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Supporting Information

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Vitamin B_{12}: a Methyl Group without a Job?

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Materials. Cobyric acid (**7**), alpha-ribazole-3'-phosphate and alpha-ribazole-3',5'-cyclo phosphate were prepared from the methods of Bonnet et al.,[S1] Brown & Hakimi,[S2] and Friedrich et al.,[S3] respectively. Cyanocobalamin, *Hoffmann-La Roche*; water purified using *Epure, Barnstead Co.*; acetone, CH_2Cl_2, CH_3OH, Hg,$(Bu_4N)PF_6$, CH_3COOH, KCl, KCN, KH_2PO_4, K_2HPO_4, NaH2PO4, Na_2HPO_4, $NaOOCH_3$, CH_3CN, ethanolamine, triethylamine, hydrochloric acid fuming 37%, all *Fluka puriss. p.a.*, or *Fluka MicroSelect*; DMF *Fluka, puriss., absolute, over molecular sieves (H_2O ≤ 0.01 %);* benzoic acid, methyl 4-toluene-sulfonate, ethyl chloroformate all *Fluka purum*; H_3PO_4, *Fluka*, crystallized *puriss.* O_2-sensitive reactions were done in a glove box (*Mecaplex GB-80*, < 10 ppm O_2). The electrochemical syntheses were carried out in an electrolysis cell with two compartments, separated by a medium porosity glass frit; Hg pool working electrode; Pt-wire counter electrode; 0.1 N calomel electrode (0.1 N CE) as reference electrode; potentiostat *Amel 550*. pH values were measured with a *WTW SenTix 41* electrode connected to a *WTW inoLab digital pH meter*. TLC: *RP18 F254s* TLC plates (*Merck* No. 105559). Preparative TLC: RP18 F254s TLC plates 20 x 20 cm (*Merck* No. 115389). Column Chromatography: *LiChroprep* RP-18 (25-40μm) (*Merck* No. 109303).UV/Vis Spectra: *Hitachi-U3000*; λmax(log ε) in nm. CD Spectra: *Jasco J715*; λmax or λmin ($\Delta\varepsilon$) in nm. NMR Spectra of B_{12}-derivatives were collected at 25° C on a *500 MHz Varian Unity Inova spectrometer* equipped with 5 mm triple-resonance probe with z-gradients. Sample concentrations were 3.7 mM (norvitamin B_{12}) and 2.5 mM (methyl-norcobalamin) in 0.6 ml D_2O. Norvitamin B_{12} (**1**): TOCSY with presaturation of the water signal: 2k x 256 complex data points. Spectral width in direct (SW2) and indirect dimension (SW1) 9 ppm centered around the residual water signal. 16 scans per increment. 80 ms DIPSI-2 spinlock with B_1-field strength of 7 kHz. HSQC: 2k x 512 complex data points. SW2 9 ppm centered around the residual water signal, SW1 0-200 ppm. 80 scans per increment. HMBC: 2k x 256 data points (real). SW2 9 ppm centered around residual water signal, SW1 0-200 ppm. 64 scans per increment. Methyl-norcobalamin (**2**): TOCSY with presaturation of the water signal: 2374 x 256 complex data points. SW1,2 10 ppm centered around the residual water signal. 16 scans per increment. 80 ms DIPSI-2 spinlock with B_1-field strength of 7 kHz. HSQC: 2k x 256 complex data points. SW2 9 ppm, SW1 0-200 ppm. 32 scans per increment. HSQC: 2k x 64 complex data points. SW2 9 ppm, SW1 25-75 ppm. 256 scans per increment. HMBC: 2k x 256 data points (real). SW2 9 ppm, SW1 0-200 ppm. 64 scans per increment. Spectra were shifted as to give the least cumulative chemical shift differences (sum of the absolute values of the chemical shift differences in each dimension) with respect to the corresponding methyl group bearing compound (vitamin B_{12} or methylcobalamin). For norvitamin B_{12}, differences between chemical shifts obtained by this procedure and by referencing to the water signal are negligible. For methylcobalamin the shifts, if referenced to water, change by +0.03 ppm for ^1H and +0.6 ppm for ^{13}C. FABMS: *Finnigan MAT 95S*, positive-ion mode; glycerine; Cs gun.

Syntheses: (2-aminoethyl)-3'-(alpha-ribazolyl)-diphosphate (**8**). Potassium (117 mg, 3.0 mmol) was added to pre-distilled ethanolamine (1 ml) until H_2 ceased to evolve. To this the cyclic alpha-ribazole-phosphate (50 mg, 0.15 mmol) in ethanolamine (0.5 ml) was added dropwise. After two hours the reaction was quenched by adding H_2O (1 ml), this was then directly loaded onto an RP-18 column (1cm x 10cm) which was washed with a column length of water, to remove the excess ethanolamine. The product was eluted with a H_2O:CH_3CN (6:2) solvent system to give 44 mg (73 %) of the mixture of the two isomers. NMR showed the two isomers to be formed in a 5:1 ratio of 3'- and 2'-phosphates, respectively. The mixture of **8** and its 2'-isomer were separated by preparative TLC on RP-18 plates (H_2O:CH_3CN; 6:2). Two chromatographically well separated fractions were eluted from the absorbant. Both spots showed a positive test after dipping in a ninhydrin dip. Yield of **8** = 32 mg (52 %).

TLC (RP-18, H_2O:CH_3CN, 6:2): R_f = 0.32. ^{1}H NMR (300 MHz, D_2O): 2.37 (3H, singlet, CH_3), 2.39 (3H, singlet, CH_3), 3.19 (2H, triplet, $H_2N\underline{CH_2}CH_2O$), 3.83 (1H, double doublet, *J*= 4.2 Hz, *J*= 12.6 Hz, H_aC5R), 3.95 (1H, double doublet, *J*= 2.7 Hz, *J*= 12.6 Hz, H_bC5R), 4.08 (2H, multiplet, $H_2NCH_2\underline{CH_2}O$), 4.62 (1H, multiplet, HC4R), 4.7-4.9 (water signal superimposes signals of HC2R, HC3R), 6.41 (1H, doublet, *J*= 4.5 Hz, HC1R), 7.45 (1H, singlet, aromatic CH), 7.53 (1H, singlet, aromatic CH), 8.34 (1H, singlet, HC2N). UV/Vis (c = 9.97*10^{-4}M, 0.1 M phosphate buffer, pH 7.25): 286.5(4.05), 278.5(4.07), 248(4.21), (c = 9.97*10^{-4}M, 0.1 M HCl, pH 1.00): 284(4.22), 276(4.25). ESI-MS: 439.97 (20, $[M + K]^+$), 423.97 (5, $[M + Na]^+$), 404.03 (5), 403.02 (20), 402.01 (100, $[M + H]^+$), 146.97 (10).

Norvitamin B_{12} (**1**). Alpha-aqua-beta-cyano cobyric acid (**7**) (10.0 mg, 10.2 µmol) was dissolved in dry DMF (3.5 ml) and cooled to ≈ 10° C in an ice-salt water bath, before triethylamine (6.2 mg, 61.5 µmol) and ethylchloroformate (3.4 mg, 30.8 µmol) were added. After 20 mins **8** (5.0 mg, 12.5 µmol) in DMF (1.0 ml) was added and the reaction was stirred for 30 min at 0^{o}C and a further 1 hour at room temperature. The reaction was quenched with H_2O (10 ml) (with a trace of cyanide) and the coloured aqueous phase was washed with CH_2Cl_2 (3*20 ml) before the solvent was removed. The crude product was precipitated with H_2O/acetone and further material was obtained from precipitating the mother liquor. The material was purified by preparative TLC (RP-18 plate) (H_2O:CH_3CN; 6:2). The red residue was crystallised from H_2O/acetone to give dark red crystals, 10 mg (73 %) of norvitamin B_{12} (**1**).

Norvitamin B_{12} (**1**): UV/Vis (c = 5.59*10^{-4} M, H_2O): 548(3.85), 518.5(3.80), 407(3.47), 360(4.36), 321.5(3.81), 304.5(3.88), 277.5(4.10). FAB-MS: 1343.5 (13), 1342.5 (22), 1341.5 (24, $[M+H]^+$), 1317.6 (57), 1316.4 (100), 1315.5 (94, $[M+H-CN]^+$). ^{1}H- and ^{13}C-NMR: see Table S1.

Methyl-norcobalamin (**2**). In the glove box (<10 ppm O_2) norvitamin B_{12} (**1**) (10 mg, 7.45 μmol) and benzoic acid (1.8 mg, 15 μmol) were dissolved in 0.1 M tetrabutylammonium hexafluorophosphate (TBAHFP) in methanol and added to the cathode chamber of the electrolysis cell. **1** was then reduced at a Hg-pool electrode, with magnetic stirring, at –1.1 V *vs.* 0.1 N CE to norcob(I)alamin (under UV control; the colour went from dark red to brown and finally to black-green). The mixture was then protected from light and a solution of methyl tosylate (13.9 mg, 75 μmol) in 0.1 M TBAHFP in methanol was added (while keeping the applied potential at –1.1 V *vs.* 0.1 N CE). After 3h, a UV-spectrum showed complete conversion to the product. The mixture was then transferred into a dark room, taken up in H_2O (10 ml) and extracted with dichloromethane (3 * 20 ml). The aqueous phase was evaporated to give 9.2 mg of raw product (93 %). The material was then crystallised from H_2O/acetone to give 7.9 mg (80%) of red crystalline **2**.

Methyl-norcobalamin (**2**): UV/Vis (c = 4.51*10^{-4} M, 0.1 M phosphate buffer, pH = 7.25): 518.5(3.83), 373.5(3.93), 339(4.01), 314.5(4.00), 279(4.15), 265(4.18). FAB-MS: 1332.6 (24), 1331.6 (43), 1330.6 (52, $[M+H]^+$), 1317.6 (68), 1316.5 (100), 1315.6 (76, $[M+H-CN]^+$). ^{1}H- and ^{13}C-NMR: see Table S1.

Determination of the pK_a of **2-H$^+$** (see Figure 5 in main text). Aqueous solutions of methyl-norcobalamin (**2**) (c = 4.51*10^{-4} M), 0.1 M buffer (HCl, acetate or phosphate) and KCl (total ionic strength 1.0M) were prepared with pH values 7.25, 3.67, 3.31, 3.14, 2.96, 2.59. 1.00. Absorbance spectra of these solutions obtained at room temperature showed isobestic points at 490 nm, 385 nm and 334 nm. The spectral changes at 533 nm, 453 nm and 303 nm were analysed according to *Equation 1* by the method of least squares, where A_x is the absorbance at a given wavelength of the solution at pH_x, and A_{AH} and A_A- are the corresponding absorbencies of **2-H$^+$** and **2**, determined (in duplicate) at the titration end points. The intercepts resulting from these fits of data determined at 533 nm, 453 nm and 303 nm, respectively, were 3.243, 3.240 and 3.241, yielding a pK_a value of 3.24 ± 0.008 for **2-H$^+$**.

$$pH_x = pK_a + \log\left(|A_x - A_{AH}| \,/\, |A_A\text{-} - A_x|\right) \qquad \textit{Equation 1}$$

Structure determination of norvitamin B_{12} (1). Crystals of norvitamin B_{12} were grown from water/acetone. A crystal specimen was immersed in hydrocarbon oil, picked up with a rayon loop,

and quickly cooled to cryotemperature by immersing in liquid nitrogen. Diffraction experiments were carried out on the EMBL beamline BW7b at DESY in Hamburg (Germany), which was equipped with a MAR imaging plate detector and a gas-stream low temperature (103(2) K) device. Data pertaining to the data collection and structure refinement are summarized in Table S2.

Indexing of diffraction images, intensity integration, and data scaling were performed with programs Denzo/Scalepack.[S6] The structure was solved by direct methods to yield the Co-atoms plus most remaining atoms of the structure. Missing atoms (mostly in the solvent region) were located in subsequent electron-density maps. Full-matrix least-squares refinement on F^2 was performed with the program SHELXL-97.[S7] No absorption correction was applied to the data. Scattering factors including real and imaginary dispersion corrections were taken from the 'International Tables of Crystallography'. H-Atom positions were calculated and refined as 'riding' on their respective non-H-atom. The isotropic adp for each H-atom was set to 1.5 times the equiv. isotropic adp of the adjacent non-H-atom. The solvent electron density was modeled with one acetone, 5 fully occupied and 18 partially occupied H_2O sites. Crystallographic residuals at the close of the refinement are also given in Table S2.

The B_{12} moiety was very well defined except for the hydroxymethylene group (C5R, O5R) of the ribose in the nucleotide loop, which was found to be disordered over two alternate conformations.

CCDC 278 482 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

Table S1: Comparison of NMR chemical shift values for **1**[a)] and **2**[a)] with those of **4**[S4] and **5**[S5]

	δ ^{1}H[ppm]				δ ^{13}C[ppm]				Δδ (2-5)		Δδ (1-4)	
	1	**2**	**4**	**5**	**1**	**2**	**4**	**5**	^{1}H	^{13}C	^{1}H	^{13}C
β-CH_3		-0.01		-0.05		9.7		9.5	0.04	0.2		
C1					88.0	87.9	87.9	87.6		0.3		0.1
C1A	0.47	0.50	0.45	0.47	22.1	23.2	22.2	22.9	0.03	0.3	0.02	-0.1
C2					50.2	49.0	50.1	48.9		0.1		0.1
C2A	1.41	1.35	1.40	1.35	19.5	19.6	19.6	19.4	0.00	0.2	0.01	-0.1
C3	4.19	4.06	4.17	4.09	59.1	58.2	59.2	58.0	-0.04	0.2	0.02	-0.1
C4					183.0	178.0	182.8	177.7		0.3		0.2
C5					110.4	108.0	110.3	108.0		0.0		0.1
C6					168.3	165.9	168.1	165.7		0.2		0.2
C7					54.4	52.7	54.2	52.6		0.1		0.2
C7A	1.86	1.76	1.87	1.77	21.7	22.1	21.9	21.7	-0.01	0.4	-0.01	-0.2
C8	3.41	3.32	3.42	3.38	58.5	57.4	58.5	57.1	-0.06	0.3	-0.01	0.0
C9					176.6	172.5	176.4	172.3		0.2		0.2
C10	6.08	5.92	6.09	5.91	97.6	96.7	97.7	96.5	0.01	0.2	-0.01	-0.1
C11					179.8	176.1	179.7	175.9		0.2		0.1
C12					51.1	49.4	50.9	49.2		0.2		0.2
C12A	1.45	1.40	1.45	1.40	21.9	22.1	22.1	22.3	0.00	-0.2	0.00	-0.3
C12B	1.19	0.95	1.20	0.94	34.0	34.4	34.1	34.6	0.01	-0.2	-0.01	-0.1
C13	3.33	3.07	3.33	3.05	56.5	56.0	56.5	55.8	0.02	0.2	0.00	0.0
C14					168.9	166.4	168.8	166.2		0.2		0.1
C15					107.1	106.3	106.9	106.0		0.3		0.2
C16					181.9	177.7	181.7	177.4		0.3		0.2
C17					62.1	60.5	62.0	60.4		0.1		0.1
C17B	1.40	1.33	1.39	1.33	18.5	19.3	18.8	19.2	0.00	0.1	0.01	-0.3
C18	2.76	2.63		2.62	41.8	41.8	41.9	41.6	0.01	0.2		-0.1
C19	4.10	3.97	4.11	3.96	77.7	76.5	77.7	76.3	0.01	0.2	-0.01	0.0
C21	2.41	2.31	2.40	2.30	45.6	45.9	45.6	45.9	0.01	0.0	0.00	0.0
C31	2.03/1.98	2.02/1.97	1.95	2.0/1.95	28.8	28.8	28.8	28.7	0.02/0.02	0.1	0.08/0.03[b)]	0.0
C32	2.57/2.50	2.50/2.43	2.58/2.50	2.48/2.42	37.7	37.8	37.8	37.8	0.02/0.01	0.0	-0.02/0.0	-0.1
C51	2.54	2.47	2.54	2.48	18.2	18.1	18.2	18.0	-0.01	0.1	0.00	0.0
C71	2.57/2.18	2.40/1.98	2.59/2.20	2.41/1.93	45.7	45.7	45.9	45.6	-0.01/0.05	0.1	-0.02/-0.02	-0.2
C81	2.00/0.99	1.83/0.87	2.02	1.85/0.94	28.8	28.5	28.8	28.5	-0.02/-0.07	0.0	-0.02	0.0
C82	1.82/1.01	1.70/1.03	1.85/1.02	1.72/1.05	34.4	34.4	34.6	34.5	-0.02/-0.02	-0.1	-0.03/-0.01	-0.2
C131	2.03/1.96	2.04/1.96	1.98	2.02/1.95	30.8	30.4	30.8	30.4	0.02/0.01	0.0	0.05/-0.02[b)]	0.0
C132	2.64	2.58	2.63	2.59	37.5	37.6	37.5	37.5	-0.01	0.1	0.01	0.0
C151	2.56	2.43	2.57	2.45	17.9	18.0	18.0	18.1	-0.02	-0.1	-0.01	-0.1
C171	2.51/2.16	2.4/2.12	2.51/1.82	2.47/1.78	35.0	34.1	35.3	34.5	-0.07/0.34	-0.4	0.00/0.34	-0.3
C172	2.62/1.86	2.48/1.81	2.66/2.12	2.43/2.09	34.6	34.2	34.3	34.4	0.05/-0.28	-0.2	-0.04/-0.26	0.3
C175	3.64/3.26	3.56/3.33	3.61/2.96	3.55/3.08	43.1	42.7	48.3	47.5	0.01/0.25	-4.8	0.03/0.30	-5.2
C176	4.04/3.95	4.04/3.89	4.30	4.32	68.1	68.2	75.8	75.5	-0.28/-0.43[b)]	-7.3	-0.26/-0.35[b)]	-7.7
C181	2.75/2.68	2.69/2.64	2.75/2.68	2.67/2.64	34.4	34.6	35.0	34.6	0.02/0.00	0.0	0.00/0.00	-0.6
C1R	6.33	6.22	6.36	6.26	89.4	88.6	89.8	89.5	-0.04	-0.9	-0.04	-0.4
C2R	4.32	4.26	4.28	4.25	71.7	71.9	71.7	71.6	0.01	0.3	0.04	0.0
C3R	4.77	4.73	4.73	4.71	76.0	76.0	75.9	75.7	0.02	0.3	0.04	0.1
C4R	4.11	4.12	4.04	4.07	84.6	83.9	84.9	84.0	0.05	-0.1	0.07	-0.3
C5R	3.92/3.77	3.87/3.74	3.93/3.75	3.9/3.75	63.1	63.0	63.3	63.0	-0.03/-0.01	0.0	-0.01/0.02	-0.2
C2N	7.14	7.05	7.10	6.98	144.7	144.5	144.7	144.4	0.07		0.04	
C4N	6.50	6.28	6.51	6.29	119.2	121.0	119.3	120.9	-0.01	0.1	-0.01	-0.1
C5N					136.1	134.3	135.8	134.1		0.2		0.3
C6N					138.0	136.5	137.9	136.3		0.2		0.1
C7N	7.28	7.17	7.28	7.19	114.2	113.1	114.3	113.1	-0.02	0.0	0.00	-0.1
C8N					132.9	133.5	132.8	133.0		0.5		0.1
C9N					139.5	140.9	139.5	140.7		0.2		0.0
C10N	2.26	2.21	2.27	2.21	22.7	22.2	22.8	22.3	0.00	-0.1	-0.01	-0.1
C11N	2.26	2.21	2.26	2.23	22.0	22.0	22.1	22.2	-0.02	-0.2	0.00	-0.1

[a)] carboxamide resonances C22, C33, C72, C83, C133, C173, C182 were not completely assigned.
[b)] These diastereotopic protons were not assigned specifically; both possible differences are shown.

Table S2: Crystallographic data for norvitamin B_{12} (**1**)

	norvitamin B_{12}
empirical formula	$C_{62}H_{86}N_{14}O_{14}PCo \cdot C_3H_6O \cdot 14\ H_2O$
acetone sites	1
H_2O sites	5 fully, 18 partially occ.
formula weight	1651.65
crystal system	orthorhombic
space group	$P2_12_12_1$
unit cell dimensions	
a [Å]	15.573(3)
b [Å]	22.846(5)
c [Å]	24.583(5)
V [Å^3]	8746(3)
Z	4
D_{calc} [g cm^{-3}]	1.254
μ [mm^{-1}]	0.295
F(000)	3528
crystal size [mm^3]	0.3 x 0.1 x 0.1
θ-range for data collection [°]	1.84-29.74 (0.85 Å resol.)
wavelength [Å]	0.8426
reflections collected	40787
data reduction programs	Denzo/Scalepack
independent reflections	7744
R(int)	0.039
completeness to θ = 29.74° (0.85 Å)	94.9%
data/restraints/parameters	7744/1693/1131
final R indices (all data)	
R_1	0.0796
wR_2	0.2137
largest diff. peak/hole (e Å^{-3})	0.89/–1.00

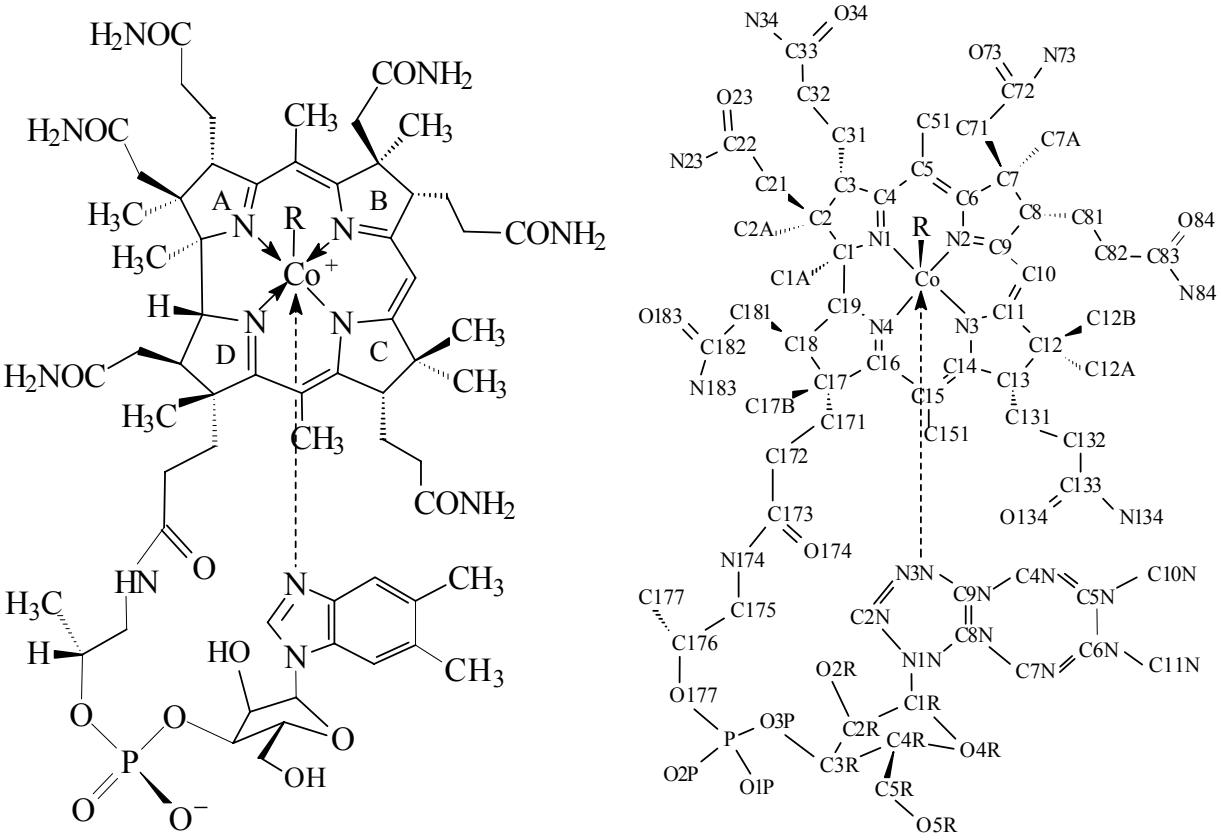

Figure S1: Structural formula of cobalamins and atom numbering used here (see [S8]).

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