SUPPORTING INFORMATION

Guided cobamide biosynthesis for heterologous production of reductive dehalogenases

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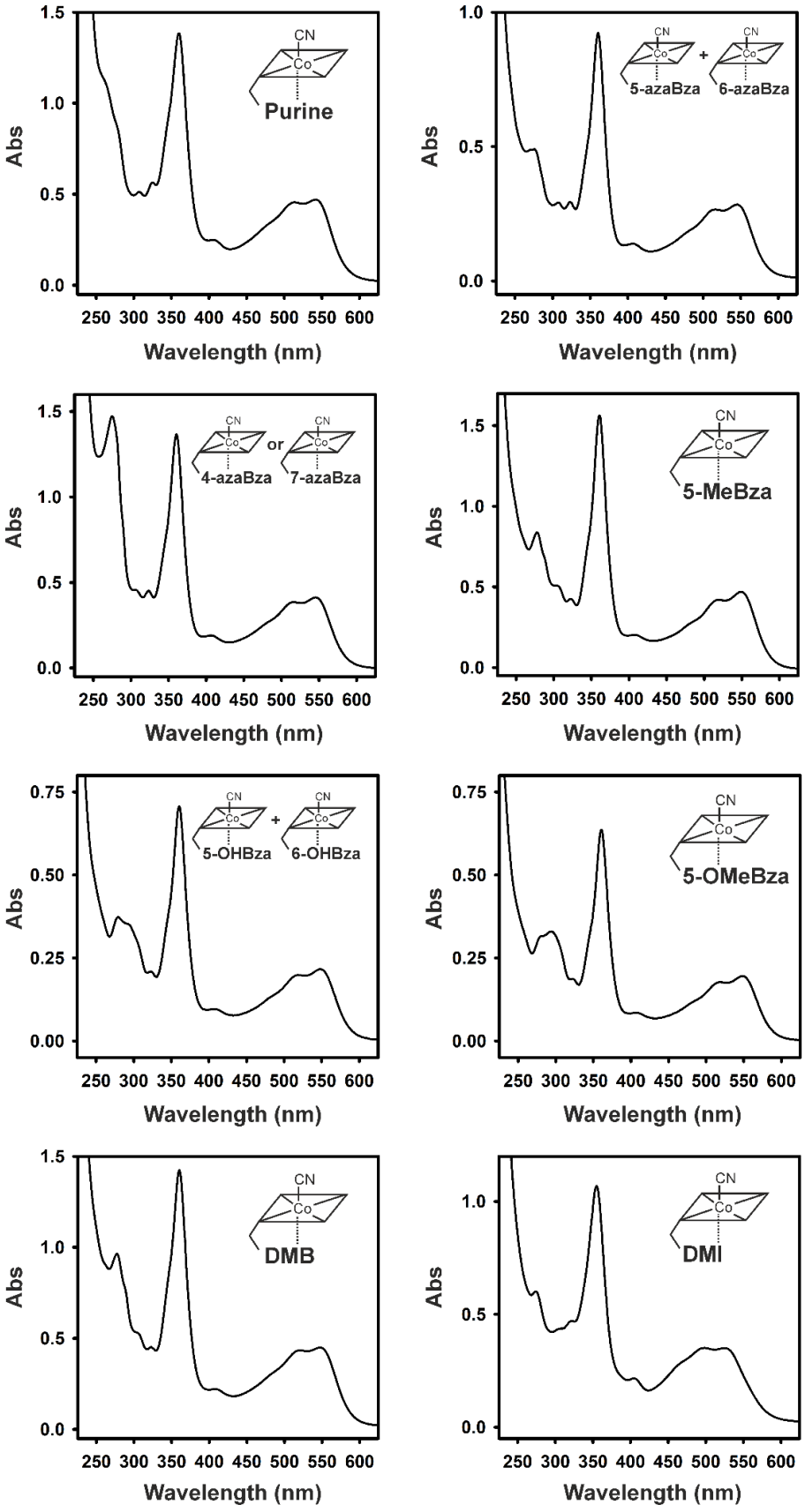


Figure S1: UV/Vis-absorbance spectra of the purified Cbas from *Desulfitobacterium hafniense* strain DCB-2.

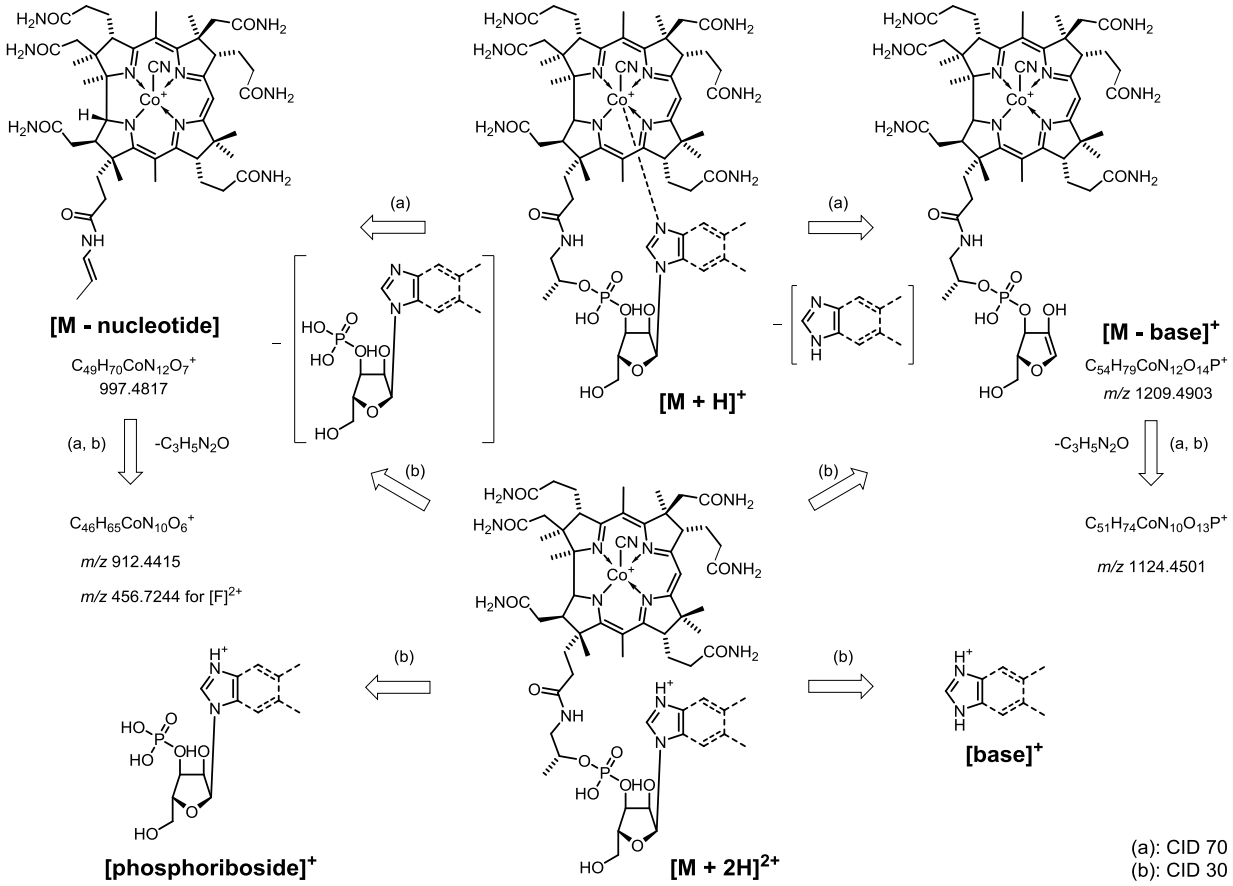


Figure S2. MS/MS fragmentation of cobamides using $[M+H]^+$ and $[M+2H]^{2+}$ precursor ions with a CID energy of 70 and 30, respectively.

Table S1: HPLC-ESI-(+)-HR-MS/MS data of a vitamin B_{12} standard.

	Molecular formula	calc. *m/z*	obs. *m/z*	Δ (ppm)
$[M+H]^+$	$[C_{63}H_{89}CoN_{14}O_{14}P]^+$	1355.5747	1355.5738	0.7
$[M+2H]^{2+}$	$[C_{63}H_{90}CoN_{14}O_{14}P]^{2+}$	678.2910	678.2912	0.3
MS/MS[a]	$[C_{54}H_{79}CoN_{12}O_{14}P]^+$	1209.4903	1209.4893	0.8
MS/MS[a]	$[C_{51}H_{74}CoN_{10}O_{13}P]^+$	1124.4501	1124.4490	1.0
MS/MS[a]	$[C_{49}H_{70}CoN_{12}O_7]^+$	997.4817	997.4805	1.2
MS/MS[a]	$[C_{46}H_{65}CoN_{10}O_6]^+$	912.4415	912.4403	1.3
MS/MS[b]	$[C_{46}H_{66}CoN_{10}O_6]^{2+}$	456.7244	456.7246	0.4
MS/MS[b]	$[C_{14}H_{20}N_2O_7P]^+$	359.1003	359.1003	0.0
MS/MS[b]	$[C_9H_{11}N_2]^+$	147.0917	147.0918	0.7

a: MS/MS of $[M+H]^+$ at CID 70; b: MS/MS of $[M+2H]^{2+}$ at CID 30.

Table S2: HPLC-ESI-(+)-HR-MS/MS data of the purinyl cobamide (signal **1** in Fig. 1) from *D. hafniense* strain DCB-2 supplemented with YE, but without other additives.

	Molecular formula	calc. *m/z*	obs. *m/z*	Δ (ppm)
$[M+H]^+$	$[C_{59}H_{83}CoN_{16}O_{14}P]^+$	1329.5339	1329.5326	1.0
$[M+2H]^{2+}$	$[C_{59}H_{84}CoN_{16}O_{14}P]^{2+}$	665.2726	665.2706	3.0
MS/MS[a]	$[C_{54}H_{79}CoN_{12}O_{14}P]^+$	1209.4903	1209.4890	1.1
MS/MS[a]	$[C_{51}H_{74}CoN_{10}O_{13}P]^+$	1124.4501	1124.4485	1.4
MS/MS[a]	$[C_{49}H_{70}CoN_{12}O_7]^+$	997.4817	997.4800	1.7
MS/MS[a]	$[C_{46}H_{65}CoN_{10}O_6]^+$	912.4415	912.4403	1.3
MS/MS[b]	$[C_{46}H_{66}CoN_{10}O_6]^{2+}$	456.7244	456.7242	0.4
MS/MS[b]	$[C_{10}H_{14}N_4O_7P]^+$	333.0595	333.0596	0.3
MS/MS[b]	$[C_5H_5N_4]^+$	121.0509	121.0504	4.1

a: MS/MS of $[M+H]^+$ at CID 70; b: MS/MS of $[M+2H]^{2+}$ at CID 30.

Table S3: HPLC-ESI-(+)-HR-MS/MS data of the 5-azabenzimidazolyl cobamide (signal **2** in Fig. 1) from *D. hafniense* strain DCB-2 supplemented with YE, but without other additives.

	Molecular formula	calc. *m/z*	obs. *m/z*	Δ (ppm)
$[M+H]^+$	$[C_{60}H_{84}CoN_{15}O_{14}P]^+$	1328.5386	1328.5376	0.8
$[M+2H]^{2+}$	$[C_{60}H_{85}CoN_{15}O_{14}P]^{2+}$	664.7730	664.7729	0.2
MS/MS[a]	$[C_{54}H_{79}CoN_{12}O_{14}P]^+$	1209.4903	1209.4896	0.6
MS/MS[a]	$[C_{51}H_{74}CoN_{10}O_{13}P]^+$	1124.4501	1124.4487	1.2
MS/MS[a]	$[C_{49}H_{70}CoN_{12}O_7]^+$	997.4817	997.4811	0.6
MS/MS[a]	$[C_{46}H_{65}CoN_{10}O_6]^+$	912.4415	912.4405	1.1
MS/MS[b]	$[C_{46}H_{66}CoN_{10}O_6]^{2+}$	456.7244	456.7241	0.7
MS/MS[b]	$[C_{11}H_{15}N_3O_7P]^+$	332.0642	332.0640	0.6
MS/MS[b]	$[C_6H_6N_3]^+$	120.0556	120.0551	4.2

a: MS/MS of $[M+H]^+$ at CID 70; b: MS/MS of $[M+2H]^{2+}$ at CID 30.

Table S4: HPLC-ESI-(+)-HR-MS/MS data of the 5,6-dimethylbenzimidazolyl cobamide from *D. hafniense* strain DCB-2 supplemented with YE and 5,6-dimethylbenzimidazole (DMB).

	Molecular formula	calc. *m/z*	obs. *m/z*	Δ (ppm)
$[M+H]^+$	$[C_{63}H_{89}CoN_{14}O_{14}P]^+$	1355.5747	1355.5745	0.1
$[M+2H]^{2+}$	$[C_{63}H_{90}CoN_{14}O_{14}P]^{2+}$	678.2910	678.2922	1.8
MS/MS[a]	$[C_{54}H_{79}CoN_{12}O_{14}P]^+$	1209.4903	1209.4891	1.0
MS/MS[a]	$[C_{51}H_{74}CoN_{10}O_{13}P]^+$	1124.4501	1124.4493	0.7
MS/MS[a]	$[C_{49}H_{70}CoN_{12}O_7]^+$	997.4817	997.4802	1.5
MS/MS[a]	$[C_{46}H_{65}CoN_{10}O_6]^+$	912.4415	912.4407	0.9
MS/MS[b]	$[C_{46}H_{66}CoN_{10}O_6]^{2+}$	456.7244	456.7246	0.4
MS/MS[b]	$[C_{14}H_{20}N_2O_7P]^+$	359.1003	359.1007	1.1
MS/MS[b]	$[C_9H_{11}N_2]^+$	147.0917	147.0920	2.0

a: MS/MS of $[M+H]^+$ at CID 70; b: MS/MS of $[M+2H]^{2+}$ at CID 30.

Table S5: HPLC-ESI-(+)-HR-MS/MS data of the benzimidazolyl cobamide from *D. hafniense* strain DCB-2 supplemented with YE and benzimidazole (Bza).

	Molecular formula	calc. *m/z*	obs. *m/z*	Δ (ppm)
$[M+H]^+$	$[C_{61}H_{85}CoN_{14}O_{14}P]^+$	1327.5434	1327.5420	1.1
$[M+2H]^{2+}$	$[C_{61}H_{86}CoN_{14}O_{14}P]^{2+}$	664.2753	664.2754	0.2
MS/MS[a]	$[C_{54}H_{79}CoN_{12}O_{14}P]^+$	1209.4903	1209.4886	1.4
MS/MS[a]	$[C_{51}H_{74}CoN_{10}O_{13}P]^+$	1124.4501	1124.4485	1.4
MS/MS[a]	$[C_{49}H_{70}CoN_{12}O_7]^+$	997.4817	997.4801	1.6
MS/MS[a]	$[C_{46}H_{65}CoN_{10}O_6]^+$	912.4415	912.4400	1.6
MS/MS[b]	$[C_{46}H_{66}CoN_{10}O_6]^{2+}$	456.7244	456.7243	0.2
MS/MS[b]	$[C_{12}H_{16}N_2O_7P]^+$	331.0690	331.0682	2.4
MS/MS[b]	$[C_7H_7N_2]^+$	119.0604	119.0600	3.4

a: MS/MS of $[M+H]^+$ at CID 70; b: MS/MS of $[M+2H]^{2+}$ at CID 30.

Table S6: HPLC-ESI-(+)-HR-MS/MS data of the 5-methylbenzimidazolyl cobamide from *D. hafniense* strain DCB-2 supplemented with YE and 5-methylbenzimidazole (5-MeBza).

	Molecular formula	calc. *m/z*	obs. *m/z*	Δ (ppm)
$[M+H]^+$	$[C_{62}H_{87}CoN_{14}O_{14}P]^+$	1341.5590	1341.5573	1.3
$[M+2H]^{2+}$	$[C_{62}H_{88}CoN_{14}O_{14}P]^{2+}$	671.2832	671.2836	0.6
MS/MS[a]	$[C_{54}H_{79}CoN_{12}O_{14}P]^+$	1209.4903	1209.4882	1.7
MS/MS[a]	$[C_{51}H_{74}CoN_{10}O_{13}P]^+$	1124.4501	1124.4478	2.0
MS/MS[a]	$[C_{49}H_{70}CoN_{12}O_7]^+$	997.4817	997.4800	1.7
MS/MS[a]	$[C_{46}H_{65}CoN_{10}O_6]^+$	912.4415	912.4399	1.8
MS/MS[b]	$[C_{46}H_{66}CoN_{10}O_6]^{2+}$	456.7244	456.7251	1.5
MS/MS[b]	$[C_{13}H_{18}N_2O_7P]^+$	345.0846	345.0850	1.2
MS/MS[b]	$[C_8H_9N_2]^+$	133.0760	133.0755	3.8

a: MS/MS of $[M+H]^+$ at CID 70; b: MS/MS of $[M+2H]^{2+}$ at CID 30.

Table S7: HPLC-ESI-(+)-HR-MS/MS data of the 5-methoxybenzimidazolyl cobamide from *D. hafniense* strain DCB-2 supplemented with YE and 5-methoxybenzimidazole (5-OMeBza).

	Molecular formula	calc. *m/z*	obs. *m/z*	Δ (ppm)
$[M+H]^+$	$[C_{62}H_{87}CoN_{14}O_{15}P]^+$	1357.5539	1357.5529	0.7
$[M+2H]^{2+}$	$[C_{62}H_{88}CoN_{14}O_{15}P]^{2+}$	679.2806	679.2811	0.7
MS/MS[a]	$[C_{54}H_{79}CoN_{12}O_{14}P]^+$	1209.4903	1209.4890	1.1
MS/MS[a]	$[C_{51}H_{74}CoN_{10}O_{13}P]^+$	1124.4501	1124.4490	1.0
MS/MS[a]	$[C_{49}H_{70}CoN_{12}O_7]^+$	997.4817	997.4802	1.5
MS/MS[a]	$[C_{46}H_{65}CoN_{10}O_6]^+$	912.4415	912.4400	1.6
MS/MS[b]	$[C_{46}H_{66}CoN_{10}O_6]^{2+}$	456.7244	456.7250	1.3
MS/MS[b]	$[C_{13}H_{18}N_2O_8P]^+$	361.0795	361.0793	0.6
MS/MS[b]	$[C_8H_9N_2O]^+$	149.0709	149.0715	4.0

a: MS/MS of $[M+H]^+$ at CID 70; b: MS/MS of $[M+2H]^{2+}$ at CID 30.

Table S8: HPLC-ESI-(+)-HR-MS/MS data of the 5-/6-hydroxybenzimidazolyl cobamide(s) from *D. hafniense* strain DCB-2 supplemented with YE and 5-hydroxybenzimidazole (5-OHBza)

	Molecular formula	**calc. *m/z***	**obs. *m/z***	**Δ (ppm)**
$[M+H]^+$	$[C_{61}H_{85}CoN_{14}O_{15}P]^+$	1343.5383	1343.5375	0.6
$[M+2H]^{2+}$	$[C_{61}H_{86}CoN_{14}O_{15}P]^{2+}$	672.2728	672.2728	0.0
MS/MS[a]	$[C_{54}H_{79}CoN_{12}O_{14}P]^+$	1209.4903	1209.4887	1.3
MS/MS[a]	$[C_{51}H_{74}CoN_{10}O_{13}P]^+$	1124.4501	1124.4484	1.5
MS/MS[a]	$[C_{49}H_{70}CoN_{12}O_7]^+$	997.4817	997.4802	1.5
MS/MS[a]	$[C_{46}H_{65}CoN_{10}O_6]^+$	912.4415	912.4399	1.8
MS/MS[b]	$[C_{46}H_{66}CoN_{10}O_6]^{2+}$	456.7244	456.7243	0.2
MS/MS[b]	$[C_{12}H_{16}N_2O_8P]^+$	347.0639	347.0639	0.0
MS/MS[b]	$[C_7H_7N_2O]^+$	135.0553	135.0551	1.5

a: MS/MS of $[M+H]^+$ at CID 70; b: MS/MS of $[M+2H]^{2+}$ at CID 30.

Table S9: HPLC-ESI-(+)-HR-MS/MS data of the purinyl cobamide (**2**) from *D. hafniense* DCB-2 supplemented with YE and purine

	Molecular formula	**calc. *m/z***	**obs. *m/z***	**Δ (ppm)**
$[M+H]^+$	$[C_{59}H_{83}CoN_{16}O_{14}P]^+$	1329.5339	1329.5340	0.1
$[M+2H]^{2+}$	$[C_{59}H_{84}CoN_{16}O_{14}P]^{2+}$	665.2726	665.2723	0.5
MS/MS[a]	$[C_{54}H_{79}CoN_{12}O_{14}P]^+$	1209.4903	1209.4898	0.4
MS/MS[a]	$[C_{51}H_{74}CoN_{10}O_{13}P]^+$	1124.4501	1124.4492	0.8
MS/MS[a]	$[C_{49}H_{70}CoN_{12}O_7]^+$	997.4817	997.4810	0.7
MS/MS[a]	$[C_{46}H_{65}CoN_{10}O_6]^+$	912.4415	912.4407	0.9
MS/MS[b]	$[C_{46}H_{66}CoN_{10}O_6]^{2+}$	456.7244	456.7251	1.5
MS/MS[b]	$[C_{10}H_{14}N_4O_7P]^+$	333.0595	333.0592	0.9
MS/MS[b]	$[C_5H_5N_4]^+$	121.0509	121.0511	1.7

a: MS/MS of $[M+H]^+$ at CID 70; b: MS/MS of $[M+2H]^{2+}$ at CID 30.

Table S10: HPLC-ESI-(+)-HR-MS/MS data of the 5-azabenzimidazolyl cobamide (**2**) from *D. hafniense* DCB-2 supplemented with YE and 5-azabenzimidazole (5-azaBza).

	Molecular formula	**calc. *m/z***	**obs. *m/z***	**Δ (ppm)**
$[M+H]^+$	$[C_{60}H_{84}CoN_{15}O_{14}P]^+$	1328.5386	1328.5384	0.2
$[M+2H]^{2+}$	$[C_{60}H_{85}CoN_{15}O_{14}P]^{2+}$	664.7730	664.7752	3.3
MS/MS[a]	$[C_{54}H_{79}CoN_{12}O_{14}P]^+$	1209.4903	1209.4894	0.7
MS/MS[a]	$[C_{51}H_{74}CoN_{10}O_{13}P]^+$	1124.4501	1124.4489	1.1
MS/MS[a]	$[C_{49}H_{70}CoN_{12}O_7]^+$	997.4817	997.4813	0.4
MS/MS[a]	$[C_{46}H_{65}CoN_{10}O_6]^+$	912.4415	912.4412	0.3
MS/MS[a]	$[C_{46}H_{66}CoN_{10}O_6]^{2+}$	456.7244	456.7244	0.0
MS/MS[a]	$[C_{11}H_{15}N_3O_7P]^+$	332.0642	332.0642	0.0
MS/MS[a]	$[C_6H_6N_3]^+$	120.0556	120.0557	0.8

a: MS/MS of $[M+H]^+$ at CID 70; b: MS/MS of $[M+2H]^{2+}$ at CID 30.

Table S11: HPLC-ESI-(+)-HR-MS/MS data of the putative 6-azabenzimidazolyl cobamide (**3**) from *D. hafniense* DCB-2 supplemented with YE and 5-azabenzimidazole (5-azaBza).

	Molecular formula	**calc. *m/z***	**obs. *m/z***	**Δ (ppm)**
$[M+H]^+$	$[C_{60}H_{84}CoN_{15}O_{14}P]^+$	1328.5386	1328.5376	0.8
$[M+2H]^{2+}$	$[C_{60}H_{85}CoN_{15}O_{14}P]^{2+}$	664.7730	664.7749	2.9
MS/MS[a]	$[C_{54}H_{79}CoN_{12}O_{14}P]^+$	1209.4903	1209.4886	1.4
MS/MS[a]	$[C_{51}H_{74}CoN_{10}O_{13}P]^+$	1124.4501	1124.4479	2.0
MS/MS[a]	$[C_{49}H_{70}CoN_{12}O_7]^+$	997.4817	997.4812	0.5
MS/MS[a]	$[C_{46}H_{65}CoN_{10}O_6]^+$	912.4415	912.4406	1.0
MS/MS[a]	$[C_{46}H_{66}CoN_{10}O_6]^{2+}$	456.7244	456.7241	0.7
MS/MS[a]	$[C_{11}H_{15}N_3O_7P]^+$	332.0642	332.0641	0.3
MS/MS[a]	$[C_6H_6N_3]^+$	120.0556	120.0559	2.5

a: MS/MS of $[M+H]^+$ at CID 70; b: MS/MS of $[M+2H]^{2+}$ at CID 30.

Table S12: HPLC-ESI-(+)-HR-MS/MS data of the 4-azabenzimidazolyl cobamide (**4**) from *D. hafniense* DCB-2 supplemented with YE and 4-azabenzimidazole (4-azaBza).

	Molecular formula	**calc. *m/z***	**obs. *m/z***	**Δ (ppm)**
$[M+H]^+$	$[C_{60}H_{84}CoN_{15}O_{14}P]^+$	1328.5386	1328.5395	0.7
$[M+2H]^{2+}$	$[C_{60}H_{85}CoN_{15}O_{14}P]^{2+}$	664.7730	664.7754	3.6
MS/MS[a]	$[C_{54}H_{79}CoN_{12}O_{14}P]^+$	1209.4903	1209.4899	0.3
MS/MS[a]	$[C_{51}H_{74}CoN_{10}O_{13}P]^+$	1124.4501	1124.4488	1.2
MS/MS[a]	$[C_{49}H_{70}CoN_{12}O_7]^+$	997.4817	997.4810	0.7
MS/MS[a]	$[C_{46}H_{65}CoN_{10}O_6]^+$	912.4415	912.4405	1.1
MS/MS[a]	$[C_{46}H_{66}CoN_{10}O_6]^{2+}$	456.7244	456.7244	0.0
MS/MS[a]	$[C_{11}H_{15}N_3O_7P]^+$	332.0642	332.0638	1.2
MS/MS[a]	$[C_6H_6N_3]^+$	120.0556	120.0553	2.5

a: MS/MS of $[M+H]^+$ at CID 70; b: MS/MS of $[M+2H]^{2+}$ at CID 30.

Table S13: NMR data (700 MHz, D_2O) for the purinyl cobamide (**1**) and the 5-azabenzimidazolyl cobamide (**2**) isolated from *D. hafniense* strain DCB-2.

		purinyl cobamide (1)			**5-azabenzimidazolyl cobamide (2)**		
		^{1}H	**^{13}C**	**HMBC**	**^{1}H**	**^{13}C**	**HMBC**
Corrinoid	1	-	85.3 *Cq*	-	-	85.3 *Cq*	-
	1a	0.44 *s*	19.8 *CH_3*	1, 2, 19	0.42 *s*	19.62 *CH_3*	1, 2, 19
	2	-	47.1 *Cq*	-	-	47.2 *Cq*	-
	2a	2.39 *s*	42.8 *CH_2*	1, 2, 2a', 2b, 3	2.40 *s*	42.9 *CH_2*	2, 2a', 2b, 3
	2b	-	175.5 *Cq*	-	-	175.6 *Cq*	-
	2a'	1.40 *s*	16.5 *CH_3*	1, 2, 2a, 3	1.40 *s*	16.6 *CH_3*	1, 2, 2a, 3
	3	4.20 *m*	56.2 *CH*	1, 2, 2a, 3a, 3b, 4	4.19 *m*	56.2 *CH*	1, 3a, 3b
	3a	1.98 *m*	25.5 *CH_2*	…	1.98 *m*	25.9 *CH_2*	…
	3b	2.53 *m*	34.3 *CH_2*	3c	2.53 *m*	34.6 *CH_2*	3c
	3c	-	177.9 *Cq*	-	-	177.7 *Cq*	-
	4	-	180.7 *Cq*	-	-	180.4 *Cq*	-
	5	-	107.6 *Cq*	-	-	107.5 *Cq*	-
	5a	2.53 *s*	15.2 *CH_3*	4, 5, 6	2.53 *s*	15.4 *CH_3*	4, 5, 6
	6	-	165.5 *Cq*	-	-	165.7 *Cq*	-
	7	-	51.5 *Cq*	-	-	51.3 *Cq*	-
	7a	2.18 *d* 13.7 Hz	42.7 *CH_2*	6, 7, 7a', 7b, 8	2.18 *d* 13.8 Hz	42.3 *CH_2*	6, 7, 7a', 7b, 8
	7a	2.59 *m*	42.7 *CH_2*	7, 7a', 7b, 8	2.59 *m*	42.3 *CH_2*	7, 7a', 7b, 8
	7b	-	174.8 *Cq*	-	-	174.9 *Cq*	-
	7a'	1.88 *s*	18.8 *CH_3*	6, 7, 7a, 8, 8a	1.87 *s*	19.0 *CH_3*	6, 7, 7a, 8
	8	3.54 *dd* 11 Hz, 5 Hz	55.7 *CH*	6, 7, 7a, 8a,	3.51 *dd* 11 Hz, 4.8 Hz	55.7 *CH*	7, 7a, 8a
	8a	2.07 *m* 0.92 *m*	26.3 *CH_2*	…	2.03 *m* 0.91 *m*	26.7 *CH_2*	…
	8b	1.51 *m* 1.95 *m*	31.9 *CH_2*	8c	1.82 *m* 1.32 *m*	32.0 *CH_2*	8c
	8c	-	177.2 *Cq*	-	-	177.1 *Cq*	-
	9	-	174.1 *Cq*	-	-	173.7 *Cq*	-
	10	6.23 *s*	95.4 *CH*	8, 9, 11, 12	6.17 *s*	95.5 *CH*	8, 9, 11, 12
	11	-	177.7 *Cq*	-	-	177.7 *Cq*	-
	12	-	48.5 *Cq*	-	-	48.4 *Cq*	-
	12a	1.48 *s*	18.9 *CH_3*	11, 12, 12a', 13	1.47 *s*	19.0 *CH_3*	11, 12, 12a', 13
	12a'	1.18 *s*	30.8 *CH_3*	11, 12, 12a, 13	1.19 *s*	31.0 *CH_3*	11, 12, 12a, 13
	13	3.39 *d* 9.6 Hz	53.8 *CH*	11, 12, 12a', 13a, 14	3.37 *m*	53.8 *CH*	11, 13a, 13b
	13a	1.95 *m*	28.1 *CH_2*	…	1.96 *m*	28.1 *CH_2*	13c

	13b	2.63 *m*	34.5 *CH₂*	13c	2.63 *m*	34.2 *CH₂*	13c
	13c	-	177.9 *Cq*	-	-	178.*0 Cq*	-
	14	-	166.3 *Cq*	-	-	166.2 *Cq*	-
	15	-	104.5 *Cq*	-	-	104.6 *Cq*	-
	15a	2.60 *s*	14.9 *CH₃*	13, 14, 15, 16, 17	2.60 *s*	15.0 *CH₃*	14, 15, 16
	16	-	179.6 *Cq*	-	-	179.4 *Cq*	-
	17	-	59.5 *Cq*	-	-	59.5 *Cq*	-
	17a	1.82 *m* 2.67 *m*	32.3 *CH₂*	…	1.83 *m* 2.66 *m*	32.3 *CH₂*	…
	17b	2.09 *m* 2.55 *m*	32.5 *CH₂*	17c	2.10 *m* 2.54 *m*	32.8 *CH₂*	17c
	17c	-	177.5 *Cq*	-	-	178.0 *Cq*	-
	17a’	1.39 *s*	15.9 *CH₃*	16, 17, 17a, 18	1.39 *s*	16.0 *CH₃*	16, 17, 17a, 18
	18	2.80 *m*	38.8 *CH*	17, 18a	2.79 *m*	38.9 *CH*	17a’, 19
	18a	2.69 *m* 2.76 *m*	31.5 *CH₂*	17, 18, 18b	2.69 *m* 2.75 *m*	31.5 *CH₂*	17, 17a’, 18, 18b,19
	18b	-	175.3 *Cq*	-	-	175.2 *Cq*	-
	19	4.13 *d* 11.0 Hz	75.2 *CH*	1, 1a’, 16, 18	4.12 *d* 10.7 Hz	75.2 *CH*	…
C3-linker	1’	2.93 *m* 3.59 *m*	45.3 *CH₂*	…	2.92 *m* 3.61 *m*	45.4 *CH₂*	…
	2’	4.30 *m*	72.9 *CH*	…	4.30 *m*	72.9 *CH*	…
	3’	1.25 *d* 6.1 Hz	18.8 *CH₃*	1’, 2’	1.25 *d* 6.0 Hz	19.0 *CH₃*	1’, 2’
RIBOSE	1’’	6.61 *d* 3 Hz	86.5 *CH*	…	6.47 *d*	87.5 *CH*	2’’, 2’’’
	2’’	4.31 *m*	69.0 *CH*	…	4.31 *m*	67.4 *CH*	…
	3’’	4.74 *m*	72.9 *CH*	…	4.72 *m*	73.0 *CH*	…
	4’’	4.10 *m*	82.6 *CH*	…	4.09 *m*	82.5 *CH*	…
	5’’	3.74 *dd* 13.0, 3.6 Hz 3.92 *d* 12.3 Hz	60.2 *CH₂*	…	3.76 *dd* 3.93 *dd*	60.4 *CH₂*	3’’ 3’’
LIGAND	2’’’	7.41 *s*	146.5 *CH*	3a’’’, 7a’’’	7.30 *s*	144.8.2 *CH*	3a’’’, 7a’’’
	3a’’’	-	129.5 *Cq*	-	-	135.5 *Cq*	-
	4’’’	-	-	-	8.00 *s*	138.2 *CH*	3a’’’, 7a’’’, 6’’’
	5’’’	8.19 *s*	145.3 *CH*	3a’’’, 7a’’’, 4’’’	-	-	-
	6’’’	-	-	-	8.37 *d* 5.0 Hz	143.0 *CH*	7’’’, 7a’’’
	7’’’	8.95 *s*	153.5 *CH*	6’’’	7.61 *d* 5.2 Hz	107.9 *CH*	3a’’’
	7a’’’	-	149.5 *Cq*	-	-	137.3 *Cq*	-

Obtained from 700 MHz ^{1}H PURGE NMR, PRESAT-*dqf*-COSY, HSQC, HMBC, and PRESAT-ROESY spectra; Referenced to water at δ_H 4.79 ppm and acetate at δ_H 1.91

Figure S3: Section of the HMBC spectrum of the purinyl cobamide (**1**) isolated from *D. hafniense* strain DCB-2 showing H,C-correlations in the purine unit.

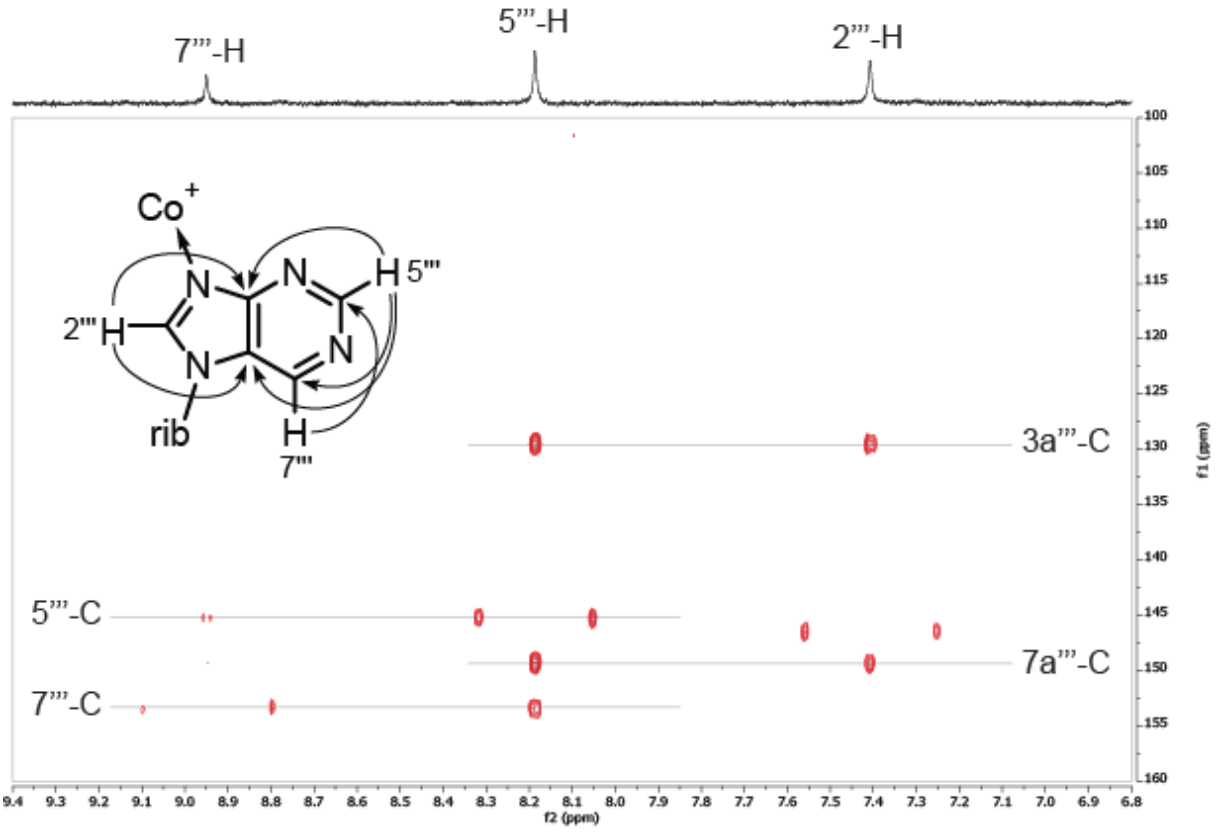
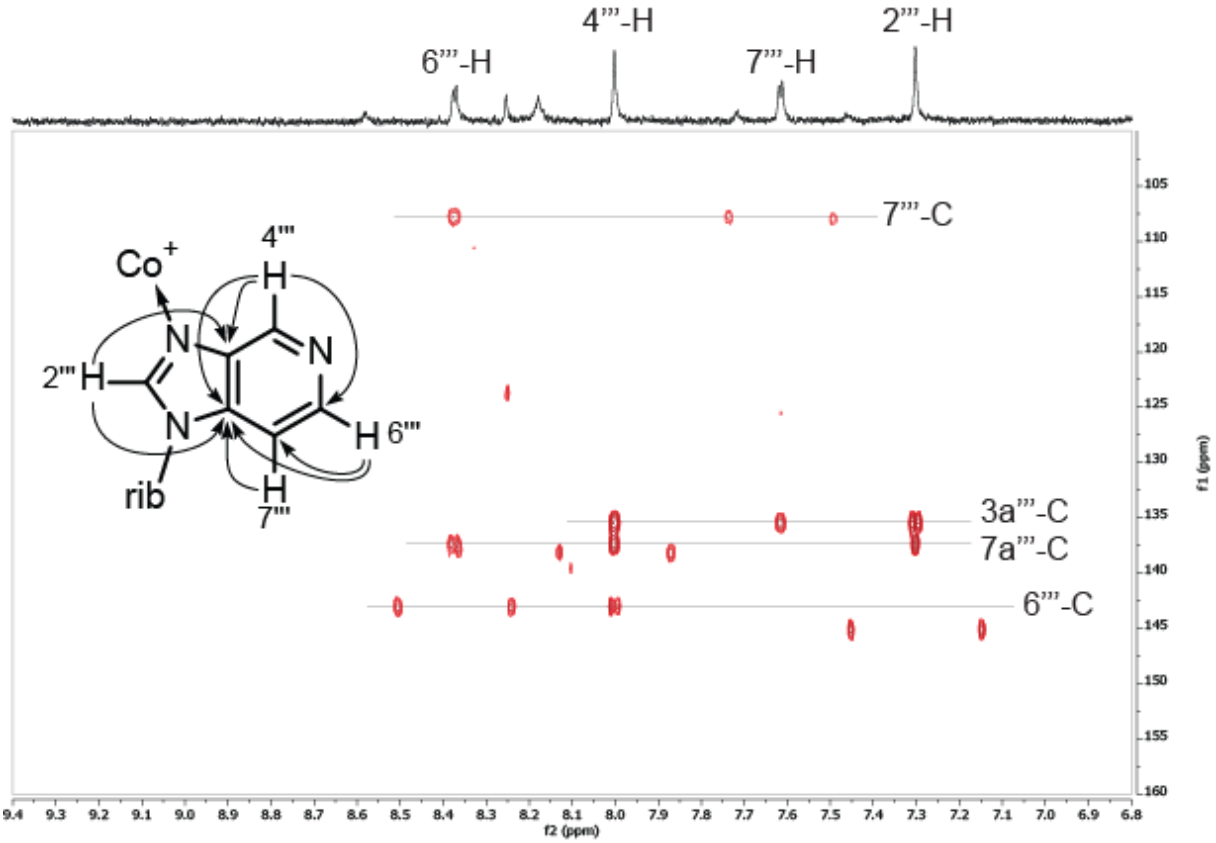
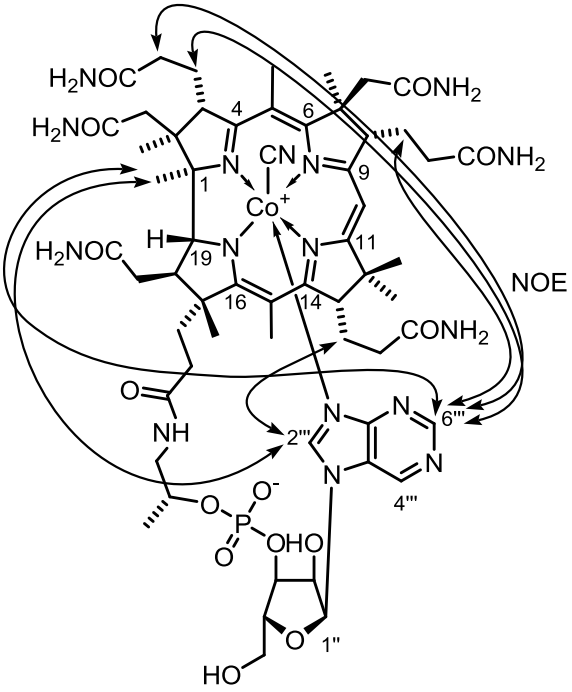


Figure S4: Section of the HMBC spectrum of the 5-azabenzimidazolyl cobamide (**2**) isolated from *D. hafniense* strain DCB-2 showing H,C-correlations in the 5-azabenzimidazole unit.



purinyl cobamide (**1**)



5-azabenzimidazolyl cobamide (**2**)

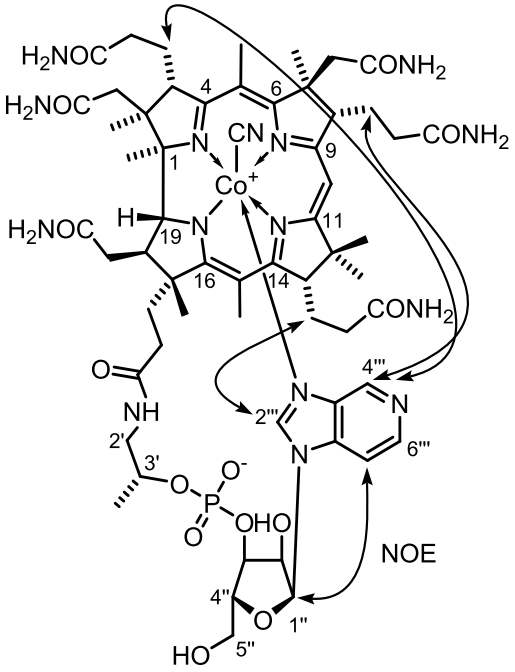


Figure S5: Orientation of the heteroaromatic ligands as deduced from analysis of NOE-correlations observed in the 700 MHz ROESY spectrum.

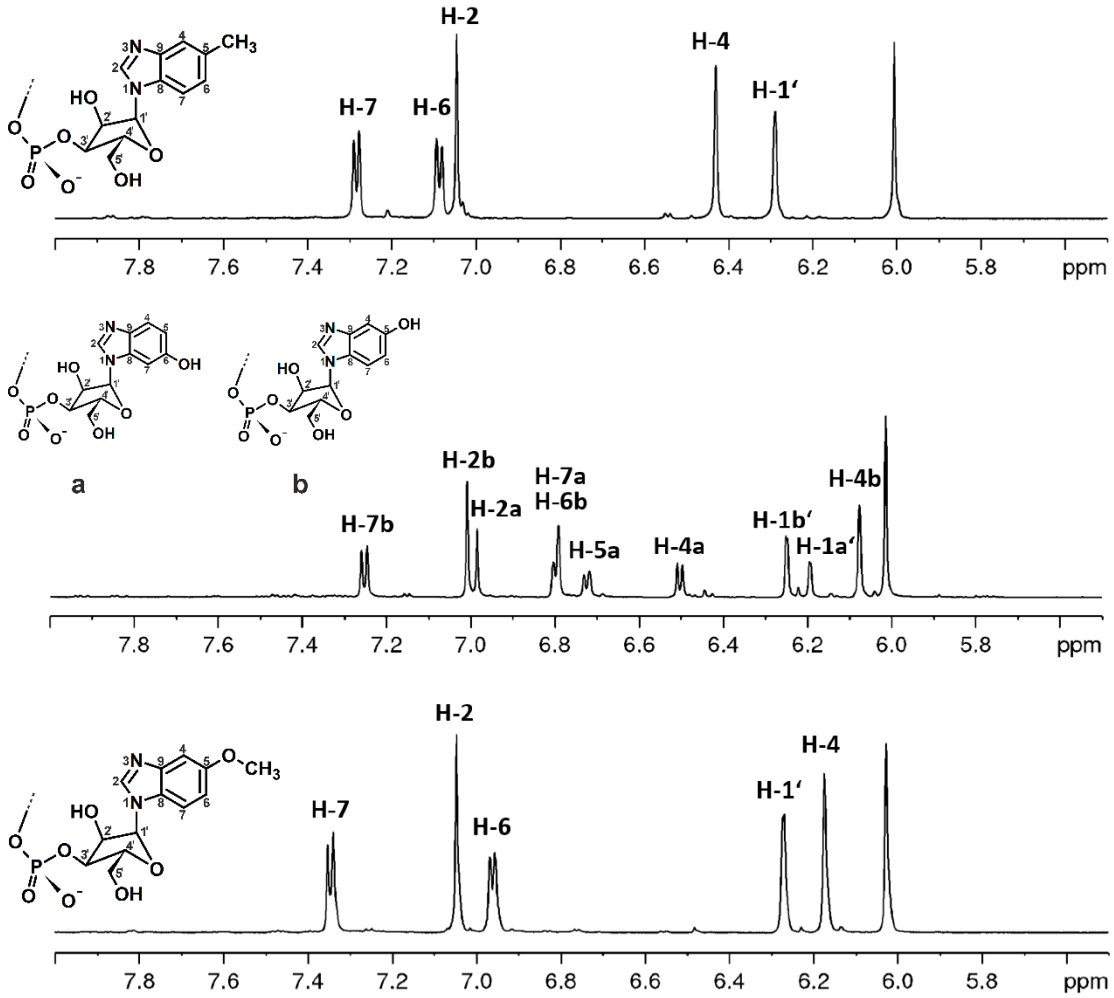


Figure S6. Low field range of ^{1}H-NMR spectra of 5-MeBza-Cba, 6-OHBza-Cba and 5-OHBza-Cba, and 5-OMeBza-Cba. The depicted sections show the signals for the respective benzimidazolyl moieties and the signal for the anomeric position of the α-ribosyl unit.

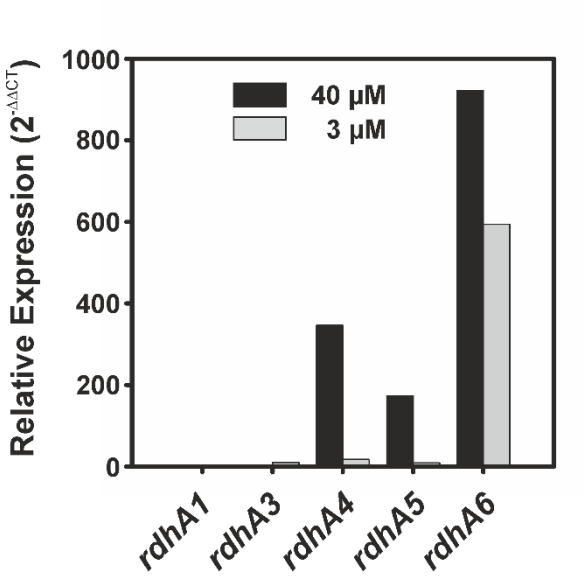


Figure S7. Relative transcript levels of the *rdhA1*, *rdhA3*, *rdhA4*, *rdhA5*, and *rdhA6* genes in two cultures of *D. hafniense* strain DCB-2. Cells were cultivated with pyruvate. When the cultures reached an OD_{578} = 0.2, 3-chloro-4-hydroxy-phenylacetate (ClOHPA; 100 µM) was added. The concentration of ClOHPA in the cultures before the harvest of the cells (OD_{578} = 0.3) is given in the figure.

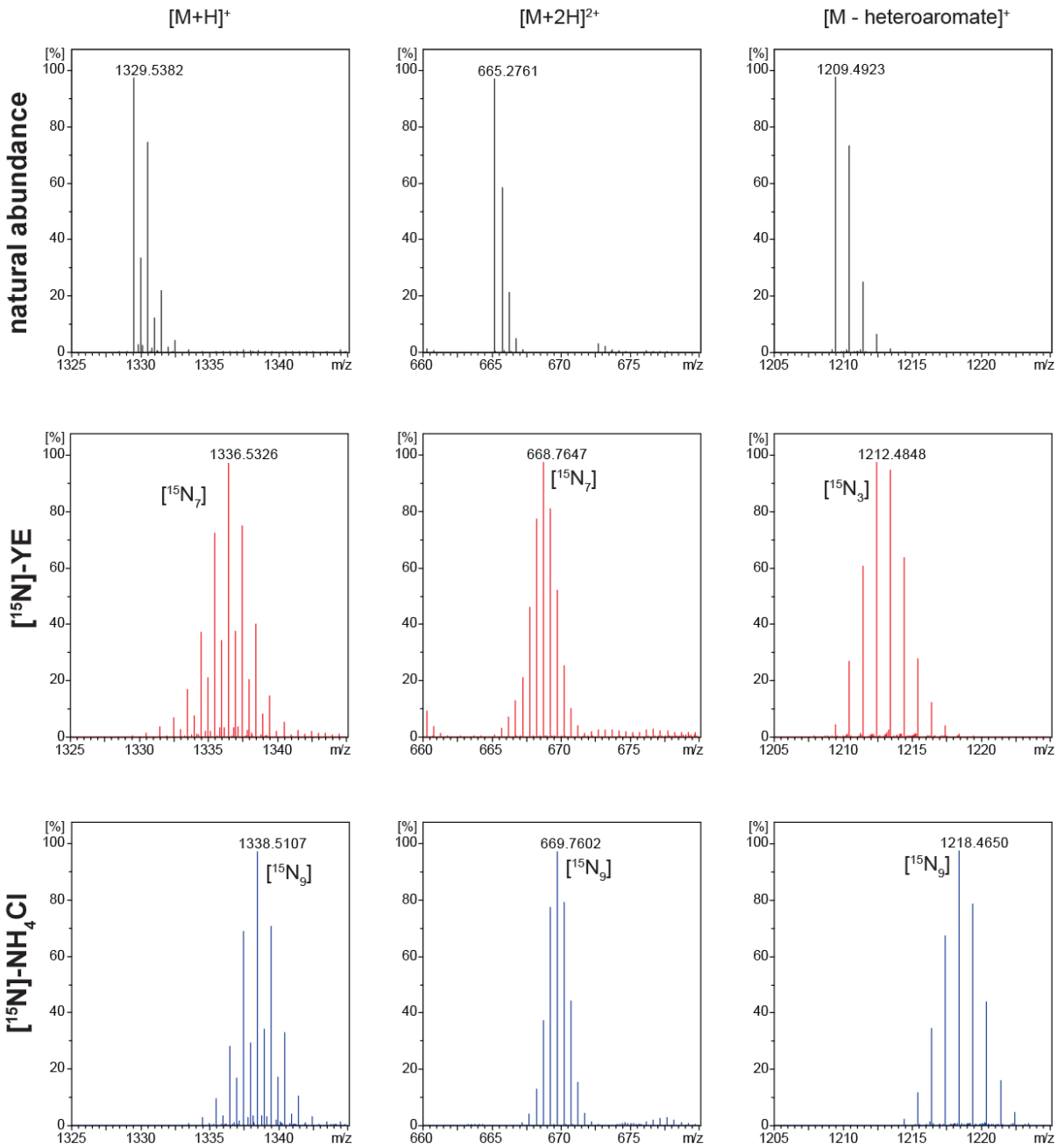


Figure S8A: HR-MS/MS analysis of the purinyl cobamide (**1**) from *D. hafniense* strain DCB-2 supplemented with [^{15}N]-enriched yeast extract or [^{15}N]-enriched NH_4Cl.

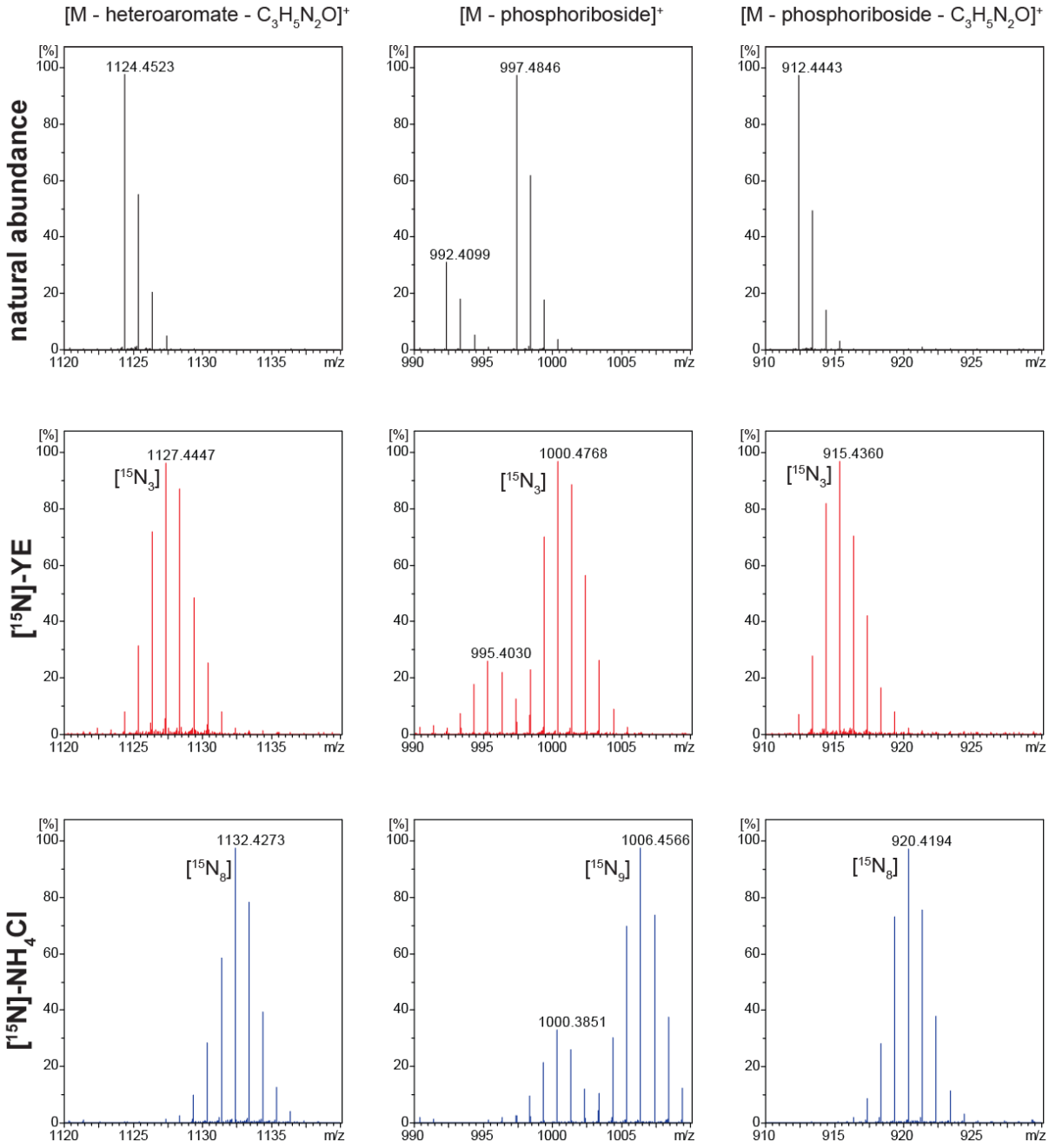


Figure S8B: HR-MS/MS analysis of the purinyl cobamide (**1**) from *D. hafniense* strain DCB-2 supplemented with [^{15}N]-enriched yeast extract or [^{15}N]-enriched NH_4Cl.

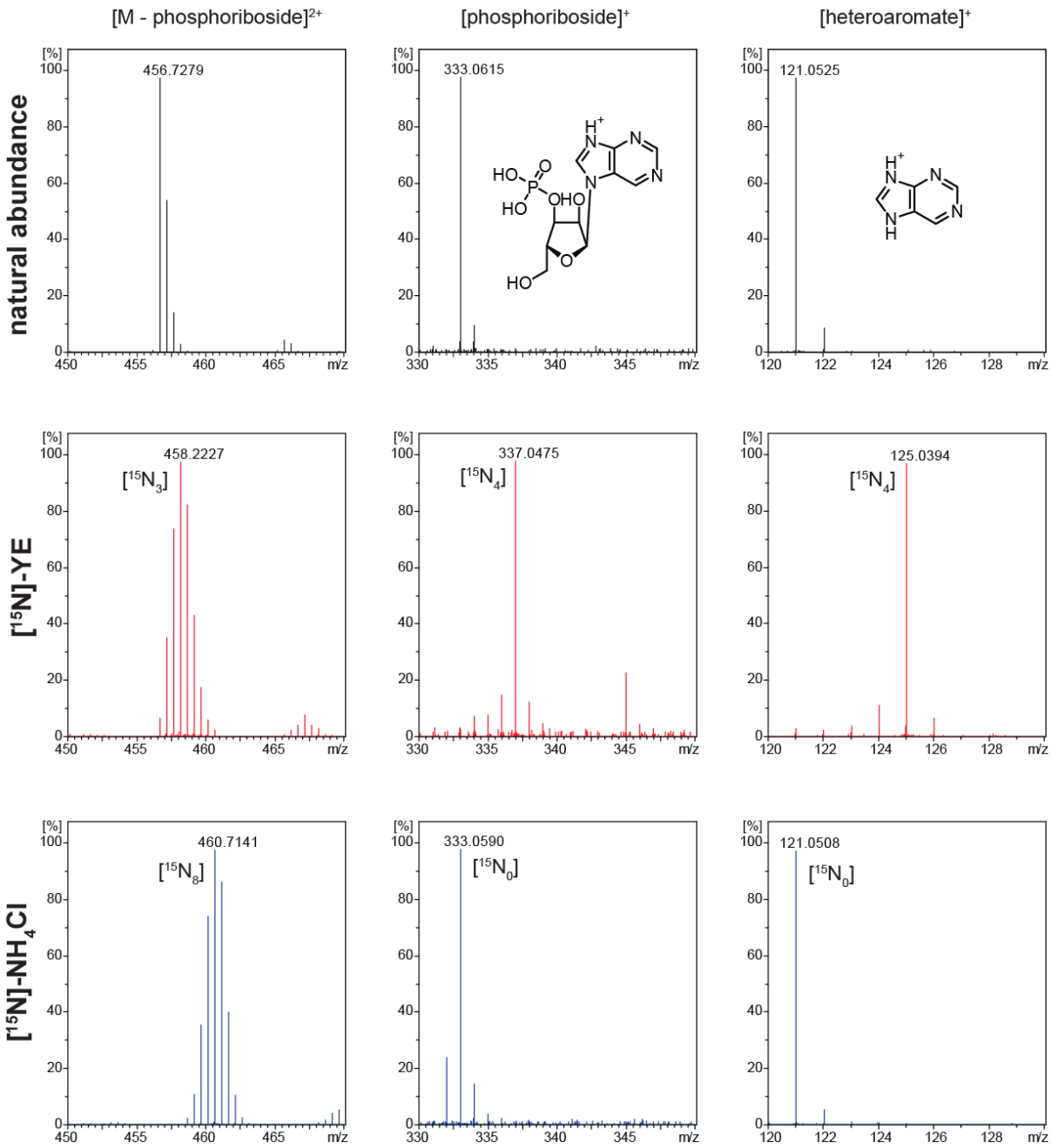


Figure S8C: HR-MS/MS analysis of the purinyl cobamide (**1**) from *D. hafniense* strain DCB-2 supplemented with [^{15}N]-enriched yeast extract or [^{15}N]-enriched NH_4Cl.

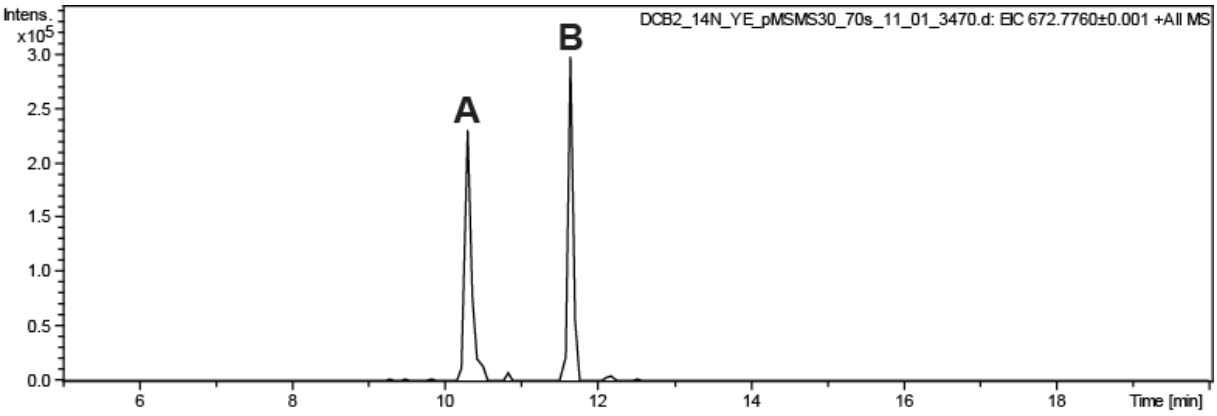


Figure S9: HPLC-ESI-(+)-HR-MS chromatogram of $[M+2H]^{2+}$ signals corresponding to putative adeninyl cobamides from *D. hafniense* strain DCB-2 supplemented with the ^{15}N-enriched YE.

Table S14: HPLC-ESI-(+)-HR-MS/MS data of putative adeninyl cobamides from *D. hafniense* strain DCB-2 supplemented with the ^{15}N-enriched YE.

A	**Molecular formula**	**calc. *m/z***	**obs. *m/z***	**Δ (ppm)**
$[M+H]^+$	$[C_{59}H_{84}CoN_{17}O_{14}P]^+$	1344.5448	1344.5434	1.1
$[M+2H]^{2+}$	$[C_{59}H_{85}CoN_{17}O_{14}P]^{2+}$	672.7760	672.7766	0.9
MS/MS[b]	$[C_{54}H_{79}CoN_{12}O_{14}P]^+$	1209.4903	1209.4880	1.9
MS/MS[b]	$[C_{51}H_{74}CoN_{10}O_{13}P]^+$	1124.4501	1124.4487	1.2
MS/MS[b]	$[C_{49}H_{70}CoN_{12}O_7]^+$	997.4817	997.4820	0.3
MS/MS[b]	$[C_{46}H_{65}CoN_{10}O_6]^+$	912.4415	912.4417	0.2
MS/MS[b]	$[C_{46}H_{66}CoN_{10}O_6]^{2+}$	456.7244	456.7243	0.2
MS/MS[b]	$[C_{10}H_{15}N_5O_7P]^+$	348.0704	348.0704	0.2
MS/MS[b]	$[C_5H_6N_5]^+$	136.0618	136.0615	1.8

B	**Molecular formula**	**calc. *m/z***	**obs. *m/z***	**Δ (ppm)**
$[M+H]^+$	$[C_{59}H_{84}CoN_{17}O_{14}P]^+$	1344.5448	1344.5421	2.0
$[M+2H]^{2+}$	$[C_{59}H_{85}CoN_{17}O_{14}P]^{2+}$	672.7760	672.7762	0.3
MS/MS[b]	$[C_{54}H_{79}CoN_{12}O_{14}P]^+$	1209.4903	1209.4875	2.3
MS/MS[b]	$[C_{51}H_{74}CoN_{10}O_{13}P]^+$	1124.4501	1124.4494	0.6
MS/MS[b]	$[C_{49}H_{70}CoN_{12}O_7]^+$	997.4817	997.4797	2.0
MS/MS[b]	$[C_{46}H_{65}CoN_{10}O_6]^+$	912.4415	912.4401	1.5
MS/MS[b]	$[C_{46}H_{66}CoN_{10}O_6]^{2+}$	456.7244	456.7238	1.3
MS/MS[b]	$[C_{10}H_{15}N_5O_7P]^+$	348.0704	348.0703	0.0
MS/MS[b]	$[C_5H_6N_5]^+$	136.0618	136.0620	1.7

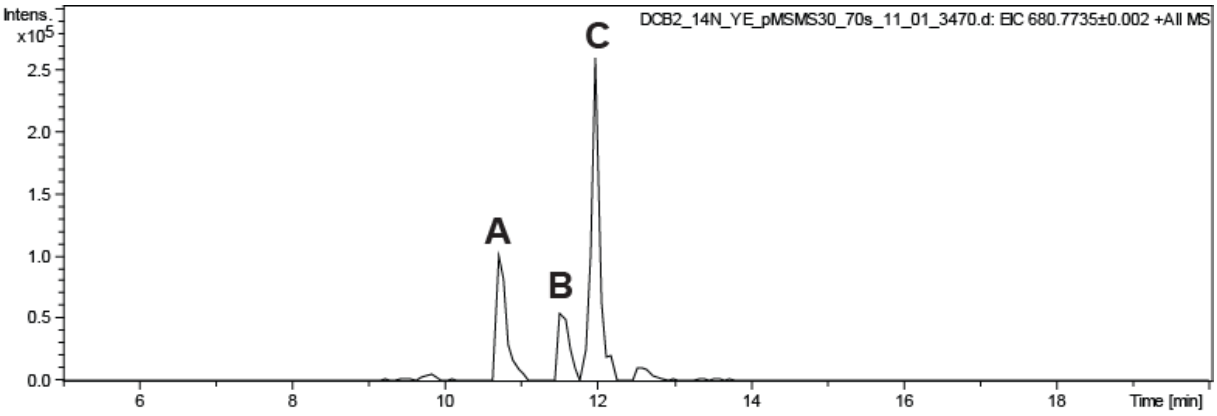


Figure S10: HPLC-ESI-(+)-HR-MS chromatogram of $[M+2H]^{2+}$ signals corresponding to putative guaninyl cobamides from *D. hafniense* strain DCB-2 supplemented with the ^{15}N-enriched YE.

Table S15: HPLC-ESI-(+)-HR-MS/MS data of putative guaninyl cobamides from *D. hafniense* strain DCB-2 supplemented with the ^{15}N-enriched YE.

A	**Molecular formula**	**calc. *m/z***	**obs. *m/z***	**Δ (ppm)**
$[M+H]^+$	$[C_{59}H_{84}CoN_{17}O_{15}P]^+$	1360.5397	1360.5431	2.5
$[M+2H]^{2+}$	$[C_{59}H_{85}CoN_{17}O_{15}P]^{2+}$	680.7735	680.7725	1.4
MS/MS[b]	$[C_{54}H_{79}CoN_{12}O_{14}P]^+$	1209.4903	1209.4896	0.6
MS/MS[b]	$[C_{51}H_{74}CoN_{10}O_{13}P]^+$	1124.4501	1124.4479	2.0
MS/MS[b]	$[C_{49}H_{70}CoN_{12}O_7]^+$	997.4817	997.4811	0.6
MS/MS[b]	$[C_{46}H_{65}CoN_{10}O_6]^+$	912.4415	912.4411	0.4
MS/MS[b]	$[C_{46}H_{66}CoN_{10}O_6]^{2+}$	456.7244	456.7256	2.6
MS/MS[b]	$[C_{10}H_{15}N_5O_8P]^+$	364.0653	364.0641	3.3
MS/MS[b]	$[C_5H_6N_5O]^+$	152.0567	152.0564	1.6

B	**Molecular formula**	**calc. *m/z***	**obs. *m/z***	**Δ (ppm)**
$[M+H]^+$	$[C_{59}H_{84}CoN_{17}O_{15}P]^+$	1360.5397	1360.5411	1.1
$[M+2H]^{2+}$	$[C_{59}H_{85}CoN_{17}O_{15}P]^{2+}$	680.7735	680.7743	1.1
MS/MS[b]	$[C_{54}H_{79}CoN_{12}O_{14}P]^+$	1209.4903	nd	-
MS/MS[b]	$[C_{51}H_{74}CoN_{10}O_{13}P]^+$	1124.4501	nd	-
MS/MS[b]	$[C_{49}H_{70}CoN_{12}O_7]^+$	997.4817	nd	-
MS/MS[b]	$[C_{46}H_{65}CoN_{10}O_6]^+$	912.4415	nd	-
MS/MS[b]	$[C_{46}H_{66}CoN_{10}O_6]^{2+}$	456.7244	nd	-
MS/MS[b]	$[C_{10}H_{15}N_5O_8P]^+$	364.0653	nd	-
MS/MS[b]	$[C_5H_6N_5O]^+$	152.0567	nd	-

C	Molecular formula	calc. *m/z*	obs. *m/z*	Δ (ppm)
$[M+H]^+$	$[C_{59}H_{84}CoN_{17}O_{15}P]^+$	1360.5397	1360.5413	1.2
$[M+2H]^{2+}$	$[C_{59}H_{85}CoN_{17}O_{15}P]^{2+}$	680.7735	680.7745	1.4
MS/MS[b]	$[C_{54}H_{79}CoN_{12}O_{14}P]^+$	1209.4903	1209.4885	1.5
MS/MS[b]	$[C_{51}H_{74}CoN_{10}O_{13}P]^+$	1124.4501	1124.4466	3.1
MS/MS[b]	$[C_{49}H_{70}CoN_{12}O_7]^+$	997.4817	997.4805	1.2
MS/MS[b]	$[C_{46}H_{65}CoN_{10}O_6]^+$	912.4415	912.4396	2.1
MS/MS[b]	$[C_{46}H_{66}CoN_{10}O_6]^{2+}$	456.7244	456.7251	1.5
MS/MS[b]	$[C_{10}H_{15}N_5O_8P]^+$	364.0653	364.0640	3.4
MS/MS[b]	$[C_5H_6N_5O]^+$	152.0567	152.0569	1.6

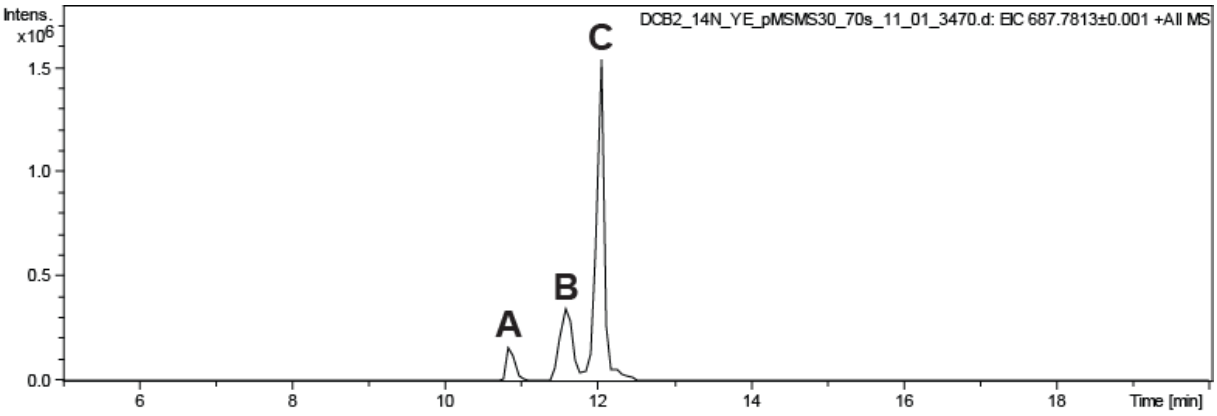


Figure S11: HPLC-ESI-(+)-HR-MS chromatogram of $[M+2H]^{2+}$ signals corresponding to putative methylguaninyl cobamides from *D. hafniense* strain DCB-2 supplemented with the ^{15}N-enriched YE.

Table S16: HPLC-ESI-(+)-HR-MS/MS data of putative methylguaninyl cobamides from *D. hafniense* strain DCB-2 supplemented with the ^{15}N-enriched YE.

A	**Molecular formula**	**calc. *m/z***	**obs. *m/z***	**Δ (ppm)**
$[M+H]^+$	$[C_{60}H_{86}CoN_{17}O_{15}P]^+$	1374.5553	1374.5549	0.3
$[M+2H]^{2+}$	$[C_{60}H_{87}CoN_{17}O_{15}P]^{2+}$	687.7813	687.7821	1.2
MS/MS[b]	$[C_{54}H_{79}CoN_{12}O_{14}P]^+$	1209.4903	1209.4903	0.0
MS/MS[b]	$[C_{51}H_{74}CoN_{10}O_{13}P]^+$	1124.4501	1124.4508	0.6
MS/MS[b]	$[C_{49}H_{70}CoN_{12}O_7]^+$	997.4817	997.4810	0.7
MS/MS[b]	$[C_{46}H_{65}CoN_{10}O_6]^+$	912.4415	912.4417	0.2
MS/MS[b]	$[C_{46}H_{66}CoN_{10}O_6]^{2+}$	456.7244	456.7243	0.2
MS/MS[b]	$[C_{11}H_{17}N_5O_8P]^+$	378.0809	378.0816	1.7
MS/MS[b]	$[C_6H_8N_5O]^+$	166.0723	166.0724	0.3

B	**Molecular formula**	**calc. *m/z***	**obs. *m/z***	**Δ (ppm)**
$[M+H]^+$	$[C_{60}H_{86}CoN_{17}O_{15}P]^+$	1374.5553	1374.5531	1.7
$[M+2H]^{2+}$	$[C_{60}H_{87}CoN_{17}O_{15}P]^{2+}$	687.7813	687.7819	0.9
MS/MS[b]	$[C_{54}H_{79}CoN_{12}O_{14}P]^+$	1209.4903	1209.4888	1.2
MS/MS[b]	$[C_{51}H_{74}CoN_{10}O_{13}P]^+$	1124.4501	1124.4479	2.0
MS/MS[b]	$[C_{49}H_{70}CoN_{12}O_7]^+$	997.4817	997.4803	1.4
MS/MS[b]	$[C_{46}H_{65}CoN_{10}O_6]^+$	912.4415	912.4406	1.0
MS/MS[b]	$[C_{46}H_{66}CoN_{10}O_6]^{2+}$	456.7244	456.7255	2.4
MS/MS[b]	$[C_{11}H_{17}N_5O_8P]^+$	378.0809	378.0824	2.3
MS/MS[b]	$[C_6H_8N_5O]^+$	166.0723	166.0724	0.3

C	Molecular formula	calc. *m/z*	obs. *m/z*	Δ (ppm)
$[M+H]^+$	$[C_{60}H_{86}CoN_{17}O_{15}P]^+$	1374.5553	1374.5544	2.8
$[M+2H]^{2+}$	$[C_{60}H_{87}CoN_{17}O_{15}P]^{2+}$	687.7813	687.7813	0.0
MS/MS[b]	$[C_{54}H_{79}CoN_{12}O_{14}P]^+$	1209.4903	1209.4891	1.0
MS/MS[b]	$[C_{51}H_{74}CoN_{10}O_{13}P]^+$	1124.4501	1124.4481	1.8
MS/MS[b]	$[C_{49}H_{70}CoN_{12}O_7]^+$	997.4817	997.4804	1.3
MS/MS[b]	$[C_{46}H_{65}CoN_{10}O_6]^+$	912.4415	912.4400	1.6
MS/MS[b]	$[C_{46}H_{66}CoN_{10}O_6]^{2+}$	456.7244	456.7244	0.0
MS/MS[b]	$[C_{11}H_{17}N_5O_8P]^+$	378.0809	378.0816	0.1
MS/MS[b]	$[C_6H_8N_5O]^+$	166.0723	166.0725	0.8

a: MS/MS of $[M+H]^+$ at CID 70; b: MS/MS of $[M+2H]^{2+}$ at CID 30.

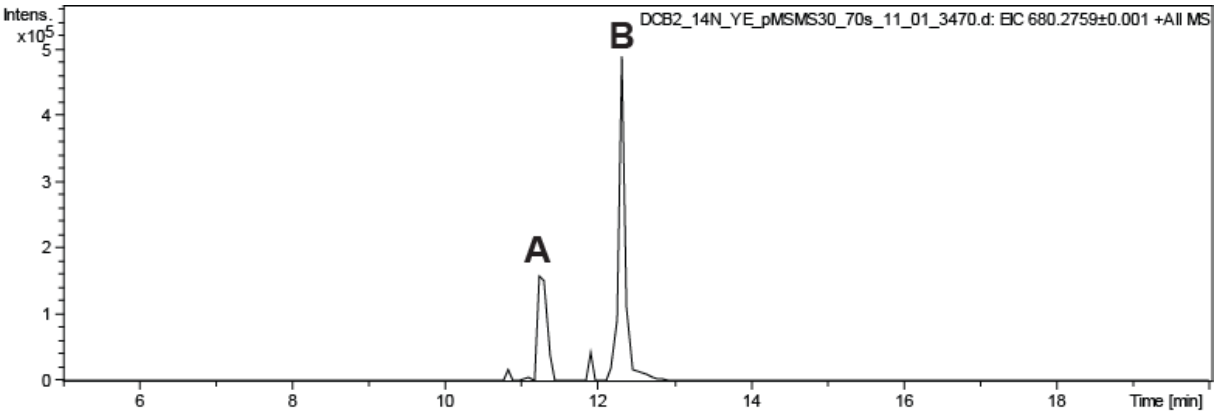


Figure S12: HPLC-ESI-(+)-HR-MS chromatogram of $[M+2H]^{2+}$ signals corresponding to putative methylhypoxanthinyl cobamides from *D. hafniense* strain DCB-2 supplemented with the ^{15}N-enriched YE.

Table S17: HPLC-ESI-(+)-HR-MS/MS data of putative methylhypoxanthinyl cobamides from *D. hafniense* strain DCB-2 supplemented with the ^{15}N-enriched YE.

A	Molecular formula	calc. *m/z*	obs. *m/z*	Δ (ppm)
$[M+H]^+$	$[C_{60}H_{85}CoN_{16}O_{15}P]^+$	1359.5444	1359.5419	1.8
$[M+2H]^{2+}$	$[C_{60}H_{86}CoN_{16}O_{15}P]^{2+}$	680.2759	680.2766	1.1
MS/MS[b]	$[C_{54}H_{79}CoN_{12}O_{14}P]^+$	1209.4903	1209.4896	0.6
MS/MS[b]	$[C_{51}H_{74}CoN_{10}O_{13}P]^+$	1124.4501	1124.4479	2.0
MS/MS[b]	$[C_{49}H_{70}CoN_{12}O_7]^+$	997.4817	997.4811	0.6
MS/MS[b]	$[C_{46}H_{65}CoN_{10}O_6]^+$	912.4415	912.4411	0.4
MS/MS[b]	$[C_{46}H_{66}CoN_{10}O_6]^{2+}$	456.7244	456.7256	2.6
MS/MS[b]	$[C_{11}H_{16}N_4O_8P]^+$	363.0700	363.0697	0.9
MS/MS[b]	$[C_6H_7N_4O]^+$	151.0614	151.0615	0.6

B	Molecular formula	calc. *m/z*	obs. *m/z*	Δ (ppm)
$[M+H]^+$	$[C_{60}H_{85}CoN_{16}O_{15}P]^+$	1359.5444	1359.5401	3.2
$[M+2H]^{2+}$	$[C_{60}H_{86}CoN_{16}O_{15}P]^{2+}$	680.2759	680.2761	0.3
MS/MS[b]	$[C_{54}H_{79}CoN_{12}O_{14}P]^+$	1209.4903	1209.4874	2.4
MS/MS[b]	$[C_{51}H_{74}CoN_{10}O_{13}P]^+$	1124.4501	1124.4468	2.9
MS/MS[b]	$[C_{49}H_{70}CoN_{12}O_7]^+$	997.4817	997.4796	2.1
MS/MS[b]	$[C_{46}H_{65}CoN_{10}O_6]^+$	912.4415	912.4390	2.7
MS/MS[b]	$[C_{46}H_{66}CoN_{10}O_6]^{2+}$	456.7244	456.7242	0.4
MS/MS[b]	$[C_{11}H_{16}N_4O_8P]^+$	363.0700	363.0695	1.5
MS/MS[b]	$[C_6H_7N_4O]^+$	151.0614	151.0613	1.2

a: MS/MS of $[M+H]^+$ at CID 70; b: MS/MS of $[M+2H]^{2+}$ at CID 30.

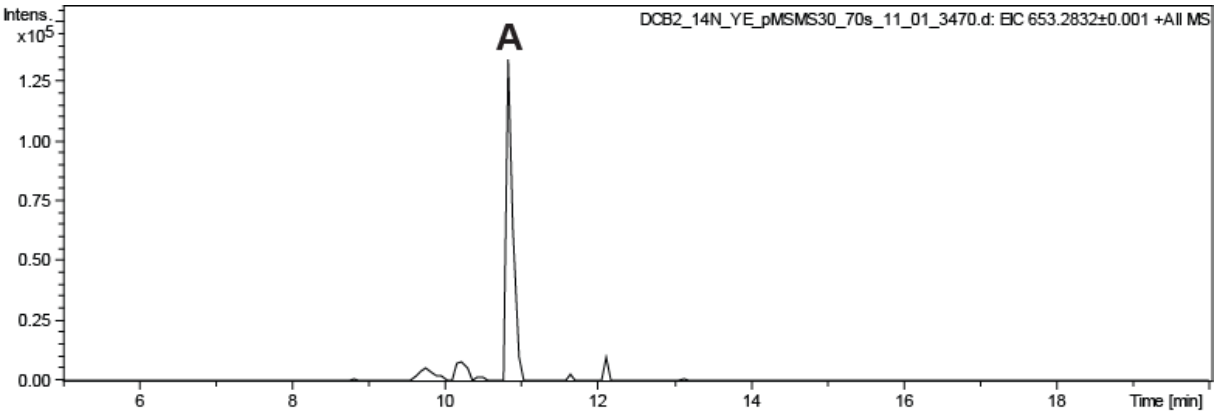


Figure S13: HPLC-ESI-(+)-HR-MS chromatogram of $[M+2H]^{2+}$ signals and HPLC-ESI-(+)-HR-MS/MS data of dimethylimidazolyl cobamide from *D. hafniense* strain DCB-2 supplemented with the ^{15}N-enriched YE.

Table S18: HPLC-ESI-(+)-HR-MS data of putative dimethylimidazolyl cobamide from *D. hafniense* strain DCB-2 supplemented with the ^{15}N-enriched YE.

A	Molecular formula	calc. *m/z*	obs. *m/z*	Δ (ppm)
$[M+H]^+$	$[C_{59}H_{87}CoN_{14}O_{14}P]^+$	1305.5590	1305.5579	0.9
$[M+2H]^{2+}$	$[C_{59}H_{88}CoN_{14}O_{14}P]^{2+}$	653.2832	653.2838	1.1
MS/MS[b]	$[C_{54}H_{79}CoN_{12}O_{14}P]^+$	1209.4903	1209.4886	1.4
MS/MS[b]	$[C_{51}H_{74}CoN_{10}O_{13}P]^+$	1124.4501	1124.4510	0.8
MS/MS[b]	$[C_{49}H_{70}CoN_{12}O_7]^+$	997.4817	997.4807	1.0
MS/MS[b]	$[C_{46}H_{65}CoN_{10}O_6]^+$	912.4415	912.4373	4.6
MS/MS[b]	$[C_{46}H_{66}CoN_{10}O_6]^{2+}$	456.7244	456.7225	4.2
MS/MS[b]	$[C_{10}H_{18}N_2O_7P]^+$	309.0846	309.0842	1.2
MS/MS[b]	$[C_5H_9N_2]^+$	97.0760	nd	-

a: MS/MS of $[M+H]^+$ at CID 70; b: MS/MS of $[M+2H]^{2+}$ at CID 30.

Table S19: HPLC-ESI-(+)-HR-MS/MS data of the 4,5-dimethylimidazolyl cobamide (signal **5** in Fig. 4) from *D. hafniense* strain DCB-2 supplemented with 4,5-dimethylimidazole (DMI).

	Molecular formula	calc. *m/z*	obs. *m/z*	Δ (ppm)
$[M+H]^+$	$[C_{59}H_{87}CoN_{14}O_{14}P]^+$	1305.5590	1305.5605	1.1
$[M+2H]^{2+}$	$[C_{59}H_{88}CoN_{14}O_{14}P]^{2+}$	653.2832	653.2858	4.0
MS/MS[a]	$[C_{54}H_{79}CoN_{12}O_{14}P]^+$	1209.4903	1209.4909	0.5
MS/MS[a]	$[C_{51}H_{74}CoN_{10}O_{13}P]^+$	1124.4501	1124.4518	1.5
MS/MS[a]	$[C_{49}H_{70}CoN_{12}O_7]^+$	997.4817	997.4837	2.0
MS/MS[a]	$[C_{46}H_{65}CoN_{10}O_6]^+$	912.4415	912.4435	2.2
MS/MS[b]	$[C_{46}H_{66}CoN_{10}O_6]^{2+}$	456.7244	456.7251	1.5
MS/MS[b]	$[C_{10}H_{18}N_2O_7P]^+$	309.0846	309.0850	1.3
MS/MS[b]	$[C_5H_9N_2]^+$	97.0760	97.0753	3.1

a: MS/MS of $[M+H]^+$ at CID 70; b: MS/MS of $[M+2H]^{2+}$ at CID 30.

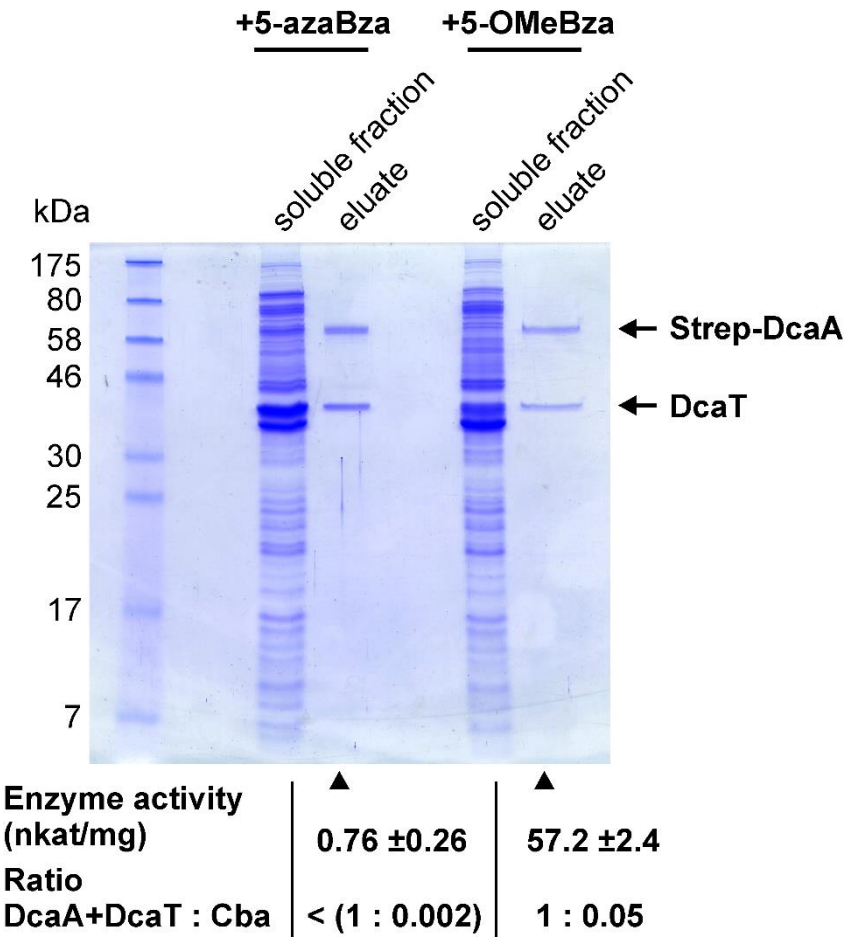


Figure S14: Purification of recombinant Strep-DcaA. The soluble fractions (10 µg protein) and the eluates (1 µg protein) were separated on a 12.5% SDS/PAGE (Coomassie-stained).