**Electronic Supporting Information for** 

## Mechanistic studies of the reactions of the reduced vitamin B<sub>12</sub> derivatives with the HNO donor Piloty's acid: further evidence for oxidation of cob(I)alamin by (H)NO

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**Fig. S1.** Plot of absorbance at 510 nm versus time for the reaction of Cbl(II) (1.00 x  $10^{-4}$  M) with 1.0 mol equiv PA at pH 10.00 ± 0.02 (25.0 °C, 0.10 M phosphate buffer, I = 1.0 M (NaCF<sub>3</sub>SO<sub>3</sub>)) under anaerobic conditions. The first order fit of the data gives observed rate constant ( $k_{obs}$ ) = (3.20 ± 0.01) x  $10^{-4}$  s<sup>-1</sup>.



**Fig. S2.** Plot of absorbance at 510 nm versus time for the reaction of Cbl(II) ( $5.00 \times 10^{-5} \text{ M}$ ) with excess PA ( $1.00 \times 10^{-3} \text{ M}$ ) at pH 9.00 ± 0.02 (25.0 °C, 0.10 M phosphate buffer, I = 1.0 M (NaCF<sub>3</sub>SO<sub>3</sub>)) under anaerobic conditions. The linear trend indicates that the rate of the reaction is independent of the Cbl(II) concentration.



**Fig. S3.** Plot of absorbance at 510 nm versus time for the reaction of Cbl(II) (1.00 x  $10^{-4}$  M) with 1.0 mol equiv PA at pH 9.00 ± 0.02 (25.0 °C, 0.10 M phosphate buffer, I = 1.0 M (NaCF<sub>3</sub>SO<sub>3</sub>)) under anaerobic conditions. The data fit to a first–order rate equation giving  $k_{obs} = (1.50 \pm 0.01)$  x  $10^{-4}$  s<sup>-1</sup>.



**Fig. S4.** Plot of absorbance at 510 nm versus time for the reaction of Cbl(II) (1.00 x  $10^{-4}$  M) with 1.0 mol equiv PA at pH 8.60 ± 0.02 (25.0 °C, 0.10 M phosphate buffer, I = 1.0 M (NaCF<sub>3</sub>SO<sub>3</sub>)) under anaerobic conditions. The data fit to a first–order rate equation giving  $k_{obs} = (0.58 \pm 0.01)$  x  $10^{-4}$  s<sup>-1</sup>.



**Fig. S5.** Plot of absorbance at 510 nm versus time for the reaction of Cbl(II) ( $5.00 \times 10^{-5} \text{ M}$ ) with excess PA ( $6.00 \times 10^{-3} \text{ M}$ ) at pH 8.00 ± 0.02 (25.0 °C, 0.10 M phosphate buffer, I = 1.0 M (NaCF<sub>3</sub>SO<sub>3</sub>)) under anaerobic conditions. The linear trend indicates that the rate of the reaction is independent of the Cbl(II) concentration.



**Fig. S6.** Plot of absorbance at 255 nm versus time for the reaction of Cbl(II) (1.00 x  $10^{-4}$  M) with 1.0 mol equiv PA at pH 8.00 ± 0.02 (25.0 °C, 0.10 M phosphate buffer, I = 1.0 M (NaCF<sub>3</sub>SO<sub>3</sub>)) under anaerobic conditions. The data fit to a first–order rate equation giving observed rate constant ( $k_{obs}$ ) = (0.15 ± 0.01) x  $10^{-4}$  s<sup>-1</sup>.



**Fig. S7.** Plot of absorbance at 510 nm versus time for the reaction of Cbl(II) ( $5.00 \times 10^{-5}$  M) with excess PA ( $1.00 \times 10^{-3}$  M) at pH 12.00 ± 0.02 ( $25.0 \degree$ C, 0.10 M phosphate buffer, I = 1.0 M (NaCF<sub>3</sub>SO<sub>3</sub>)) under anaerobic conditions. The linear trend indicates that the rate of the reaction is independent of the Cbl(II) concentration.



**Fig. S8.** Plot of absorbance at 510 nm versus time for the reaction of Cbl(II) (1.00 x  $10^{-4}$  M) with 1.0 mol equiv PA at pH 12.00 ± 0.02 (25.0 °C, 0.10 M phosphate buffer, I = 1.0 M (NaCF<sub>3</sub>SO<sub>3</sub>)) under anaerobic conditions. The data fit to a first–order rate equation giving  $k_{obs} = (3.93 \pm 0.01)$  x  $10^{-4}$  s<sup>-1</sup>.



**Fig. S9.** UV–vis spectra for equilibrated solutions of Cbl(II) ( $5.00 \times 10^{-5}$  M) with PA (0, 0.5–3.5 mol equiv) at pH 10.00 ( $25.0 \,^{\circ}$ C, 0.10 M phosphate buffer) under strictly anaerobic conditions. The product solutions were equilibrated for at least 15 min at 25.0  $^{\circ}$ C prior to UV–vis measurements.



**Fig. S10.** Plot of absorbance at **a**) 312 nm and **b**) 510 nm versus mol equiv PA for the reaction of Cbl(II) (5.00 x  $10^{-5}$  M) with PA (0, 0.5–3.5 mol equiv) at pH 10.00 (25.0 °C, 0.10 M phosphate buffer) under anaerobic conditions.



**Fig. S11.** UV–vis spectra recorded as a function of time (spectra recorded every 0.5 min) for the reaction between Cbl(I)<sup>–</sup> (2.00 x  $10^{-4}$  M) and 5.0 mol equiv PA at pH 10.00 (25.0 °C, 0.01 M carbonate buffer, I = 1.0 M (NaCF<sub>3</sub>SO<sub>3</sub>)) under anaerobic conditions. The reaction occurs via Cbl(II) intermediate ultimately leading to the formation of the final product, NOCbl. Isosbestic points occurring at 347, 417 and 542 nm are for the Cbl(I)<sup>–</sup>/Cbl(II) conversion<sup>1,2</sup> whereas those occurring at 377, 488 and 546 nm are for the formation of NOCbl product.<sup>3,4</sup>



**Fig. S12.** UV–vis spectra recorded as a function of time (spectra recorded every 0.5 min) for the reaction of Cbl(I)<sup>–</sup> (2.00 x  $10^{-4}$  M) with 1.0 mol equiv PA at pH 10.00 (25.0 °C, 0.01 M carbonate buffer, I = 1.0 M NaCF<sub>3</sub>SO<sub>3</sub>)) under strictly anaerobic conditions. Isosbestic points at 418 and 543 nm indicate the formation of Cbl(II) from Cbl(I)<sup>–</sup>. With close observation of the spectra, another set of isosbestics (374 and 545 nm) is evident corresponding to very slight formation of NOCbl.



**Fig. S13.** UV–vis spectra recorded as a function of time (spectra recorded every 1 min) for the reaction of excess Cbl(I)<sup>–</sup> (2.00 x  $10^{-4}$  M) with PA (2.00 x  $10^{-5}$  M) at pH 10.00 ± 0.02 (25.0 °C, 0.01 M carbonate buffer, I = 1.0 M (NaCF<sub>3</sub>SO<sub>3</sub>)) under strictly anaerobic conditions. The clean isosbestic points occurring at 344, 416 and 541 nm indicate the direct conversion of Cbl(I)<sup>–</sup> to Cbl(II).



**Fig. S14.** Plot of absorbance at 475 nm versus time for the reaction of excess Cbl(I)<sup>–</sup> (2.00 x 10<sup>-4</sup> M) with PA (2.00 x 10<sup>-5</sup> M) at pH 10.00 ± 0.02 (25.0 °C, 0.01 M carbonate buffer, I = 1.0 M (NaCF<sub>3</sub>SO<sub>3</sub>)) under strictly anaerobic conditions. The data fit well to a first order rate equation giving  $k_{obs} = (2.38 \pm 0.01) \times 10^{-4} \text{ s}^{-1}$ .



**Fig. S15.** Plot of absorbance at 250 nm versus time for the spontaneous decomposition of PA (1.00 x  $10^{-4}$  M) at pH 10.00 ± 0.1 (25.0 °C, 0.01 M carbonate buffer, I = 1.0 M (NaCF<sub>3</sub>SO<sub>3</sub>)) under strictly anaerobic conditions. The first–order fit of the data gives  $k_{obs} = (3.27 \pm 0.01) \times 10^{-4}$  s<sup>-1</sup>.



**Fig. S16. a)** UV–vis spectra of equilibrated solutions of Cbl(I)<sup>–</sup> (200  $\mu$ M) with PA (0, 1.0–5.0 mol equiv) at pH 10.00 (0.01 M carbonate buffer) under strictly anaerobic conditions. **b**) Corresponding plot of absorbance at 362 nm versus mol equiv PA for the same reaction. Note that Cbl(II) (blue trace) is formed with the addition of 1.0 equiv PA to the Cbl(I)<sup>–</sup> (red trace). The product solutions were equilibrated for at least 15 min at 25.0 °C prior to UV–vis measurements.



**Fig. S17.** UV–vis spectrum of the product mixture of the reaction between Cbl(I)<sup>–</sup> (200  $\mu$ M) with 1.0 mol equiv PA in the presence of 50.0 mol equiv NO<sub>2</sub><sup>–</sup> at pH 10.00 (25.0 °C, 0.10 M carbonate buffer) under strictly anaerobic conditions. The final spectrum of the product matches well with the spectrum of NOCbl (inset to Fig. S17). High Cbl(I)<sup>–</sup> concentrations are required to stabilize this complex in aqueous solution.



**Fig. S18.** UV–vis spectrum of the products of the reaction of Cbl(III) (HOCbl, 50.0  $\mu$ M) with 1.1 mol equiv PA in the presence of 50.0 mol equiv NO<sub>2</sub><sup>-</sup> at pH 10.00 (25.0 °C, 0.10 M carbonate buffer) under strictly anaerobic conditions. The product spectrum does not match with the spectrum of NOCbl (inset to Fig. S18), and instead resembles the reactant HOCbl.



## References

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