ELSEVIER

Journal of Molecular Catalysis A: Chemical 116 (1997) 147–155

JOURNAL OF MOLECULAR CATALYSIS A: CHEMICAL

An efficient method for the preparation of methylcobalamin, nature's organometallic methyl transfer catalyst

Martin Tollinger, Tomas Dérer, Robert Konrat, Bernhard Kräutler *

Institute of Organic Chemistry, University of Innsbruck, Innrain 52a, A-6020 Innsbruck, Austria

Received 29 December 1995; revised 3 June 1996; accepted 10 June 1996

Abstract

Methylcobalamin (**1**), the structurally simplest organocobalamin and nature's organometallic methyl transfer catalyst, was prepared from aquocob(III)alamin and methyl iodide in > 90% yield in a previously unexplored, but experimentally simple procedure, using formate as reducing agent and exploiting the previously established tendency of cob(II)alamin to be alkylated by methyliodide. A thorough NMR-spectroscopic analysis of aqueous solutions of this organometallic B_{12}-derivative was carried out at ambient temperature, which allowed the unambiguous and nearly complete assignment of the proton, nitrogen and carbon signals.

Keywords: Alkylation reaction; Biocatalyst; Bioorganometallic chemistry; NMR-spectroscopy; Organometallic cofactor

1. Introduction

The organometallic B_{12}-derivative methylcobalamin (**1**) was first made available by partial synthesis from vitamin B_{12} (**2**) [1,2], soon after X-ray analysis had revealed the organometallic nature of the coenzyme B_{12} (**3**) (Figs. 1 and 2) [3]. Shortly thereafter, methylcobalamin (**1**) was isolated from natural sources [4] and recognized to be another B_{12}-coenzyme. In the meantime methylcorrinoids, such as **1**,

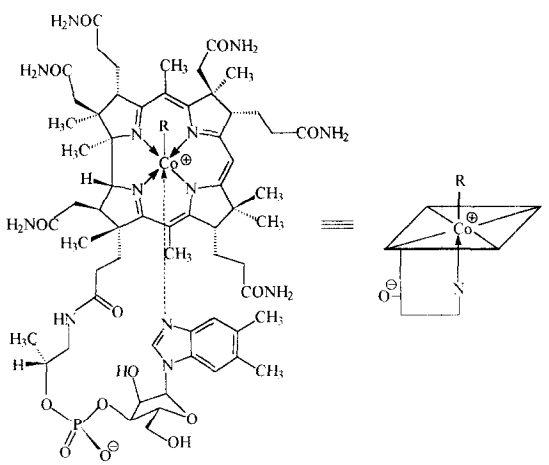


Fig. 1. Structural formulae of vitamin B_{12}-derivatives. **1**: R = CH_3, methyl-cob(III)alamin; **2**: R = CN, vitamin B_{12}; **3**: R = 5′-deoxy-5′adenosyl, coenzyme B_{12}; **5**: R = H_2O^+ (Cl^-), aquocob(III)alamin(chloride); **6**: R = e^-, cob(II)alamin.

* Corresponding author. Tel.: +43-512-5075200; fax: +43-512-5072892.

PII S1381-1169(96)00229-4

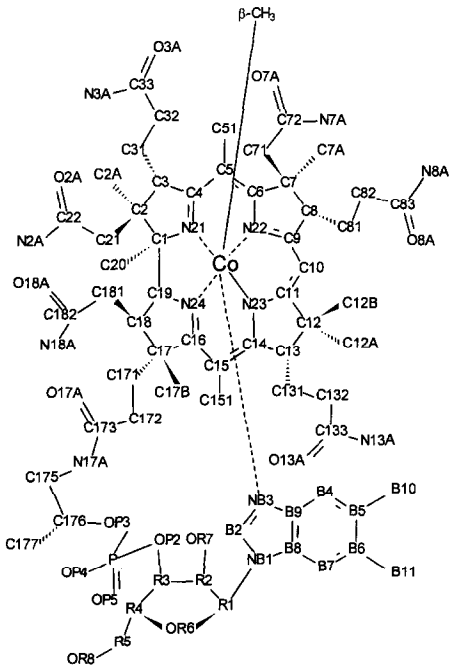


Fig. 2. Atom-numbering of methylcob(III)alamin (**1**) used for the description of the NMR-results.

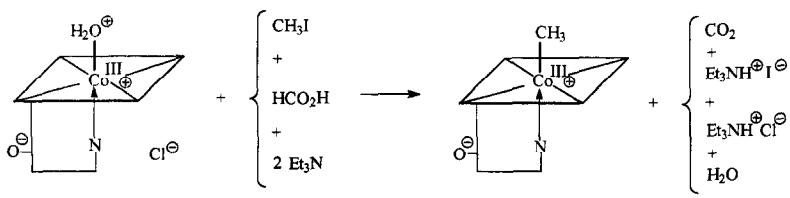
have been shown to be important cofactors in some enzymatic methyl transfer reactions [5]. Among the latter, it is in the newly discovered, organometallic pathway of fixation of carbon dioxide via acetyl coenzyme A [6], used in some anaerobically growing microorganisms [7], that the metabolic function of methyl-corrinoids as natural methyl transfer catalysts appears to be particularly fundamental [8].

So far, the standard preparation of methylcobalamin (**1**) has relied on the rapid alkylation of the strongly nucleophilic cob(I)alamin (**4**) [1,2,9–11]. The original investigations, concerned with the synthesis of methylcobalamin (**1**) from the vitamin **2**, were explored as model reactions for the preparation of the coenzyme **3** [1,2]. Indeed, the early methods for the preparation of **1** were quite involved with respect to work-up with an extraction with phenol [9]. More modern procedures for the preparation of **1** also rely on the formation of the strongly reduced and highly nucleophilic cob(I)alamin (**4**) and still require a rather tedious work-up [10,11]. The Co(I)corrinoid (**4**) can be prepared in situ by reduction with sodium borohydride (or with metallic zinc) either of **2** or of aquocob(III)alamin (**5**), a second commercially available B_{12}-form, that is more readily reducible than the vitamin **2**.

Here we report on an alternative and efficient method for the preparation of methyl-cobalamin (**1**), using triethyl ammonium formate and methyl iodide as reducing and methylating agents, respectively. In addition, we present extensive NMR-spectroscopic data of the title compound in its 'base-on' form (at pH 5.5) in aqueous solution.

2. Results and discussion

The method for the synthesis of methylcobalamin (**1**), reported here, depends on the known methylation reaction of the Co(II)-form



Scheme 1.

cob(II)alamin (**6**) with methyl iodide [12,13]. This has been investigated in kinetic experiments, but has not been used, so far, for preparative purposes [12,13]. Blaser and Halpern [12] have suggested a mechanism for the methylation of **6** with methyl iodide in aqueous solution, involving the reaction of **6** with a complex of the iodide and **6** (corresponding to a third order rate law). Our preparative experiments, in a qualitative sense, are consistent with such a mechanism. The use of cob(II)alamin (**6**) as the corrinoid reaction partner in a preparative experiment, in turn, is made attractive by the ease of its preparation by the reduction of aquocobalamin (**5**) [14] and of other corrinoids [15] with formic acid or formate salts. (Scheme 1)

In a typical experiment, aquocobalamin (**5**) was dissolved in a deaerated aqueous solution of triethyl ammonium formate. The Co(III)corrin **5** was rapidly reduced to cob(II)alamin (**6**), as tested UV/Vis-spectroscopically, and the resulting solution of **6** then was treated with an excess of methyl iodide. At room temperature and with protection from light the deaerated reaction mixture was left to stand for 5.5 h, after which time the conversion to **1** was practically complete, as judged by a UV/Vis-spectrum. After work-up, the organocobalamin **1** was obtained as red crystals in 92–95% yield.

The method reported here for the preparation of **1** proved to be useful also for the preparation of **1**-^{13}C, as well as of **1**-d_3, isotopically labeled at the cobalt-bound methyl ($^{13}CH_3$, CD_3).

A thorough NMR-spectroscopic analysis (of the 'base-on' form) of methylcobalamin (**1**) was carried out, in order to obtain a complete set of spectroscopic data that would provide the basis for a detailed analysis of the solution structure of this 'complete' organometallic B_{12}-derivative [16]. Earlier, Rossi et al. [11] have assigned selected proton and ^{13}C-signals of this B_{12}-coenzyme, in connection with a report on its crystal structure. More recently, Calafat and Marzilli have listed an extensive set of signal assignments for the carbons in the base-on form of **1** [17] in connection with a discussion of the NMR-spectra of several cob(III)alamins carrying 'inorganic' axial ligands [1].

Here we report a thorough analysis of **1** in aqueous solution (pH 5.5) and on a complete assignment of all (but one of the two exchange labile hydroxyl) protons (65 signals accommodating for 90 protons), of all (but two of the corrin-ligand) nitrogens and of all 63 carbons of a molecule of **1.** Signal assignments and chemical shifts for ^{1}H, ^{13}C and ^{15}N are listed in Table 1 and were deduced from the following sets of two-dimensional spectra [2]: Assignment of both ^{1}H and ^{13}C signals was obtained from two-dimensional gradient-enhanced heteronuclear experiments (gradient-enhanced heteronuclear sin-

[1] After submission of the original manuscript a publication by Brown et al. [18] (= BEZV) reported on an independent signal assignment of the carbons and the non-exchange labile protons of 1 (in D_2O). Our signal assignments agree largely with these assignments in D_2O solution and assignments concerning carbons published earlier [17]. However, our use of band-selective gradient-enhanced experiments allowed for the fist time to resolve ambiguities concerning the carbons and protons of certain propionamide side chains. Using this approach in combination with ROESY data we arrived at a different assignment of the methylene protons at C171 and C172. Our assignment concerning the distinction these methylene groups is based on the observation of only one long range correlation ($^3J_{CH}$) in a band-selective gradient-enhanced HMBC experiment between the methyl protons H(C17B) and one of the two carbons in question, i.e. C171, which gives rise to a signal at 34.5 ppm. In a band-selective gradient-enhanced HSQC experiment we observed the attached protons H(C171) at 1.78 and 2.47 ppm (reported at 2.07 and 2.45 ppm (BEZV)). Thus, of the two carbon resonances, the one observed at higher field indeed must be assigned to C172, but its protons H(C172) give rise to signals at 2.09 and 2.43 ppm (but were reported both at 1.80 ppm (BEZV)). Our assignment is supported by the observation of NOEs between one of the diastereotopic protons H(C171) at 1.78 ppm and the protons H(C17B) and H(C18) as well as of NOEs between a proton giving the signal at 2.09 ppm and assigned here to be bound to C172, and H(R4), H(N17A) and H(OR7), which are all located in the nucleotide-loop 'cavity'. This assignment (of the proton at 2.09 ppm to H(C172)) is further corroborated by a lack of an NOE between it and H(C17B) (the near isochronicity of the signals at 2.43 and 2.47 ppm with that of the C(151) methyl group protons at 2.45 ppm prevents an unambiguous distinction of their NOEs).

[2] In order to unambiguously assign the amide protons it was necessary to apply experimental techniques which do not presaturate the water signal, because otherwise the intensity of the amide protons would be significantly decreased due to chemical exchange.

Table 1
^{1}H-, ^{13}C- and ^{15}N-NMR chemical shifts [a] and signal assignments for methyl-cobalamin

Assignment	δ (^{1}H)	δ (^{13}C) [b]	δ (^{15}N)
β-CH_3	− 0.05	9.5	—
C1	—	87.6	—
C2	—	48.9	—
C21	2.31/2.29	45.9	—
C22	—	179.0	—
N2A	7.76 (H_E)/7.07 (H_Z)	—	110.9
C2A	1.35	19.4	—
C3	4.09	58.0	—
C31	2.00/1.95	28.7	—
C32	2.48/2.42	37.8	—
C33	—	180.7	—
N3A	7.56 (H_E)/6.83 (H_Z)	—	104.3
C4	—	177.7	—
C5	—	108.0	—
C51	2.48	18.0	—
C6	—	165.7	—
C7	—	52.6	—
C71	2.41/1.93	45.6	—
C72	—	177.8	—
N7A	7.66 (H_E)/6.93 (H_Z)	—	109.7
C7A	1.77	21.7	—
C8	3.38	57.1	—
C81	1.85/0.94	28.5	—
C82	1.72/1.05	34.5	—
C83	—	180.1	—
N8A	6.35 (H_Z)/6.16 (H_E)	—	101.9
C9	—	172.3	—
C10	5.91	96.5	—
C11	—	175.9	—
C12	—	49.2	—
C12A	1.40	22.3	—
C12B	0.94	34.6	—
C13	3.05	55.8	—
C131	2.02/1.95	30.4	—
C132	2.59	37.5	—
C133	—	180.9	—
N13A	7.74 (H_E)/7.00 (H_Z)	—	105.4
C14	—	166.2	—
C15	—	106.0	—
C151	2.45	18.1	—
C16	—	177.4	—
C17	—	60.4	—
C171	2.47/1.78	34.5	—
C172	2.43/2.09	34.4	—
C173	—	177.8	—
N17A	8.18	—	111.6
C17B	1.33	19.2	—
C18	2.62	41.6	—
C181	2.67/2.64	34.6	—
C182	—	178.9	—
N18A	7.87 (H_E)/7.01 (H_Z)	—	106.4
C19	3.96	76.3	—
C20	0.47	22.9	—
C175	3.55/3.08	47.5[1]	—
C176	4.32	75.5[2]	—
C177	1.21	21.4[3]	—
R1	6.26	89.5	—
R2	4.25	71.6[4]	—
R3	4.71	75.7[5]	—
R4	4.07	84.0[6]	—
R5	3.90/3.75	63.0	—
OR7	5.49	—	—
B2	6.98	144.4	—
B4	6.29	120.9	—
B5	—	134.1	—
B6	—	136.3	—
B7	7.19	113.1	—
B8	—	133.0	—
B9	—	140.7	—
B10	2.21	22.3	—
B11	2.23	22.2	—
N22/N23	—	—	188.6/190.1
NB1	—	—	149.5
NB3	—	—	218.8

[a] ^{1}H-NMR: δ with $\delta(TSP)_{int} = 0$ ppm. ^{13}C-NMR: δ with $\delta(TSP)_{int} = 0$ ppm. ^{15}N-NMR: δ with $\delta(NH_3(l))_{ext} = 0$ ppm.
[b] ^{31}P–^{13}C couplings: (1) $J_{CP} = 3.0$ Hz, (2) $J_{CP} = 6.6$ Hz, (3) $J_{CP} = 4.4$ Hz, (4) $J_{CP} < 1$ Hz, (5) $J_{CP} = 4.5$ Hz, (6) $J_{CP} = 8.5$ Hz.

gle-quantum coherence, PFG-HSQC [19,20]; gradient-enhanced ^{1}H-detected multiple-bond heteronuclear multiple-quantum coherence, PFG-HMBC [19,21]; gradient-enhanced heteronuclear single-quantum coherence total correlation spectroscopy, PFG-HSQC-TOCSY [22,23]) and conventional homonuclear experiments like total correlation spectroscopy ('watergate'-TOCSY [24,25]) and rotating frame Overhauser enhancement spectroscopy ('watergate'-ROESY [25,26]). To resolve residual ambiguities in the signal assignment of the carbons and protons, we have also performed ^{13}C band-selective gradient-enhanced heteronuclear correlation experiments, such as band-selective gradient-enhanced HMBC [19,21,27] and band-selective gradient-enhanced HSQC [19,20] as well as band-selective gradient-enhanced HSQC-TOCSY [22,23]. These techniques exploit the very high two-dimensional resolution obtained by selective excitation of the

carbon region of interest. Based on a series of homonuclear and ^{1}H, ^{13}C-heteronuclear correlations, the complete assignment of the signals of the hydrogens of **1** was achieved, extending the range of identified signals to those of exchange labile protons in aqueous solution [18].

Furthermore, 'watergate'-ROESY and ^{1}H–^{15}N PFG-HSQC experiments in aqueous solution allowed for the assignment of the signals of the H(OR7) hydroxyl proton and of all thirteen amide protons, together with their (directly bonded) amide nitrogens at natural abundance in **1**. Fig. 3 shows the ^{1}H–^{15}N PFG-HSQC spectrum of **1**. For each amide group except the

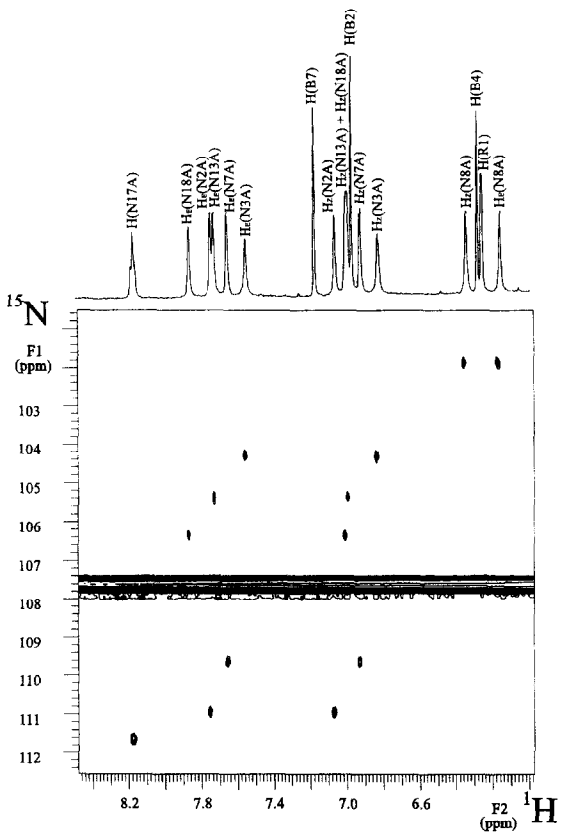


Fig. 3. Part of the ^{1}H–^{15}N PFG-HSQC spectrum of **1**, containing the cross peaks between the amide protons and the directly bonded amide nitrogens. The connectivities between the amide ^{15}N resonances and their attached protons are shown using the accompanying downfield portion of the ^{1}H NMR spectrum. The amide protons are labelled by their side chain designations (Fig. 2). Other protons resonating in this spectral area of the ^{1}H NMR spectrum are also labelled.

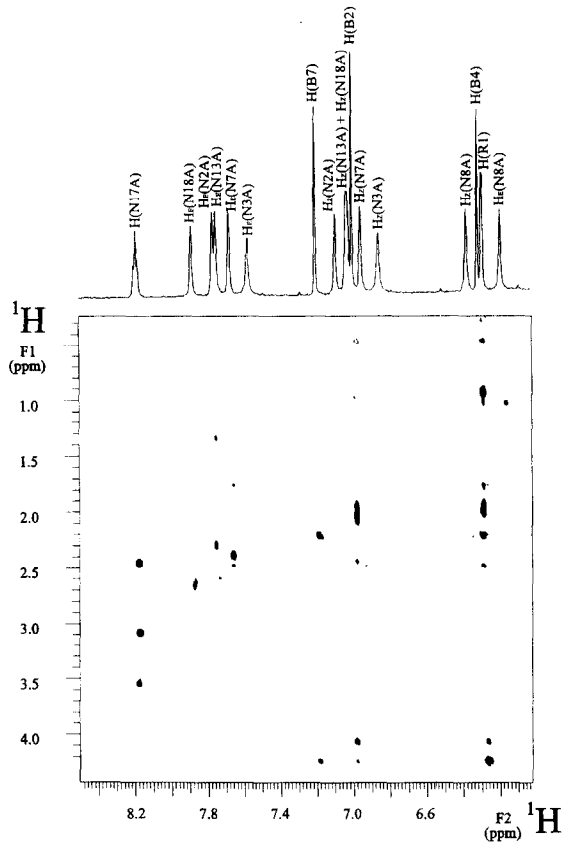


Fig. 4. Part of the 200 ms 'watergate'-ROESY spectrum of **1** containing NOEs between amide protons and protons in the highfield region of the ^{1}H NMR spectrum of **1**. The amide protons are labeled by their side chain designations (Fig. 2) in the accompanying downfield portion of the ^{1}H NMR spectrum. Other protons resonating in this part of the ^{1}H NMR spectrum are also labeled.

d-side chain protons, the low-field proton signal was assigned to H_E due to ROESY cross peaks between the amide protons and side chain methylene protons, since only H_E can be close in space to the α-methylene protons of the carboxamide functions (see Fig. 4). The inverse assignment (i.e. H_E at higher field than H_Z) concerning the d-side chain amide protons is compatible with a specific shielding effect of the nearby coordinated dimethylbenzimidazole base affecting both amide protons and causing remarkable highfield shifts of both signals. Comparing our ^{15}N chemical shift data with values reported for a dimethyl sulfoxide solution of **1** [28], the sequence of the amide nitrogen signals

was found to be dependent upon the solvent. One striking feature in these data is that the ^{15}N resonance of the f-amide occurs at lowest field in aqueous solution (Fig. 3), resulting in the chemical shift sequence *f*–a–c–g–e–b–d, while the sequence in dimethyl sulfoxide solution is a–c–*f*–g–e–b–d. This *relative* shift of the ^{15}N resonance of the f-amide is consistent with the formation of a hydrogen bond to a water molecule in aqueous solution, assigned to an intramolecular water bridge linking the H(OR7) proton and the phosphate [16]. In addition, the sequence of the amide resonances in the spectrum of **1** differs characteristically from that of **5** [29] also: a notable low field shift of the ^{15}N signal of the c-acetamide occurs in the spectrum of **5** that has been traced back to an intramolecular hydrogen bond specific to this acetamide side chain [29].

From ^{1}H–^{15}N PFG-HMBC spectra the ^{15}N resonances for the benzimidazole base of **1** were detected indirectly due to long range couplings to H(B2) and were further assigned in analogy to literature data [30]. Likewise, the ^{15}N resonances for the corrin nitrogens N22 and N23 were detected due to their long range couplings to the vinylic H(C10), but an individual assignment was not made.

Elsewhere, we will use the information from these spectra to analyze thoroughly the solution structure of **1**, with emphasis on conformational properties of the side chains and of the nucleotide loop of **1** [16].

3. Conclusion and outlook

Here we have presented a new method for the preparation of the organometallic B_{12} derivative methylcobalamin, the organometallic methylating agent and cofactor of enzymes involved in methylation reactions. It does not require the formation of the strongly reducing, nucleophilic cob(I)alamin and is based on the radical-like reactivity of cob(II)alamin and therefore may be of relevance also with respect to more recent considerations concerning the methylation of enzyme bound Co(II)corrin [31]. Accordingly, the efficient preparation of reductively degradable organocobalamins has also been achieved in this laboratory [32], based on the reaction of organic halides with cob(II)alamin [12].

In addition, the signals in the ^{1}H-, ^{13}C- and ^{15}N-NMR-spectra of **1** in aqueous solution have been assigned, so that the stage is set for a structural analysis in aqueous solution of **1** [16], which is intended to probe the relevance of the aqueous surroundings with respect to the structure of this B_{12} coenzyme in solution,

4. Experimental part

4.1. General data: reagents and solvents

Aquocobalamin hydrochloride (from Roussel UCLAF); formic acid puriss. p.a., triethylamine puriss. p.a., methyl iodide puriss. p.a., methanol puriss. p.a., acetone puriss. p.a. (all from Fluka); ^{13}C-methyl iodide (90% ^{13}C, Stohler Isotope Chemicals, Innerberg, Switzerland); water was purified by mixed-bed deionization, carbon adsorption and filtration (Barnstead NANOpure system).

4.2. Preparation of crystalline methylcobalamin (1)

All solvents and liquid reagents were degassed prior to the synthesis which was performed at room temperature in a glovebox (under nitrogen). Aquocobalamin hydrochloride (**5**) (1000.6 mg, 7.24×10^{-4} mol) was dissolved in 20 mL of water; 0.50 mL of formic acid (1.3×10^{-2} mol) and 1.85 mL of triethylamine (1.3×10^{-2} mol) were added to the solution. Due to the reduction of aquocobalamin the deep red solution immediately changed its color to brown. 1.0 mL of methyl iodide (1.6×10^{-2} mol) was added and the solution was stirred vigorously for 5.5 h in the dark, recovering its red color.

All further operations were carried out in a dark room. The reaction mixture was applied on a column of silica gel (3 cm × 15 cm) and the corrinoid was eluted with methanol:water (9:1) as the first (red) fraction. After removal of the solvents with the help of a rotary evaporator at $T \leq 35$°C the so-obtained red residue was dissolved in ca. 5 mL of water and crystallized by dropwise addition of ca. 3 mL of acetone. The crystalline methylcobalamin was separated from its mother liquor, washed with acetone and dried (high vacuum (ca. 0.01 mbar), room temperature). After recrystallization from a similar solvent mixture 901.8 mg (6.70×10^{-4} mol, 92.5%) of crystalline methylcobalamin (**1**) was obtained, that was used for the subsequent spectral analysis by mass spectrometry (see below) and NMR spectroscopy (see text).

FAB-MS (Finnigan MAT 95, nitrobenzyl alcohol (NOBA) matrix, Cs^+ bombardment, positive ion spectrum): *m/z* (%) 1347.6 (30.9), 1346.6 (74.6), 1345.6 (100.0) [MH^+], 1343.6 (10.1), 1332.6 (24.2), 1331.6 (56.3), 1330.7 (78.9) [MH^+-CH_3], 1328.7 (15.5).

4.3. Preparation of $^{13}CH_3$-cob(III)alamin (1-^{13}C)

In an experiment, carried out in an analogous fashion, but using 1001.5 mg of (**5**) (7.24×10^{-4} mol), 0.50 mL of formic acid (1.3×10^{-2} mol), 1.85 mL of triethylamine (1.3×10^{-2} mol) and 0.44 mL of ^{13}C-methyl iodide (2.8×10^{-3} mol) 887.8 mg crystalline $^{13}CH_3$-cob(III)alamin (**1**-^{13}C) was obtained (6.59×10^{-4} mol, 91.0%), to be used for the subsequent spectral analysis by mass spectrometry and NMR spectroscopy.

FAB-MS (Finnigan MAT 95, nitrobenzyl alcohol (NOBA) matrix, Cs^+ bombardment, positive ion spectrum): *m/z* (%) 1348.3 (24.0), 1347.3 (62.2), 1346,3 (92.0) [MH^+], 1345.3 (19.6), 1332.4 (30.2), 1331.3 (64.6), 1330.3 (100.0) [MH^+-$^{13}CH_3$], 1329.3 (19.0), 1328.3 (14.1); calculated ^{13}C-content of (**1**-^{13}C): 86% (± 10%); ^{13}C-content determined from integrals of high field signals in the 500 MHz ^{1}H-NMR-spectrum of (**1**-^{13}C): 86% (± 2%).

4.4. NMR experiments

All NMR experiments were carried out on a Varian 500 UNITY plus spectrometer with a 5 mm indirect detection probe equipped with field gradient facilities and a 5 mm broadband direct detection probe: 499.887 MHz (^{1}H)/125.15 MHz (^{13}C)/50.66 MHz (^{15}N). NMR solutions: 10 mM solutions, sample size 0.7 ml, pH = 5.5, 100 mM phosphate buffer, 26°C.

4.5. 2D-NMR experiments

Gradient-enhanced heteronuclear single-quantum coherence experiment (PFG-HSQC) [19,20]; gradient-enhanced ^{1}H-detected multiple-bond heteronuclear multiple-quantum coherence experiment (PFG-HMBC) [19,21]; band-selective gradient-enhanced ^{1}H-detected multiple-bond heteronuclear multiple-quantum coherence experiment (band-selective PFG-HMBC) [19,21,27]; 'watergate' total correlation spectroscopy ('watergate'-TOCSY) [24,25]; 'watergate' spin-locked NOE spectroscopy ('watergate'-ROESY) [25,26]. All experiments parametrized as described in [29].

4.6. Gradient-enhanced proton-relayed X, H correlation spectroscopy (PFG-HSQC-TOCSY) [23,24]

The HSQC-TOCSY spectrum of methylcobalamin resulted from a 512 × 1024 data matrix before and a 1024 × 2048 data matrix after zero filling, with 64 scans per t_1 value and a delay time between scans of 1 s. A MLEV-17 mixing sequence of 70 ms at 6.5 kHz rf field strength (corresponding to a 90° pulse length of 38.2 μs) was applied along the *x*-axis. Gradient strengths and durations were as follows: 20.0 G/cm, 2 ms; −15.0 G/cm, 1 ms; 15 G/cm, 1

ms. All gradients were rectangular and applied along the z-axis. Decoupling (during acquisition) was achieved with the use of the GARP [33] decoupling sequence, using a 3030 Hz radio frequency field. Quadrature detection in F_1 was achieved by quadrature phase shift of the carbon excitation pulse. Cosine bell squared filters were used both in t_1 and t_2 dimensions. Low-frequency substraction in the time domain was applied to improve the spectra, using standard Varian Vnmr software (lfs).

4.7. Gradient-enhanced band-selective proton-relayed X, H correlation spectroscopy (band-selective PFG-HSQC-TOCSY) [23,24]

The selective HSQC-TOCSY spectrum of methylcobalamin resulted from a 512×1024 data matrix before and a 1024×2048 data matrix after zero filling, with 64 scans per t_1 value and a delay time between scans of 1 s, using a 2 ms EBURP-2 [34] band-selective excitation pulse for the excitation of the carbon region of interest. This band-selective excitation pulse was created by using the VARIAN Vnmr software package *pulsetools*. Bloch equation simulations were used to define an excitation pulse with proper pulse length and excitation bandwidth. A MLEV-17 mixing sequence of 70 ms at 6.5 kHz rf field strength (corresponding to a 90° pulse length of 38.2 μs) was applied along the x-axis. Gradient strengths and durations were as follows: 20.0 G/cm, 2 ms; −15.0 G/cm, 1 ms; 15 G/cm, 1 ms. All gradients were rectangular and applied along the z-axis. Decoupling (during acquisition) was achieved with the use of the GARP [32] decoupling sequence, using a 3030 Hz radio frequency field. Quadrature detection in F_1 was achieved by quadrature phase shift of the selective carbon excitation pulse. Cosine bell squared filters were used both in t_1 and t_2 dimensions. Low-frequency substraction in the time domain was applied to improve the spectra, using standard Varian Vnmr software (lfs).

Acknowledgements

We thank Doz. Dr. K.-H. Ongania for measuring FAB-MS spectra and Dr. G. Färber for help with computer modelling. This work was supported by the Austrian Science Foundation (Proj. No. P10816-GEN)

References

[1] K. Bernhauer, O. Müller and G. Müller, Biochem. Z. 336 (1962) 102.
[2] E.L. Smith, L. Mervyn, A.W. Johnson and N. Shaw, Nature 194 (1962) 1175.
[3] P.G. Lenhert and D.C. Hodgkin, Nature 192 (1961) 937.
[4] K. Lindstrand and K.G. Stahlberg, Acta Med. Scand. 174 (1963) 665.
[5] B. Kräutler, in: The Biological Alkylation of Heavy Elements, P.J. Craig and F. Glockling (Eds.) (Royal Soc. Chem. London, 1988) p. 31.
[6] H.G. Wood, S.W. Ragsdale and E. Pezacka, Trends Biochem. Sci. 11 (1986) 14.
[7] R.K. Thauer, Eur. J. Biochem. 176 (1988) 497.
[8] S.W. Ragsdale, Crit. Rev. Biochem. Mol. Biol. 26 (1991) 261.
[9] D. Dolphin, in: Inorg. Synthesis, D.H. Busch (Ed.), Vol. XX (John Wiley and Sons, New York, 1980) p. 134.
[10] K.L. Brown and S. Peck, in: Organometallic Synthesis, R.B. King and J.J. Eisch (Eds.), Vol. 4 (1988).
[11] M. Rossi, J.P. Glusker, L. Randaccio, M.F. Summers, P.J. Toscano and L.G. Marzilli, J. Am. Chem. Soc. 107 (1985) 1729.
[12] H.U. Blaser and J. Halpern, J. Am. Chem. Soc. 102 (1980) 1684, and references therein.
[13] J.M. Pratt, M.S.A. Hamza and G.J. Buist, J. Chem. Soc. Chem. Commun. (1993) 701, and references therein.
[14] D.E. Linn, Jr. and E.S. Gould, Inorg. Chem. 27 (1988) 1625.
[15] B. Kräutler and C. Caderas, Helv. Chim. Acta 67 (1984) 1891.
[16] M. Tollinger, R. Konrat and B. Kräutler, unpublished.
[17] A.M. Calafat and L.G. Marzilli, J. Am. Chem. Soc. 115 (1993) 9182.
[18] K.L. Brown, D.R. Evans, J.D. Zubkowski and E.J. Valente, Inorg. Chem. 35 (1996) 415.
[19] R.E. Hurd and B.K. John, J. Magn. Reson. 91 (1991) 648.
[20] A.L. Davis, J. Keeler, E.D. Laue and D. Moskau, J. Magn. Reson. 98 (1992) 207.
[21] A. Bax and M.F. Summers, J. Am. Chem. Soc. 108 (1986) 2093.
[22] T.J. Norwood, J. Boyd, J.E. Heritage, N. Soffe and I.D. Campbell, J. Magn. Reson. 87 (1990) 488; J. Cavanagh, A.G. Palmer, III, P.E. Wright and M. Rance, J. Magn. Reson. 91 (1991) 429.
[23] G. Wider and K. Wüthrich, J. Magn. Reson. B 102 (1993) 239; A. Bax and S. Pochapsky, J. Magn. Reson. 99 (1992)

638; A. Majumdar and E.R.P. Zuiderweg, J. Magn. Reson. B 102 (1993) 242.
[24] L. Braunschweiler and R.R. Ernst, J. Magn. Reson. 53 (1983) 521; A. Bax and D.G. Davies, J. Magn. Reson. 65 (1985) 355; D.G. Davies and A. Bax, J. Am. Chem. Soc. 107 (1985) 2820.
[25] M. Piotto, V. Sautek and V. Sklenar, J. Biomol. NMR 2 (1992) 661.
[26] A.A. Bothner-By, R.L. Stephens, C.D. Warren and R.W.J. Jeanloz, J. Am. Chem. Soc. 106 (1984) 811; A. Bax and D.G. Davies, J. Magn. Reson. 63 (1985) 207.
[27] R. Konrat, manuscript in preparation.
[28] K.L. Brown and D.R. Evans, Inorg. Chem. 32 (1993) 2544.
[29] C. Kratky, G. Färber, K. Gruber, K. Wilson, Z. Dauter, H.-F. Nolting, R. Konrat and B. Kräutler, J. Am. Chem. Soc. 117 (1995) 4654.
[30] K.L. Brown and D.R. Evans, Inorg. Chem. 33 (1994) 525.
[31] J.T. Jarret, M. Amaratunga, C.L. Drennan, J.D.Scholten, R.H. Sands, M.L. Ludwig and R.G. Matthews, Biochemistry 35 (1996) 2464.
[32] M. Puchberger and B. Kräutler, unpublished.
[33] A.J. Shaka, P.B. Barker and R. Freeman, J. Magn. Reson. 64 (1985) 547.
[34] H. Geen and R. Freeman, J. Magn. Reson. 87 (1990) 415.