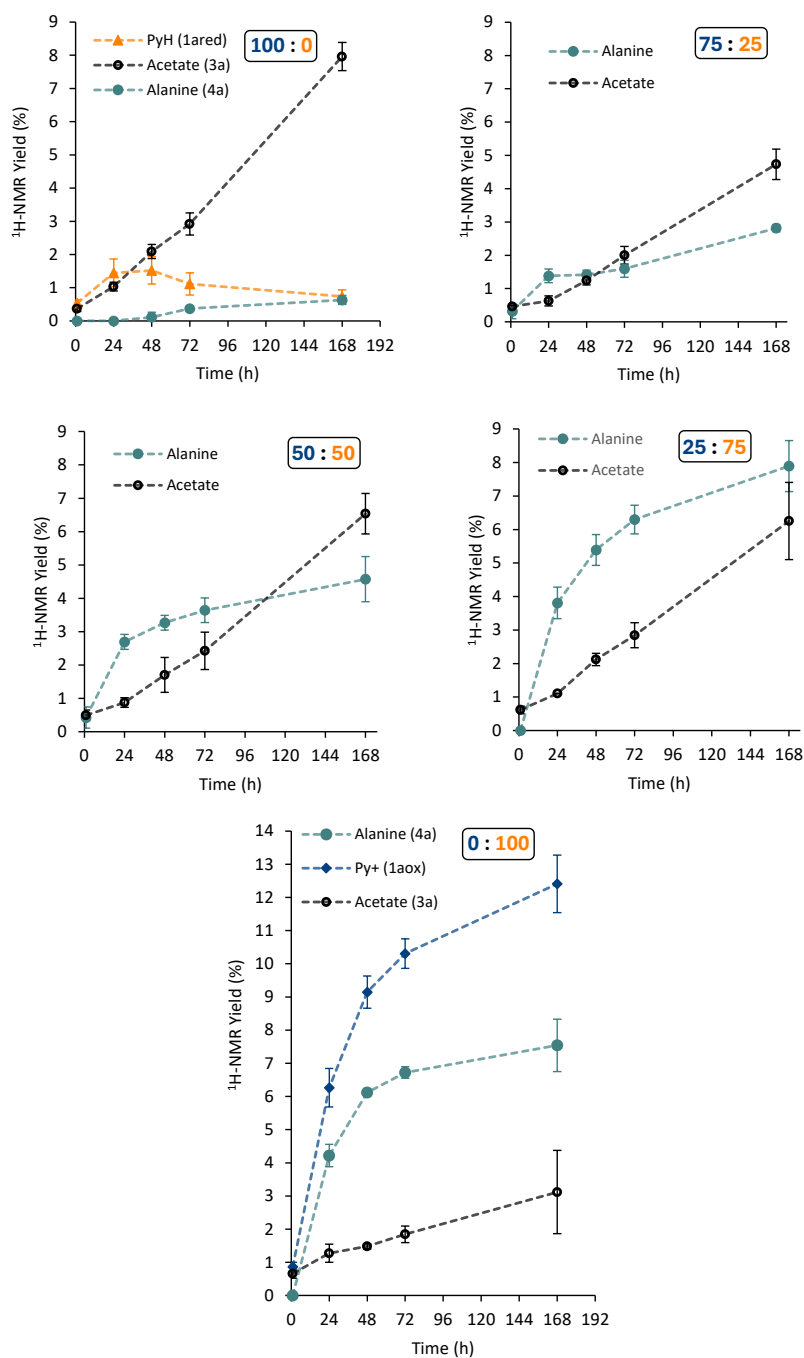


Figure S55. ^1H -NMR monitoring of acetate (**3a**) and alanine (**4a**) in the reaction of pyruvate (**1a**, 30 mM) with different ratios of pyridinium **1a_{ox}** : dihydropyridine **1a_{red}** (30 mM total concentration) in aqueous $\text{NH}_3/\text{NH}_4\text{Cl}$ solution (600 mM, pH = 8.5). Monitoring of **1a_{red}** and **1a_{ox}** have been shown in the cases where they were not initially present and were formed exclusively as products. All numerical data are presented in Table S41. Errors bars represent $\pm$ S.D. In the case of the initial ratio 100:0, formation of alanine has been confirmed by GC-MS, see section 11.

Control reactions

As was shown in Table S1, control experiment of pyruvate in these conditions (aq. $\text{NH}_3/\text{NH}_4\text{Cl}$, 600 mM, pH = 8.5) exhibited no detectable alanine (**4a**) background formation. Accordingly, the alanine (**4a**) detected in the reaction conducted at a $\mathbf{1a_{ox}}:\mathbf{1a_{red}}$ ratio of 100:0 can be attributed to the action of the *in situ* generated $\mathbf{1a_{red}}$.

Nevertheless, there is a low but appreciable background formation of acetate (**3a**) in the control reactions of pyruvate (**2a**) in $\text{NH}_3/\text{NH}_4\text{Cl}$ (600 mM) solutions, being slightly higher at pH 8.5 (Figure S56) than at pH 8.0 (see above, Figure S7). However, in both cases, a comparison with reactions of pyruvate (**2**) in the presence of $\mathbf{1a_{red}}$ leads to the conclusion that the acetate formation (**3a**) arises predominantly from the action of the *in situ* generated $\mathbf{1a_{ox}}$, with a minor contribution from background process.

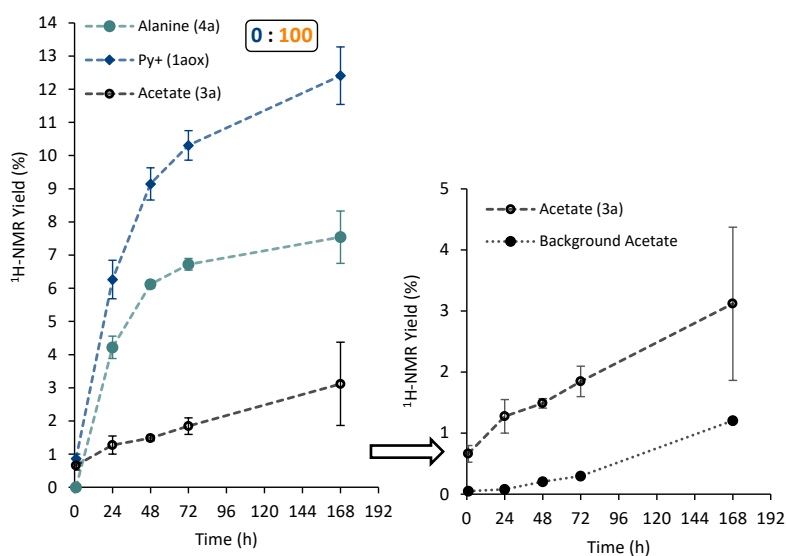


Figure S56. Reaction of $\mathbf{1a_{red}}$ (30 mM) and $\mathbf{2a}$ (30 mM) in aq. $\text{NH}_3/\text{NH}_4\text{Cl}$ (600 mM) at pH 8.5. **A)** Plots of ^1H -NMR monitoring of alanine (**4a**), pyridinium $\mathbf{1a_{ox}}$ and acetate (**3a**) formation (data from Table S41). **B)** Comparative of the acetate formed in previous reaction with the background acetate (**3a**) formation in the control reaction of pyruvate ($\mathbf{2a}$, 30 mM) in $\text{NH}_4\text{Cl}/\text{NH}_3$ (600 mM, pH = 8.5) (data from Table S1). Errors bars represent $\pm$ S.D.

10. Interaction of metal cations in the studied reactions

The activity of metal ions in the reactions promoted by the pyridinium/dihydropyridine system was studied.

The presence of CsCl, CaCl₂ or MgCl₂, either in catalytic amounts or stoichiometric amounts, in the reaction **1a_{red}** + **2a** under reductive amination conditions (NH₄Cl/NH₃, 600 mM, pH 8) allowed the reaction to proceed, with the formation of **1a_{ox}**, alanine (**4a**), and acetate (**3a**) being observed, although with slightly inhibited kinetics (Figure S57B) (for example, in the presence of 1 equivalent of MgCl₂ at 168 h, a 6.8 % of alanine is obtained against the 11.6 % in metal-free conditions).

We took MgCl₂ as a representative example to study oxidative decarboxylation conditions in both carbonate and ammonia media. We observed the next effects:

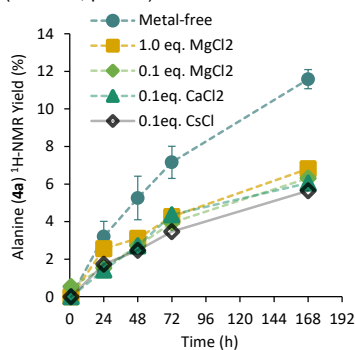
- The presence of 1 equivalent of MgCl₂ in NH₄Cl/NH₃ (600 mM) increments the background formation of acetate compared to metal-free conditions, with the effect being more pronounced at pH 8.5 than at pH 8.0. In contrast, this increase is not observed in carbonate buffer, where this background formation remains negligible (Figure S57A).
- The addition of 1 equiv. of MgCl₂, in both ammonia and carbonate media, catalyses the formation of the adduct **Py⁺-Pyruv** and, consequently, the **1a_{red}** amount specifically generated by this process (Figure S57C and Figure S57D).
- The presence of 1 equiv. of MgCl₂ in carbonate medium partially inhibits acetate formation by **1a_{ox}** (Figure S57C).
- In solution, the equilibrium between parapyruvate (**2a**) and its other forms (**2a.II**, **2a.III**, and **2a.IV**) is shifted further toward parapyruvate (**2a.III**) in the presence 1 equiv. of MgCl₂ (in both carbonate and ammonia media) compared to metal-free conditions (Figure S57A).

These experiments reveal that metal ions interact with the redox pair and substrates, influencing several aspects of the observed reactivity. While a comprehensive investigation of metal-cofactor interactions is beyond the scope of the present work, our preliminary findings demonstrate that the redox activity of **1a_{ox}/1a_{red}** is compatible with the presence of prebiotically plausible metal ions and provide a basis for identifying further beneficial metal-mediated effects.

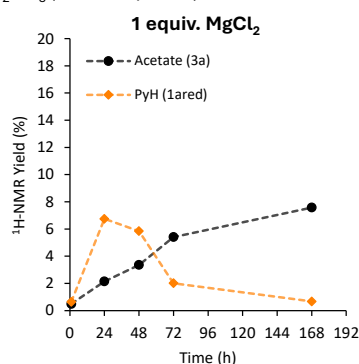
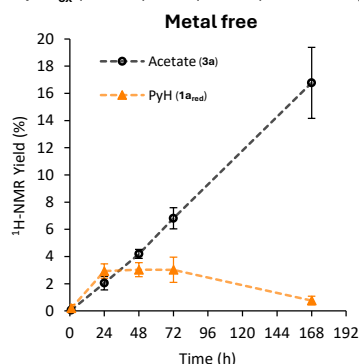
A) Control experiments of 2a (30 mM) in different conditions

Conditions	Time (h)	Pyruvate (2a)	Hydrate (2a.II)	Parapyruv. (2a.III)	(2a.IV)	Acetate (3a)	Acetate (3a) yield (%)
NaHCO ₃ /Na ₂ CO ₃ (200 mM, pH = 9.0) 1eq. MgCl ₂	1	11.6	0.9	43.6	0.00	0.1	0.13
	24	12.5	1.1	43.1	0.00	0.1	0.09
	48	13.3	1.2	42.7	0.00	0.1	0.06
	72	7.8	0.8	45.6	0.00	0.1	0.14
	168	54.5	4.7	20.4	0.00	0.0	0.00
NH ₄ Cl/NH ₃ (600 mM, pH = 8.0) 1eq. MgCl ₂	1	13.1	1.8	33.7	8.3	0.95	0.29
	24	44.1	4.7	19.4	6.1	0.08	0.08
	48	44.7	4.9	15.8	9.2	0.39	0.42
	72	17.9	2.4	12.7	25.8	2.73	1.28
	168	42.4	4.6	12.0	12.8	3.32	2.93
NH ₄ Cl/NH ₃ (600 mM, pH = 8.5) 1eq. MgCl ₂	1	22.1	2.5	30.2	6.9	1.26	0.56
	24	20.1	3.3	19.5	18.5	0.62	0.42
	48	15.7	7.6	20.7	17.0	1.35	0.79
	72	16.5	8.4	14.0	21.9	3.27	1.72
	168	37.1	5.9	11.4	10.9	12.34	6.72

B) 1a_{red} (30 mM) + 2a (30 mM) in NH₃/NH₄Cl (600 mM, pH 8.0)



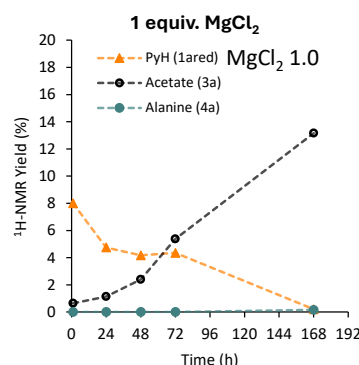
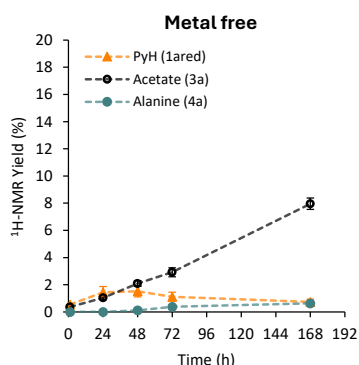
C) 1a_{ox} (30 mM) + 2a (30 mM) in NaHCO₃/Na₂CO₃ (200 mM, pH 9.0)



Py⁺-Pyruv formation in this conditions

Time (h)	Metal-free	1eq. MgCl ₂
	Py ⁺ -Pyruv	Py ⁺ -Pyruv
1	0.0 ±0.0	1.3
24	5.6 ±1.9	10.8
48	7.4 ±2.5	12.1
72	8.2 ±2.7	10.2
168	12.2 ±4.0	20.5

D) 1a_{ox} (30 mM) + 2a (30 mM) in NH₃/NH₄Cl (600 mM, pH 8.5)



Py⁺-Pyruv formation in this conditions

Time (h)	Metal-free	1eq. MgCl ₂
	Py ⁺ -Pyruv	Py ⁺ -Pyruv
1	0.7 ±0.2	7.8
24	2.5 ±0.8	6.5
48	3.7 ±1.2	7.9
72	4.0 ±1.3	10.2
168	7.0 ±2.3	11.1

Figure S57. Studies about the interaction of metal ions in the reactions promoted the pyridine/dihydropyridine system. See previous page for discussion.

11. GC-MS measurements

Gas chromatography – mass spectrometry was used as an additional qualitative technique to confirm the formation of some of the studied products. A sample of 700 μL was taken from the reaction at 168 hours and a protocol of derivatization was carried out, based on the literature:¹⁰

1. In an eppendorf, 50 mg of KOH were added to 700 μL of sample, and the mixture was centrifuged for 3 min at 6000 rpm.
2. To 600 μL of supernatant, 300 μL of ethanol, 40 μL of pyridine and 40 μL of ethyl chloroformate were added, and the mixture was vortexed for 30 s.
3. Another 40 μL of ethyl chloroformate were added, and the mixture was vortexed for 30 s.
4. 200 μL of chloroform were added, and the mixture was vortexed for 10 s.
5. 600 μL of sat. NaHCO_3 solution were added, and the mixture was vortexed for 10 s.
6. The organic phase was separated and dried over anhydrous Na_2SO_4 .
7. 50 μL of the dried organic phase and 150 μL of ethyl acetate were added to a GC-MS vial with insert.

The derivatized sample was analysed by GC-MS according to the method described in section 1. The most representative fragmentations of the derivatized analytes are shown in Figure S58.

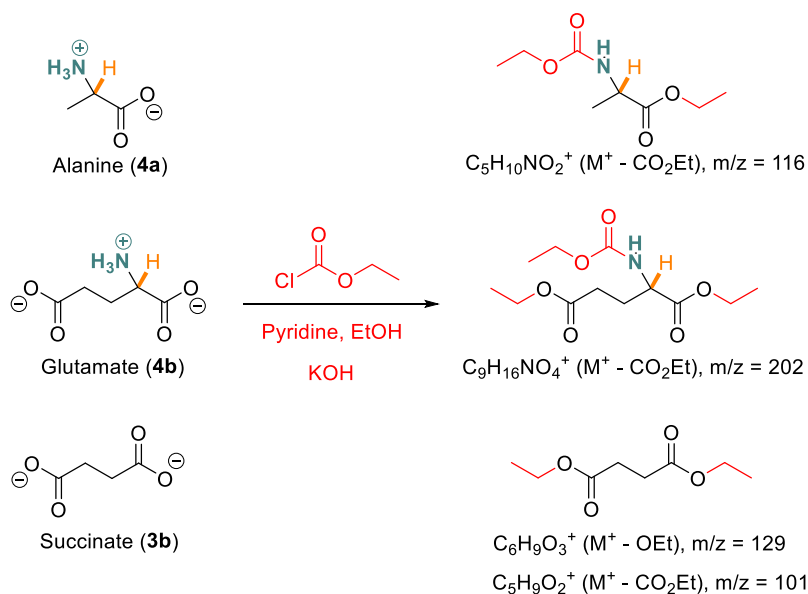
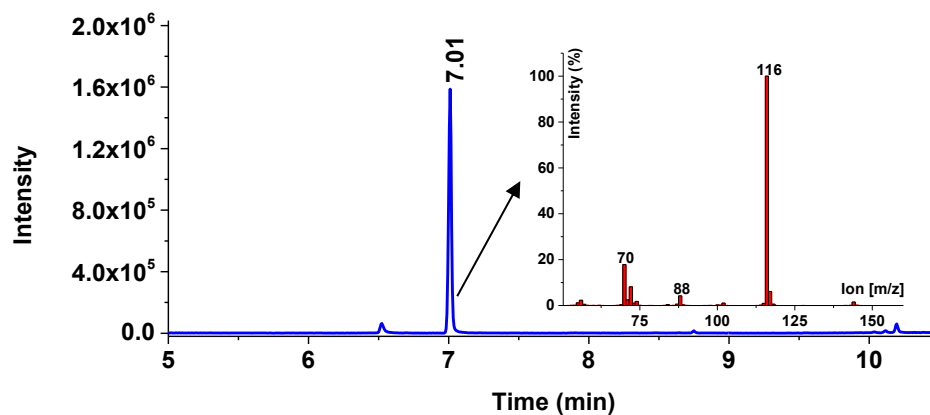
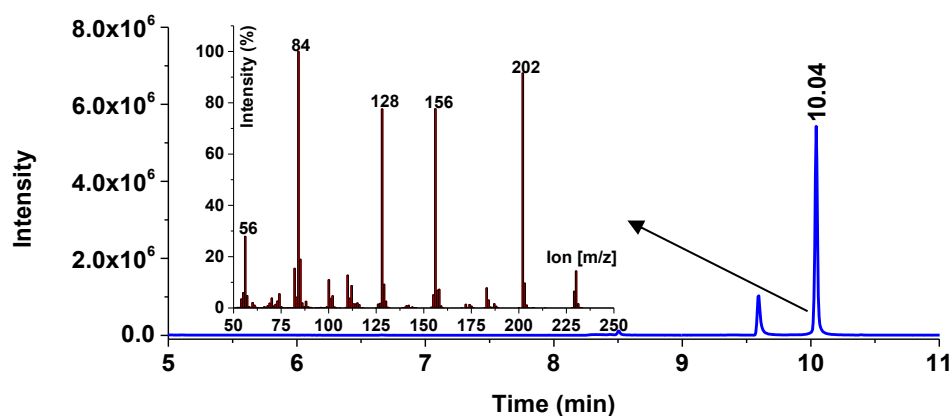


Figure S58. Chemical structure of the derivatized analytes and their most representative fragmentations in mass spectrometry.

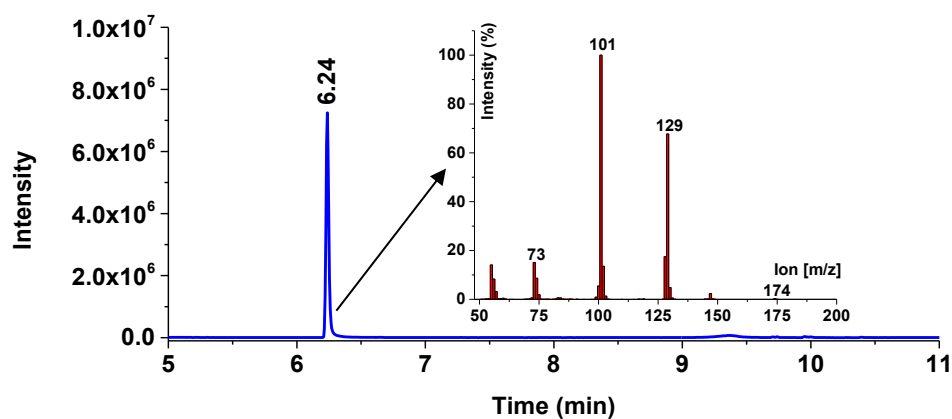
Gas chromatograms and mass spectra (insets) of derivatized commercial analytes



Commercial alanine (**4a**). Representative signal: m/z = 116 in peak 7.0 min.

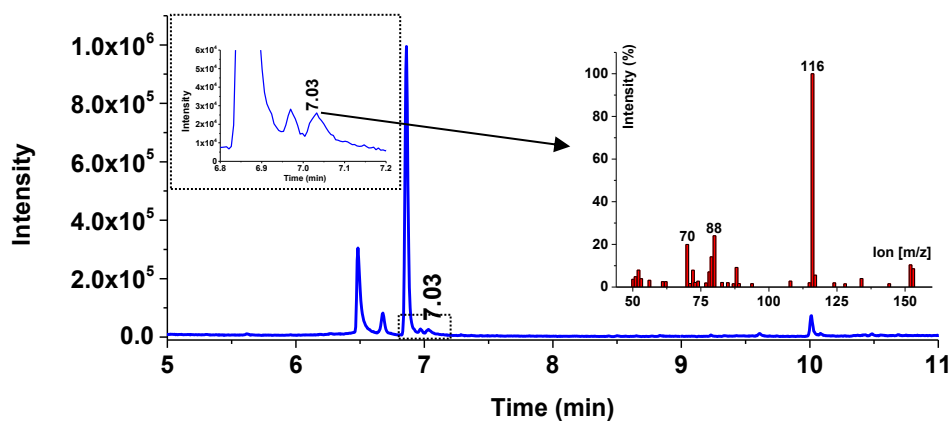


Commercial glutamate (**4b**). Representative signal: m/z = 202 in peak 10.0 min.

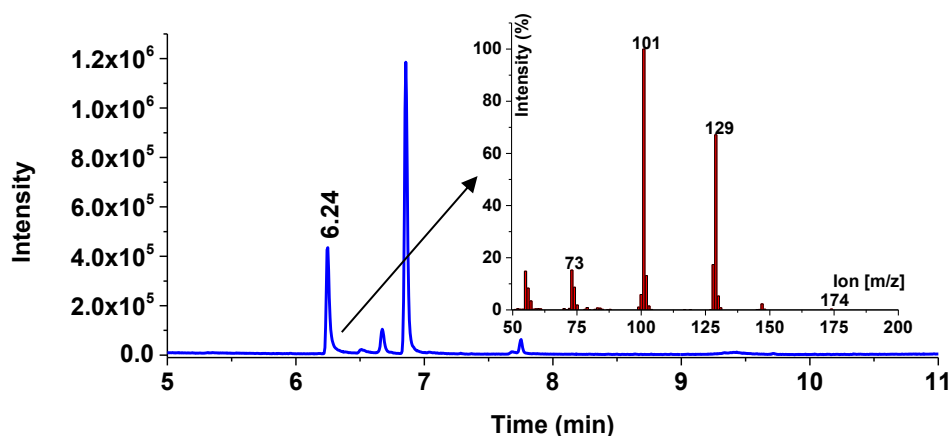


Commercial succinate (**3b**). Representative signal: m/z = 129, 101 in peak 6.2 min.

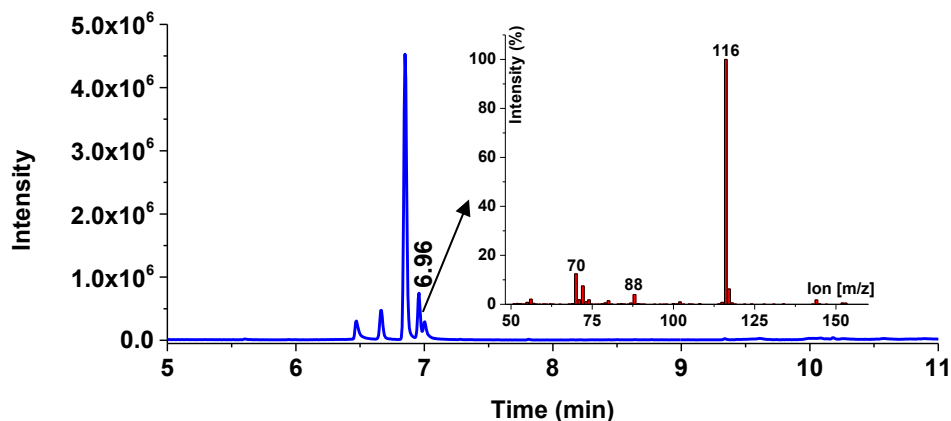
Gas chromatograms and mass spectra (insets) of derivatized reaction samples



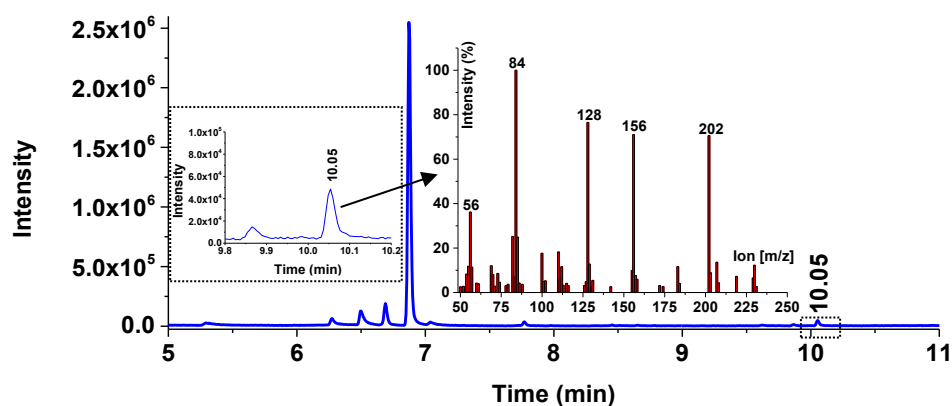
Reaction of **1a_{ox}** (30 mM) and pyruvate **2a** (30 mM) in $\text{NH}_3/\text{NH}_4\text{Cl}$ (600 mM, pH 8.5) at 168 h. Detection of alanine (**4a**) was confirmed: $m/z = 116$ in peak 7.0 min.



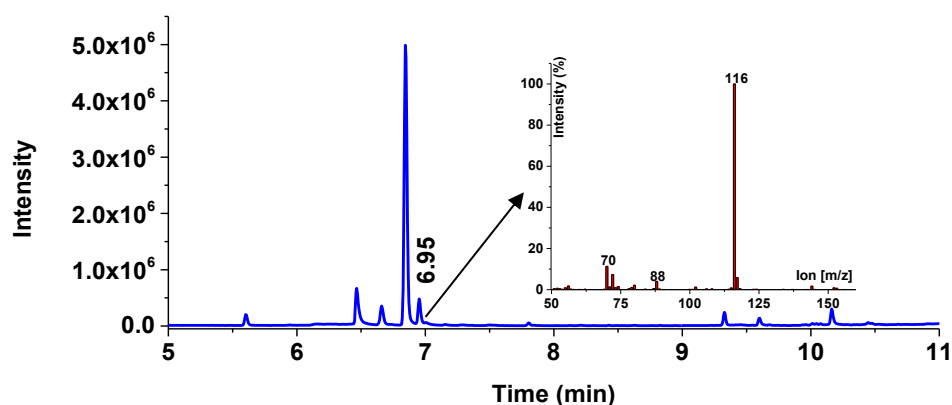
Reaction of **1a_{ox}** (30 mM) and α -ketoglutarate **2b** (30 mM) in $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ (200 mM, pH 9.0) at 168 h. Detection of succinate (**3b**) was confirmed: $m/z = 129, 101$ in peak 6.2 min.



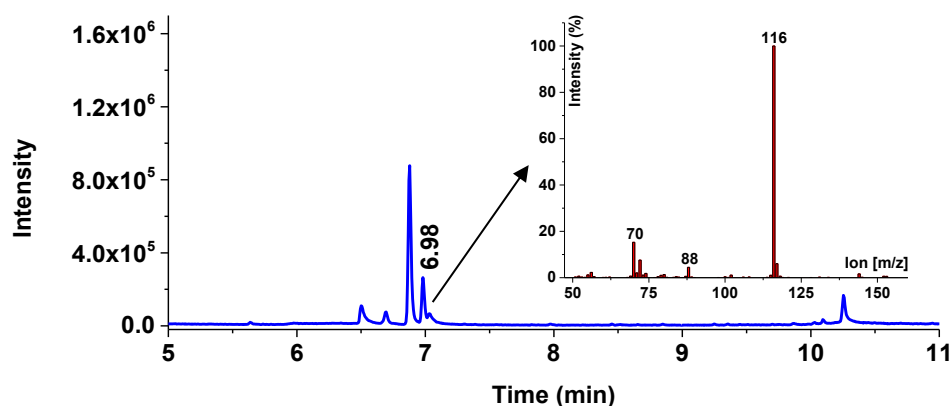
Reaction of **1a_{red}** (30 mM) and pyruvate **2a** (30 mM) in $\text{NH}_3/\text{NH}_4\text{Cl}$ (600 mM, pH 8.0) at 168 h. Detection of alanine (**4a**) was confirmed: $m/z = 116$ in peak 7.0 min.



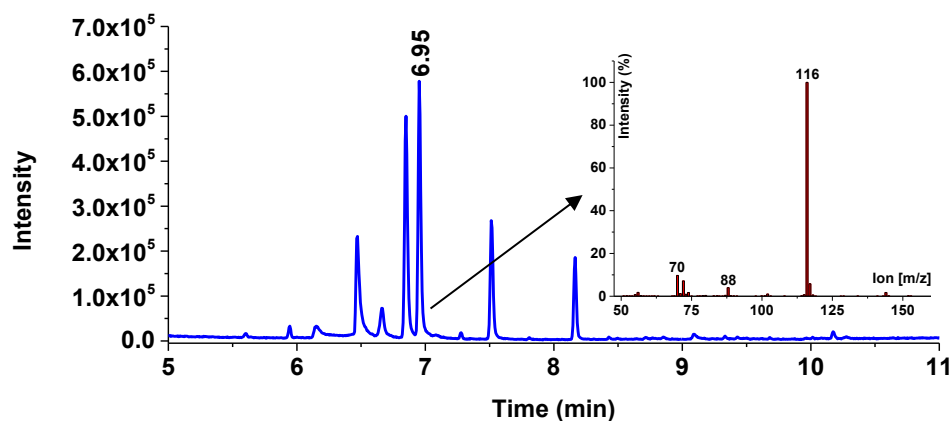
Reaction of **1a_{red}** (30 mM) and α -ketoglutarate **2b** (30 mM) in $\text{NH}_3/\text{NH}_4\text{Cl}$ (600 mM, pH 8.0) at 168 h. Detection of glutamate (**4b**) was confirmed: $m/z = 202$ in peak 10.1 min.



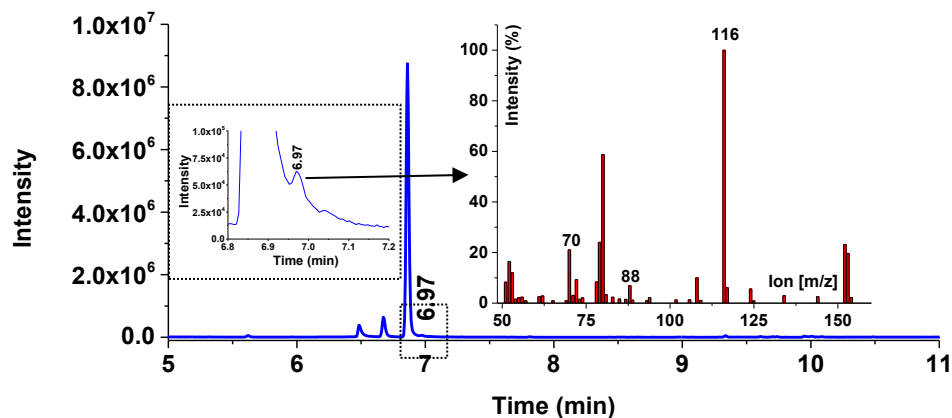
Reaction of **1b_{red}** (30 mM) and pyruvate **2a** (30 mM) in $\text{NH}_3/\text{NH}_4\text{Cl}$ (600 mM, pH 8.0) at 168 h. Detection of alanine (**4a**) was confirmed: $m/z = 116$ in peak 7.0 min.



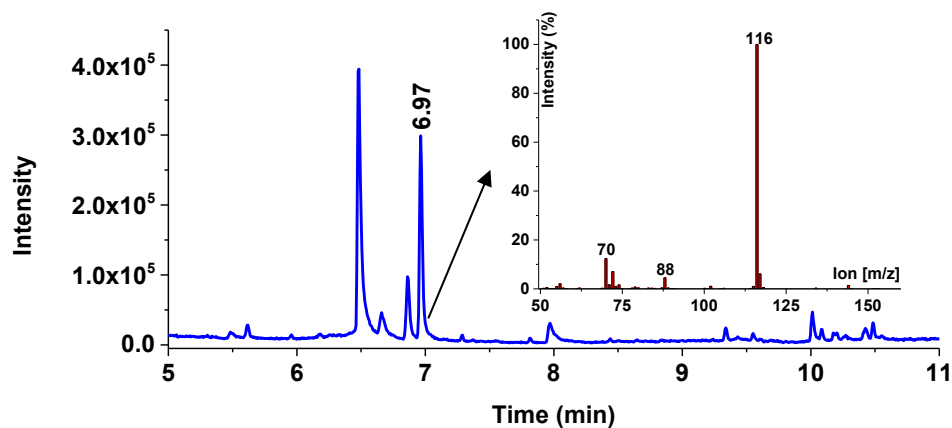
Reaction of **1c_{red}** (30 mM) and pyruvate **2a** (30 mM) in $\text{NH}_3/\text{NH}_4\text{Cl}$ (600 mM, pH 8.0) at 168 h. Detection of alanine (**4a**) was confirmed: $m/z = 116$ in peak 7.0 min.



Reaction of **1e_{red}** (30 mM) and pyruvate **2a** (30 mM) in NH₃/NH₄Cl (600 mM, pH 8.0) at 168 h. Detection of alanine (**4a**) was confirmed: m/z = 116 in peak 7.0 min.



Reaction of **1f_{red}** (30 mM) and pyruvate **2a** (30 mM) in NH₃/NH₄Cl (600 mM, pH 8.0) at 168 h. Detection of alanine (**4a**) was confirmed: m/z = 116 in peak 7.0 min.

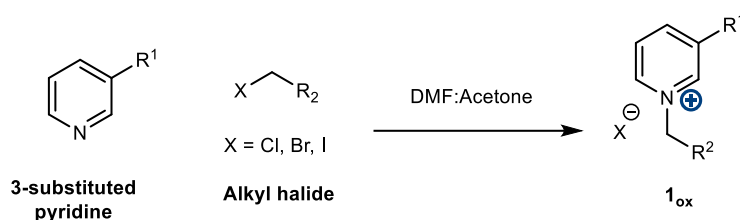


Reaction of **1g_{red}** (30 mM) and pyruvate **2a** (30 mM) in NH₃/NH₄Cl (600 mM, pH 8.0) at 168 h. Detection of alanine (**4a**) was confirmed: m/z = 116 in peak 7.0 min.

12. Synthesis and characterization of pyridiniums (**1_{ox}**) and dihydropyridines (**1_{red}**)

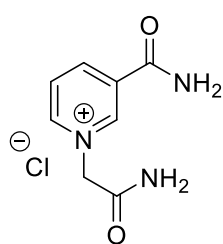
NMR characterization has been performed with DMSO-*d*₆ as solvent, using the residual peak of the DMSO-*d*₅ as reference (2.50 ppm in ¹H-NMR and 39.52 ppm in ¹³C-NMR). Signals of ¹H-NMR spectra in water have also been presented, since they have been used to identify and quantify these compounds in the reactions of oxidative decarboxylation and reductive amination. They were acquired in H₂O:D₂O 9:1 (in Na₂CO₃/NaHCO₂ 200 mM pH 9 for **1_{ox}** or in NH₃/NH₄Cl 600 mM pH 8 for **1_{red}**) with water suppression or in pure D₂O, in all cases referenced with the internal standard TMSP-*d*₄ (0.00 ppm). Abbreviations used: s (singlet), d (doublet), t (triplet), q (quartet), br (broad signal), app. (apparent), δ (chemical shift, given in ppm). For ¹³C-NMR, proton-decoupled spectra were recorded; for each spectrum, a DEPT135 experiment was also acquired to assign the multiplicity of each signal.

General procedure A for the synthesis of pyridinium salts (**1_{ox}**)



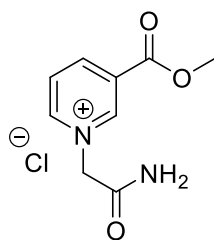
This general procedure is based on a previously reported protocol.¹¹ In a sealed tube equipped with a stirring bar, 30 mmol of the corresponding 3-substituted pyridine was dissolved in 15 mL of a mixture of acetone and DMF (see volumetric ratio in each case). If necessary, an ultrasound bath was applied for complete dissolution. Then, 30 mmol of the corresponding alkyl halide was added and the tube was sealed. The reaction was stirred at the indicated temperature for 24 hours. A precipitate appeared; it was filtered, washed with acetone and dried under vacuum.

1-(2-Amino-2-oxoethyl)-3-carbamoylpyridin-1-ium chloride (**1a_{ox}**)



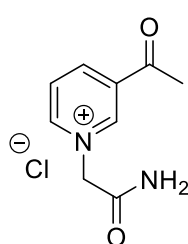
Following general procedure A using acetone:DMF (5:1) at 80 °C, the title compound was obtained as a white solid (4.41 g, 20.4 mmol, 68%). **¹H-NMR (500 MHz, DMSO)** δ 9.58 (br t, *J* = 1.5 Hz, 1H), 9.14 (dt, *J* = 6.2, 1.3 Hz, 1H), 9.11 (dt, *J* = 8.2, 1.5 Hz, 1H), 8.85 (s, 1H), 8.28 (dd, *J* = 8.2, 6.1 Hz, 1H), 8.26 (s, 1H), 8.16 (s, 1H), 7.73 (s, 1H), 5.53 (s, 2H). **¹H-NMR (300 MHz, H₂O+D₂O 9:1)** δ 9.30 (br t, *J* = 1.8 Hz, 1H), 9.05–8.96 (m, 2H), 8.28 (dd, *J* = 8.1, 6.2 Hz, 1H), 5.62 (s, 2H). **¹³C-NMR (126 MHz, DMSO)** δ 166.0 (C), 162.7 (C), 147.9 (CH), 146.4 (CH), 144.0 (CH), 133.1 (C), 127.1 (CH), 61.7 (CH₂). **HRMS (ESI)⁺**: Exact mass calculated for C₈H₁₀N₃O₂⁺ [*M* – Cl]⁺ 180.0768, found 180.0765.

1-(2-Amino-2-oxoethyl)-3-(methoxycarbonyl)pyridin-1-ium chloride (**1b_{ox}**)



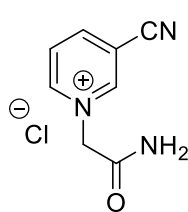
Following general procedure A using acetone:DMF (2:1) at 56 °C, the title compound was obtained as a brown solid (3.69 g, 15.98 mmol, 53%). **¹H-NMR (300 MHz, DMSO)** δ 9.61 (br t, *J* = 1.2 Hz, 1H), 9.31 (dt, *J* = 6.2, 1.3 Hz, 1H), 9.04 (dt, *J* = 8.2, 1.4 Hz, 1H), 8.39 (s, 1H), 8.34 (dd, *J* = 8.1, 6.1 Hz, 1H), 7.75 (s, 1H), 5.65 (s, 2H), 3.97 (s, 3H). **¹H-NMR (300 MHz, D₂O)** δ 9.47 (s, 1H), 9.16 (br dt, *J* = 8.2, 1.6 Hz, 1H), 9.05 (br dt, *J* = 5.7, 1.7 Hz, 1H), 8.30 (t, *J* = 7.1 Hz, 1H), 5.63 (s, 2H), 4.07 (s, 3H). **¹³C-NMR (75 MHz, DMSO)** δ 165.9 (C), 162.1 (C), 149.6 (CH), 147.2 (CH), 145.6 (CH), 129.1 (C), 127.7 (CH), 61.8 (CH₂), 53.5 (CH₃). **HRMS (ESI)⁺**: Exact mass calculated for C₉H₁₁N₂O₃⁺ [*M* - Cl]⁺ 195.0764, found 195.0758.

3-Acetyl-1-(2-amino-2-oxoethyl)pyridin-1-ium chloride (**1c_{ox}**)



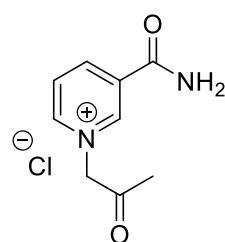
Following general procedure A using acetone:DMF (2:1) at 56 °C, the title compound was obtained as a white solid (2.12 g, 9.87 mmol, 33%). **¹H-NMR (500 MHz, DMSO)** δ 9.63 (br t, *J* = 1.6 Hz, 1H), 9.19 (dt, *J* = 6.0, 1.3 Hz, 1H), 9.06 (dt, *J* = 8.1, 1.5 Hz, 1H), 8.32 (dd, *J* = 8.1, 6.0 Hz, 1H), 8.27 (s, 1H), 7.74 (s, 1H), 5.58 (s, 2H), 2.73 (s, 3H). **¹H-NMR (300 MHz, H₂O+D₂O 9:1)** δ 9.40 (br t, *J* = 1.7 Hz, 1H), 9.14 (dt, *J* = 8.2, 1.5 Hz, 1H), 9.02 (dt, *J* = 6.1, 1.4 Hz, 1H), 8.30 (dd, *J* = 8.2, 6.1 Hz, 1H), 5.63 (s, 2H), 2.80 (s, 3H). **¹³C-NMR (126 MHz, DMSO)** δ 194.1 (C), 165.9 (C), 149.1 (CH), 146.7 (CH), 144.6 (CH), 134.8 (C), 127.4 (CH), 61.7 (CH₂), 27.2 (CH₃). **HRMS (ESI)⁺**: Exact mass calculated for C₉H₁₁N₂O₂⁺ [*M* - Cl]⁺ 179.0815, found 179.0811.

1-(2-Amino-2-oxoethyl)-3-cyanopyridin-1-ium chloride (**1d_{ox}**)



At the beginning of the procedure, NaI (0.450 g, 3 mmol, 10 mol%) was added as catalyst to the reaction tube. Following general procedure A using acetone for 42 hours at 56 °C, the solid compound was obtained. An additional wash of the solid with ethanol was performed to yield the titled compound as a white solid (3.28 g, 16.62 mmol, 55%). Alternatively, a yield of 2.4 % was obtained if only 1 mol% of NaI was added. **¹H-NMR (500 MHz, DMSO)** δ 9.86 (br t, *J* = 1.5 Hz, 1H), 9.34 (dt, *J* = 6.3, 1.3 Hz, 1H), 9.16 (dt, *J* = 8.2, 1.4 Hz, 1H), 8.41 (dd, *J* = 8.2, 6.3 Hz, 1H), 8.37 (s, 1H), 7.79 (s, 1H), 5.56 (s, 2H). **¹H-NMR (300 MHz, D₂O)** δ 9.48 (br t, *J* = 1.7 Hz, 1H), 9.15 (dt, *J* = 6.3, 1.4 Hz, 1H), 9.07 (dt, *J* = 8.2, 1.5 Hz, 1H), 8.36 (dd, *J* = 8.2, 6.3 Hz, 1H), 5.64 (s, 2H). **¹³C-NMR (126 MHz, DMSO)** δ 165.4 (C), 150.2 (CH), 150.0 (CH), 149.1 (CH), 127.8 (CH), 113.8 (C), 112.0 (C), 61.9 (CH₂). **HRMS (ESI)⁺**: Exact mass calculated for C₈H₈N₃O⁺ [*M* - Cl]⁺ 162.0662, found 162.0660.

3-Carbamoyl-1-(2-oxopropyl)pyridin-1-ium chloride (**1e_{ox}**)

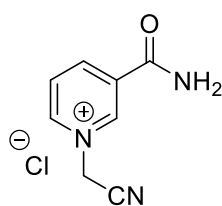


Following general procedure A using acetone:DMF (1:1) at 65 °C, the title compound was obtained as a white solid (3.84 g, 17.88 mmol, 60%). **¹H-NMR (300 MHz, DMSO)** δ 9.53 (br t, *J* = 1.3 Hz, 1H), 9.22 (dt, *J* = 8.3, 1.5 Hz, 1H), 9.13 (dt, *J* = 6.1, 1.3 Hz, 1H), 8.98 (s, 1H), 8.33 (dd, *J* = 8.1, 6.1 Hz, 1H), 8.19 (s, 1H), 5.96 (s, 2H), 2.32 (s, 3H). **¹H-NMR (500 MHz, H₂O+D₂O 9:1)** δ 9.15 (s, 1H), 9.01 (d, *J* = 8.4 Hz, 1H), 8.85 (d, *J* = 6.1 Hz, 1H), 8.27 (dd, *J* = 8.2, 6.2 Hz, 1H, app. t), 2.46 (s, 3H). **¹³C-NMR (75 MHz, DMSO)** δ 199.2 (C), 162.7 (C), 147.4 (CH), 146.3 (CH), 144.0 (CH), 133.3 (C),

*** One proton signal is missing due to overlap with the water suppression region.

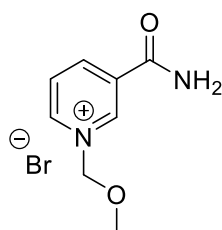
127.3(CH), 68.4(CH₂), 27.1(CH₃). **HRMS (ESI)⁺**: Exact mass calculated for C₉H₁₁N₂O₂⁺ [M – Cl]⁺ 179.0815, found 179.0812.

3-Carbamoyl-1-(cyanomethyl)pyridin-1-ium chloride (**1f_{ox}**)



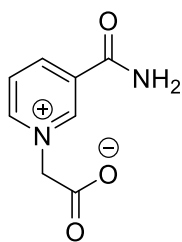
At the beginning of the procedure, NaI (0.3 mmol, 1%) was added as catalyst to the reaction tube. Following general procedure A using acetone:DMF (2:1) at 65 °C, the title compound was obtained as a pale yellow solid (2.88 g, 14.58 mmol, 49%). Alternatively, a yield of 31 % was obtained if NaI was not added. **¹H-NMR (300 MHz, DMSO)** δ 9.70 (br t, *J* = 1.7 Hz, 1H), 9.35 (dt, *J* = 6.2, 1.3 Hz, 1H), 9.14 (dt, *J* = 8.2, 1.4 Hz, 1H), 8.80 (s, 1H), 8.36 (dd, *J* = 8.1, 6.2 Hz, 1H), 8.21 (s, 1H), 6.08 (s, 2H). **¹H-NMR (300 MHz, D₂O)** δ 9.54 (td, *J* = 1.6, 0.7 Hz, 1H), 9.25 (ddd, *J* = 6.2, 1.6, 1.2 Hz, 1H, app. dt), 9.09 (ddd, *J* = 8.2, 1.8, 1.2 Hz, 1H), 8.35 (ddd, *J* = 8.2, 6.2, 0.7 Hz, 1H). **¹³C-NMR (126 MHz, DMSO)** δ 162.4(C), 146.9(CH), 146.0(CH), 145.2(CH), 133.9(C), 128.2(CH), 114.1(C), 47.8(CH₂). **HRMS (ESI)⁺**: Exact mass calculated for C₈H₈N₃O⁺ [M – Cl]⁺ 162.0662, found 162.0659.

3-Carbamoyl-1-(methoxymethyl)pyridin-1-ium bromide (**1g_{ox}**)



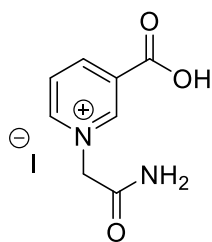
This compound was obtained following a different procedure: nicotinamide (1.83 g, 15 mmol, 1.43 equiv.) was dissolved in 54 mL of acetone. Ultrasound bath was applied for complete dissolution. Bromo(methoxy)methane (1.31 g, 10.5 mmol, 1.00 equiv.) was added dropwise for 3 minutes. The mixture was stirred at room temperature for 3 hours. A precipitate was formed, filtered, washed with acetone and dried under vacuum, obtaining the titled compound as a white solid (2.39 g, 9.68 mmol, 92%). **¹H-NMR (300 MHz, DMSO)** δ 9.52 (br t, *J* = 1.6 Hz, 1H), 9.25 (dt, *J* = 6.1, 1.3 Hz, 1H), 9.06 (dt, *J* = 8.2, 1.5 Hz, 1H), 8.64 (s, 1H), 8.35 (dd, *J* = 8.1, 6.1 Hz, 1H), 8.19 (s, 1H), 5.96 (s, 2H), 3.44 (s, 3H). **¹H-NMR (500 MHz, H₂O+D₂O 9:1)** δ 9.43 (t, *J* = 1.7 Hz, 1H, app. s), 9.15 (dt, *J* = 6.0, 1.5 Hz, 1H, app. d), 9.03 (br dt, *J* = 8.2, 1.5 Hz, 1H), 8.31 (dd, *J* = 8.2, 6.1 Hz, 1H), 5.98 (s, 2H), 3.55 (s, 3H). **¹³C-NMR (75 MHz, DMSO)** δ 162.7(C), 145.1(CH), 145.0(CH), 143.3(CH), 133.5(C), 127.9(CH), 90.0(CH₂), 58.0(CH₃). **HRMS (ESI)⁺**: Exact mass calculated for C₈H₁₁N₂O₂⁺ [M – Cl]⁺ 167.0815, found 167.0809.

2-(3-Carbamoylpyridin-1-ium-1-yl)acetate (**1h_{ox}**)



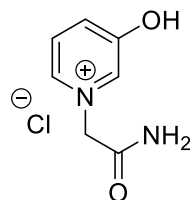
Starting from nicotinamide (30 mmol) and sodium 2-chloroacetate (30 mmol) and following general procedure A using 15 mL of MeOH (instead of acetone:DMF) at 65 °C, the title compound was obtained as a white solid (4.46 g, 18.6 mmol, 62%). Isolated as inner salt. Characterization was performed exclusively in water. **¹H-NMR (500 MHz, H₂O+D₂O 9:1)** δ 9.27 (s, 1H), 8.99 – 8.92 (m, 2H), 8.23 (dd, *J* = 8.1, 6.2 Hz, 1H, app d), 5.32 (s, 2H). **¹³C-NMR (126 MHz, D₂O)** δ 170.2(C), 165.9(C), 147.5(CH), 145.2(CH), 144.1(CH), 133.6(C), 128.0(CH), 63.7(CH₂). **HRMS (ESI)⁺**: Exact mass calculated for C₈H₉N₂O₃⁺ [M + H]⁺ 181.0608, found 181.0604.

1-(2-Amino-2-oxoethyl)-3-carboxypyridin-1-ium iodide (**1i_{ox}**)



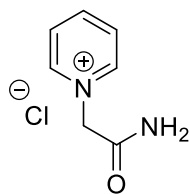
Starting from nicotinic acid (10 mmol) and 2-iodoacetamide (10 mmol) and following general procedure A using DMF at 70 °C, the solid compound was obtained. Two additional washes of the solid with boiling methanol and with boiling dichloromethane were performed to yield the titled compound as a yellow solid (377.7 mg, 1.2 mmol, 12%). **¹H-NMR (300 MHz, DMSO)** δ 9.37 (br t, *J* = 1.4 Hz, 1H), 9.01 (dt, *J* = 6.1, 1.4 Hz, 1H), 8.93 (dt, *J* = 8.0, 1.4 Hz, 1H), 8.18 (dd, *J* = 8.0, 6.1 Hz, 1H), 8.04 (s, 1H), 7.69 (s, 1H), 5.48 (s, 2H). **¹H-NMR (500 MHz, H₂O+D₂O 9:1)** δ 9.16 (d, *J* = 1.8 Hz, 1H, app. s), 8.97 (br dt, *J* = 8.2, 1.5 Hz, 1H), 8.87 (dd, *J* = 6.1, 1.6 Hz, 1H, app. d), 8.18 (dd, *J* = 8.1, 6.1 Hz, 1H), 5.59 (s, 2H). **¹³C-NMR (126 MHz, DMSO)** δ 166.0(C), 163.1(C), 148.7(CH), 147.3(CH), 145.7(CH), 131.6(C), 127.5(CH), 61.7(CH₂). **HRMS (ESI)⁺**: Exact mass calculated for C₈H₉N₂O₃⁺ [M – I]⁺ 181.0608, found 181.0605. (Although it is isolated as a protonated carboxylic acid, when dissolved in bicarbonate solution and the pH is adjusted to 9.0, the equilibrium shifts towards the carboxylate form, as shown in Figure 1B of the main text).

1-(2-Amino-2-oxoethyl)-3-hydroxypyridin-1-ium chloride (**1j_{ox}**)



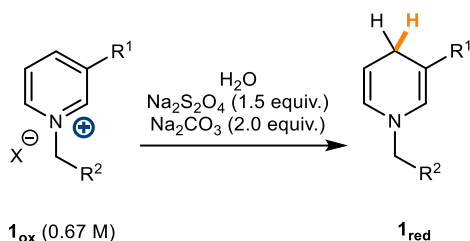
Following general procedure A using acetone at 56 °C, the solid compound was obtained. An additional wash of the solid with ethanol was performed to yield the titled compound as a white solid (2.85 g, 15.11 mmol, 50%). **¹H-NMR (500 MHz, DMSO)** δ 12.47 (br s, 1H), 8.51 (br dd, *J* = 2.5, 1.4 Hz, 1H), 8.41 (br dt, *J* = 5.9, 1.2 Hz, 1H), 8.11 (s, 1H), 8.07 (br ddd, *J* = 8.8, 2.5, 1.0 Hz, 1H), 7.91 (dd, *J* = 8.7, 5.8 Hz, 1H), 7.64 (s, 1H), 5.33 (s, 2H). **¹H-NMR (500 MHz, H₂O+D₂O 9:1)** δ 7.74 (dt, *J* = 5.7, 1.3 Hz, 1H, app. d), 7.71 (t, *J* = 2.2 Hz, 1H), 7.60 (dd, *J* = 8.9, 5.7 Hz, 1H), 7.52 (br ddd, *J* = 8.9, 2.7, 1.0 Hz, 1H), 5.22 (s, 2H). **¹³C-NMR (126 MHz, DMSO)** δ 166.1(C), 157.3(C), 136.4(CH), 134.5(CH), 131.7(CH), 127.7(CH), 61.5(CH₂). **HRMS (ESI)⁺**: Exact mass calculated for C₈H₉N₂O₃⁺ [M – Cl]⁺ 153.0659, found 153.0656.

1-(2-Amino-2-oxoethyl)pyridin-1-ium chloride (**1k_{ox}**)



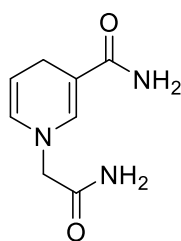
Following general procedure A using acetone at 56 °C, the title compound was obtained as a white solid (2.52 g, 14.60 mmol, 49%). **¹H-NMR (300 MHz, DMSO)** δ 9.05 – 8.97 (m, 2H), 8.65 (tt, *J* = 7.8, 1.4 Hz, 1H), 8.24 – 8.14 (m, 3H), 7.69 (s, 1H), 5.46 (s, 2H). **¹H-NMR (500 MHz, H₂O+D₂O 9:1)** δ 8.83 (d, *J* = 6.1 Hz, 2H), 8.67 (t, *J* = 7.8 Hz, 1H), 8.15 (t, *J* = 7.1 Hz, 2H), 5.54 (s, 2H). **¹³C-NMR (126 MHz, DMSO)** δ 166.1(C), 146.3(2 x CH), 145.9(CH), 127.4(2 x CH), 61.5(CH₂). **HRMS (ESI)⁺**: Exact mass calculated for C₇H₉N₂O⁺ [M – Cl]⁺ 137.0709, found 137.0704.

General procedure B for the synthesis of dihydropyridines (**1_{red}**)



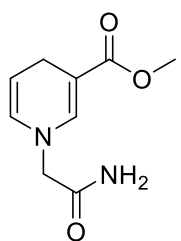
This general procedure is based on a previously reported protocol.¹¹ The corresponding pyridinium salt (1.0 equivalent) is dissolved in water (0.67 M) in a two necked flask equipped with a stirring bar. The solution is bubbled with nitrogen. A mixture of solid $\text{Na}_2\text{S}_2\text{O}_4$ (1.5 equivalents) and Na_2CO_3 (2.0 equivalents) was added in portions under a nitrogen current. The reaction was sealed with a septum and the mixture was stirred for 20 minutes. See purification for each compound. With the exception of **1a_{red}**, which was quite stable under atmospheric conditions, all dihydropyridines **1_{red}** were stored under nitrogen in the freezer (-20 °C).

1-(2-Amino-2-oxoethyl)-1,4-dihydropyridine-3-carboxamide (**1a_{red}**)



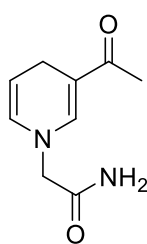
General procedure B was applied starting from 1-(2-amino-2-oxoethyl)-3-carbamoylpyridin-1-ium chloride (2.16 g, 10 mmol). After 20 minutes of reaction, a precipitate appeared; it was filtered and washed with cold water. It was recrystallized from water, obtaining the titled compound as a yellow solid (1.70 g, 9.39 mmol, 94%). **¹H-NMR (500 MHz, DMSO)** δ 7.31 (s, 1H), 7.09 (s, 1H), 6.79 (d, J = 1.7 Hz, 1H), 6.51 (s, 2H), 5.76 (dq, J = 8.0, 1.6 Hz, 1H), 4.56 (dt, J = 8.0, 3.4 Hz, 1H), 3.68 (s, 2H), 2.95 (dd, J = 3.1, 1.4 Hz, 2H). **¹H-NMR (300 MHz, H₂O+D₂O 9:1)** δ 6.97 (d, J = 1.7 Hz, 1H, app. s), 5.85 (br dt, J = 8.0, 1.8 Hz, 1H), 3.98 (s, 2H), 3.08 (dd, J = 3.7, 1.8 Hz, 2H). **¹³C-NMR (126 MHz, DMSO)** δ 170.5 (C), 169.1 (C), 138.5 (CH), 130.1 (CH), 101.1 (CH), 100.1 (C), 54.8 (CH₂), 22.2 (CH₂). **HRMS (APCI)⁺**: Exact mass calculated for $\text{C}_8\text{H}_{13}\text{N}_3\text{O}_2^+$ [$M + H$]⁺ 182.0924, found 182.0925.

Methyl 1-(2-amino-2-oxoethyl)-1,4-dihydropyridine-3-carboxylate (**1b_{red}**)



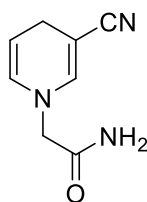
General procedure B was applied starting from 1-(2-amino-2-oxoethyl)-3-(methoxycarbonyl)pyridin-1-ium chloride (3.00 g, 13 mmol). After 20 minutes of reaction, a precipitate appeared; it was filtered and washed with cold water and dried under vacuum, obtaining the titled compound as an orange solid (2.17 g, 11.04 mmol, 85%). **¹H-NMR (300 MHz, DMSO)** δ 7.39 (s, 1H), 7.10 (s, 1H), 7.01 (d, J = 1.6 Hz, 1H), 5.77 (dq, J = 8.1, 1.7 Hz, 1H), 4.68 (dt, J = 8.1, 3.4 Hz, 1H), 3.78 (s, 2H), 3.56 (s, 3H), 2.94 (dd, J = 3.5, 1.7 Hz, 2H). **¹H-NMR (300 MHz, H₂O+D₂O 9:1)** δ 7.16 (br d, J = 1.8 Hz, 1H), 5.82 (dq, J = 8.1, 1.7 Hz, 1H), 3.98 (s, 2H), 3.70 (s, 3H), 3.03 (br dd, J = 3.7, 1.8 Hz, 2H). **¹³C-NMR (75 MHz, DMSO)** δ 170.2 (C), 167.6 (C), 142.9 (CH), 129.7 (CH), 103.0 (CH), 95.5 (C), 54.7 (CH₂), 50.5 (CH₃), 21.7 (CH₂). **HRMS (ESI)⁺**: Exact mass calculated for $\text{C}_9\text{H}_{13}\text{N}_2\text{O}_3^+$ [$M + H$]⁺ 197.0921, found 197.0919.

2-(3-Acetylpyridin-1(4*H*)-yl)acetamide (**1c_{red}**)



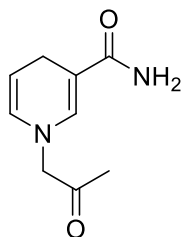
General procedure B was applied starting from 3-acetyl-1-(2-amino-2-oxoethyl)pyridin-1-ium chloride (1.72 g, 8 mmol). After 20 minutes of reaction, CH₂Cl₂ was added (20 mL per mmol of reactant) and the biphasic mixture was vigorously stirred under nitrogen for 24 hours. The organic phase was separated from the aqueous phase, then dried over anhydrous Na₂SO₄ and filtered. The solvent was reduced under vacuum until 10% of the initial volume and finally dried bubbling nitrogen, obtaining the titled compound as a yellow solid (390.2 mg, 2.16 mmol, 27%). **¹H-NMR (300 MHz, DMSO)** δ 7.39 (s, 1H), 7.23 (d, *J* = 1.5 Hz, 1H), 7.14 (s, 1H), 5.81 (dq, *J* = 7.7, 1.8 Hz, 1H), 4.77 (dt, *J* = 7.5, 3.4 Hz, 1H), 3.85 (s, 2H), 2.86 (br dd, *J* = 3.6, 1.8 Hz, 2H), 2.06 (s, 3H). **¹H-NMR (300 MHz, H₂O+D₂O 9:1)** δ 7.40 (d, *J* = 1.6 Hz, 1H), 5.88 (dq, *J* = 8.1, 1.8 Hz, 1H), 5.13 (dt, *J* = 7.6, 3.6 Hz, 1H), 4.10 (s, 2H), 2.98 (br dd, *J* = 3.9, 1.9 Hz, 2H), 2.22 (s, 3H). **¹³C-NMR (75 MHz, DMSO)** δ 193.8 (C), 170.1 (C), 145.2 (CH), 129.3 (CH), 107.8 (C), 104.6 (CH), 54.9 (CH₂), 23.9 (CH₃), 21.1 (CH₂). **HRMS (ESI)⁺**: Exact mass calculated for C₉H₁₃N₂O₂⁺ [*M* + *H*]⁺ 181.0972, found 181.0969.

2-(3-Cyanopyridin-1(4*H*)-yl)acetamide (**1d_{red}**)



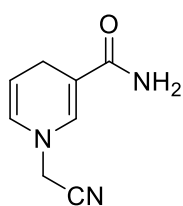
General procedure B was applied starting from 1-(2-amino-2-oxoethyl)-3-cyanopyridin-1-ium chloride (700 mg, 3.54 mmol). After 20 minutes of reaction, CH₂Cl₂ was added (20 mL per mmol of reactant) and the biphasic mixture was vigorously stirred under nitrogen for 24 hours. The organic phase was separated from the aqueous phase, then dried over anhydrous Na₂SO₄ and filtered. The solvent was reduced under vacuum until 10% of the initial volume and finally dried bubbling nitrogen, obtaining the titled compound as a red solid (56.6 mg, 0.35 mmol, 10%). **¹H-NMR (300 MHz, DMSO)** δ 7.37 (s, 1H), 7.12 (s, 1H), 6.80 (d, *J* = 1.5 Hz, 1H), 5.76 (dq, *J* = 8.2, 1.6 Hz, 1H), 4.57 (dt, *J* = 8.1, 3.4 Hz, 1H), 3.74 (s, 2H), 2.97 (dd, *J* = 3.3, 1.5 Hz, 2H). **¹H-NMR (300 MHz, H₂O+D₂O 9:1)** δ 6.76 (d, *J* = 1.6 Hz, 1H, app. s), 5.79 (dq, *J* = 8.2, 1.8 Hz, 1H), 3.94 (s, 2H), 3.05 (br dd, *J* = 3.5, 1.7 Hz, 2H). **¹³C-NMR (75 MHz, DMSO)** δ 170.0 (C), 145.5 (CH), 129.8 (CH), 121.7 (C), 100.4 (CH), 75.3 (C), 54.4 (CH₂), 22.4 (CH₂). **HRMS (APCI)⁺**: Exact mass calculated for C₈H₁₀N₃O⁺ [*M* + *H*]⁺ 164.0818, found 164.0815.

1-(2-Oxopropyl)-1,4-dihydropyridine-3-carboxamide (**1e_{red}**)



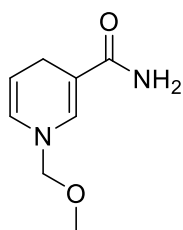
General procedure B was applied starting from 3-carbamoyl-1-(2-oxopropyl)pyridin-1-ium chloride (2.15 g, 10 mmol). After 20 minutes of reaction, CH₂Cl₂ was added (20 mL per mmol of reactant) and the biphasic mixture was vigorously stirred under nitrogen for 24 hours. The organic phase was separated from the aqueous phase, then dried over anhydrous Na₂SO₄ and filtered. The solvent was reduced under vacuum until 10% of the initial volume and finally dried bubbling nitrogen, obtaining the titled compound as an orange solid (679.6 mg, 3.7 mmol, 37%). **¹H-NMR (300 MHz, DMSO)** δ 6.74 (d, *J* = 1.5 Hz, 1H), 6.52 (s, 2H), 5.72 (dq, *J* = 8.0, 1.6 Hz, 1H), 4.57 (dt, *J* = 8.0, 3.4 Hz, 1H), 4.06 (s, 2H), 2.96 (dd, *J* = 3.5, 1.7 Hz, 2H), 2.04 (s, 3H). **¹H-NMR (300 MHz, D₂O)** δ 6.86 (d, *J* = 1.6 Hz, 1H), 5.75 (dq, *J* = 8.1, 1.8 Hz, 1H), 4.90 (dt, *J* = 7.9, 3.5 Hz, 1H), 4.23 (s, 2H), 3.08 (dd, *J* = 3.6, 1.8 Hz, 2H), 2.20 (s, 3H). **¹³C-NMR (75 MHz, DMSO)** δ 205.2 (C), 169.1 (C), 138.5 (CH), 130.1 (CH), 101.0 (CH), 100.1 (C), 61.3 (CH₂), 26.6 (CH₃), 22.2 (CH₂). **HRMS (APCI)⁺**: Exact mass calculated for C₉H₁₃N₂O₂⁺ [*M* + *H*]⁺ 181.0972, found 181.0974.

1-(Cyanomethyl)-1,4-dihydropyridine-3-carboxamide (**1f_{red}**)



General procedure B was applied starting from 3-carbamoyl-1-(cyanomethyl)pyridin-1-ium chloride (197.6 mg, 10 mmol). After 20 minutes of reaction, CH₂Cl₂ was added (20 mL per mmol of reactant) and the biphasic mixture was vigorously stirred under nitrogen for 24 hours. The organic phase was separated from the aqueous phase, then dried over anhydrous Na₂SO₄ and filtered. The solvent was reduced under vacuum until 10% of the initial volume and finally dried bubbling nitrogen, obtaining the titled compound as a yellow solid (464 mg, 2.84 mmol, 28%). **¹H-NMR (500 MHz, DMSO)** δ 6.91 (d, *J* = 1.7 Hz, 1H), 6.73 (s, 2H), 5.93 (dq, *J* = 8.0, 1.7 Hz, 1H), 4.78 (dt, *J* = 8.0, 3.5 Hz, 1H), 4.39 (s, 2H), 2.92 (br dd, *J* = 4.1, 1.5 Hz, 2H). **¹H-NMR (300 MHz, H₂O+D₂O 9:1)** δ 7.01 (d, *J* = 1.6 Hz, 1H), 5.96 (dq, *J* = 8.0, 1.8 Hz, 1H), 5.05 (dt, *J* = 8.0, 3.5 Hz, 1H), 4.35 (s, 2H), 3.05 (dd, *J* = 3.6, 1.8 Hz, 2H). **¹³C-NMR (126 MHz, DMSO)** δ 168.4(C), 136.4(CH), 128.5(CH), 117.4(C), 104.3(C), 103.9(CH), 40.7(CH₂), 21.8(CH₂). **HRMS (APCI)⁺**: Exact mass calculated for C₈H₁₀N₃O⁺ [*M* + *H*]⁺ 164.0818, found 164.0822.

1-(Methoxymethyl)-1,4-dihydropyridine-3-carboxamide (**1g_{red}**)



General procedure B was applied starting from 3-carbamoyl-1-(methoxymethyl)pyridin-1-ium bromide (1000 mg, 4.05 mmol). After 20 minutes of reaction, CH₂Cl₂ was added (20 mL per mmol of reactant) and the biphasic mixture was vigorously stirred under nitrogen for 24 hours. The organic phase was separated from the aqueous phase, then dried over anhydrous Na₂SO₄ and filtered. The solvent was reduced under vacuum until 10% of the initial volume and finally dried bubbling nitrogen, obtaining the titled compound as a yellow solid (210 mg, 1.24 mmol, 31%). **¹H-NMR (500 MHz, DMSO)** δ 6.99 (dt, *J* = 1.7, 0.8 Hz, 1H), 6.65 (s, 2H), 6.00 (dq, *J* = 8.0, 1.6 Hz, 1H), 4.66 (dt, *J* = 8.0, 3.4 Hz, 1H), 4.42 (s, 2H), 3.19 (s, 3H), 2.95 (ddd, *J* = 3.5, 1.7, 0.8 Hz, 2H). **¹H-NMR (300 MHz, H₂O+D₂O 9:1)** δ 7.12 (br d, *J* = 1.7 Hz, 1H), 6.05 (dq, *J* = 8.1, 1.7 Hz, 1H), 4.97 (dt, *J* = 8.1, 3.5 Hz, 1H), 4.57 (s, 2H), 3.35 (s, 3H), 3.07 (dd, *J* = 3.6, 1.8 Hz, 2H). **¹³C-NMR (126 MHz, DMSO)** δ 168.9(C), 137.0(CH), 129.0(CH), 102.0(CH), 101.8(C), 83.7(CH₂), 54.7(CH₃), 22.2(CH₂). **HRMS (APCI)⁺**: Exact mass calculated for C₈H₁₀N₃O⁺ [*M* + *H*]⁺ 169.0972, found 169.0973.

13. Electrochemical measurements

Cyclic voltammetry (CV) and RDE measurements

Prior to each measurement a glassy carbon electrode (diameter = 0.3 cm, area = 0.0707 cm²) was polished using 1 μm and 0.3 μm alumina pastes with subsequent rinsed with distilled water in an ultrasonic bath. The glassy carbon electrode was activated in 0.1M H₂SO₄ solution that had been previously deoxygenated by bubbling N₂ for 15 minutes. The activation involved performing 20 cycles CV at a scan rate of 100 mV/s from -1.3 V to 1.0 V in a three-neck electrochemical cell, using Ag/AgCl/KCl (sat) as the reference electrode and platinum as the counter electrode. Then, CV was performed for 2 cycles of the phosphate buffer (Na₂HPO₄/NaH₂PO₄, 100 mM, pH = 7) after deoxygenation with N₂ for 15 minutes, from -1.3 V to 1.0 V at a sweep speed of 100 mV/s. All measurements with the different compounds under study were carried out in an oxygen free solution, which was purged for 15 min with N₂ before each measurement. A N₂ atmosphere above the solution was maintained during the measurements to ensure the absence of oxygen.

Rotatory Disk Electrode (RDE) measurements and kinetic analysis were employed to study the electrochemical properties of the compounds studied. The steady-state voltammograms of oxidation-reduction obtained for **NAD⁺/NADH**, **1x_{ox}/1x_{red}** (x=a-g) on Glassy Carbon (GC) electrodes, performed at 5 mV/s and different rotation speeds, are shown in Figure S63-Figure S70. In all cases, the anodic and cathodic limiting current density (*j_L*) increases with increased rotation speed (*ω*). The linearity of *j_L* vs *ω*^{1/2} relationship characteristic of a diffusion-controlled electrochemical process was observed. Considering the Levich equation for irreversible processes the diffusion coefficient, *D* (cm²/s) was calculated (Eq. S2):

$$j_L = 0.620nFD\frac{2}{3}\nu^{-\frac{1}{6}}C\omega^{\frac{1}{2}} \quad (\text{S2})$$

Where *j_L* is the limiting current density (A/cm²), *n* is the number of electrons transferred (1 for the reduction of Py⁺ and 2 for the oxidation of PyH), *F* is the Faraday constant (96485.3321 C/mol), *ν* is the kinematic viscosity (0.01 cm²/s), *C* is the bulk concentration of the electroactive species (1·10⁶ mol/cm²) and *ω* is the angular rotation rate (rad/s).

The kinetic analysis was evaluated considering the mass transport elimination, to isolate the kinetic current density, *j_K*, from the total current density according to (Eq. S3):

$$\frac{j_L \cdot j}{j_L - j} = j_K \quad (\text{S3})$$

Where *j_L* is the limiting current density, *j* is the total current density measured and *j_K* is the kinetic current density. The resulting *j_K* data were then fitted to the Butler-Volmer equation (Eq. S4) to obtain the standard rate constant, *k₀* (see Figure S63-Figure S70):

$$\log j_K = \log(nFCk_0) + \frac{\alpha nF}{2.303RT} \eta \quad (\text{S4})$$

Where *j_K* is the kinetic current density, *n* is the number of electrons transferred, *F* is the faraday constant, *C* is the concentration, *k₀* is the standard rate constant (cm/s), *α* is the charge transfer coefficient, *R* is the gas constant (8.3145 J/Kmol), *T* is the temperature (298.15 K) and *η* is the overpotential (for the calculation of *k₀*, *η*=*E*-*E_{eq}* and *E_{eq}* was considered *E_{onset}* in which the current value is ±5·10⁻⁶ A).

The diffusion coefficient D and the k_0 values are included in Table S42.

Chemical description of the CV peaks

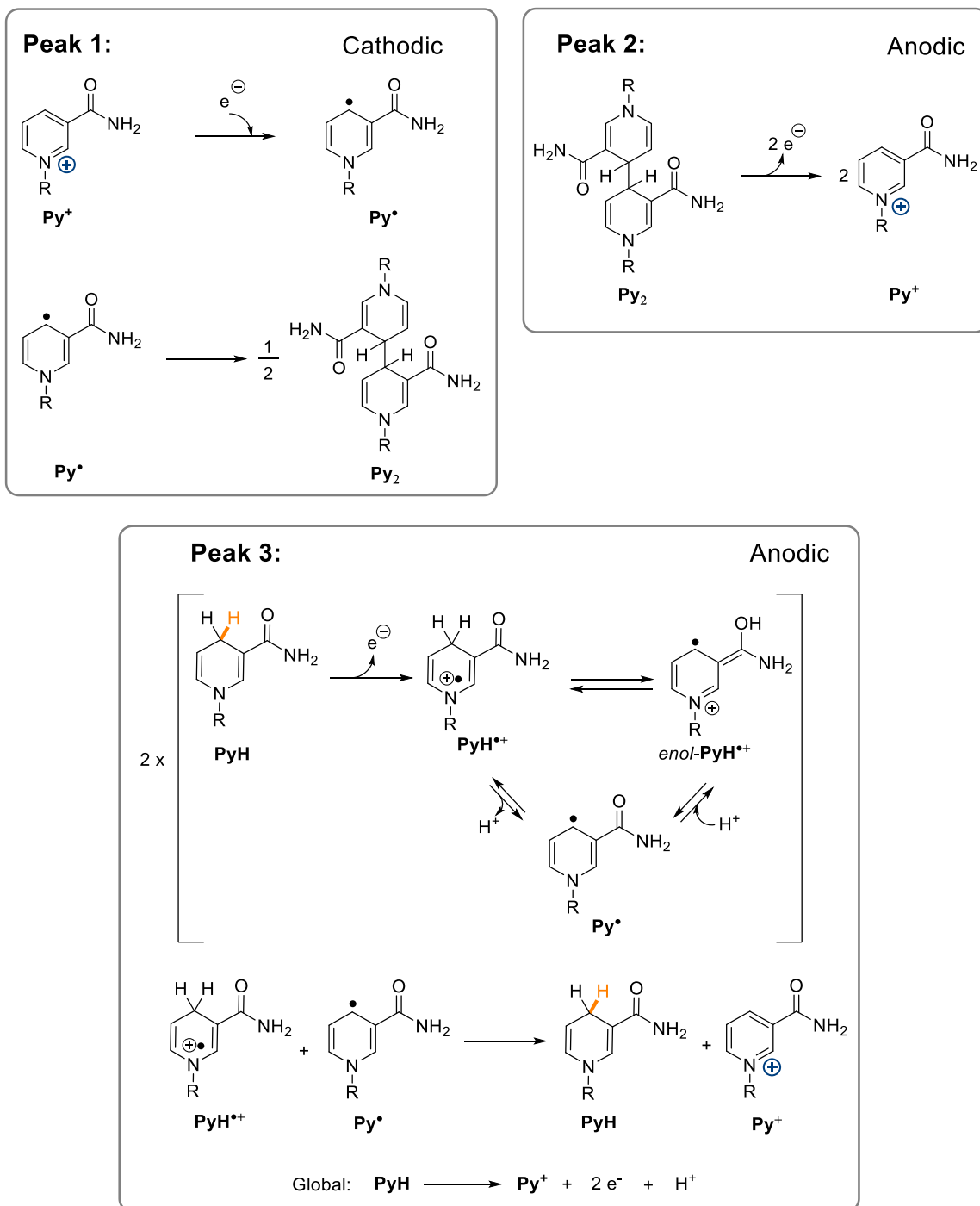


Figure S59. Chemical description of the different peaks seen in the CVs. Assignations are made based on the literature¹² and confirmed with the experiments performed in Figure S60.

Peak relationships in cyclic voltammetries

We proceeded to analyse the capacity of $\mathbf{1a_{ox}}$ and $\mathbf{1a_{red}}$ for regeneration after experiencing the redox processes described in the main text, which would facilitate their performance as a prebiotic cofactor. $\mathbf{1a_{ox}}$ is easily regenerated, as the Py_2 dimer oxidation peak (p2) and the Py^+ reduction peak (p1) remained constant over eight cycles (Figure S60a). A gradual decrease in the current density of p1 was observed with the successive scans when the anodic limit of CV was cut at -0.4 V (Figure S60b), indicating that the dimer oxidation is needed for Py^+ regeneration. The voltammograms of $\mathbf{1a_{red}}$ also showed the three peaks over eight cycles (Figure S60c). The gradual decrease in current density of p3 with the successive eight scans indicates that PyH is not electrochemical regenerated. When the anodic limit was cut at 0.20 V, *i.e.*, before the increase of p3, the reduction peak (p1) did not appear, confirming that PyH oxidation is the source of Py^+ (Figure S60d). Furthermore, cutting the cathodic scan off at -0.4V results in the elimination of p2 (Figure S60e), which is further evidence that Py_2 can only form upon Py^+ reduction.

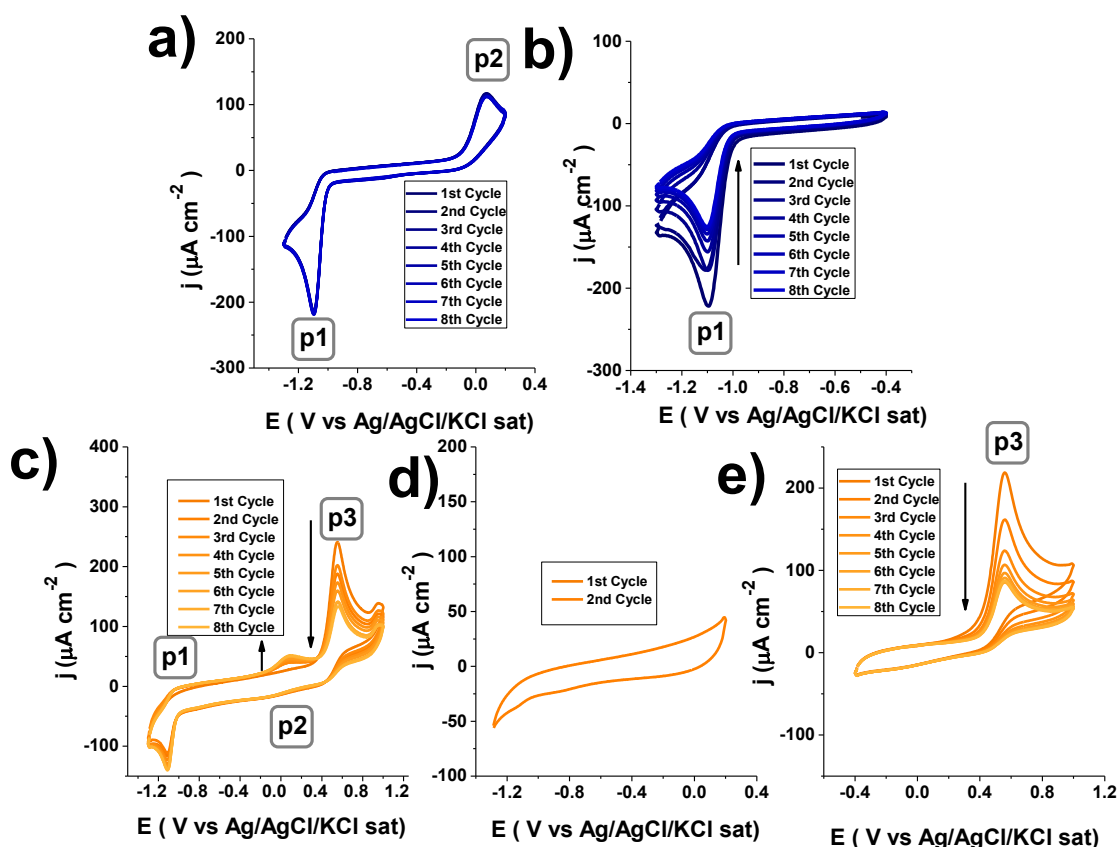


Figure S60. Electrochemical measurements of regenerative capacity of $\mathbf{1a_{ox}}$ and $\mathbf{1a_{red}}$. **a)** Cyclic voltammogram of compound $\mathbf{1a_{ox}}$ (1 mM). **b)** Cyclic voltammogram of $\mathbf{1a_{ox}}$ (1 mM) with cut-off at -0.4V. **c)** Cyclic voltammogram of $\mathbf{1a_{red}}$ (1 mM). **d)** Cyclic voltammogram of $\mathbf{1a_{red}}$ (1mM) with cut-off at 0.2V. **e)** Cyclic voltammogram of $\mathbf{1a_{red}}$ (1 mM) with cut-off at -0.4V. In all the cases, CVs were performed at 100 mV/s in phosphate buffer (pH 7.0).

Electrochemical regeneration of PyH with pyruvate in cyclic voltammeteries

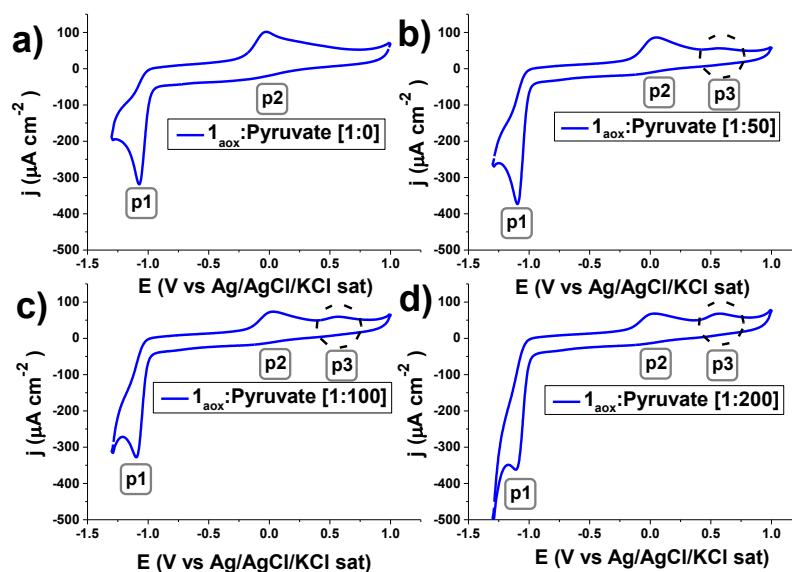


Figure S61. Cyclic voltammogram of a mixture of **1a_{ox}** (1mM) and pyruvate at 100 mV/s in phosphate buffer (pH 7.0) with **1a_{ox}**:pyruvate molar ratios of: **a)** [1:0]. **b)** [1:50]. **c)** [1:100]. **d)** [1:200].

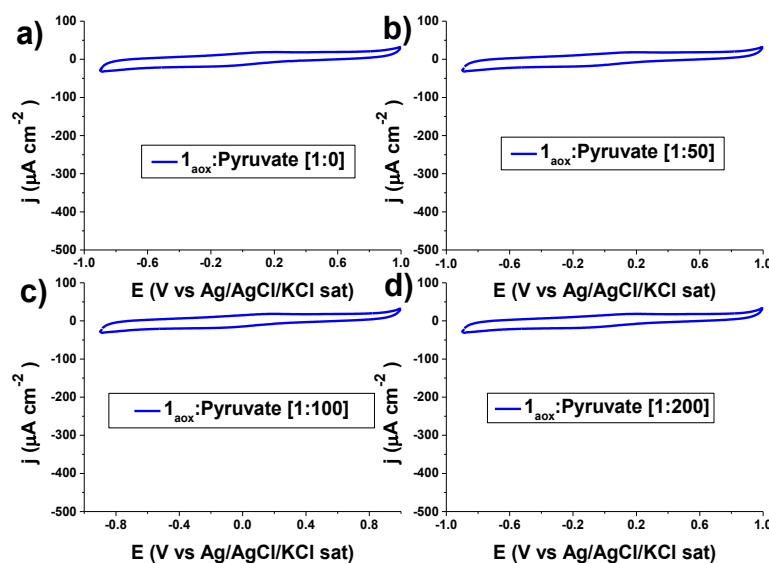


Figure S62. Cyclic voltammogram of a mixture of **1a_{ox}** (1mM) and pyruvate at 100 mV/s with cut-off at -0.9 V in phosphate buffer (pH 7.0) with **1a_{ox}**:pyruvate molar ratios of: **a)** [1:0]. **b)** [1:50]. **c)** [1:100]. **d)** [1:200]. Peak p3, which indicates the presence of PyH, is not observed. This blank experiment illustrates the need for the formation of Py[•] in the peak p1 to interact with the pyruvate and regenerate the PyH.

Since both experiments are performed at pH 7, the generation of PyH by non-electrochemical oxidative carboxylation does not occur.

Rotating Disk Electrode (RDE) measurements

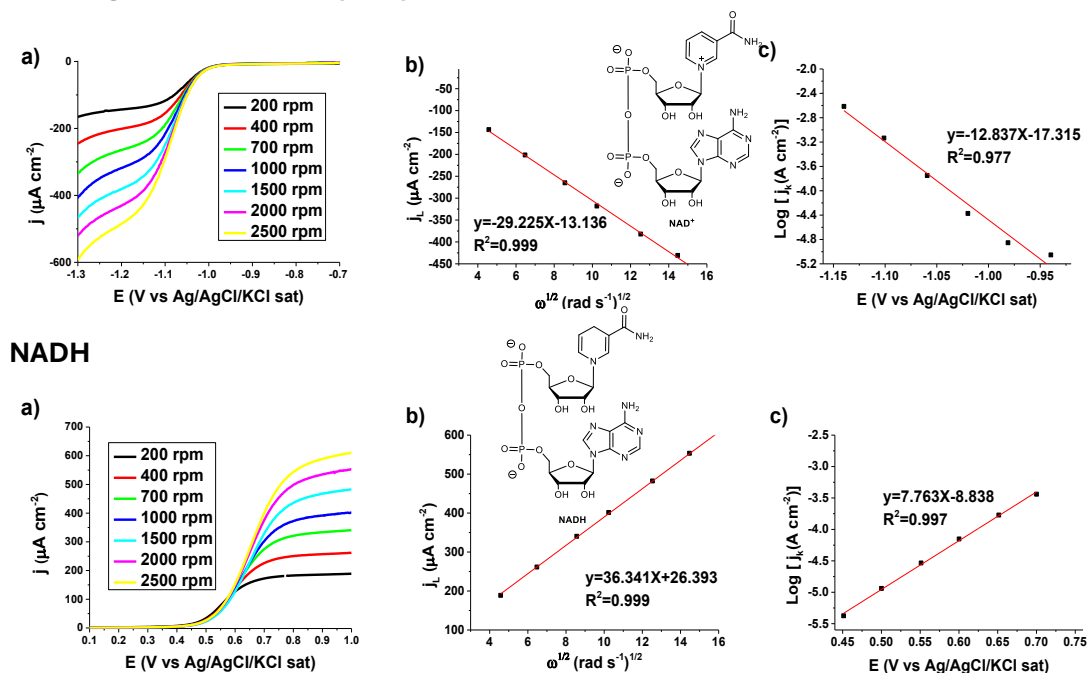


Figure S63. RDE analysis of **NAD⁺** and **NADH** in 1mM and phosphate buffer (pH= 7): (a) Linear Sweep Voltammetry (LSV) at 5 mV·s⁻¹ with different rotating speeds; (b) Levich analysis and (c) Tafel-plot for 1500 rpm without the mass transport of the reduction ($E_{\text{onset}} = -0.89$ V) or oxidation ($E_{\text{onset}} = 0.47$ V) processes.

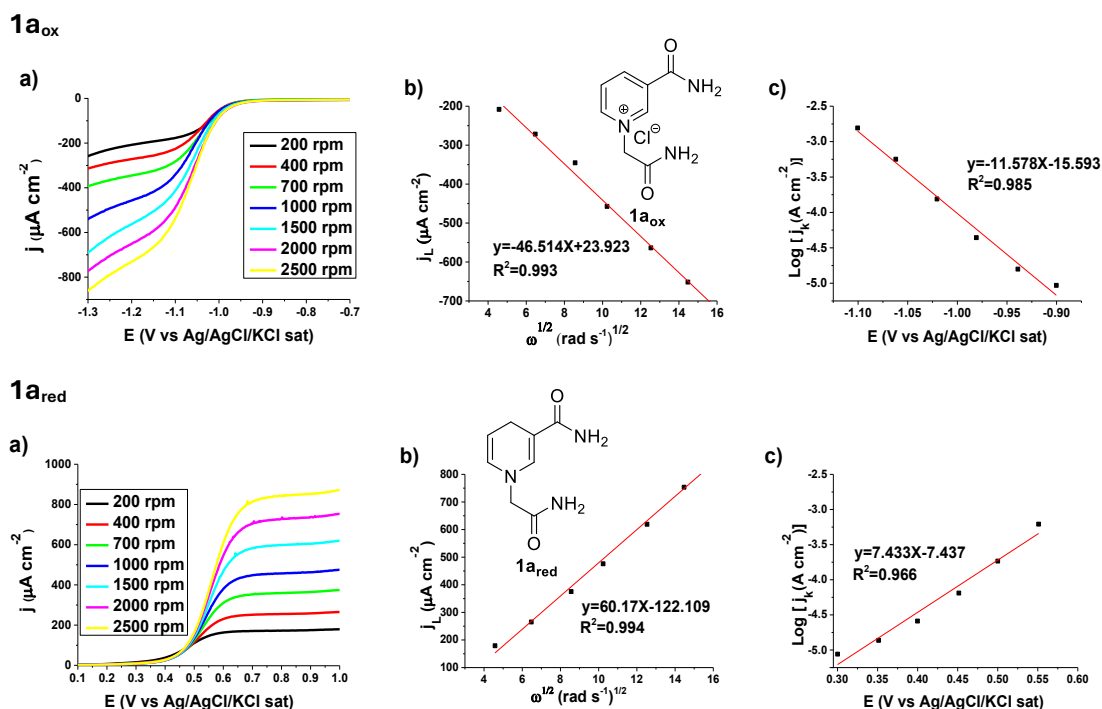


Figure S64. RDE analysis of **1a_{ox}** and **1a_{red}** in 1mM and phosphate buffer (pH= 7): (a) Linear Sweep Voltammetry (LSV) at 5 mV·s⁻¹ with different rotating speeds; (b) Levich analysis and (c) Tafel-plot for 1500 rpm without the mass transport of the reduction ($E_{\text{onset}} = -0.88$ V) or oxidation ($E_{\text{onset}} = 0.28$ V) processes.

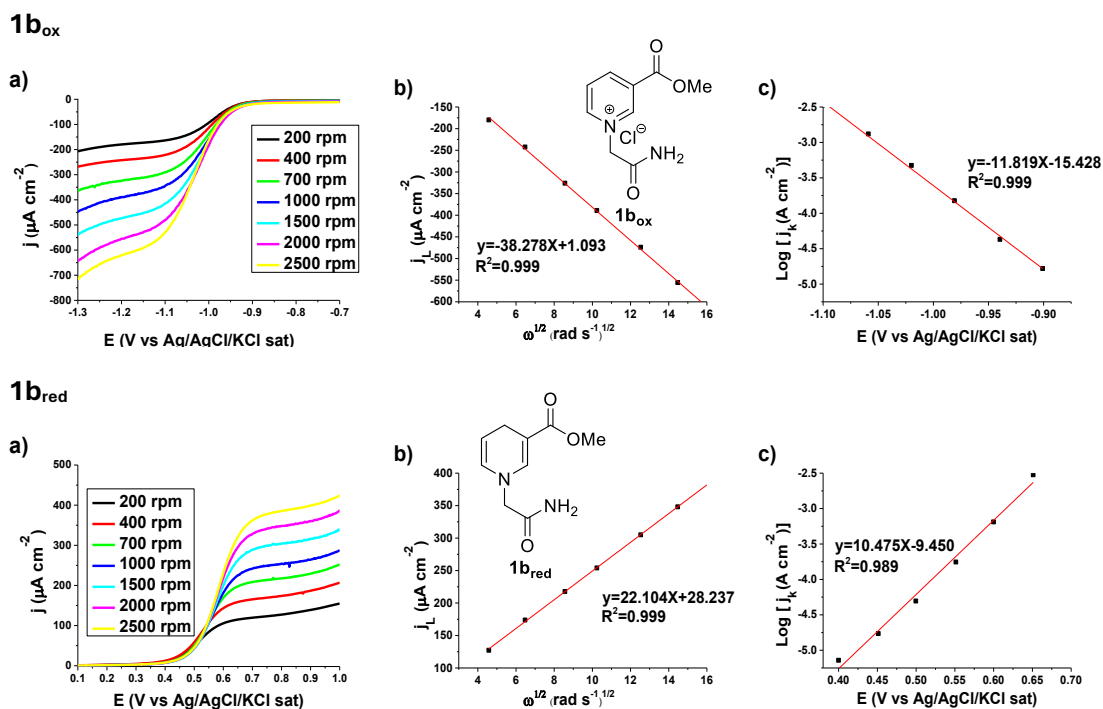


Figure S65. RDE analysis of **1b_{ox}** and **1b_{red}** in 1mM and phosphate buffer (pH= 7): (a) Linear Sweep Voltammetry (LSV) at 5 mV·s⁻¹ with different rotating speeds; (b) Levich analysis and (c) Tafel-plot for 1500 rpm without the mass transport of the reduction ($E_{\text{onset}} = -0.72$ V) or oxidation ($E_{\text{onset}} = 0.39$ V) processes.

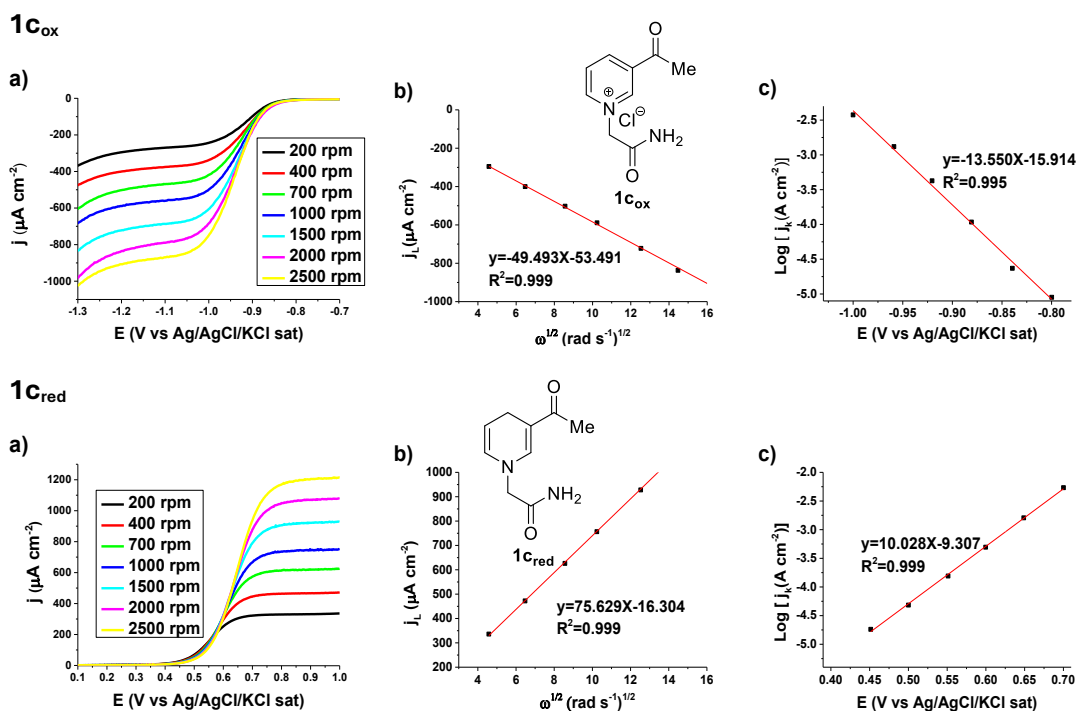


Figure S66. RDE analysis of **1c_{ox}** and **1c_{red}** in 1mM and phosphate buffer (pH= 7): (a) Linear Sweep Voltammetry (LSV) at 5 mV·s⁻¹ with different rotating speeds; (b) Levich analysis and (c) Tafel-plot for 1500 rpm without the mass transport of the reduction ($E_{\text{onset}} = -0.79$ V) or oxidation ($E_{\text{onset}} = 0.38$ V) processes.

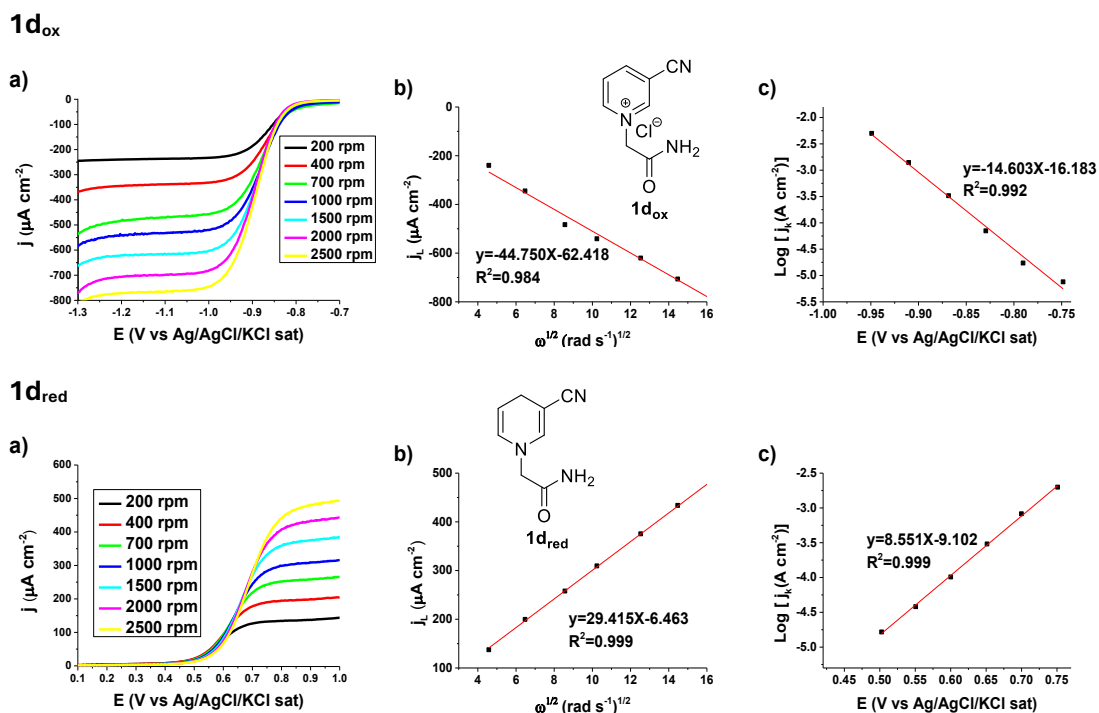


Figure S67. RDE analysis of **1d_{ox}** and **1d_{red}** in 1mM and phosphate buffer (pH= 7): (a) Linear Sweep Voltammetry (LSV) at 5 mV·s⁻¹ with different rotating speeds; (b) Levich analysis and (c) Tafel-plot for 1500 rpm without the mass transport of the reduction ($E_{\text{onset}} = -0.70$ V) or oxidation ($E_{\text{onset}} = 0.43$ V) processes.

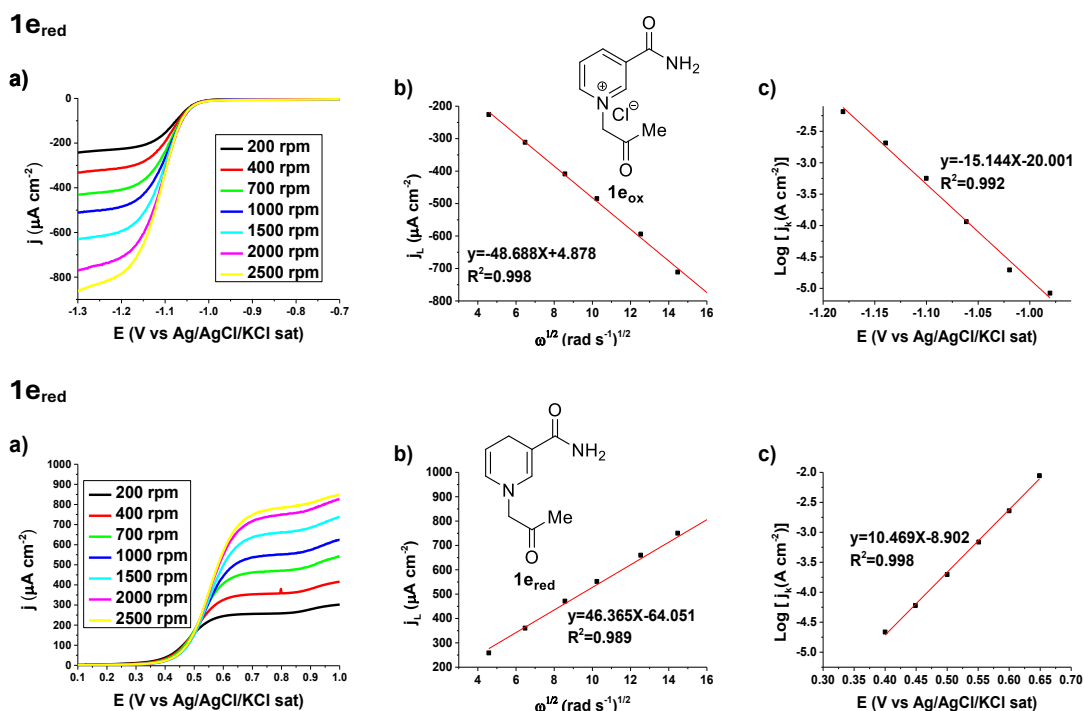


Figure S68. RDE analysis of **1e_{ox}** and **1e_{red}** in 1mM and phosphate buffer (pH= 7): (a) Linear Sweep Voltammetry (LSV) at 5 mV·s⁻¹ with different rotating speeds; (b) Levich analysis and (c) Tafel-plot for 1500 rpm without the mass transport of the reduction ($E_{\text{onset}} = -0.98$ V) or oxidation ($E_{\text{onset}} = 0.31$ V) processes.

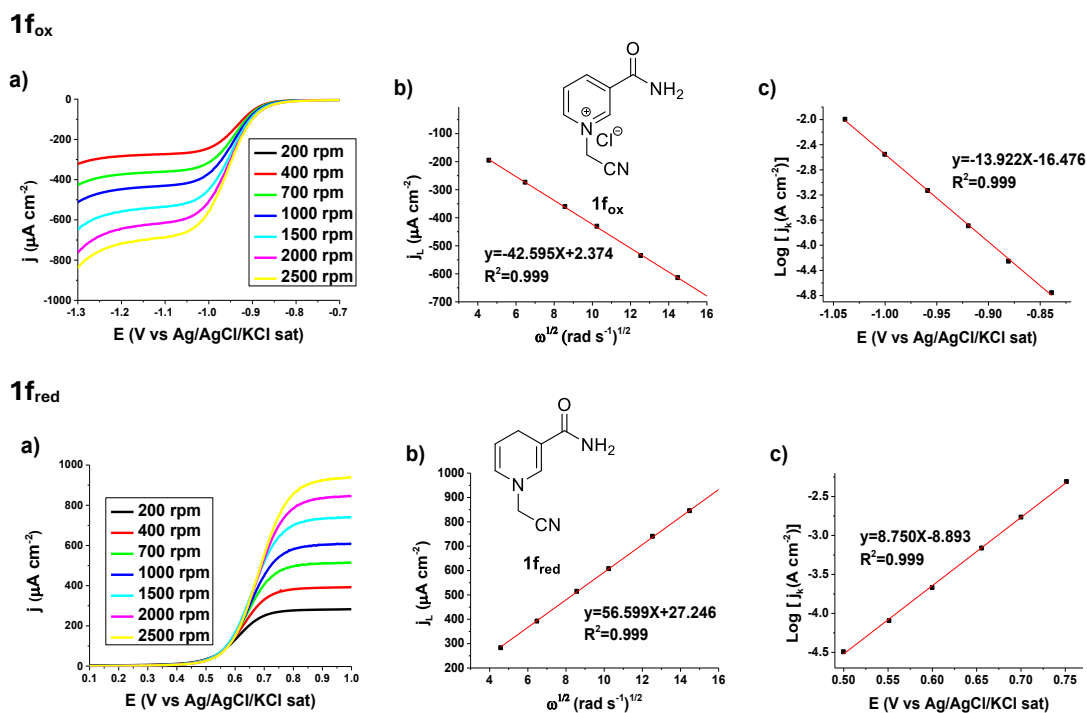


Figure S69. RDE analysis of **1f_{ox}** and **1f_{red}** in 1mM and phosphate buffer (pH= 7): (a) Linear Sweep Voltammetry (LSV) at 5 mV·s⁻¹ with different rotating speeds; (b) Levich analysis and (c) Tafel-plot for 1500 rpm without the mass transport of the reduction ($E_{\text{onset}} = -0.79$ V) or oxidation ($E_{\text{onset}} = 0.40$ V) processes.

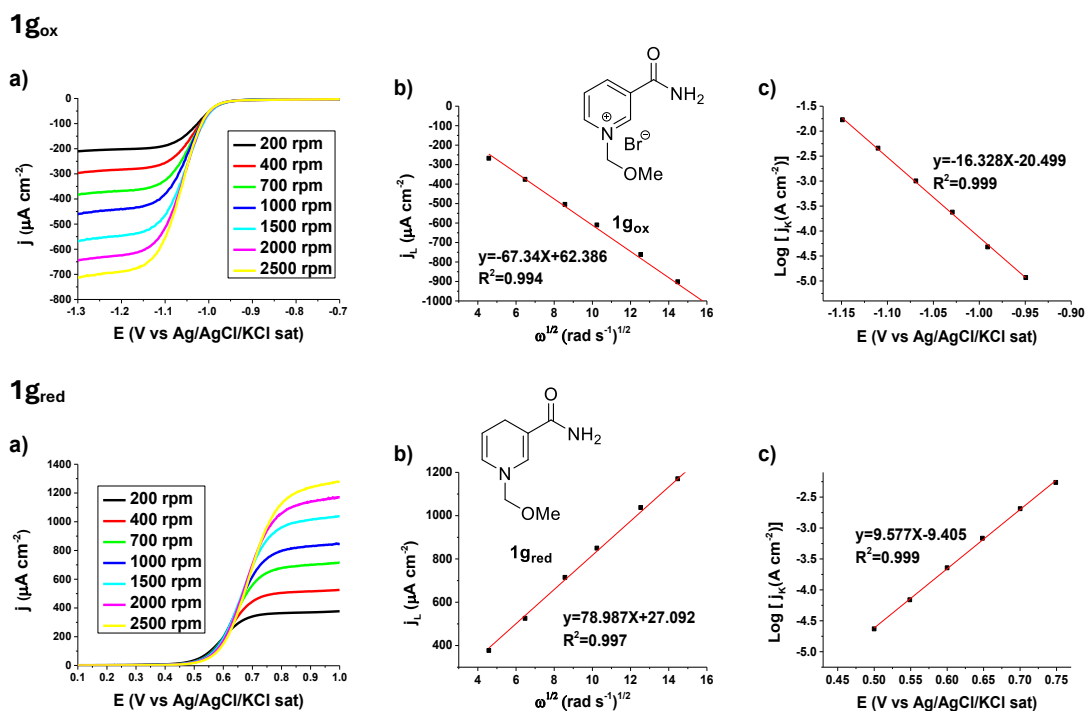


Figure S70. RDE analysis of **1g_{ox}** and **1g_{red}** in 1mM and phosphate buffer (pH= 7): (a) Linear Sweep Voltammetry (LSV) at 5 mV·s⁻¹ with different rotating speeds; (b) Levich analysis and (c) Tafel-plot for 1500 rpm without the mass transport of the reduction ($E_{\text{onset}} = -0.93$ V) or oxidation ($E_{\text{onset}} = 0.45$ V) processes.

Table S42. k_0 , E_{peak} and Diffusion coefficients (D) for the compounds studied (E_{peak} measured in a CV with a scan rate of 5 mV/s)

Py⁺	NAD⁺	1a_{ox}	1b_{ox}	1c_{ox}	1d_{ox}	1e_{ox}	1f_{ox}	1g_{ox}
k₀ (cm/s)·10⁻⁵	1.33	4.08	0.13	6.40	1.13	7.17	3.45	5.87
E_{p1} (V)	-1.10	-1.08	-1.02	-0.94	-0.87	-1.09	-0.94	-1.04
D (cm²/s) ·10⁻⁶	3.43	6.89	5.14	7.56	6.50	7.38	6.04	12.0

PyH	NADH	1a_{red}	1b_{red}	1c_{red}	1d_{red}	1e_{red}	1f_{red}	1g_{red}
k₀ (cm/s)·10⁻⁵	3.50	2.26	2.24	1.65	1.95	1.14	2.09	4.09
E_{p3} (V)	0.61	0.48	0.50	0.60	0.62	0.45	0.60	0.59
D (cm²/s) ·10⁻⁶	1.68	3.58	0.80	5.05	1.23	2.42	3.27	5.39

In the main text, k_0 values have been taken as a general approximation of the kinetics of a monoelectronic capture by Py⁺. However, a redox process on an electrode surface does not exactly match its equivalent in solution, so k_0 values provide only a qualitative indication of the kinetics of a given electron-transfer event; detailed kinetic data can only be obtained from processes studied in solution.

Reversibility analysis

Experiments at different scan rates were carried out to examine the electrochemical process's reversibility. The relationship between the scan rate (ν) and the peak potential (E_{peak}) was investigated. Despite the scan rate, E_{peak} should remain constant in a system that is completely reversible (admitted $\Delta E_{\text{peak}} = 57\text{mV/n}$). However, the increase in scan rate is usually accompanied by the variation of E_{peak} in both irreversible ($\Delta E_{\text{peak}} > 200\text{mV/n}$) and quasi-reversible processes ($57\text{mV/n} > \Delta E_{\text{peak}} > 200\text{mV/n}$).

Analysing the CVs included below for p1, p2 and p3, it can be stated that none of the studied redox processes behaves as a reversible process under the conditions studied. In all of them, E_{peak} varies with the scan rate as well as the ΔE_{peak} causing all processes to be considered irreversible (see Figure S71-Figure S78 and Table S43-Table S46).

NAD⁺/NADH

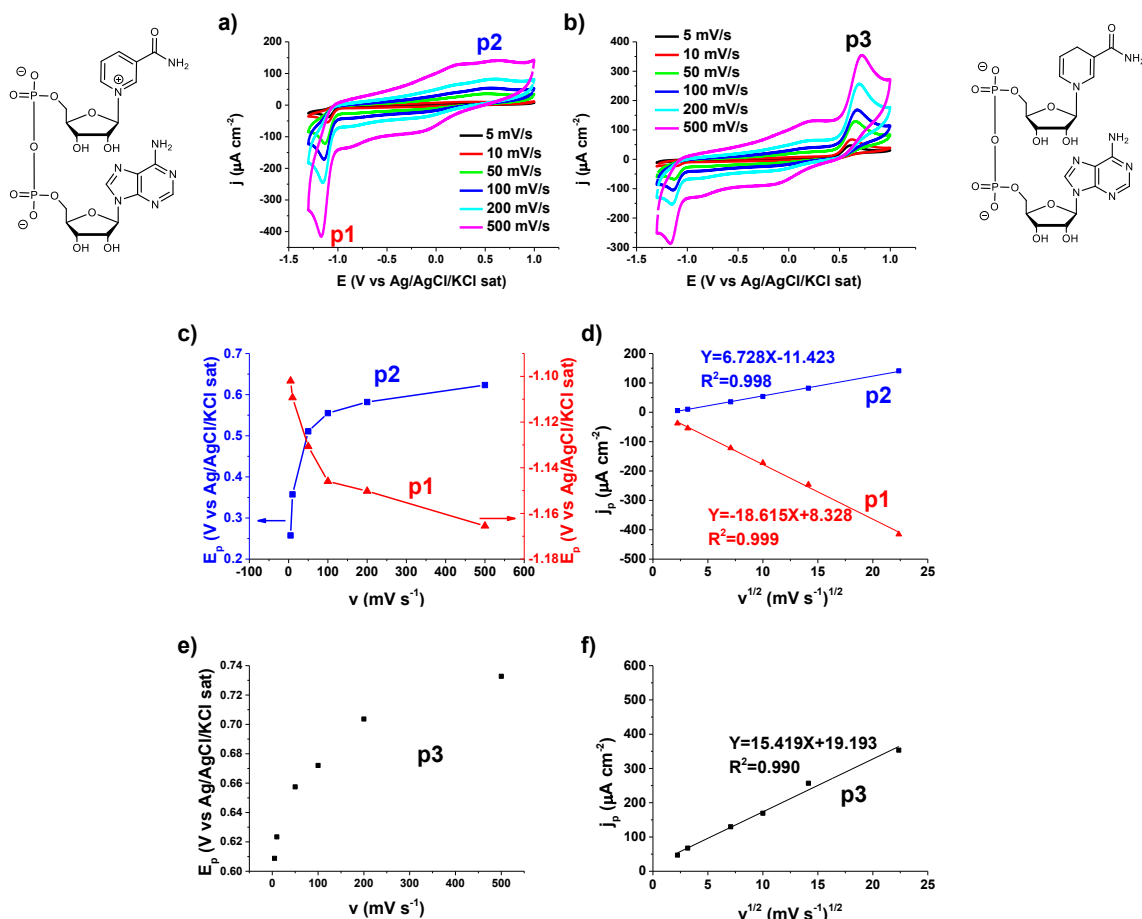


Figure S71. Reversibility analysis of NADH/NAD⁺ in 1mM and phosphate buffer (pH= 7): (a) Cyclic voltammograms at different scan rate for NAD⁺; and (b) for NADH. (c) Potential shift analysis of p1 and p2 peaks; (d) j_p vs ν^{1/2} of p1 and p2 peaks; (e) Analysis of the potential shift analysis of p3 peak and (f) j_p vs ν^{1/2} of p3 peak.

1a

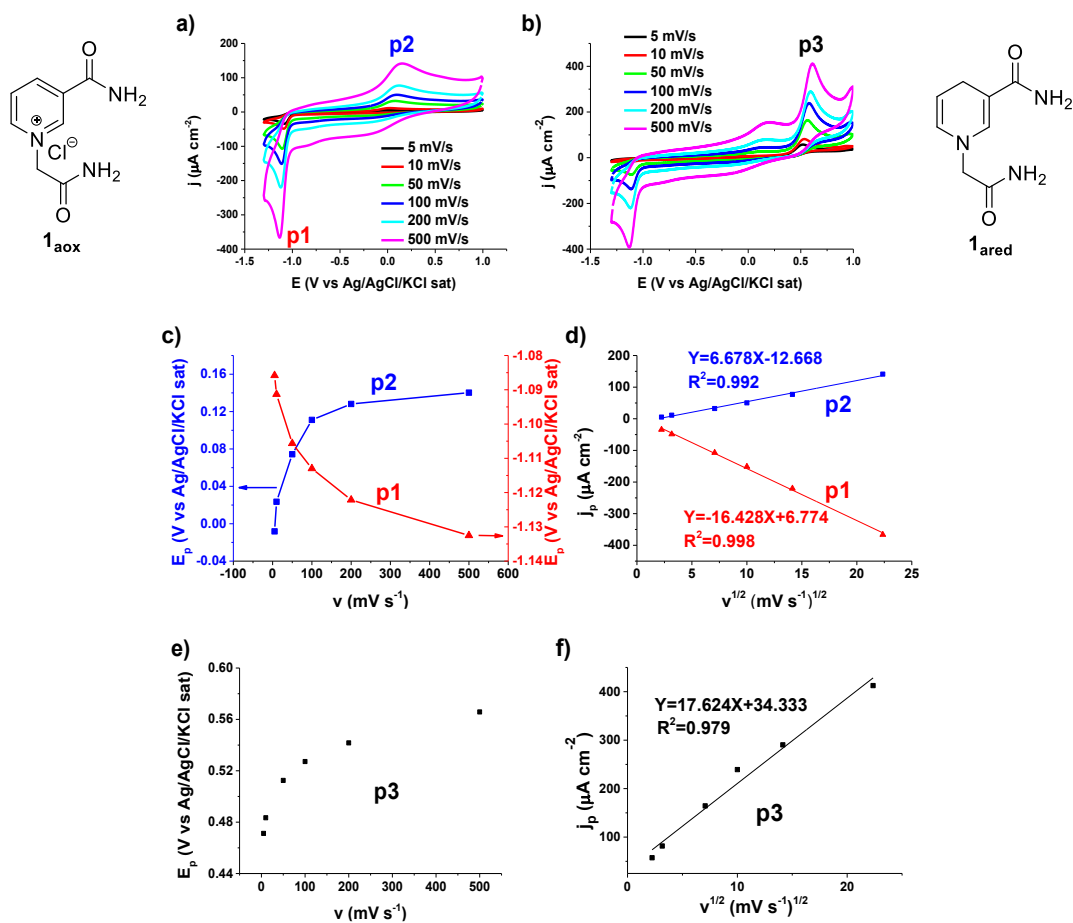


Figure S72. Reversibility analysis of **1a** in 1mM and phosphate buffer (pH= 7): (a) Cyclic voltammograms at different scan rate for **1a_{ox}**; and (b) for **1a_{red}**. (c) Potential swift analysis of p1 and p2 peaks; (d) j_p vs $v^{1/2}$ of p1 and p2 peaks; (e) Analysis of the potential swift analysis of p3 peak and (e) j_p vs $v^{1/2}$ of p3 peak.

1b

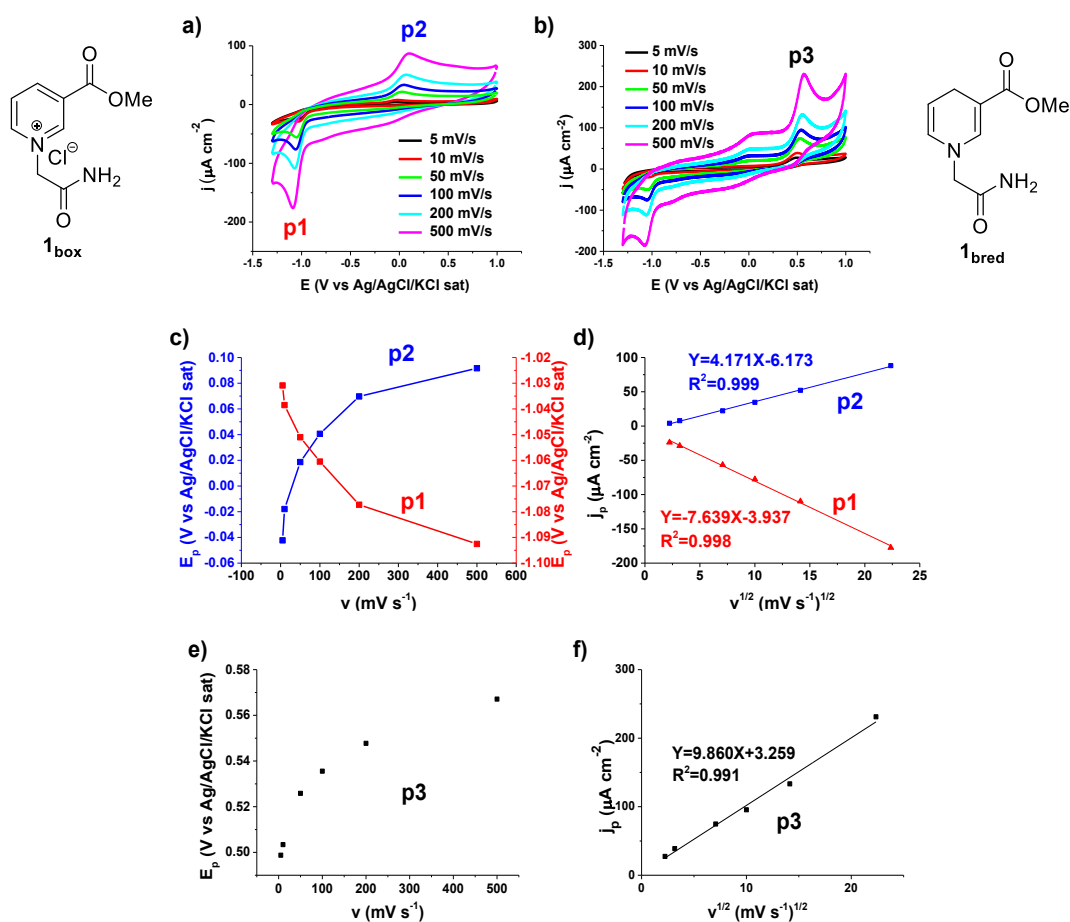


Figure S73. Reversibility analysis of **1b** in 1mM and phosphate buffer (pH= 7): (a) Cyclic voltammograms at different scan rate for **1box**; and (b) for **1bred**. (c) Potential swift analysis of p1 and p2 peaks; (d) j_p vs $v^{1/2}$ of p1 and p2 peaks; (e) Analysis of the potential swift analysis of p3 peak and (e) j_p vs $v^{1/2}$ of p3 peak.

1c

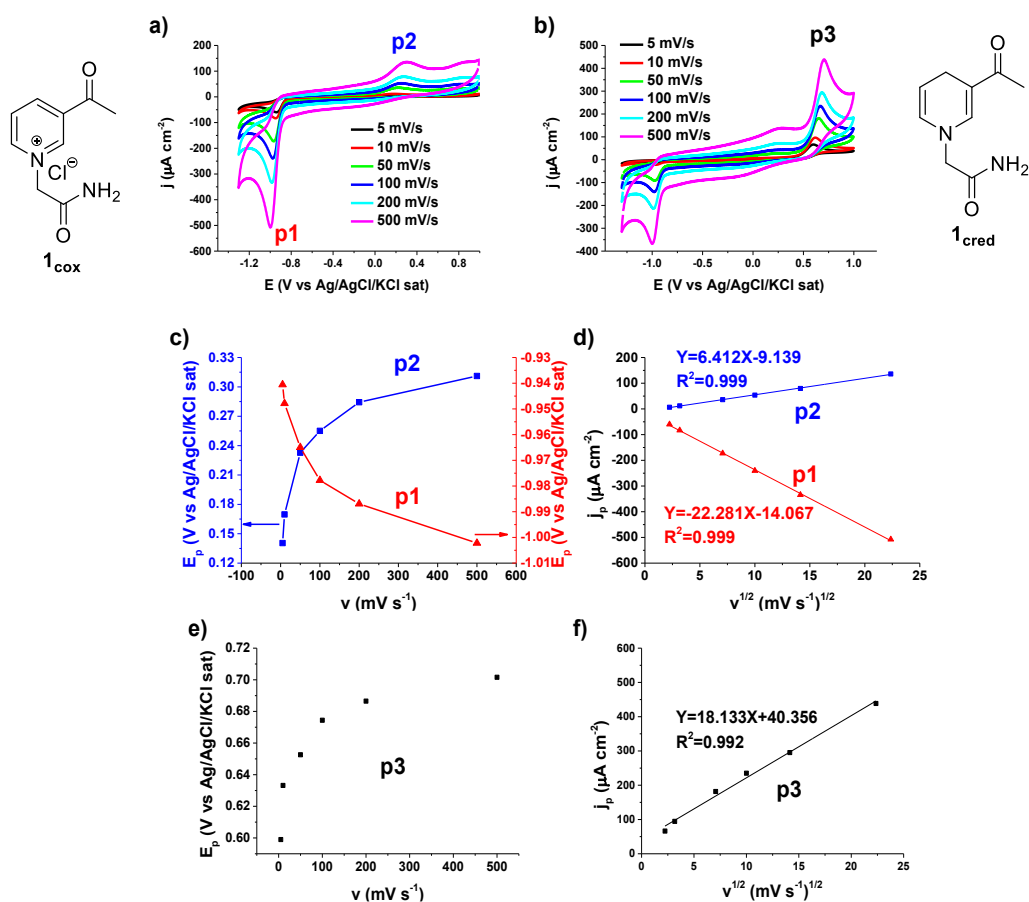


Figure S74. Reversibility analysis of **1c** in 1M and phosphate buffer (pH= 7): (a) Cyclic voltammograms at different scan rate for **1cox**; and (b) for **1cred**. (c) Potential swift analysis of p1 and p2 peaks; (d) j_p vs $v^{1/2}$ of p1 and p2 peaks; (e) Analysis of the potential swift analysis of p3 peak and (f) j_p vs $v^{1/2}$ of p3 peak.

1d

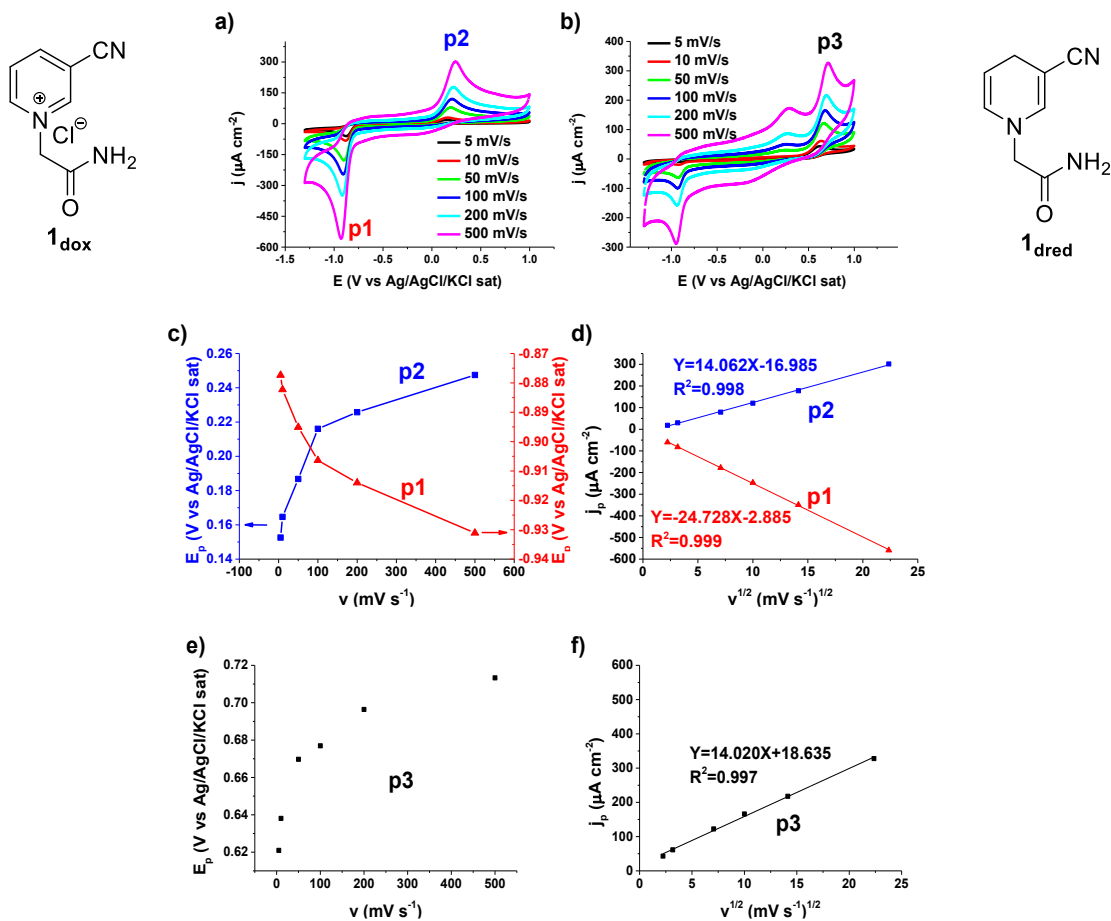


Figure S75. Reversibility analysis of **1d** in 1mM and phosphate buffer (pH= 7): (a) Cyclic voltammograms at different scan rate for **1d_{ox}**; and (b) for **1d_{red}**. (c) Potential swift analysis of p1 and p2 peaks; (d) j_p vs $v^{1/2}$ of p1 and p2 peaks; (e) Analysis of the potential swift analysis of p3 peak and (e) j_p vs $v^{1/2}$ of p3 peak.

1e

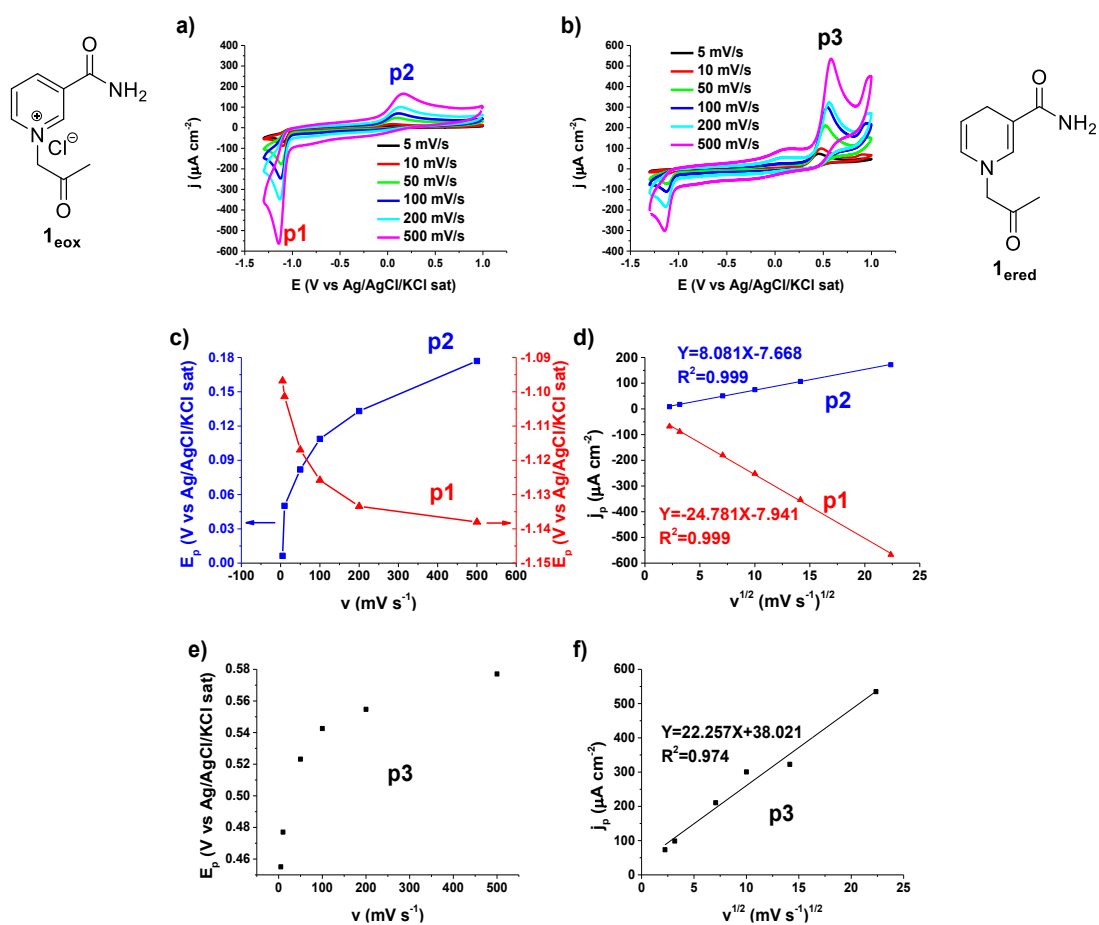


Figure S76. Reversibility analysis of **1e** in 1mM and phosphate buffer (pH= 7): (a) Cyclic voltammograms at different scan rate for **1e_{ox}**; and (b) for **1e_{red}**. (c) Potential swift analysis of p1 and p2 peaks; (d) j_p vs $v^{1/2}$ of p1 and p2 peaks; (e) Analysis of the potential swift analysis of p3 peak and (e) j_p vs $v^{1/2}$ of p3 peak.

1f

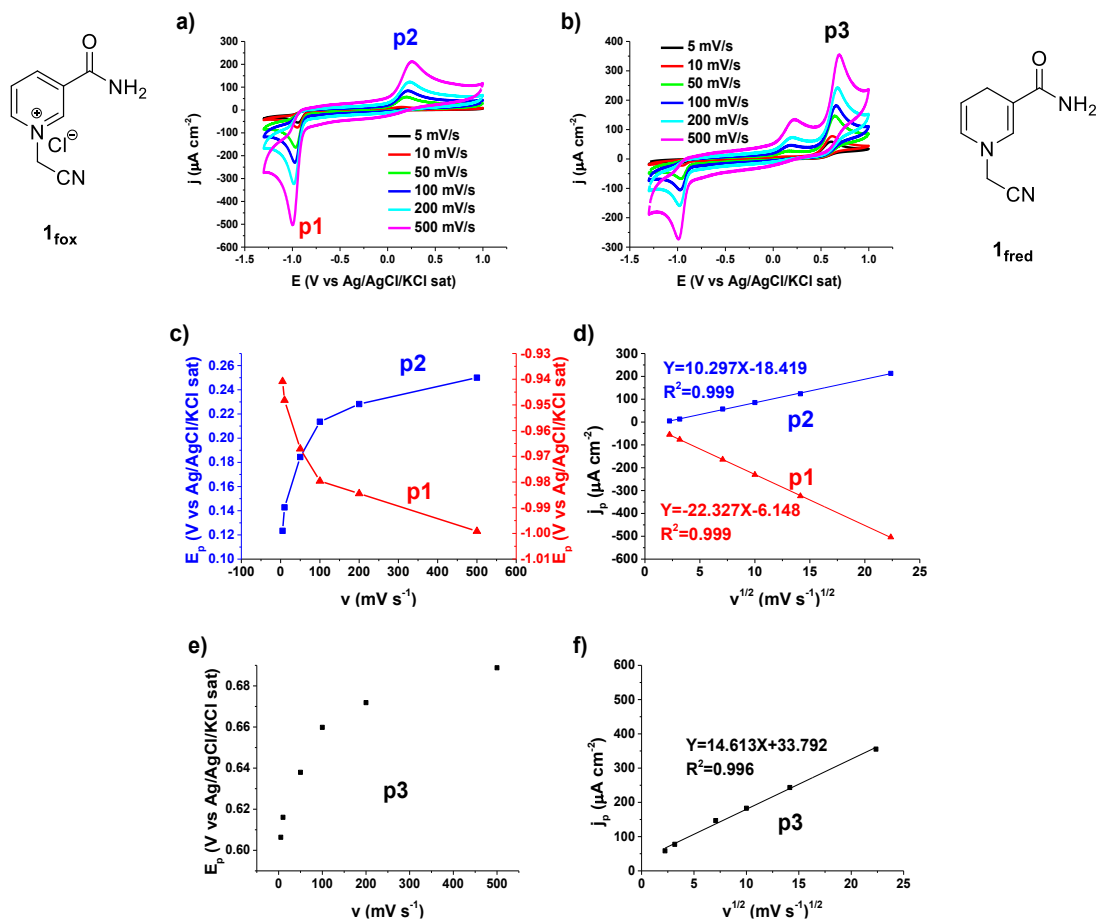


Figure S77. Reversibility analysis of **1f** in 1mM and phosphate buffer (pH= 7): (a) Cyclic voltammograms at different scan rate for **1fox**; and (b) for **1fred**. (c) Potential swift analysis of p1 and p2 peaks; (d) j_p vs $v^{1/2}$ of p1 and p2 peaks; (e) Analysis of the potential swift analysis of p3 peak and (e) j_p vs $v^{1/2}$ of p3 peak.

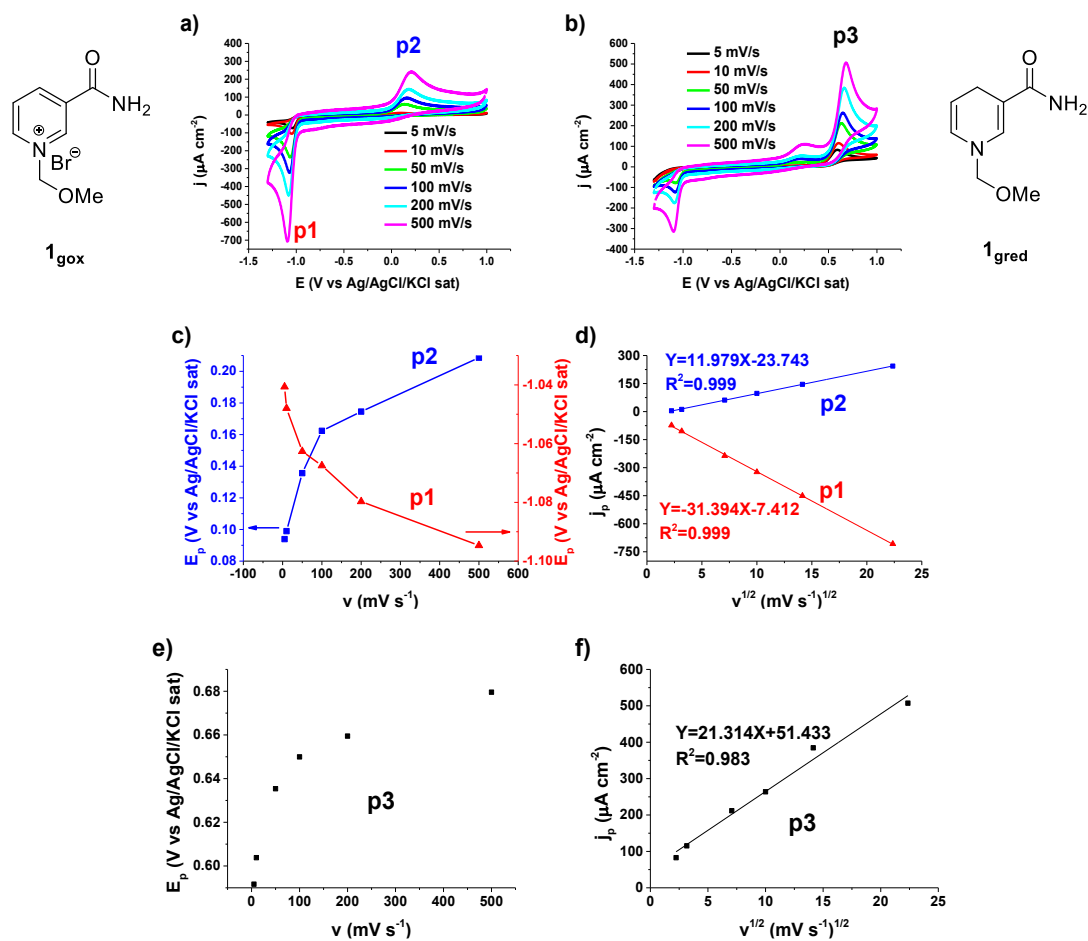
1g

Figure S78. Reversibility analysis of **1g** in 1mM and phosphate buffer (pH= 7): (a) Cyclic voltammograms at different scan rate for **1g_{ox}**; and (b) for **1g_{red}**. (c) Potential swift analysis of p1 and p2 peaks; (d) j_p vs $v^{1/2}$ of p1 and p2 peaks; (e) Analysis of the potential swift analysis of p3 peak and (f) j_p vs $v^{1/2}$ of p3 peak.

Table S43. E_{peak} reversibility analysis of p1 and p2 for compounds **NADH/NAD⁺** and **1a**.

v (mV/s)	NADH/NAD⁺			Compound 1a		
	E_{p1} (V)	E_{p2} (V)	ΔE_{p} (V)	E_{p1} (V)	E_{p2} (V)	ΔE_{p} (V)
5	-1.10	0.26	1.36	-1.09	0.00	1.09
10	-1.11	0.36	1.47	-1.09	0.02	1.11
50	-1.13	0.51	1.64	-1.11	0.07	1.18
100	-1.15	0.56	1.70	-1.11	0.11	1.22
200	-1.15	0.58	1.73	-1.12	0.13	1.25
500	-1.17	0.62	1.79	-1.13	0.14	1.27

Table S44. E_{peak} reversibility analysis of p1 and p2 for compounds **1b** and **1c**.

v (mV/s)	Compound 1b			Compound 1c		
	E_{p1} (V)	E_{p2} (V)	ΔE_{p} (V)	E_{p1} (V)	E_{p2} (V)	ΔE_{p} (V)
5	-1.03	-0.04	0.99	-0.94	0.14	1.08
10	-1.04	-0.02	1.02	-0.95	0.17	1.12
50	-1.05	0.02	1.07	-0.96	0.23	1.20
100	-1.06	0.04	1.10	-0.98	0.26	1.23
200	-1.08	0.07	1.15	-0.99	0.28	1.27
500	-1.09	0.09	1.18	-1.00	0.31	1.31

Table S45. E_{peak} reversibility analysis of p1 and p2 for compounds **1d** and **1e**.

v (mV/s)	Compound 1d			Compound 1e		
	E_{p1} (V)	E_{p2} (V)	ΔE_{p} (V)	E_{p1} (V)	E_{p2} (V)	ΔE_{p} (V)
5	-0.87	0.15	1.02	-1.10	0.01	1.11
10	-0.88	0.16	1.05	-1.10	0.05	1.15
50	-0.90	0.19	1.08	-1.12	0.08	1.20
100	-0.91	0.22	1.12	-1.13	0.11	1.24
200	-0.91	0.23	1.14	-1.13	0.13	1.26
500	-0.93	0.25	1.18	-1.14	0.18	1.32

Table S46. E_{peak} reversibility analysis of p1 and p2 for compounds **1f** and **1g**.

v (mV/s)	Compound 1f			Compound 1g		
	E_{p1} (V)	E_{p2} (V)	ΔE_{p} (V)	E_{p1} (V)	E_{p2} (V)	ΔE_{p} (V)
5	-0.94	0.12	1.06	-1.04	0.09	1.13
10	-0.95	0.14	1.09	-1.05	0.10	1.15
50	-0.97	0.18	1.15	-1.06	0.14	1.20
100	-0.98	0.21	1.19	-1.07	0.16	1.23
200	-0.98	0.22	1.20	-1.08	0.17	1.25
500	-1.00	0.25	1.25	-1.09	0.21	1.30

14. Theoretical Calculations

Computational details

All quantum chemical calculations were carried out using the Gaussian16 software¹³ in the framework of the Density Functional Theory (DFT). Geometry optimizations were performed with the M06-2X functional,¹⁴ employing the correlation-consistent triple-zeta basis set cc-pVTZ and without symmetry constraints. Solvent effects were included using the SMD implicit solvation model,¹⁵ with water as the solvent. All optimized structures were confirmed as true minima by the absence of imaginary frequencies in the vibrational analysis. Topological analyses of the electron density based in the Quantum Theory of Atoms In Molecules (QTAIM)¹⁶ were performed using the AIMAll program,¹⁷ based on wavefunctions obtained from the Gaussian calculations. Conformational search with explicit consideration of water molecules was performed with the CREST software¹⁸ using the GNF2 Hamiltonian.¹⁹

Note: The reported $\Delta G^\ddagger$ values (in Figure 3B of main text and in Figure S79B and Figure S81) come from static DFT optimizations with harmonic free-energy corrections and an implicit solvation model. This framework is useful for qualitative mechanistic insight, but it does not explicitly capture hydrogen-bond networks or solvent reorganization and it does not consider ensemble sampling of the microstates. In addition, the harmonic treatment can be inaccurate for low-frequency modes in flexible cofactors. Therefore, absolute barriers should not be regarded as quantitatively predictive in condensed phase. Here we interpret the calculations conservatively, focusing on relative trends ($\Delta\Delta G^\ddagger$) across closely related cofactors, consistent with experiments.

Calculations related to the substitution of NCH₂-position

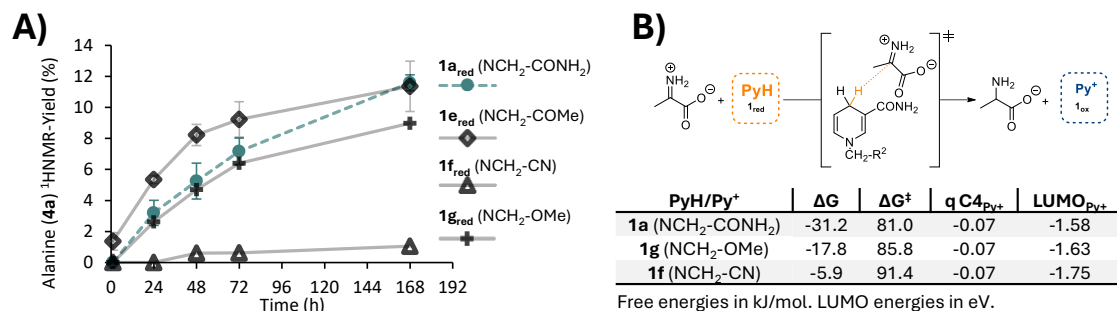
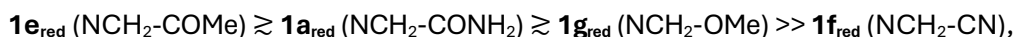


Figure S79. Experimental and computational structure-activity analysis for different substitutions at NCH₂-position in **1_{ox}**/**1_{red}** pairs. **A)** Comparison of alanine yields at different reaction times (conditions in Table 1 of main text) starting from pyruvate and various **1_{red}** derivatives (data from Table S26, Table S34, Table S36 and Table S38). **B)** Computational results for the step of hydride addition to the α-ketoacid iminium ion.

Figure S79A compares the experimental alanine (**4a**) formation for different dihydropyridines (PyH, **1_{red}**) as reductants, all of them with the group carbamoyl (-CONH₂) in 3-position of the aromatic ring but with different substituents in the *N*-methylenic position (R² in scheme of Figure S79B). The order of reactivity is:



which is inversely correlated with the order of the Taylor inductive substituent parameter (σ_I)²⁰ for these functional groups:^{†††}

$$\sigma_I \text{ COMe} (0.26) = \sigma_I \text{ CONH}_2 (0.26) < \sigma_I \text{ OMe} (0.29) \ll \sigma_I \text{ CN} (0.63).$$

DFT calculations of representative molecules also show the same tendency in the activation barriers (Figure S79B), with higher $\Delta G^\ddagger$ for the nitrile-substituted system. Examination of the Py⁺ (**1_{ox}**) parameters indicates that substituents at the NCH₂-position do not significantly alter the charge at carbon-4 (unlike substitutions at 3-position) but they modulate the LUMO energy. As expected, **1a_{ox}** (NCH₂-CONH₂) exhibits the highest LUMO energy and is therefore the most difficult to reduce.

Accordingly, both the experimental results and the theoretical calculations show that the functional group attached to the methylene carbon, despite not being directly bonded to the aromatic ring, exerts a considerable influence on the reactivity of these pyridinium/dihydropyridine systems.

^{†††} According to reference 20, several inductive parameter scales exist (Stock, Grob, Charton and Taylor), but all of them indicate a similar σ_I value for the substituents -COMe, -CONH₂ and -OMe and a larger value for -CN.

Effect of the explicit solvation

In order to study the effect of the explicit consideration of solvent molecules on the ionization potentials, we have calculated ionizations energies adding from 1 to 5 explicit water molecules (in addition to the implicit solvation model).

For every system, we have calculated the conformer distribution using CREST and we have optimized at DFT level the 10 most stable structures. Then, we calculated the vertical ionization energy of all the 150 systems (3 molecules x 5 number of water molecules x 10 structures). The results, shown in Figure S80, indicate that the explicit consideration of water molecules can increase the absolute ionization energies, although the trends are correctly predicted by implicit solvation. Therefore, all subsequent calculations reported in the Supporting Information and the main text were performed using exclusively the implicit solvation model.

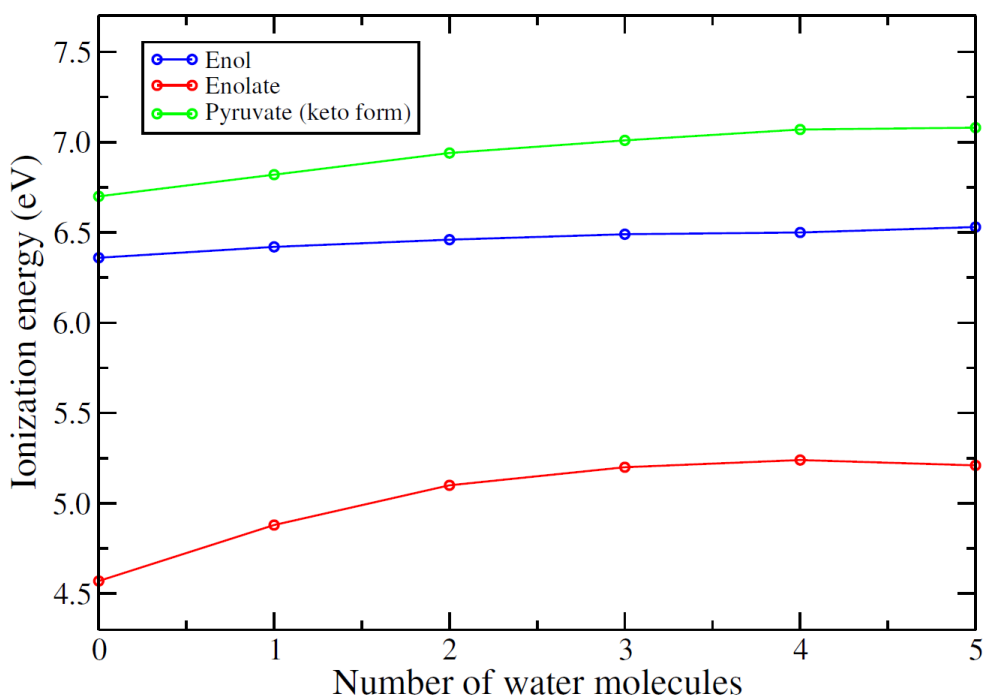


Figure S80. Ionization energy of the most stable structure as function of the number of explicit water molecules.

Ionization potentials and decarboxylation barriers

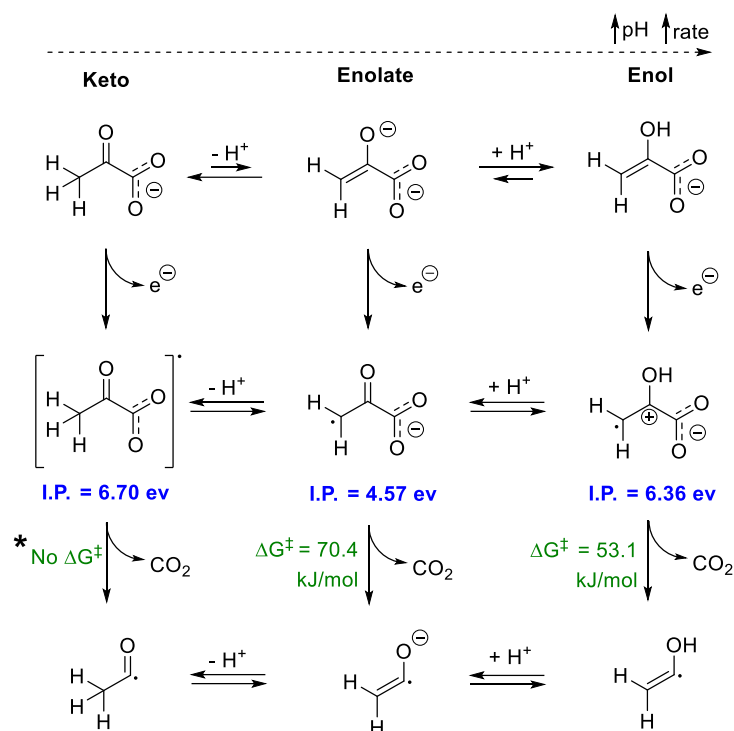


Figure S81. Vertical ionization potentials (I.P.) of the different forms of pyruvate in equilibrium. Activation free energy of decarboxylation of ionized products. The acid-base equilibrium between the ionized products is discussed in Figure S83.

*No intrinsic electronic barrier was identified on the calculated potential energy surface. Although solvent reorganization in the condensed phase may still influence the effective kinetics, the calculations suggest that C-C bond cleavage is more favorable for the keto radical form than for the other radical intermediates.

Molecular orbital analysis

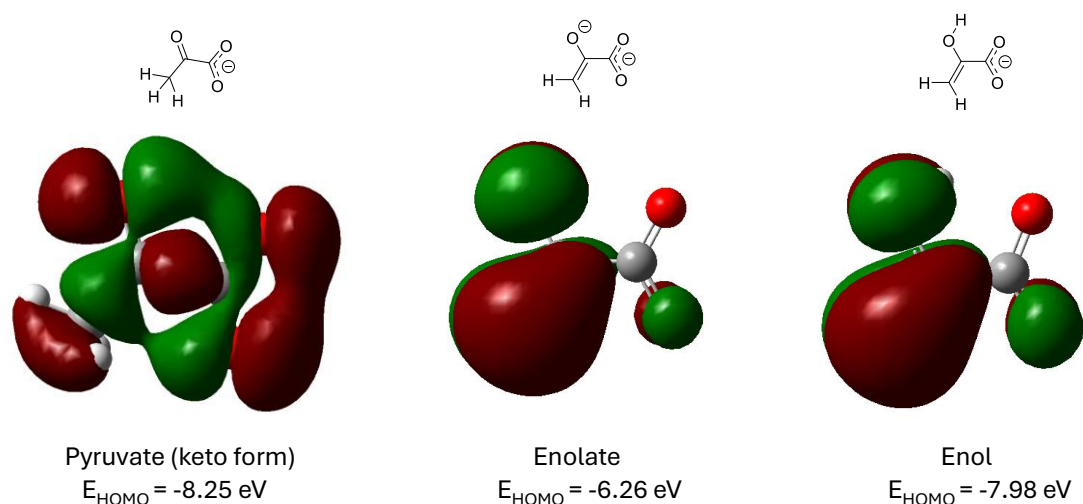


Figure S82. HOMO plot of the different forms of pyruvate in equilibrium.

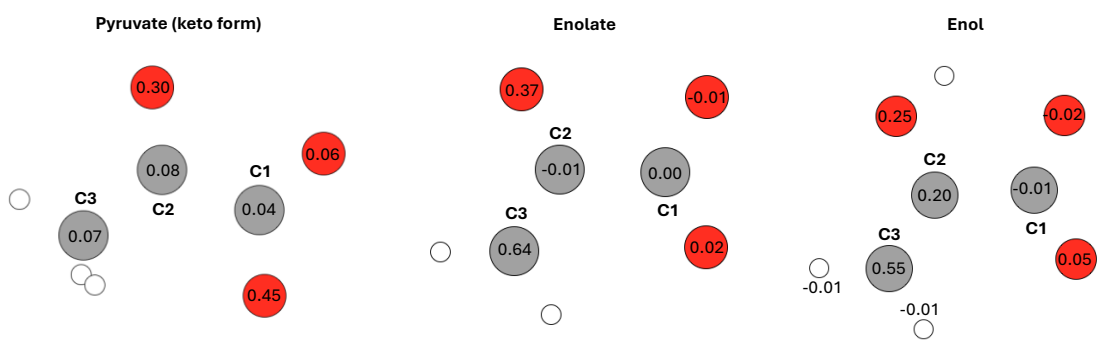
When pyruvate is dissolved under basic aqueous conditions, three species are considered to be in equilibrium: the keto form, its enol tautomer, and the corresponding enolate. Among these, the keto form exhibits the highest ionization potential, indicating that electron removal is less favourable for this species compared to the enol and enolate. This behaviour correlates with the nature of the HOMO orbital in each structure (see Figure S82).

In the keto form, the HOMO is delocalized over the entire molecule, including the carboxylate group. This extensive delocalization likely contributes to the stabilization of the electron distribution, making ionization more energetically demanding. Also, its bonding character (C-C) is consistent with the direct decarboxylation (C-C bond breaking) once the system is ionized. In contrast, the HOMO of both the enol and enolate forms are more localized, primarily over the π -system involving the C=C double bond and the adjacent hydroxyl oxygen atom. In these molecules, ionization from the HOMO orbital does not affect the C-C bond order, this being the reason why decarboxylation still requires an activation energy to be observed.

Spin density distribution

In addition to the simple chemical picture given by the analysis of the molecular orbitals of the neutral system, we have carried out Quantum Theory of Atoms In Molecules (QTAIM) calculations, using the electron density of the system after ionization (Table S47). The resulting radical in pyruvate is predominantly delocalized over the carboxylate moiety, as reflected by a spin density close to 0.5 electrons in that region. On the other hand, for both the enol and enolate species, the unpaired electron is mainly localized on the terminal carbon of the double bond, with a smaller contribution on the oxygen atom of the hydroxyl group, which is consistent with the explanation based on the molecular orbitals. The different localization of the unpaired electron is also in good agreement with the existence of an energy barrier in order to break the C-C bond.

Table S47. AIM spin density distribution in the atoms of the different forms of pyruvate in equilibrium.



	C1	C2	C3	O (C2)	O (Carboxylate) ^a
Keto form	0.04	0.08	0.07	0.30	0.51
Enolate	0.00	-0.01	0.64	0.37	0.01
Enol	-0.01	0.20	0.55	0.25	0.03

^aSum of the spin density in both oxygen atoms of the carboxylate group.

IRC of the protonation of the enolate radical

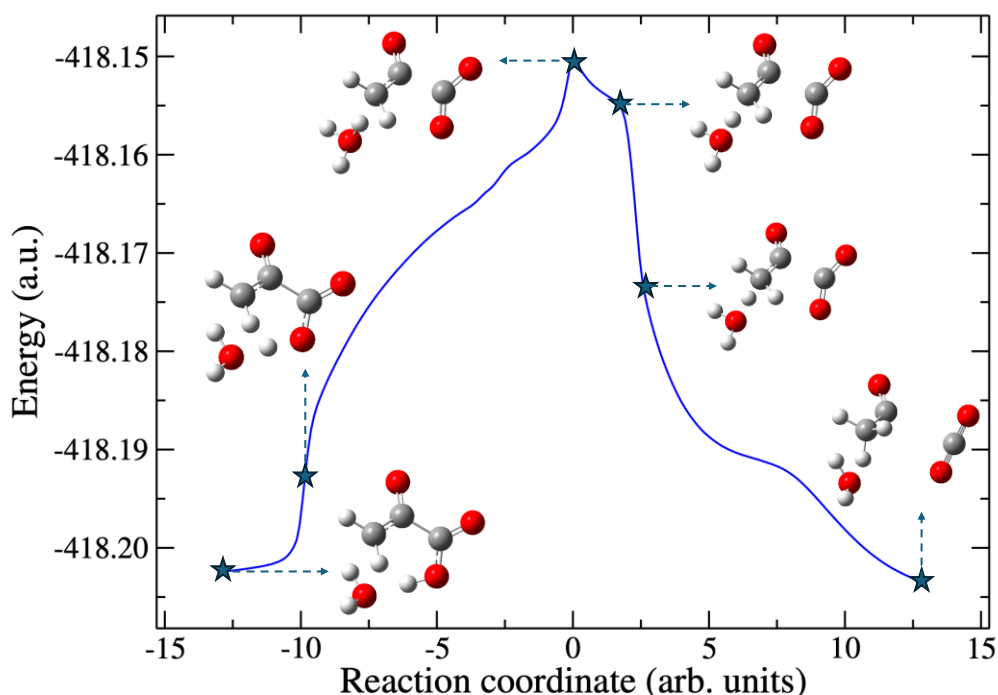


Figure S83. IRC of the protonation of the enolate radical by a model protonation source (H_3O^+).

To further explore the mechanistic aspects of the decarboxylation observed upon ionization of pyruvate, we investigated an alternative pathway involving the protonation of the ionized enolate species. This approach was motivated by the observation that, in the case of the keto form of pyruvate, spontaneous decarboxylation occurs upon ionization, with no apparent energy barrier. We thus hypothesized that a similar outcome could be achieved by first ionizing the enolate and then protonating the carbon atom to produce the radical of the keto form.

The IRC calculation for the protonation of the ionized enolate, which is shown in Figure S83, reveals a concerted mechanism: as the proton is transferred to the terminal carbon of the former enolate moiety, a simultaneous cleavage of the C–C bond leading to CO_2 release takes place (see positive values of reaction coordinate). The reaction proceeds in a single, continuous step, indicating strong coupling between the acid-base event and the decarboxylation process. No stable intermediate is formed along the path, suggesting that protonation facilitates the breaking of the carboxylate linkage, if ionization of enolate would happen.

These results support a mechanistic scenario in which selective ionization of the enolate, followed by proton transfer, can serve as a viable route to induce decarboxylation. This mechanism may be particularly relevant under basic conditions where the population of enolate form is increased, and local proton sources are available to initiate the transformation. Due to the high acidity of the H_3O^+ ion (model protonation source in this calculation), the IRC does not stop in a structure with the proton bonded to a water molecule, but to the carboxylate group (see negative values of reaction coordinate). This also reflects the existence of acid-base equilibria among this species with no barrier.

Hypothetical mechanism proposal

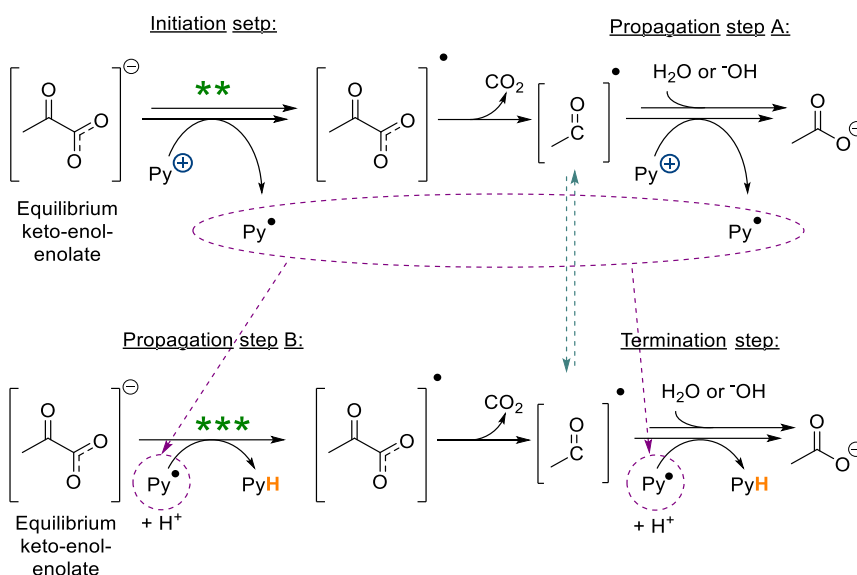


Figure S84. Suggested radical propagation mechanism of oxidative decarboxylation of pyruvate (**2a**) by pyridiniums **1_{ox}** (**Py⁺**). This scheme is a simplified representation that does not explicitly distinguish between the keto, enol, and enolate forms of pyruvate. Combination of this scheme with Figure S81 provides a broader view of the suggested mechanism. See the notes marked with (**) and (***) for considerations related to these steps.

Purple arrows indicate that the **Py[•]** that is formed in the initiation step or propagation step A can act in another oxidation, *i.e.*, the oxidation of pyruvate or the oxidation of the decarboxylated radical.

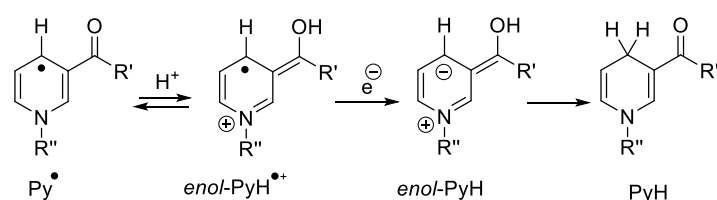
Dark teal arrows indicate that a decarboxylated radical that is generated by the action of a **Py[•]** (+ **H⁺**) can, in the second redox reaction, be oxidised by a **Py⁺** and *vice versa*.

*** Based on the calculations presented in Figure S81, this initial step is more likely to proceed via the enol or enolate form of pyruvate. The radical intermediate generated in this step can subsequently undergo proton exchange prior to the barrierless decarboxylation.

*** This step is observed in electrochemical measurements at pH 7 (Figure S61), suggesting that the oxidant may be capable of accepting an electron directly from the keto form of pyruvate without requiring conversion to the enol or enolate form.

Another plausible termination step would be the homocoupling of **Py[•]** to form **Py₂**.

About the action of **Py[•]** as oxidant: J. Gębicki and co-workers reported different works where they detected, by transient absorption spectroscopy, different radical intermediates and enol intermediates in the interconversion of **NAD⁺/NADH** (or analogues **Py⁺/PyH**).²¹ Based on these studies, a possible mode of action would involve the **Py[•]** radical being protonated to form **enol-PyH^{•+}**, which then would act as oxidant by accepting one electron and converting into **enol-PyH**, which would finally rearrange into **PyH**.



15. ^1H -NMR and ^{13}C -NMR spectra of pyridiniums (**1_{ox}**) and dihydropyridines (**1_{red}**)

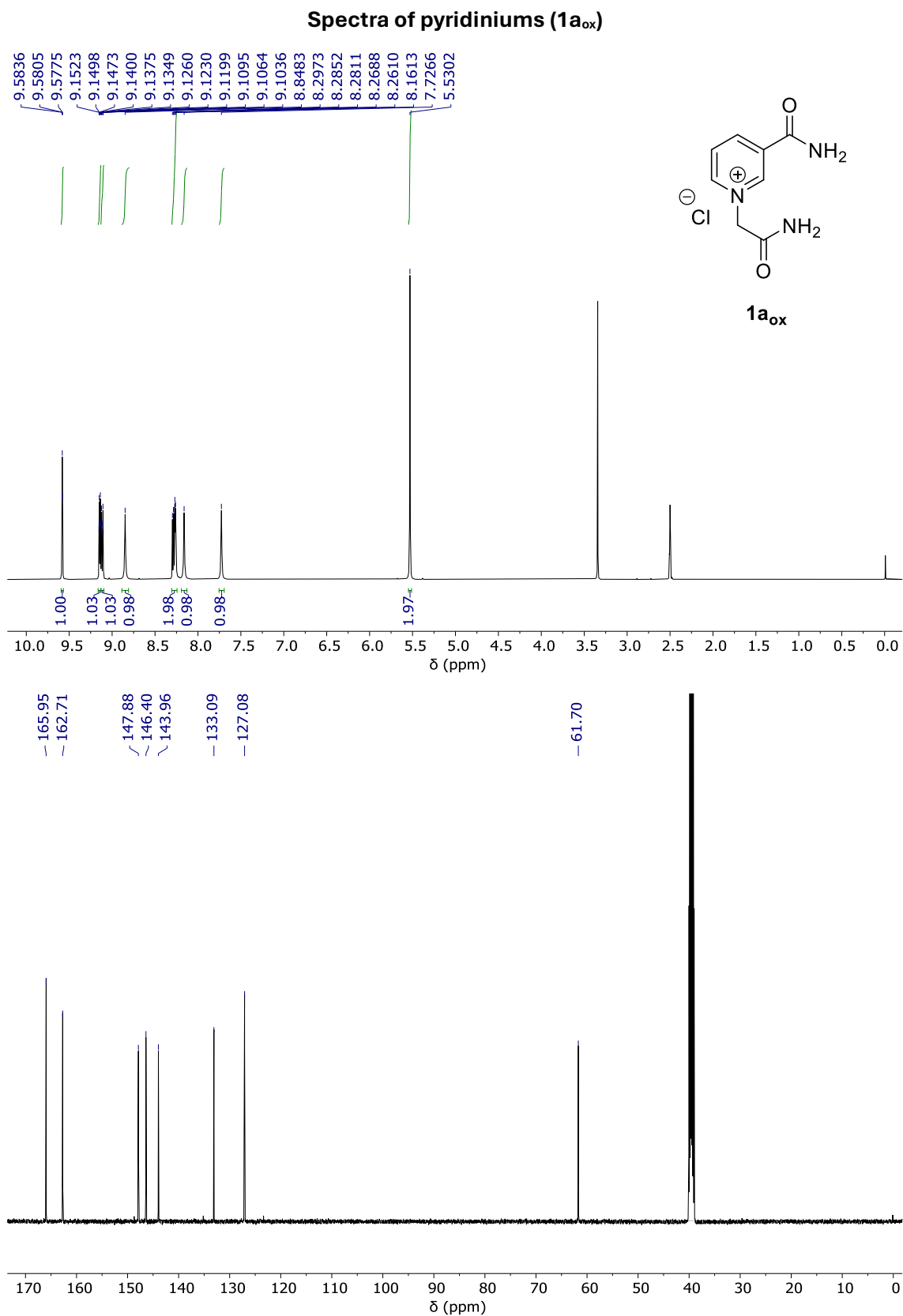


Figure S85. ^1H -NMR (500 MHz, DMSO) and ^{13}C -NMR (126 MHz, DMSO) spectra of compound **1_{ox}**.

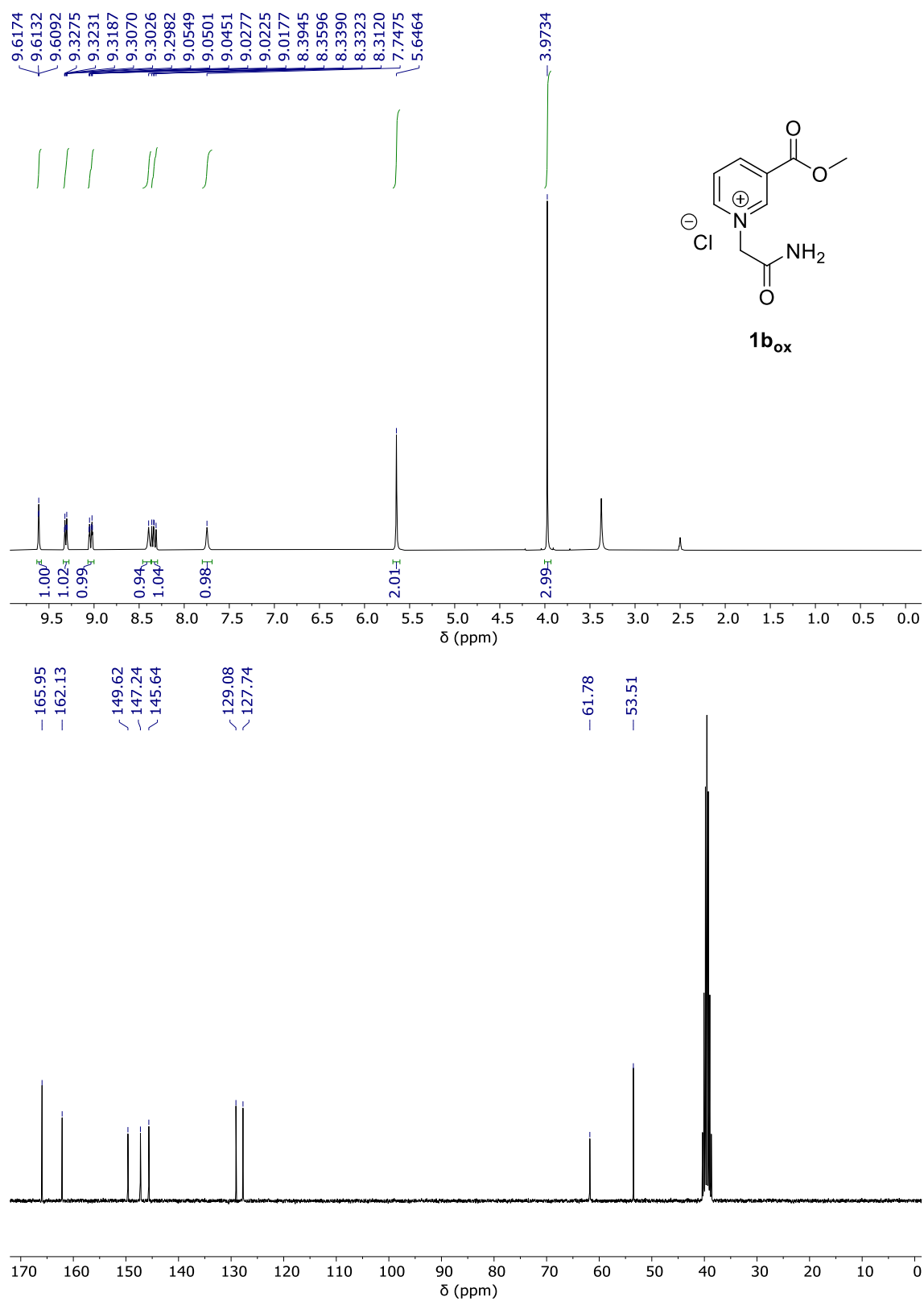


Figure S86. 1H -NMR (300 MHz, DMSO) and ^{13}C -NMR (75 MHz, DMSO) spectra of compound $1b_{ox}$.

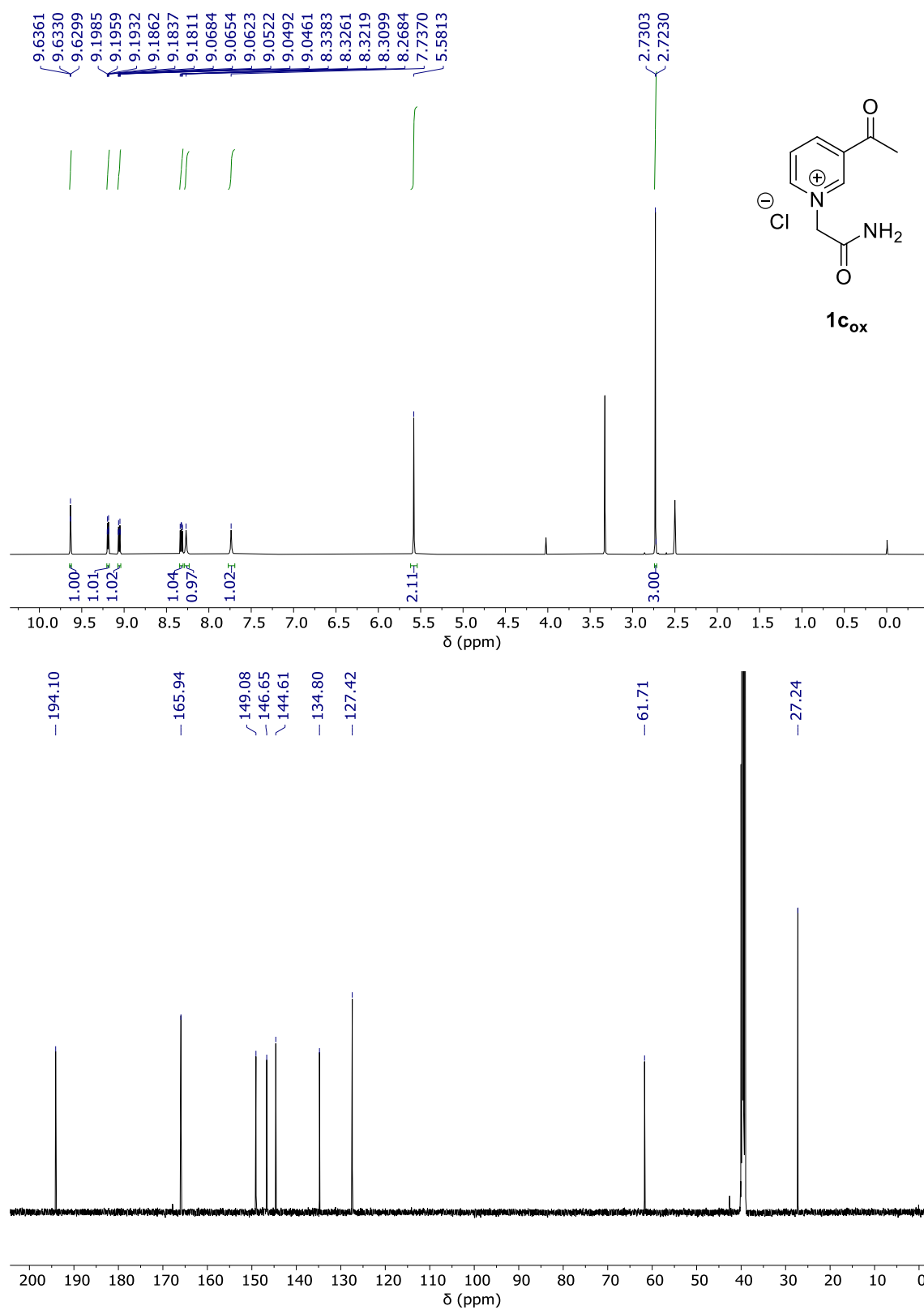


Figure S87. ¹H-NMR (500 MHz, DMSO) and ¹³C-NMR (126 MHz, DMSO) spectra of compound **1c_{ox}**.

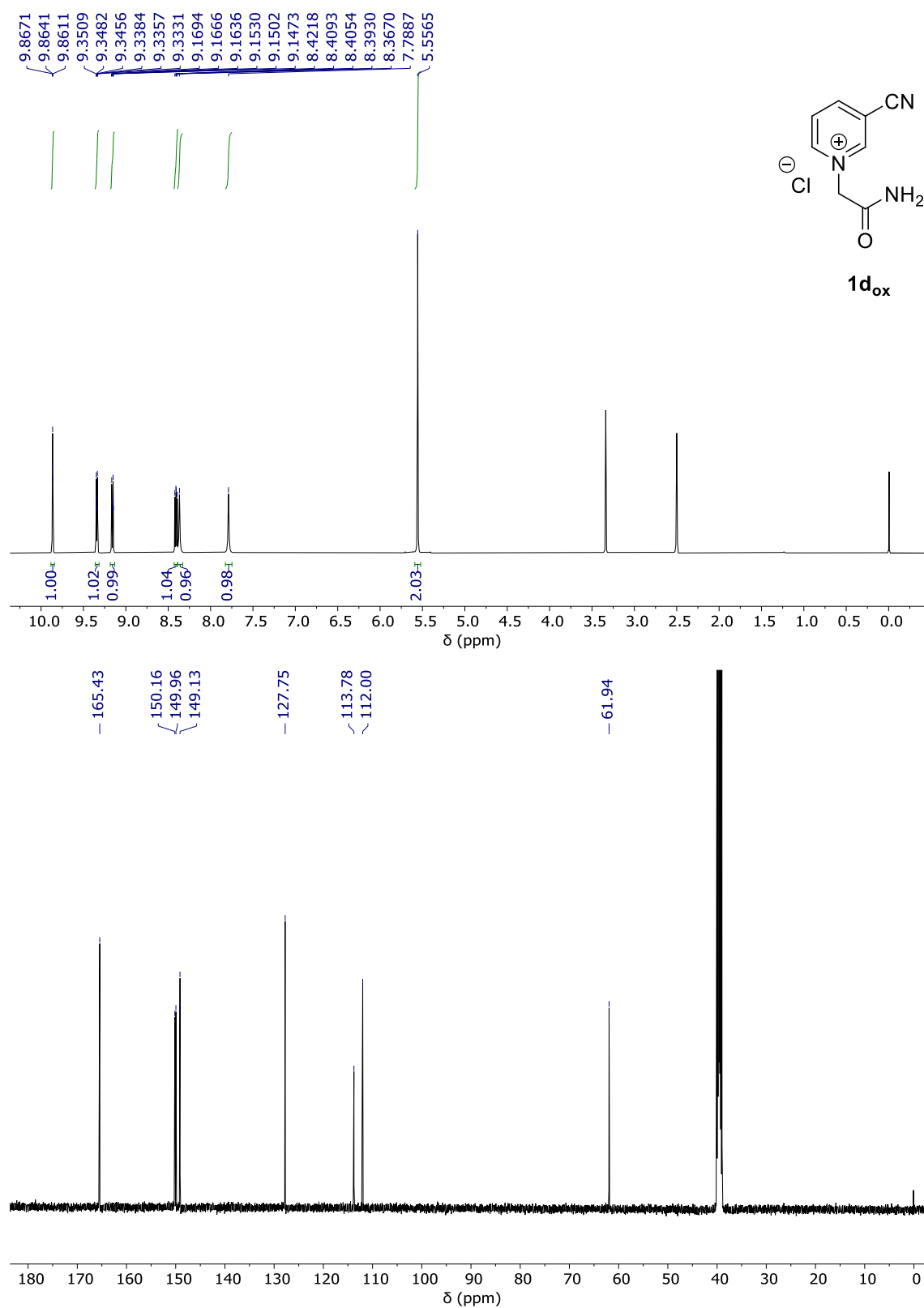


Figure S88. 1H -NMR (500 MHz, DMSO) and ^{13}C -NMR (126 MHz, DMSO) spectra of compound $1d_{ox}$.

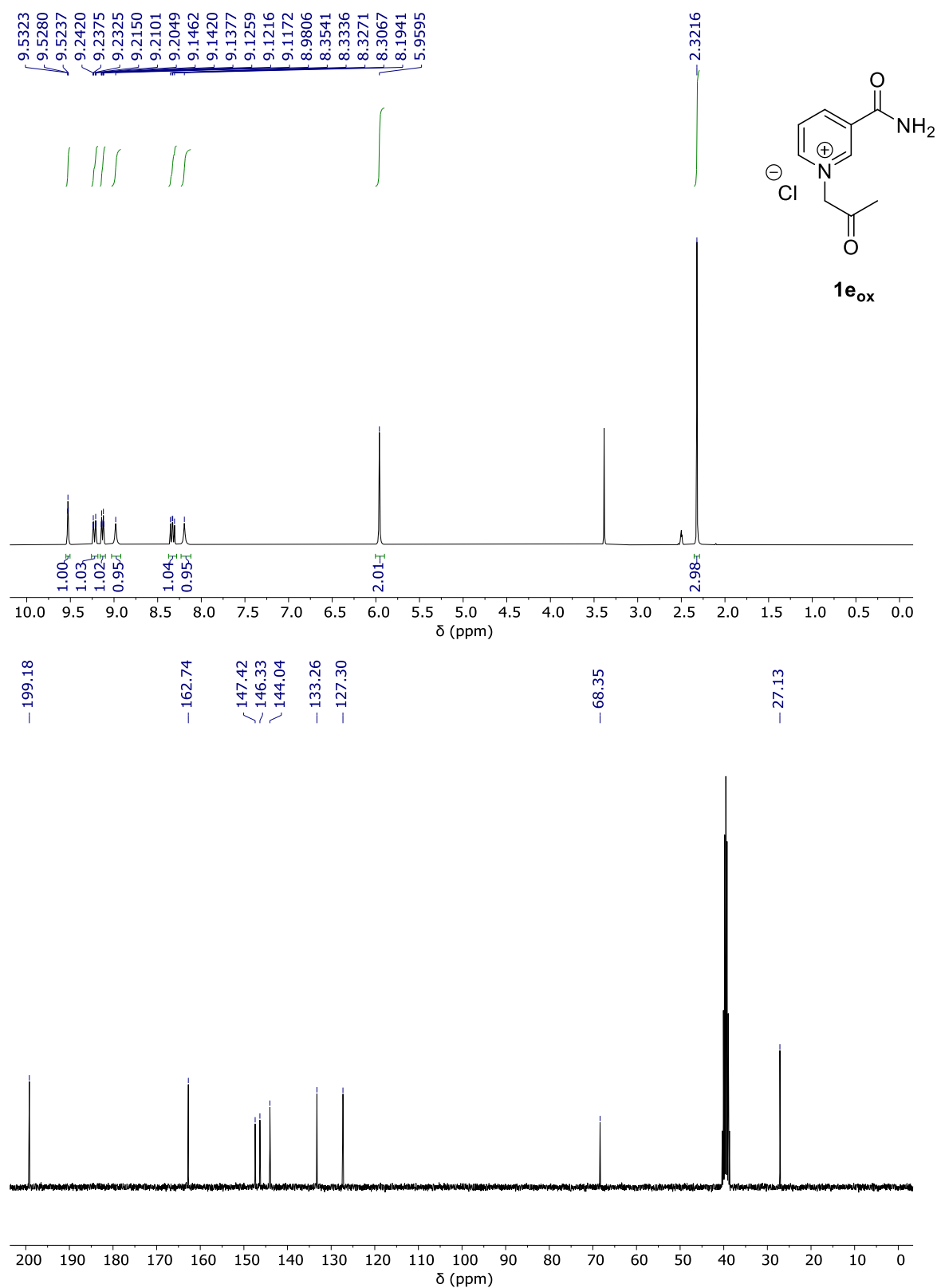


Figure S89. ¹H-NMR (300 MHz, DMSO) and ¹³C-NMR (75 MHz, DMSO) spectra of compound **1e_{ox}**.

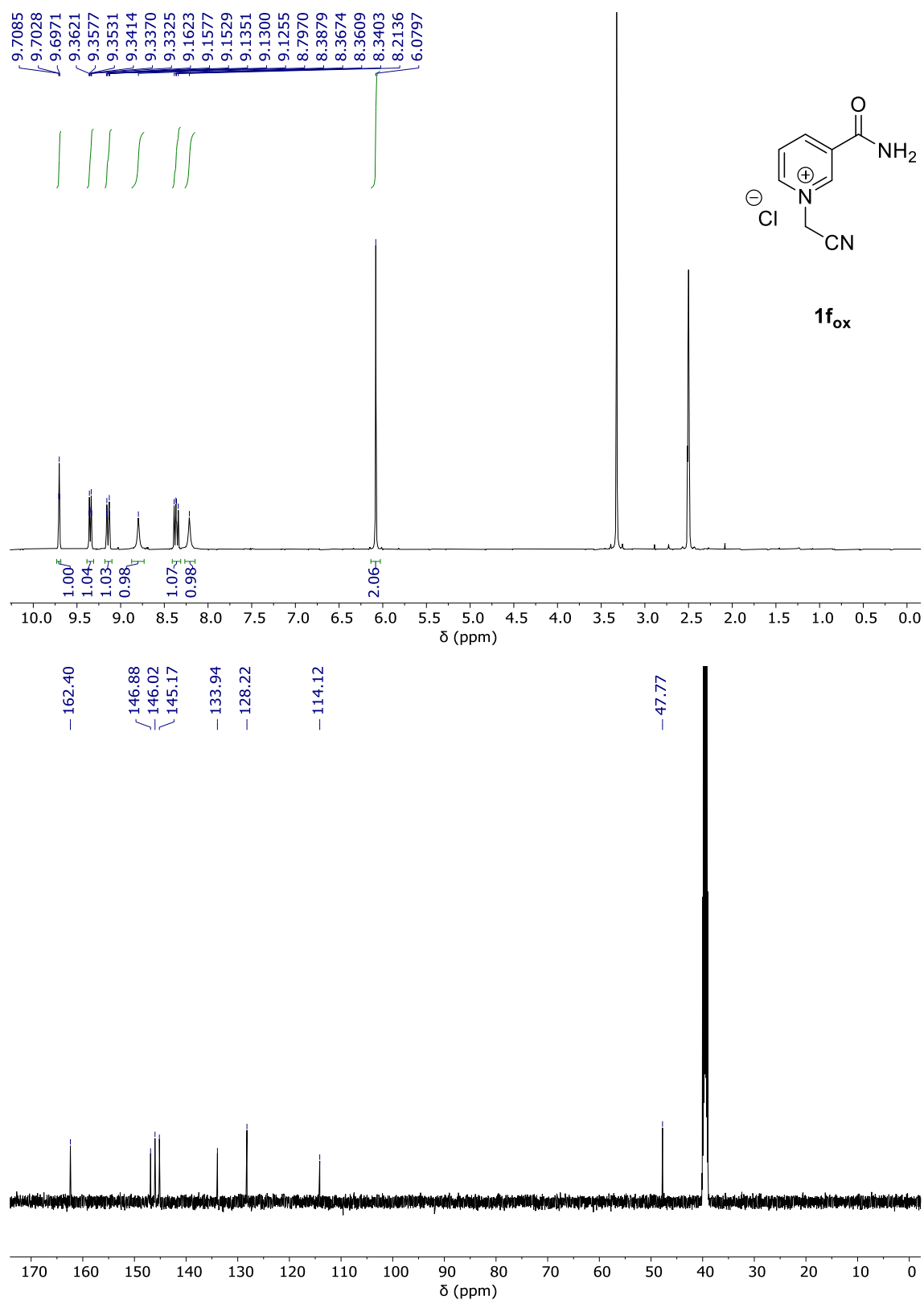


Figure S90. 1H -NMR (300 MHz, DMSO) and ^{13}C -NMR (126 MHz, DMSO) spectra of compound $1f_{ox}$.

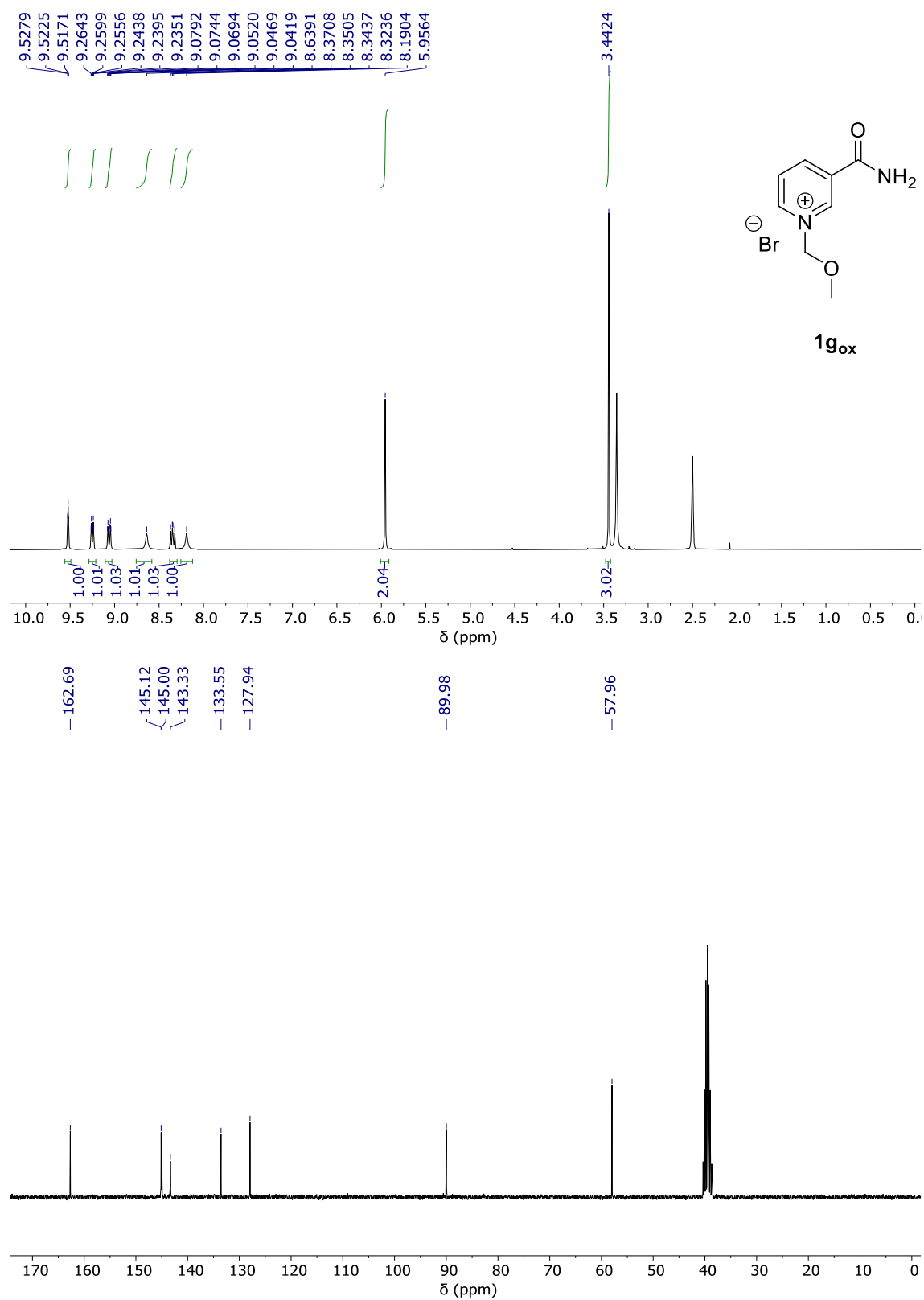


Figure S91. ¹H-NMR (300 MHz, DMSO) and ¹³C-NMR (75 MHz, DMSO) spectra of compound **19_{ox}**.

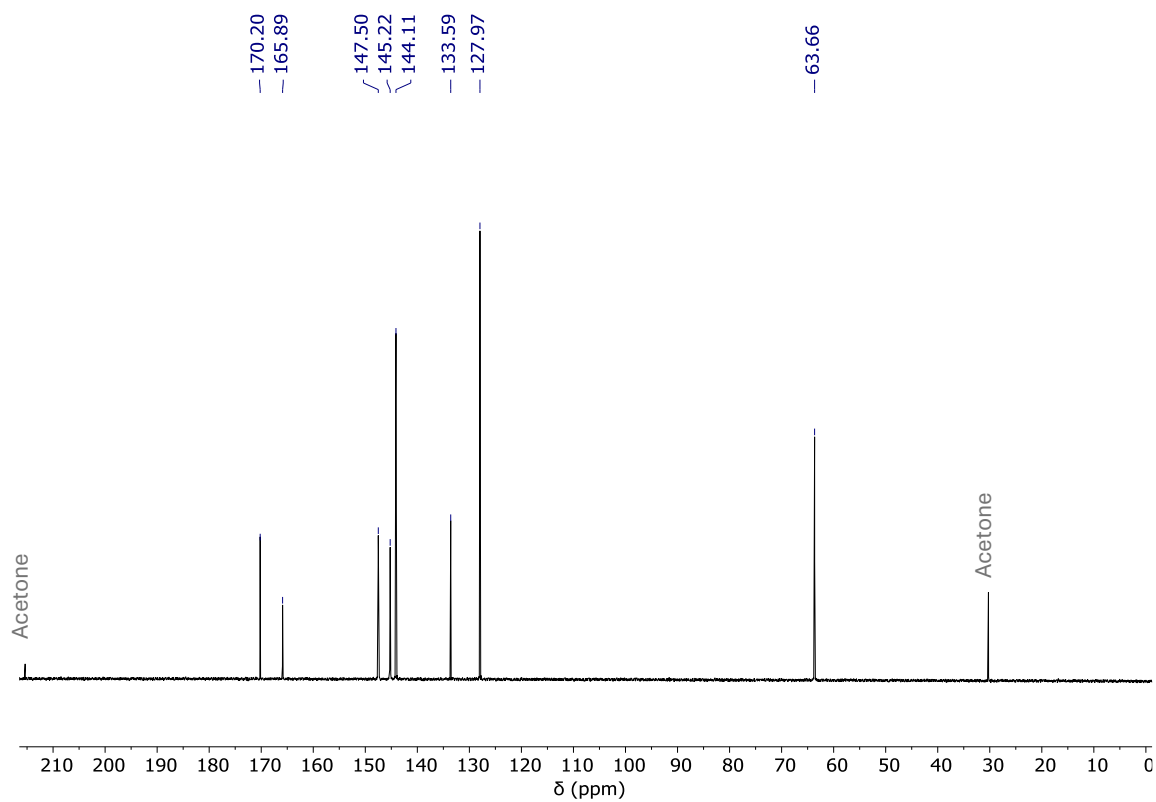
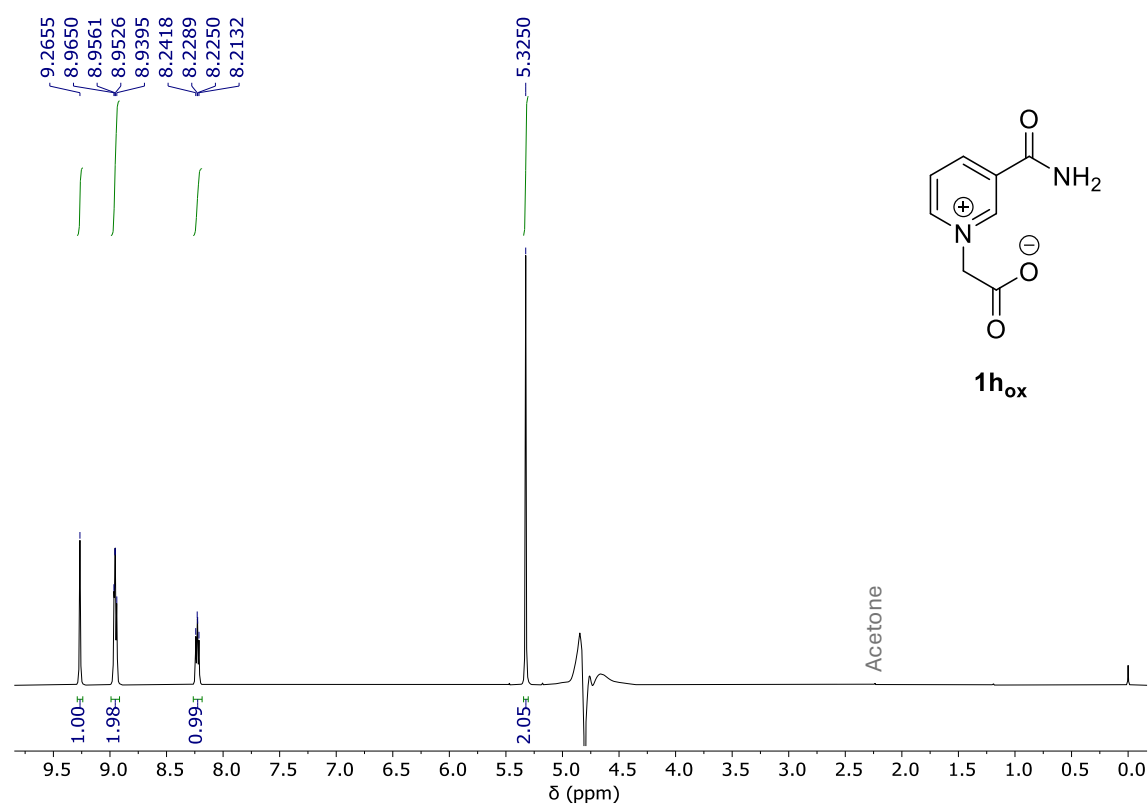


Figure S92. ¹H-NMR (500 MHz, H₂O+D₂O) and ¹³C-NMR (126 MHz, D₂O) spectra of compound **1h_{ox}**.

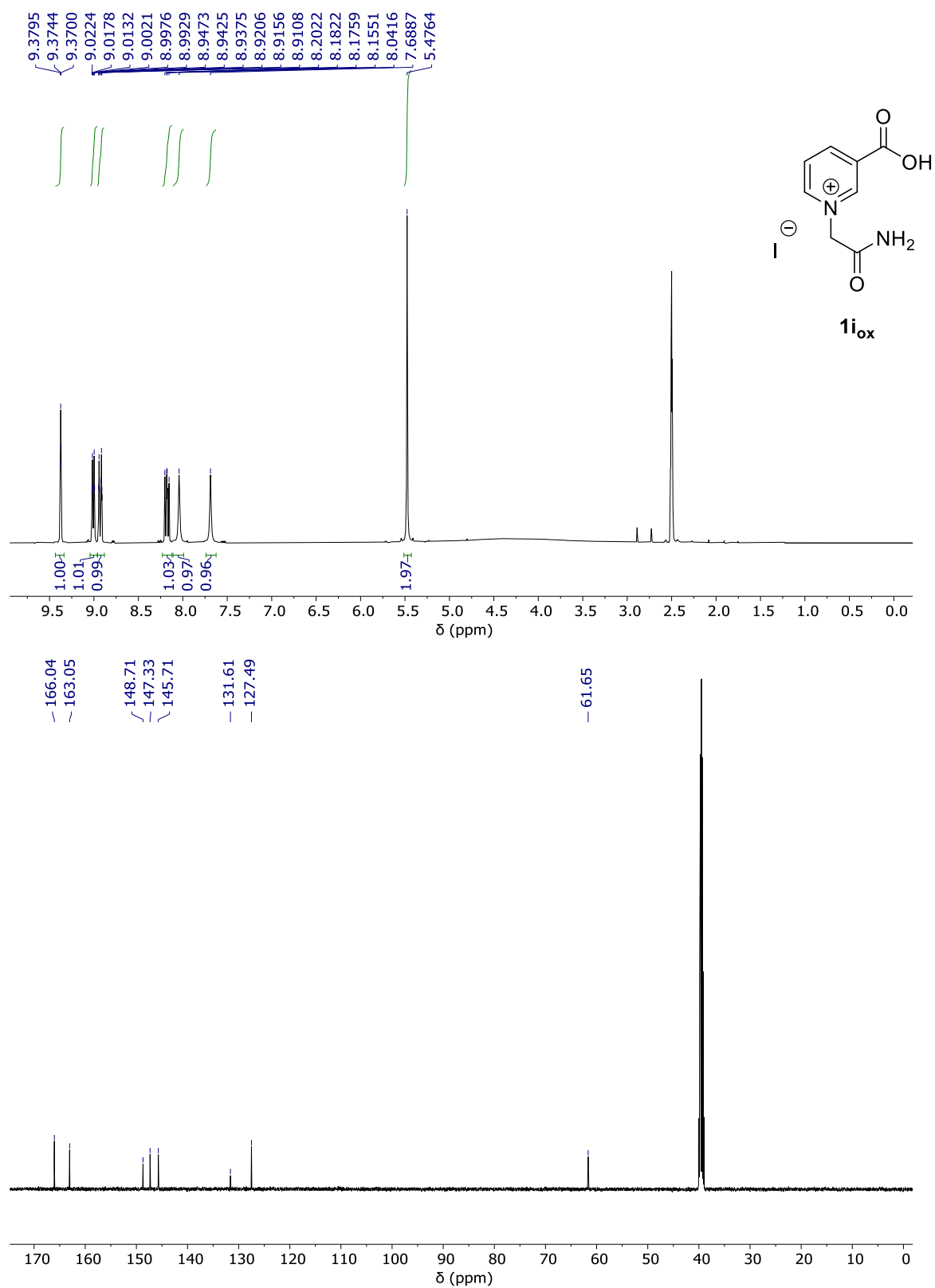


Figure S93. $^1\text{H-NMR}$ (300 MHz, DMSO) and $^{13}\text{C-NMR}$ (126 MHz, DMSO) spectra of compound $1i_{ox}$.

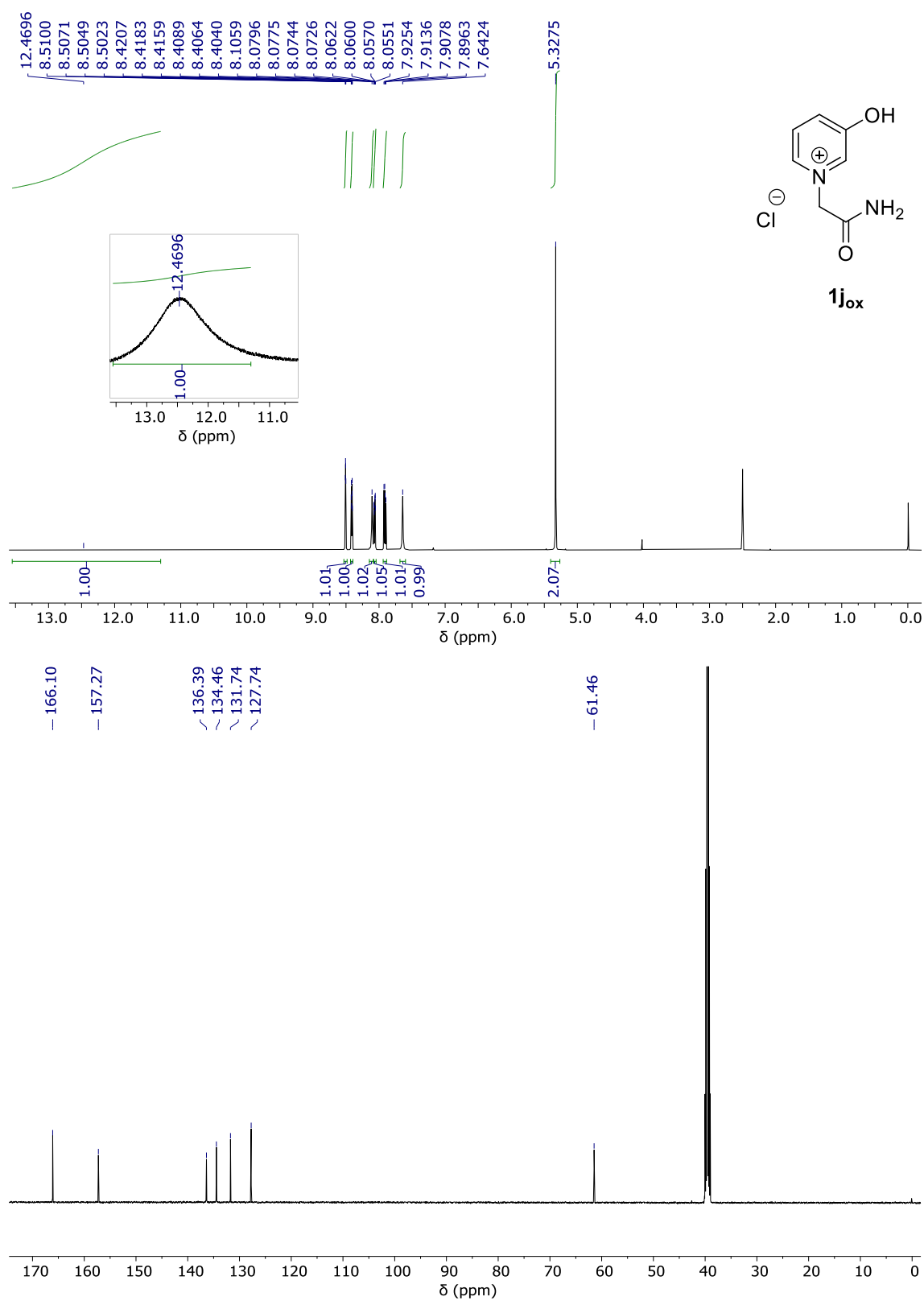


Figure S94. ¹H-NMR (500 MHz, DMSO) and ¹³C-NMR (126 MHz, DMSO) spectra of compound **1j_{ox}**.

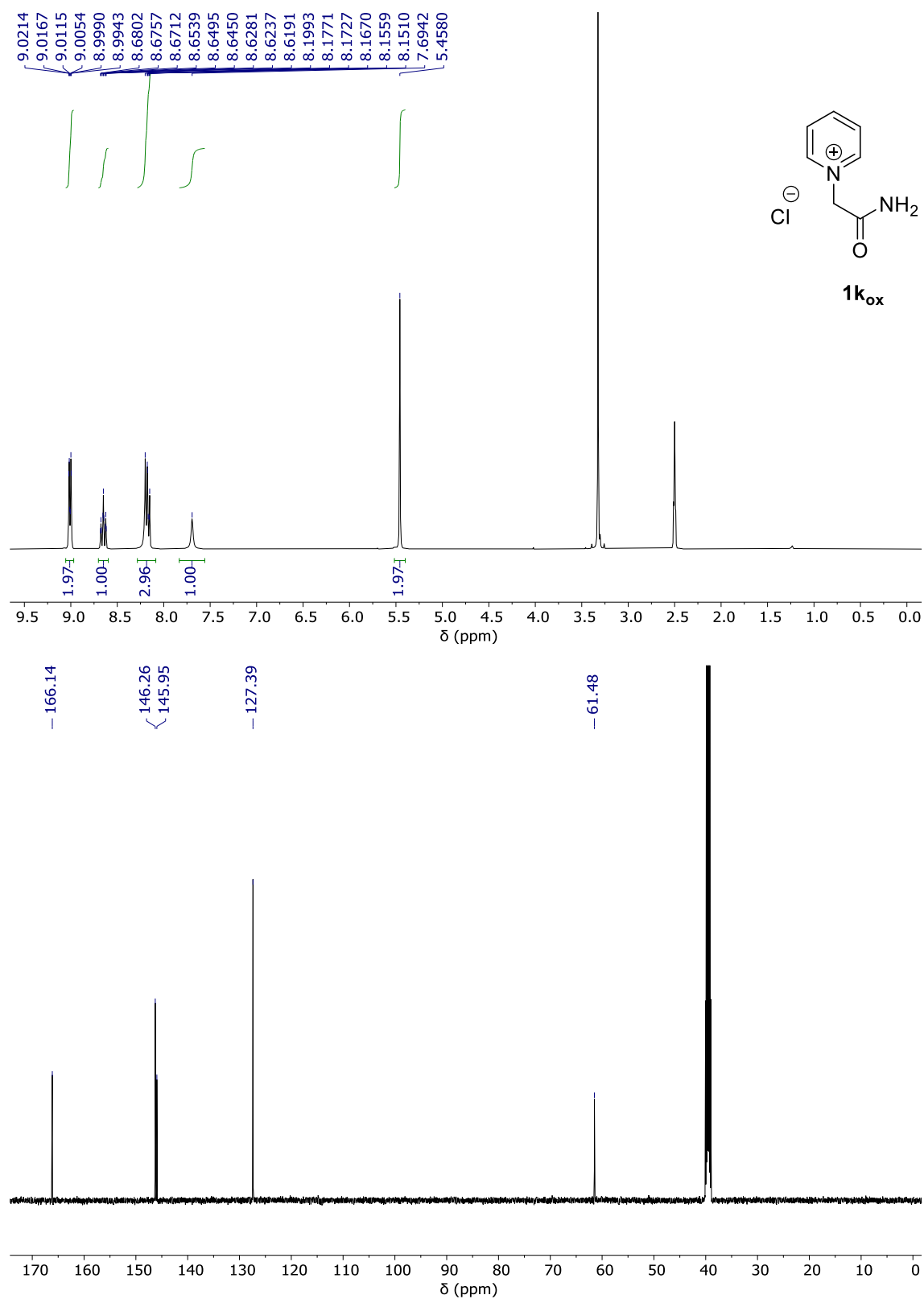


Figure S95. 1H -NMR (300 MHz, DMSO) and ^{13}C -NMR (126 MHz, DMSO) spectra of compound $1k_{ox}$.

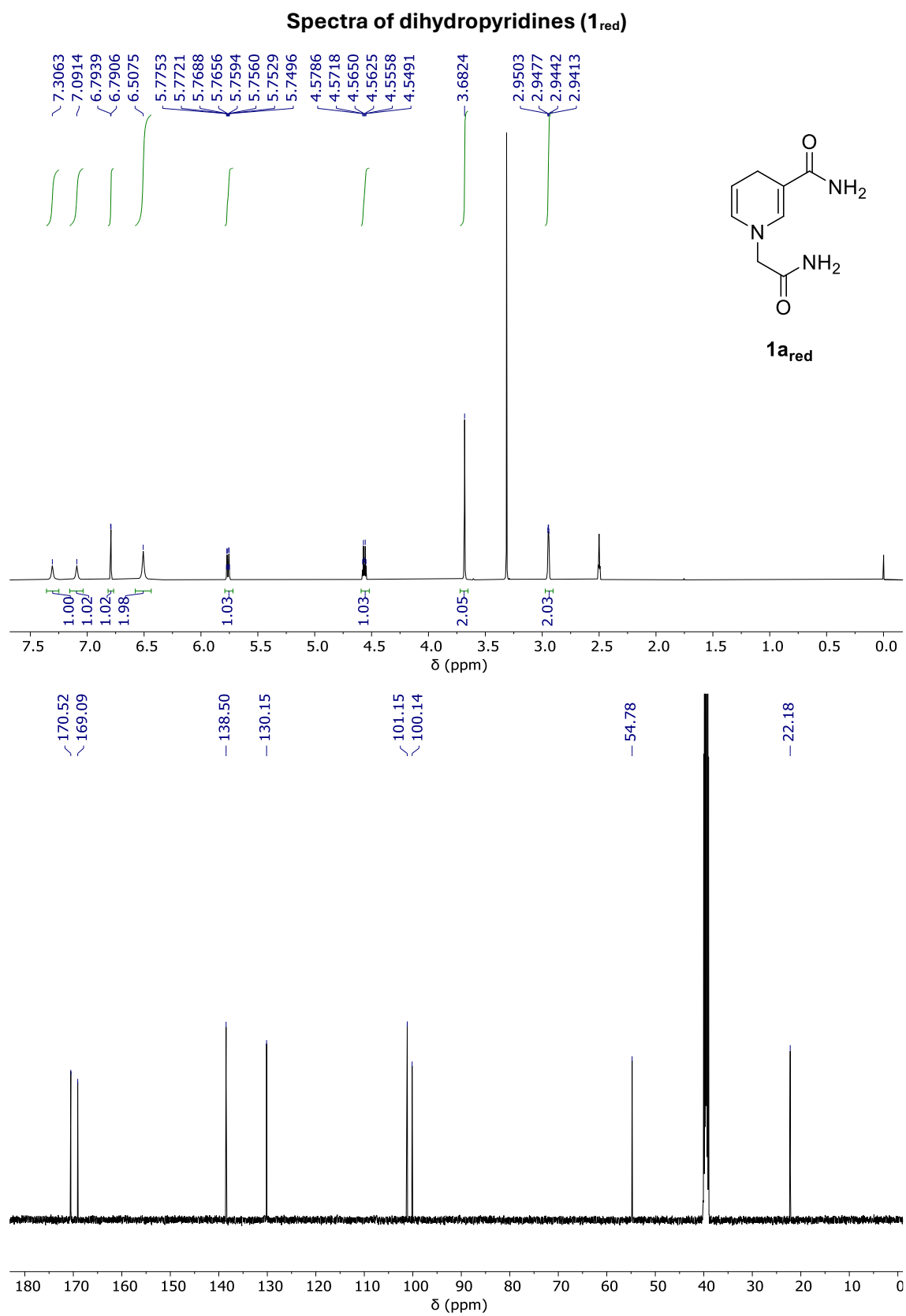


Figure S96. ^1H -NMR (500 MHz, DMSO) and ^{13}C -NMR (126 MHz, DMSO) spectra of compound **1_{red}**.

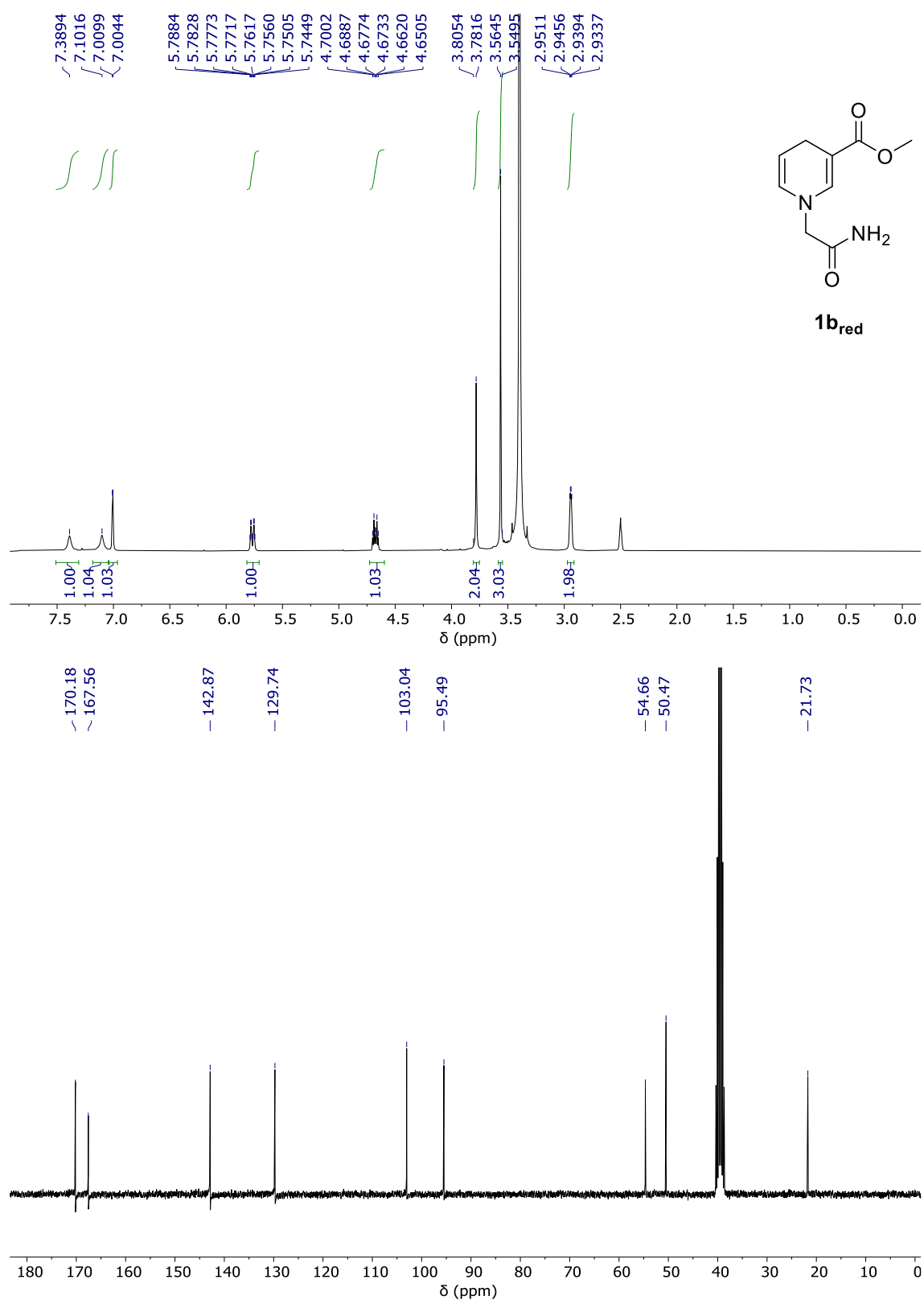


Figure S97. ¹H-NMR (300 MHz, DMSO) and ¹³C-NMR (75 MHz, DMSO) spectra of compound **1b_{red}**.

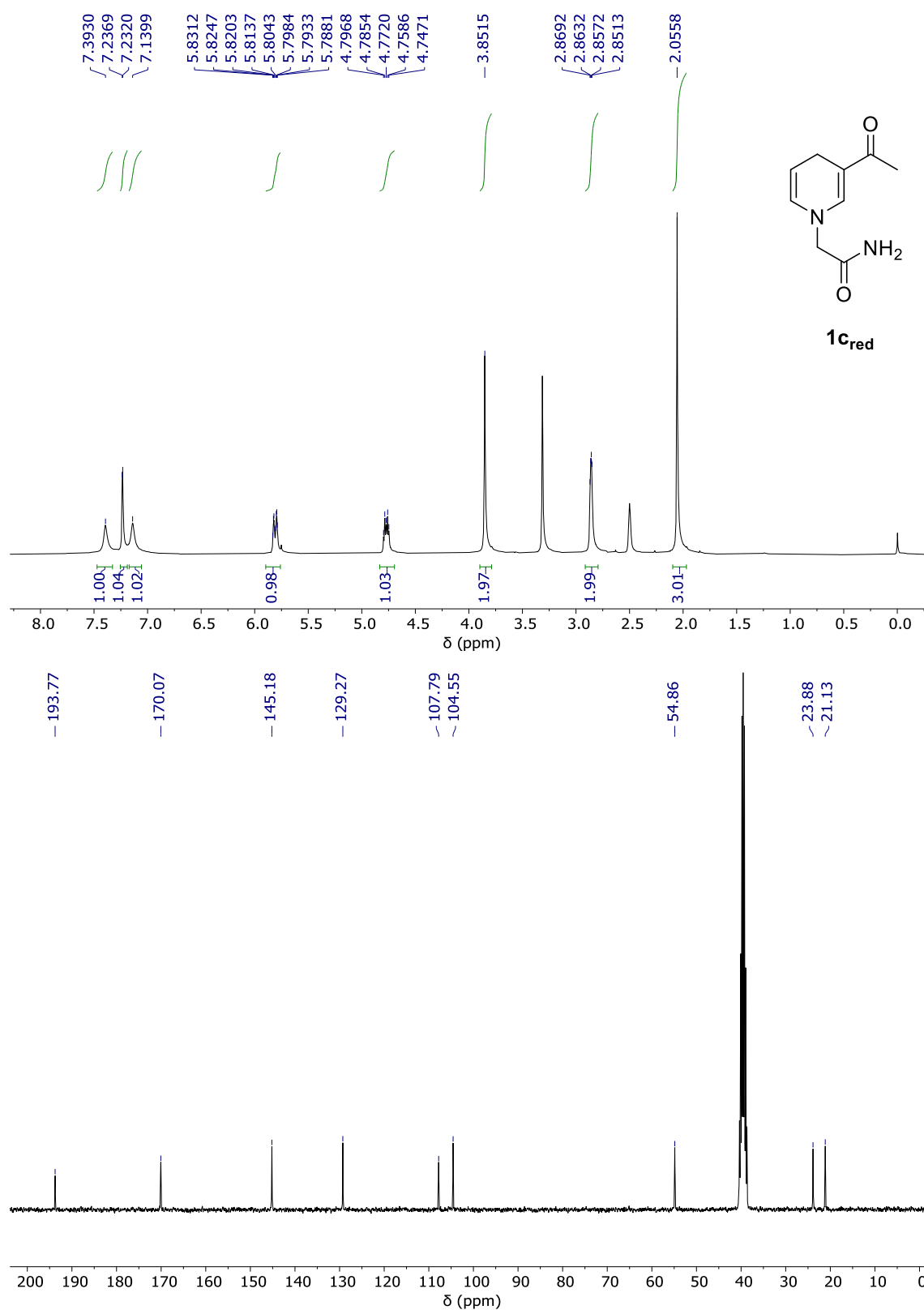


Figure S98. ¹H-NMR (300 MHz, DMSO) and ¹³C-NMR (75 MHz, DMSO) spectra of compound **1c_{red}**.

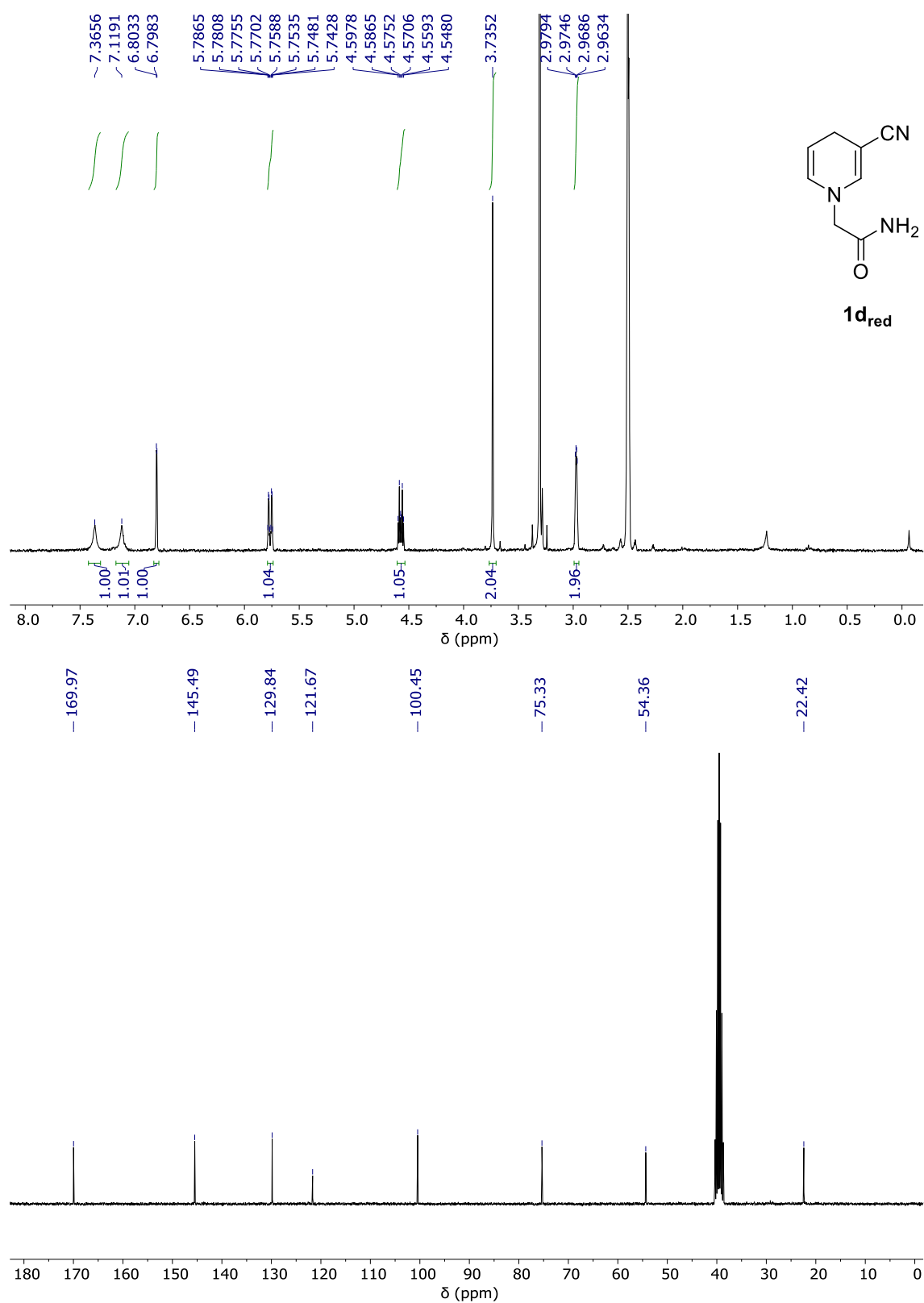


Figure S99. ¹H-NMR (300 MHz, DMSO) and ¹³C-NMR (75 MHz, DMSO) spectra of compound **1d_{red}**.

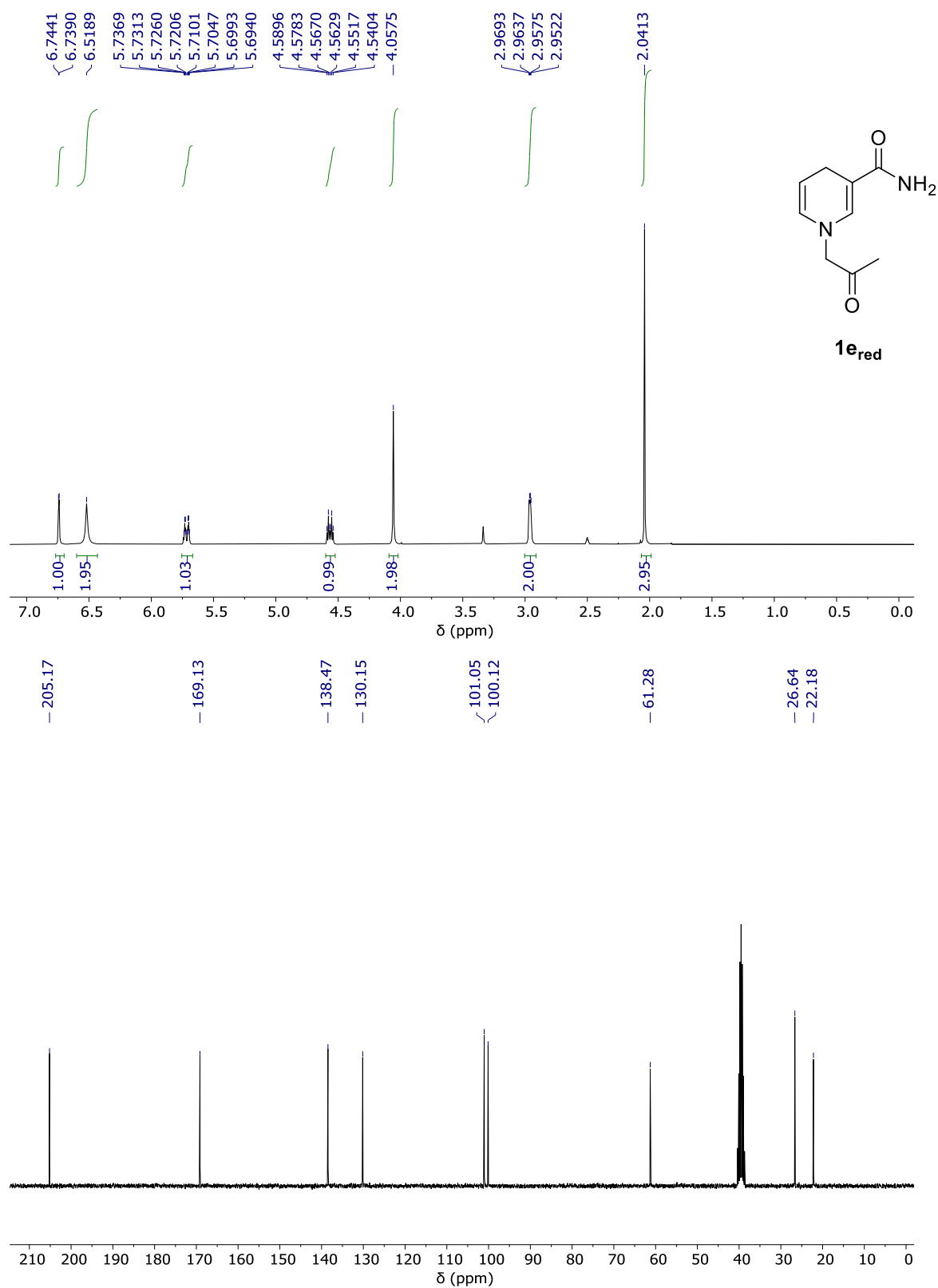


Figure S100. $^1\text{H-NMR}$ (300 MHz, DMSO) and $^{13}\text{C-NMR}$ (75 MHz, DMSO) spectra of compound $1e_{red}$.

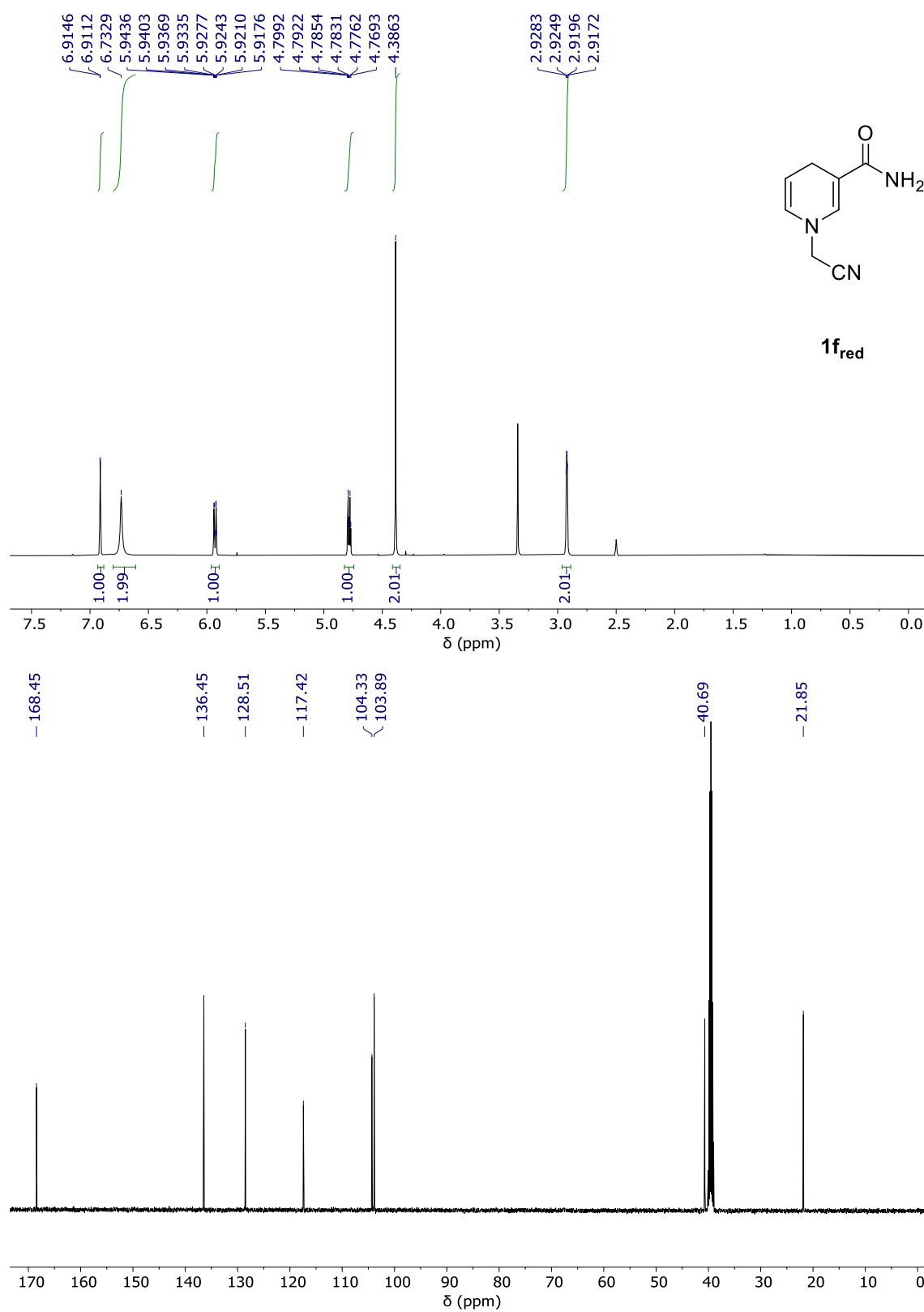


Figure S101. ¹H-NMR (500 MHz, DMSO) and ¹³C-NMR (126 MHz, DMSO) spectra of compound **1f_{red}**.

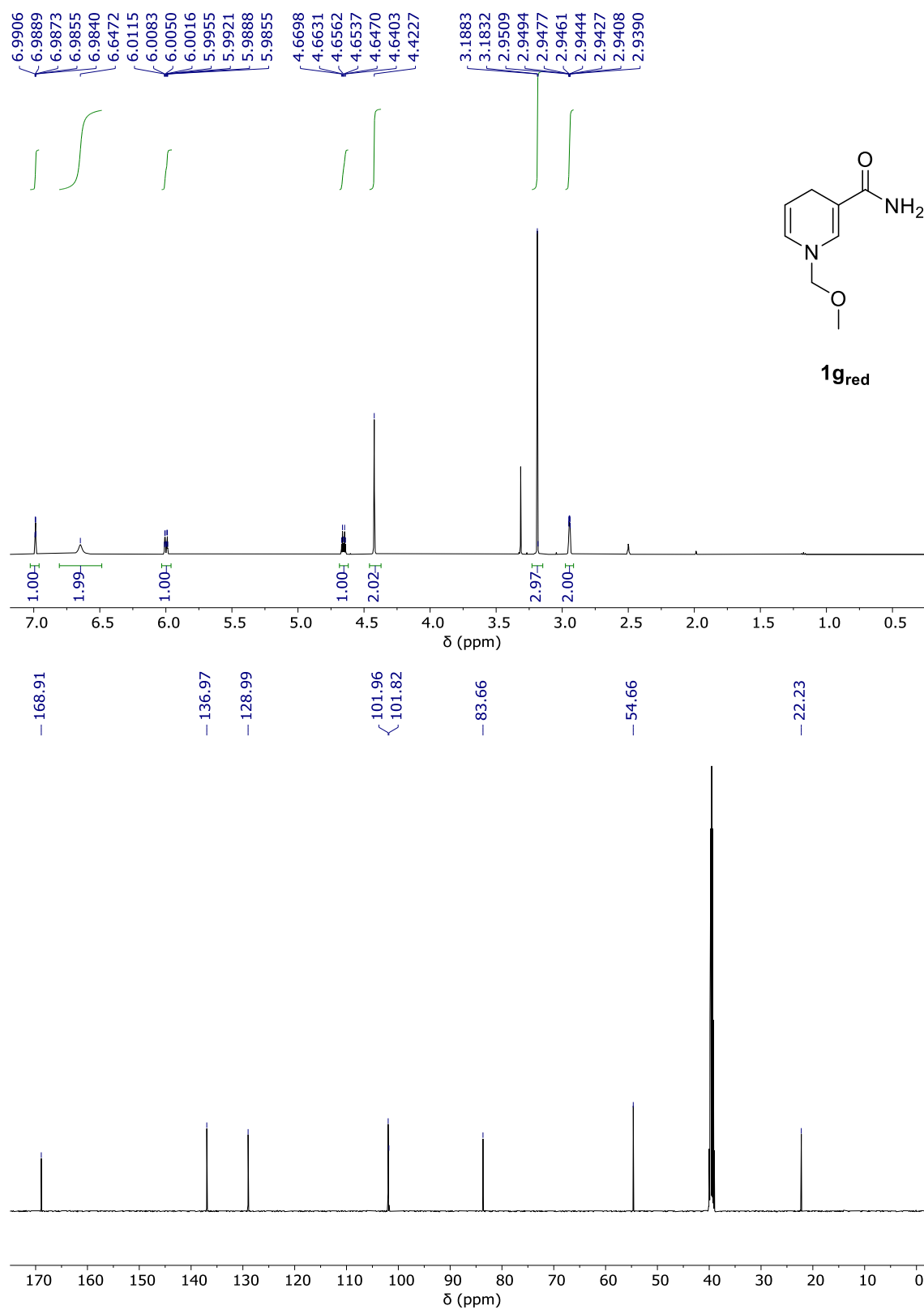


Figure S102. 1H -NMR (500 MHz, DMSO) and ^{13}C -NMR (126 MHz, DMSO) spectra of compound $1g_{red}$.

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