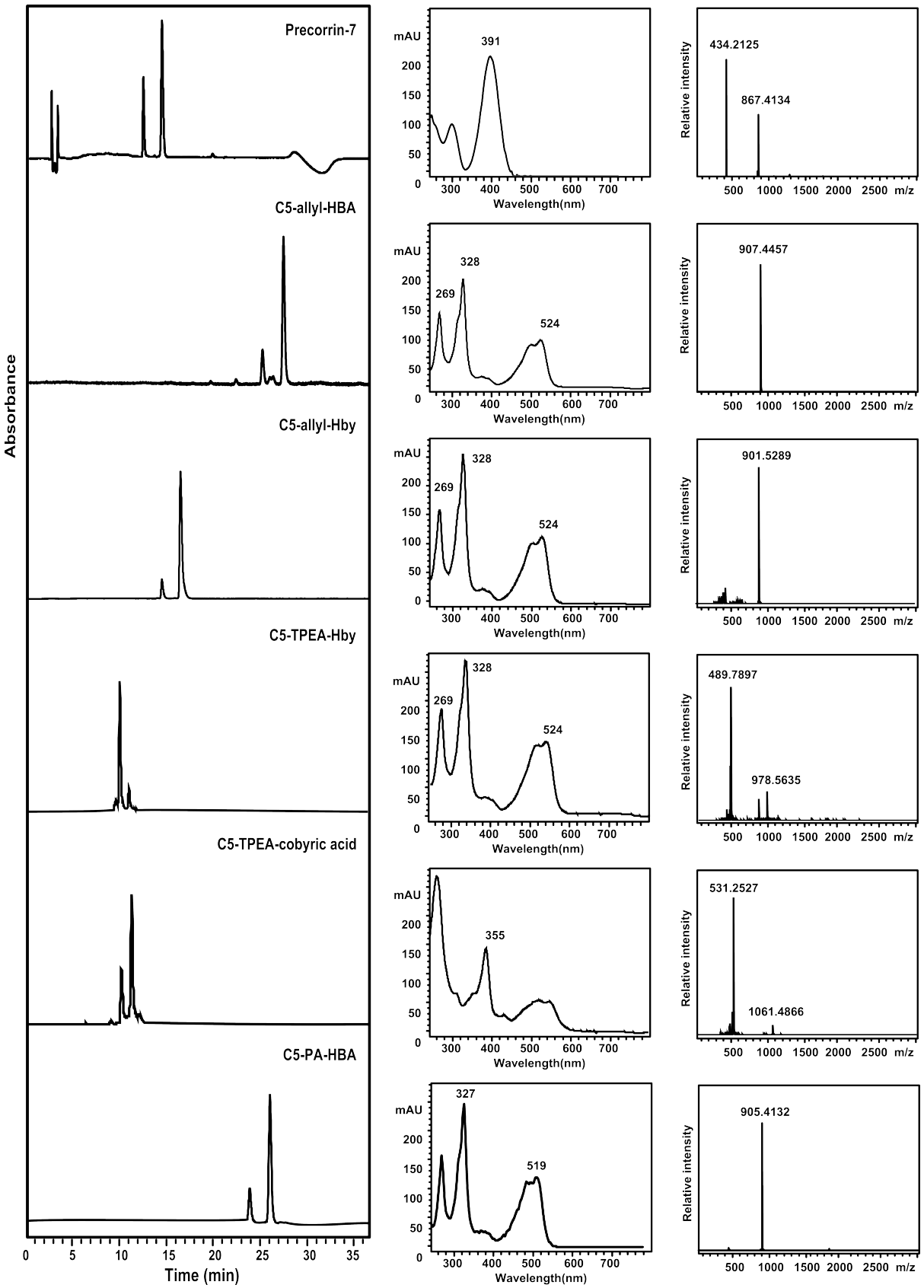
Cell Chemical Biology, Volume *25*

Supplemental Information

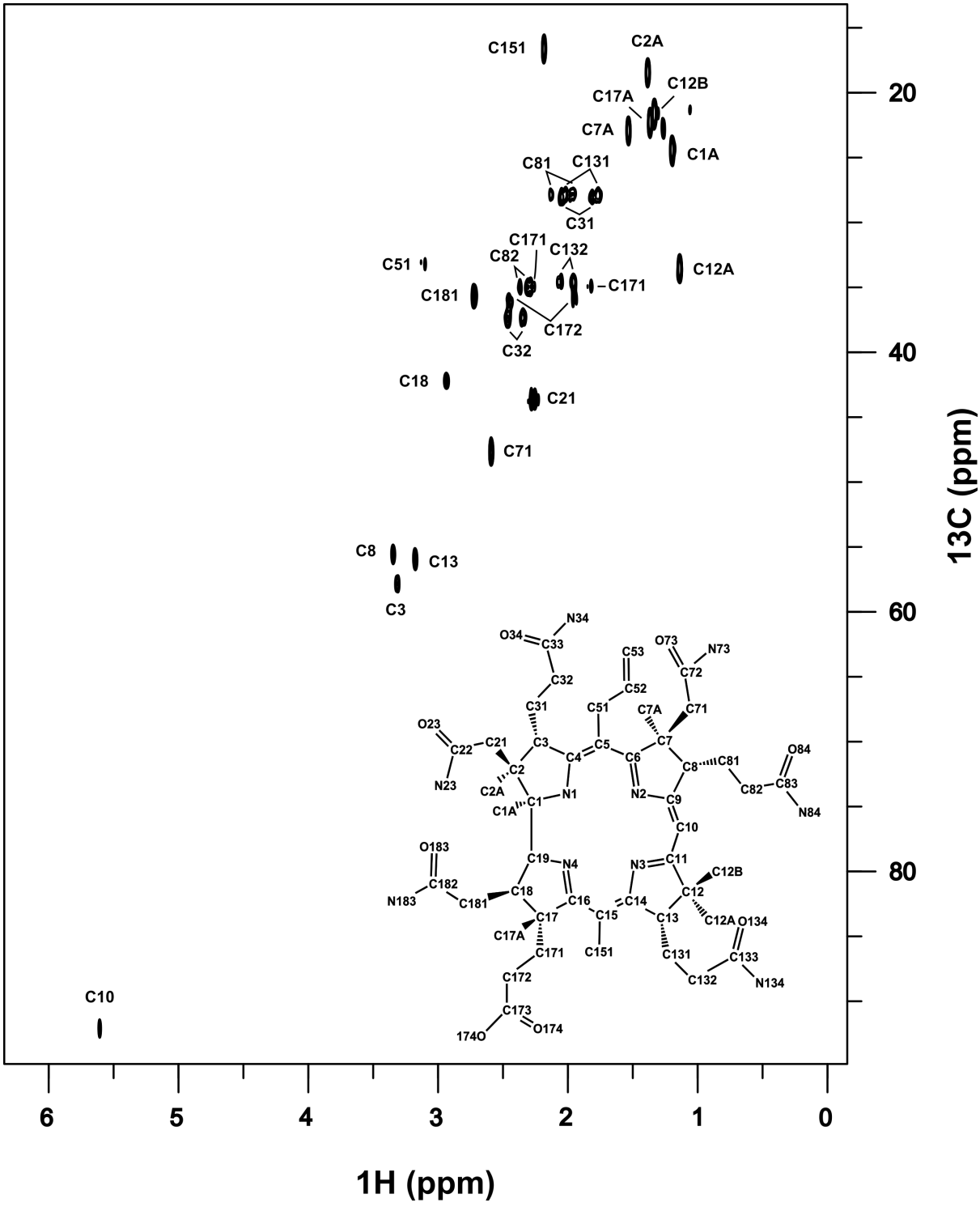
Construction of Fluorescent Analogs to Follow the Uptake and Distribution of Cobalamin (Vitamin B_{12}) in Bacteria, Worms, and Plants

Andrew D. Lawrence, Emi Nemoto-Smith, Evelyne Deery, Joseph A. Baker, Susanne Schroeder, David G. Brown, Jennifer M.A. Tullet, Mark J. Howard, Ian R. Brown, Alison G. Smith, Helena I. Boshoff, Clifton E. Barry III, and Martin J. Warren

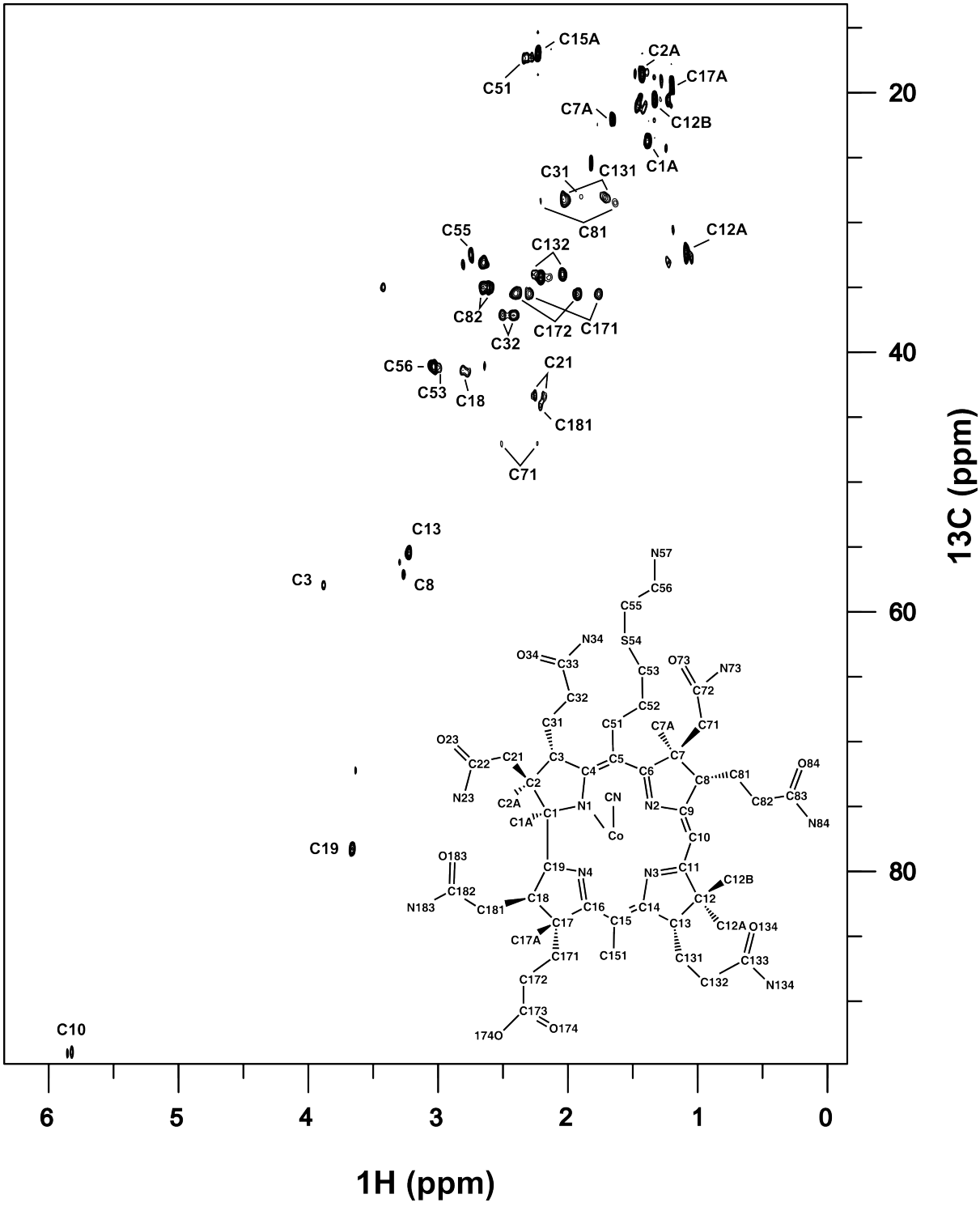
Supplementary Figure 1. HPLC-MS and UV absorption spectrum of C5 analogue synthesis intermediates. Related to Figures 2 and 4.



Supplementary Figure 2. 1H 13C HSQC of C5-allyl-Hby. Related to Figures 2 and 4.

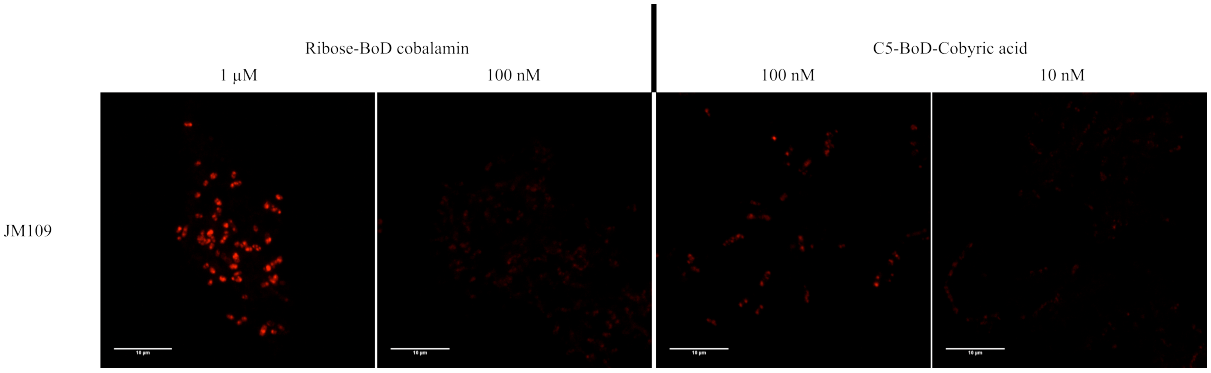


Supplementary Figure 3. 1H 13C HSQC Cyano-C5-TPEA-Cobyric acid. Related to Figures 2 and 4.

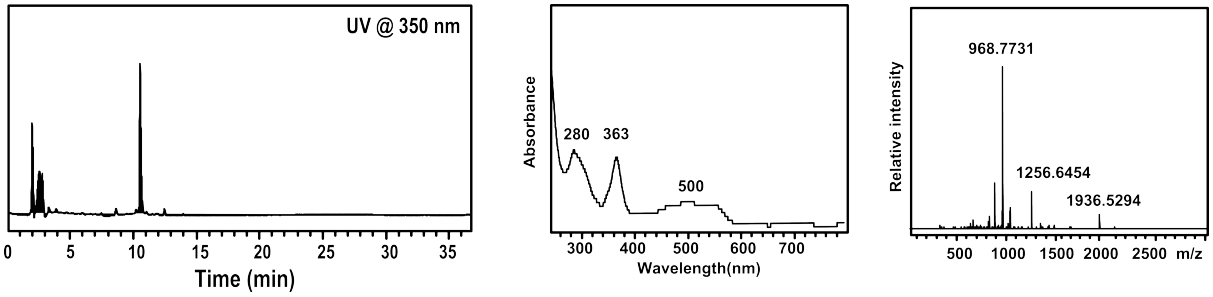


Supplementary Figure 4. Concentration dependence of the uptake of fluorescent analogues in *E. coli* JM109 – related to Figure 5.

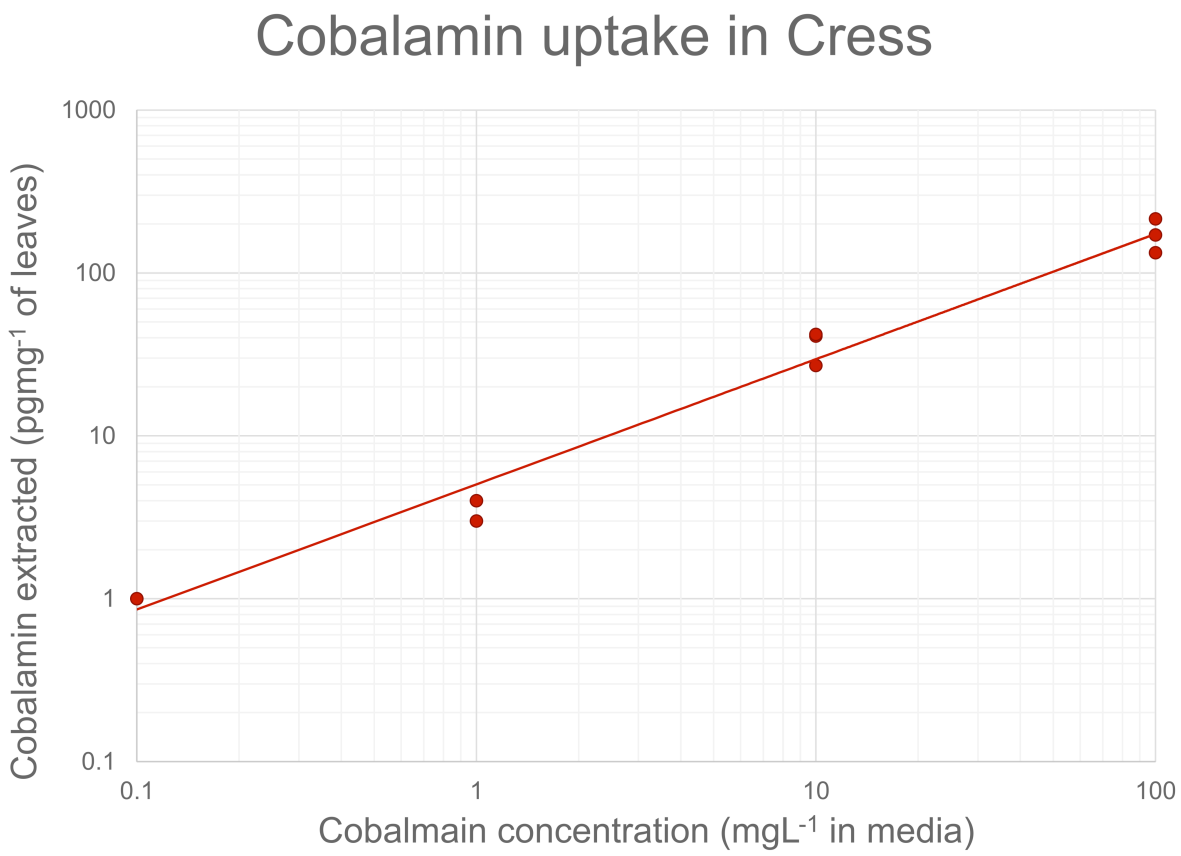
E. coli JM109 cells were grown overnight at 37°C in LB medium supplemented with either ribose-BoD-cobalamin or C5-BoD-cobyric acid at range of different concentrations. The cells were collected by centrifugation, washed 3 times in PBS, incubated for 15 minutes in 2% (w/v) formaldehyde in PBS and then washed a further 3 times in PBS. Cells (10 µL) were pipetted onto a 1.5 thickness coverslip before being inverted onto a drop of ProLong Gold antifade mountant (Life Technologies) on a glass slide. Slides were incubated at room temperature in the dark for 24 hours to cure. Cells were imaged by confocal microscopy. The red fluorescence observed within the cells indicates that *E. coli* has taken up both compounds. The ribose-BoD-cobalamin analogue could be observed down to a concentration of 100 nM but not at 10 nM. The C5-BoD-cobyric acid analogue could be observed at 10 nM supplementation but not at 1 nM.



Supplementary Figure 5. HPLC-MS and UV absorption of the fluorescent analogues extracted from *E. coli*. Related to Figure 5.



Supplementary Figure 6. Cobalamin uptake in *L. sativum*. Related to Figure 7.



Supplementary Table 1. Parameters of the crystal structure of CobH(T85A) complexed with C5-allyl-HBA. Related to Figure 3.

	Co-crystal CobH (T85A) allyl-HBA
Wavelength (Å)	0.92819
High resolution limit (Range)	1.57 (7.02 - 1.57)
Low resolution limit (Range)	48.38 (48.38 - 1.61)
Completeness	98.8
Multiplicity	3.3
$< I/\sigma(I) >$ [1]	1.37 (at 1.57Å)
R_{merge}	0.237
Unit cell dimensions: a (Å)	71.230
b (Å)	66.630
c (Å)	48.920
α (°)	90.000
β (°)	99.030
γ (°)	90.000
Spacegroup	C 1 2 1
	Final
R factor	0.1723
R free	0.2384
Rms Bond Length (Å)	0.0304
Rms Bond Angle (°)	2.7310

[1]Xtriage's analysis on translational NCS is as follows: The largest off-origin peak in the Patterson function is 8.41% of the height of the origin peak. No significant pseudotranslation is detected.

Supplementary Table 2. NMR chemical shift assignments for C5-allyl-Hby and Cyano-C5-TPEA-Cby in D_2O. Related to Figures 2 and 4.

	C5-allyl-Hby		C5-TPEA-Cby	
	δ(1H) [ppm]	δ(13C) [ppm]	δ(1H) [ppm]	δ(13C) [ppm]
C1		83.2		85.34
C1A	1.20	24.4	1.43	24.28
C2		48.8		49.27
C2A	1.39	18.4	1.46	19.19
C21	2.27	43.6	2.21, 2.28	43.74
C22				178.09
C3	3.31	57.8	3.89	58.16
C31	1.82, 2.05	28.0	1.93, 2.24	28.56
C32	2.35, 2.47	37.4	2.45, 2.53	37.55
C33				183.99
C4				179.48
C5				108.28
C51	3.11, 3.51	33.1	2.35	17.90
C52	5.97	140.2	1.61, 1.84	34.42
C53	4.96, 5.07	119.0	3.02	41.58
C55			2.77	32.96
C56			3.06	41.48
C6		162.5		166.71
C7		49.1		52.09
C7A	1.53	22.9	1.7	22.60
C71	2.59	47.7	2.28, 2.54	47.23
C72		177.7		177.67
C8	3.35	55.6	3.28	57.35
C81	1.96, 2.14	27.8	1.67, 2.24	28.96
C82	2.31, 2.37	35.0	2.64, 2.68	35.45
C83				178.83
C9		171.4		174.70
C10	5.61	92.1	5.82	93.80
C11		193.3		180.65
C12		51.7		49.78
C12A	1.33	21.6	1.37	21.07
C12B	1.14	33.6	1.13	32.89
C13	3.18	56.0	3.24	55.67
C131	1.76, 2.02	28.0	1.75, 2.06	28.68
C132	1.96, 2.07	34.6	2.07, 2.29	34.45
C133				181.83
C14				165.44
C15		105.7		106.92
C151	2.18	16.6	2.26	17.59
C16		182.1		180.71
C17		60.1		61.92
C17A	1.37	22.2	1.24	20.00
C171	1.84, 2.27	34.9	1.96, 2.32	35.96
C172	1.95, 2.45	36.1	1.80, 2.43	35.87

C173				180.63
C18	2.94	42.2	2.82	41.83
C181	2.72	35.7	2.24	44.51
C182		178.9		180.60
C19	4.29	69.5	3.68	78.28