45. Lipophilic Functionalized Cobyrinic-Acid Derivatives

Part 1

Cobester α-Monoacids (α,α,α,β,β,β-Hexamethyl α-Hydrogen *Coα,Coβ*-Dicyanocobyrinates)

by **Bernhard Kräutler**[1]* and **Christian Caderas**[2])

Laboratorium für Organische Chemie der ETH, Universitätstrasse 16, CH–8092 Zürich

and **Robert Konrat** and **Michael Puchberger**

Institut für Organische Chemie der Universität Innsbruck, Innrain 52a, A–6020 Innsbruck

and **Christoph Kratky***

Institut für Physikalische Chemie der Universität Graz, Heinrichstrasse 28, A–8010 Graz

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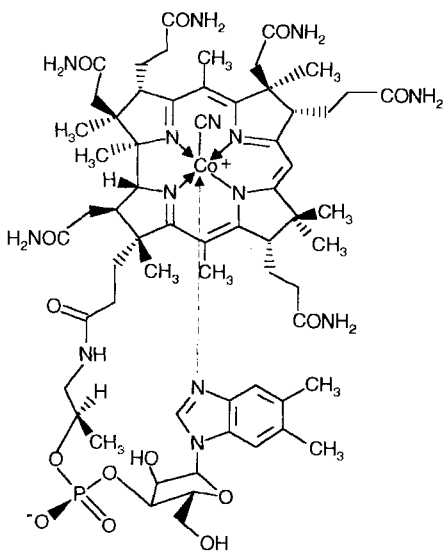
The four α,α,α, β,β,β-hexamethyl α-hydrogen *Coα,Coβ*-dicyanocobyrinates **2b, d–f**, with a free *b*-, *d*-, *e*-, and *f*-propionic-acid function, respectively, were prepared by partial hydrolysis of heptamethyl *Coα,Coβ*-dicyanocobyrinate (cobester; **1**) in aqueous sulfuric acid. The cobester monoacids **2b, d–f** were obtained as a *ca.* 1:1:1:1 mixture which was separated. The monoacids were purified by chromatography and isolated in crystalline form. The position of the free propionic-acid function was determined by an extensive analysis of **2b, d–f** using 2D-NMR techniques; an analysis of the C,H-coupling network topology resulted in an alternative assignment strategy for cobyrinic-acid derivatives, based on pattern recognition. Additional information on the structure of the most polar of the four hexamethyl cobyrinates, of the *b*-isomer **2b**, was also obtained in the solid state from a single-crystal X-ray analysis. Earlier structural assignments based on 1D-NMR spectra of the corresponding regioisomeric monoamides **3b, d–f** (obtained from crystalline samples of the monoacids **2b, d–f**) were confirmed by the present investigations.

Introduction. – In view of other structural features of vitamin B_{12} [1], its seven substituents bearing an amide function appear to be a less spectacular structural peculiarity, although the specific regio- and stereochemical arrangement of these at the corrin core provided a considerable challenge in the synthesis of cobyric acid [2] [3]. In return, however, these seven substituents provide a set of functional and polar terminal groups, which are positioned above and below the corrin core. In addition, the alternating attachment at the faces of the corrin core of the alkanoyl substituents in the cobyrinic-acid derivatives results in a remarkable facial partitioning: the three acetyl substituents are attached on the 'top' (β) face and the four propionyl chains on the 'bottom' (α) face of the corrin core [1] [4]. As a consequence, in vitamin-B_{12} derivatives, the seven peripheral alkanoyl functions form a well structured, three-dimensional array, whose chemical modification at specific sites is of considerable interest.

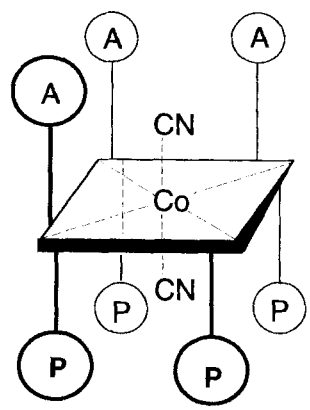
[1]) New address: Institut für Organische Chemie der Universität Innsbruck, Innrain 52a, A–6020 Innsbruck.

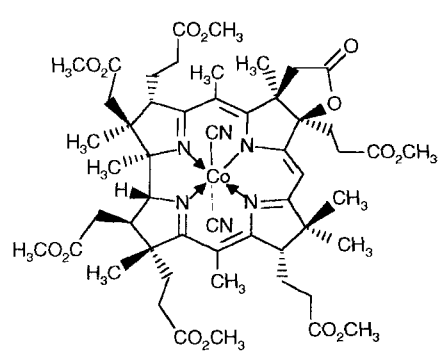
[2]) New address: *Sandoz Pharma AG*, CH–4002 Basel.

The peripheral functional groups were suggested to contribute crucially in the vitamin-B_{12} cofactors to their net of interactions with the protein[3]). Accordingly, to probe for the relevance of (any particular one of) such interactions, the chemical modification of specific vitamin-B_{12} carboxamide functions was attempted [5] [6]. The structures of the starting materials for this endeavor, three chromatographically separable monoacids from partial hydrolysis of vitamin B_{12}, were tentatively analyzed as being due to selective hydrolysis of the apparently less sterically hindered propionamide groups [7], but it was only recently that their structures became amenable to spectroscopic analysis [8]. A

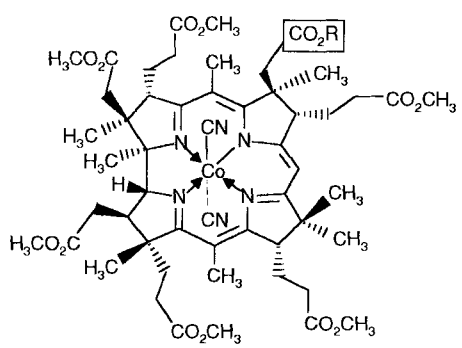


Vitamin B_{12}





4 Cobester *c*-lactone

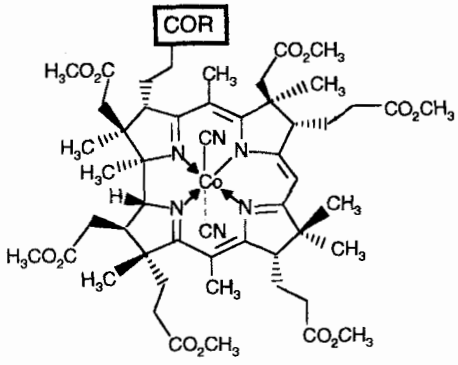


1 R = CH_3 Cobester
2c R = H Cobester *c*-acid

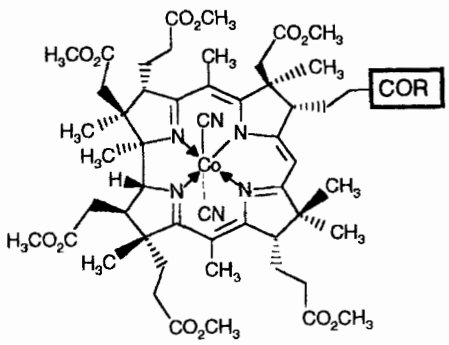
[3]) For recognition in transport proteins, such as intrinsic factor, see [5]; for activation of the coenzyme B_{12} in the vitamin-B_{12}-dependent mutases, see [6] (but also [8]).

special case in this respect are vitamin-B_{12} derivatives in which the *c*-acetyl side chain on the *β*-face is specifically modified, since the *c*-acetamide group can specifically be attacked oxidatively (to give a lactone or lactam function) [7].

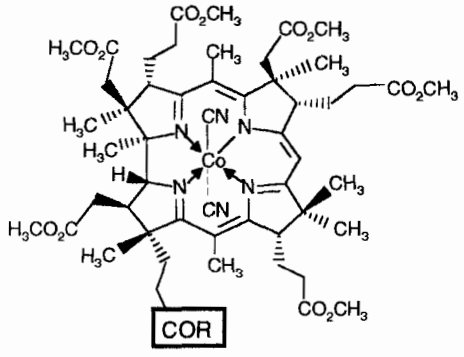
As an offspring of the work towards the total synthesis of vitamin B_{12}, the crystalline heptamethyl *Coα,Coβ*-dicyanocobyrinate (cobester; **1**) has become available by methanolysis of vitamin B_{12} [9] [10][4]). In this original context, **1** mainly served the purpose to represent an authentic cobyrinate as reference material, as well as to provide the basic structure for the preparation of cobyrinates with a differentiated function at the *f*-side chain [12] [13]: as precursor for the hexamethyl hydrogen cobyrinate **2f** [12], the corresponding monoamide **3f** was accessible by ammonolysis of **1** [13]. As expected, ammonolysis was not selective for the *f*-side chain, but occurred preferentially and with similar probability at any one of the four propionate functions: the four regioisomeric monoamides **3b**, **d**–**f** were formed as a mixture; they were separated and characterized, but (with the exception of **3f**) they could not be structurally assigned at that time [13].



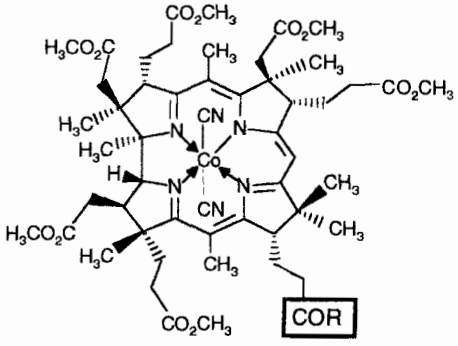
2b R = OH **3b** R = NH_2



2d R = OH **3d** R = NH_2



2f R = OH **3f** R = NH_2



2e R = OH **3e** R = NH_2

[4]) Partial methanolysis of vitamin B_{12} gave tetra-, penta-, and hexamethyl cobyrinates with a complementary set of acetamide substituents only [11], in accordance with the pattern of reactivities (propionyl > acetyl) in acid-catalyzed solvolysis.

The preparative access to specifically modified ('nonnatural') vitamin-B_{12} derivatives has also turned out to be of considerable interest. Cobester and specific compounds derived from it have helped to address mechanistic questions related to biological topics [14], as well as to develop the use of vitamin-B_{12} and of the vitamin-B_{12}-derived catalysts in organic synthesis [15]. Indeed, for the purpose of structuring the space above and below the cobalt-corrin plane, so as to modify the reactivity of the corrin-bound Co-ion in organometallic and other reactions, the seven alkanoyl side chains provide a unique foundation. For this task, derivatives of the heptamethyl ester **1** would appear particularly suitable, due to its solubility in a wide variety of conventional organic solvents. Earlier on, reductive opening of the lactone function of the 'cobester *c*-lactone' **4** [13] yielded the hexamethyl hydrogen cobyrinate **2c** with a free *c*-acetic-acid function (an 'upper' substituent) [13] [16]. The availability of **2c** subsequently was exploited in electrode coatings, as lipophilic *c*-esters in polymeric membranes [17], and, in *Scheffold*'s group, for the build-up of 'B_{12}-polymer' electrodes [18].

To date, a systematic entry to lipophilic vitamin-B_{12} derivatives with a free acid function on the 'lower' propionyl side chains is lacking. Here we report on a first step in this direction, on the preparation of the four hexamethyl hydrogen cobyrinates **2b**, **d**–**f** from **1** and on their structural characterization. Earlier, the structural assignment for **2b**, **d**–**f** has relied on that of the corresponding cobester α-monoamides **3b**, **d**–**f**[5]), which had been prepared from **2b**, **d**–**f** in *Eschenmoser*'s group [13] [19].

Results. – *Hexamethyl Hydrogen Cobyrinates* **2b**, **d**–**f** *from Acid-Catalyzed Partial Hydrolysis of* **1**. When a solution of heptamethyl *Coα,Coβ*-dicyanocobyrinate (cobester; **1**) [9] [10] was acidified with aqueous sulfuric acid, a color change to red indicated rapid loss of one of the Co-bound CN groups. Acid-catalyzed hydrolysis of the methylester functions of **1** took place slowly at 0° (TLC). In 20% (*v*/*v*) H_2SO_4/H_2O, 50% of **1** were transformed into (more polar) corrinoid hydrolysis products within *ca.* 4 h. Four new cobyrinates first built up (according to TLC) in a *ca.* 1:1:1:1 ratio, later succeeded by (and eventually consumed in part in the form of) serveral considerable more polar corrinoids. Interruption of the hydrolysis after 3 h 40 min by neutralization and separation of the mixture according to the degree of hydrolysis afforded 39% of a *ca.* 1:1:1:1 mixture of the monoacids **2b**, **2d**, **2e**, and **2f**, *ca.* 9% of a mixture of more polar hydrolysis products (diacids mainly), and 52% of unchanged starting material **1**. By quantitative HPLC analysis and based on the structural characterization discussed below, the monoacid fraction was found to consist (in order of increasing polarity) of 25.0% of **2e**, 23.6% of **2d**, 23.9% of **2f**, and 26.0% of **2b**, besides 1.5% of two other cobyrinate fractions[6]). The crude mixture **2b**/**2d**/**2e**/**2f** was separated into the chromatographically pure components by column chromatography (CC) on silica gel, and the individual monoacids were crystallized from acetone/hexane to give 19.0% of **2b**, 17.6% of **2d**, 18.1% of **2e**, and 17.8% of **2f**[7]).

The acid-catalyzed hydrolysis of **1** under less stringent conditions than reported above, in H_2O-saturated toluene/CF_3COOH 100:1 at room temperature led to slow, but clean hydrolysis of the propionate functions, with a somewhat altered selectivity: after

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[5]) See [10] [19] and ref. therein.

[6]) Due, presumably, to hexamethyl hydrogen cobyrinates with a free acetic-acid function, such as the *c*-acid **2c**.

[7]) Yield based on consumed **1**.

14 days *ca.* 40% of **1** were partially hydrolyzed, to give *ca.* 36% of **2b**/**2d**/**2e**/**2f** 1.37:1.0:1.06:2.81. Under these conditions, the *f*-acid **2f** turned out to be the most prominent of the monoacids.

The partial hydrolysis of **1** by aqueous MeOH containing KCN at 30° [20], in contrast, produced the *b*-isomer **2b** as the major monoacid, *i.e.*, **2b**/**2d**/**2e**/**2f** *ca.* 4.9:1.3:1.0:2.1.

Originally, the structures of monoacids **2b**, **d**–**f** were assigned by a comparison of their spectral properties with that of cobester (**1**) [9] [10] [13] [21], as well as by their transformation into the corresponding monoamides **3b**, **d**–**f** by *Nussberger* (see [10] [19] [22]). The structures of the amides **3b**, **d**–**f** [13] in turn were (re)assigned by *Ernst*[8]) by comparison of their ^{1}H- and ^{13}C-NMR data with that of **1** (see [19] [23]).

The constitutionally and configurationally intact cobyrinic-acid core of the regioisomeric hexamethyl hydrogen cobyrinates **2b**, **d**–**f** was indicated by their UV/VIS and CD spectra, that were virtually superimposable with that of **1** [9] [10] [13] [24]. The presence of one COOH function was consistent with the IR spectra (broad absorption at 3500–2500 cm^{-1}) and the fast atom bombardment (FAB) MS data (M^+ at m/z 1074). The bulk of the structural information was given by the ^{1}H-NMR (300, 400, and 500 MHz, $CDCl_3$) and ^{13}C-NMR (75.47 and 100.6 MHz) spectra: first of all, only the signals of six ester MeO groups were present, and the spectra were highly similar to the fully assigned ^{1}H- and ^{13}C-NMR spectra [14a] [21] of **1**. Closer scrutiny of the ^{13}C-NMR spectra of **2b**, **d**–**f** and **1**, recorded under identical conditions, provided a consistent support for the proposed structures, in spite of relatively small spectral differences. An original, tentative assignment of the individual signals was made on the basis of a correlation of the chemical shifts in these five spectra (see *Table 1*) [10]. In accordance with the expected local effect of the structural change (COOMe to COOH function) [25], chemical-shift differences considerably exceeding the standard deviations[9]) were noted systematically for signals of C-atoms along one particular propionyl substituent: *a*) a signal assigned to a carbonyl C-atom of a particular COOMe group in **1** [21] was found to be replaced by a new signal at lower field (δ *ca.* 174.5 ± 0.2 ppm, COOH function) in each of the spectra of **2b**, **d**–**f**; *b*) differences in the chemical shifts for saturated C-atoms in the spectra of **2b**, **d**–**f** and **1** were consistently observed mainly at the particular side chain and its point of attachment at the corrin core. In addition, the signal of one of the CN groups was noticeably displaced downfield (up to 3 ppm).

Complete and unambiguous assignment of the ^{1}H and ^{13}C signals was achieved in the meantime by modern homonuclear and heteronuclear 2D-NMR spectroscopy [26] (see *Tables 1* and *2*). In particular, double-quantum-filtered correlation spectroscopy (DQF-COSY) [27] was applied to establish ^{1}H-connectivities in **2b**, **d**–**f**. The signals of all H-bearing C-atoms were correlated with the corresponding ^{1}H-signals by the help of a

[8]) The amides **3b**, **d**–**f**, first prepared in *Eschenmoser*'s group by ammonolysis of **1** (*Maag* [13]), were identical to the ones made from the acids **2b**, **d**–**f** *via* a mixed anhyride (*Nussberger et al.* [22]). Samples from the cobester ammonolysis [13] were used in the NMR analysis, which was based on a careful correlation of the ^{13}C-NMR chemical-shift data of **1** with those of **3b**, **d**–**f** [23]. This correlation allowed the identification in each case of chemical-shift differences due to methylene C-atoms of one particular propionate side chain. The observed effects on the chemical shifts of selected ^{13}C signals were interpreted as a consequence of the replacement of a particular ester by a carboxamide function, whose site of attachment accordingly could be assigned [19] [22].

[9]) Calculated standard deviations of signals due to all C-atoms other than those of propionyl substituent: 0.08 ppm (β-substituents, 0.05 ppm; α-substituents, 0.08 ppm; corrin C-atoms, 0.09 ppm).

gradient-enhanced heteronuclear single-quantum coherence spectrum (PFG-HSQC) [28][10]). Gradient-enhanced heteronuclear multiple-bond coherence spectra (PFG-HMBC) [29] [30] were used to assign the complete set of the ^1H and ^{13}C signals, confirming largely the original tentative assignments.

First of all, the presence of three ester functions at the three acetyl side chains in the spectra of the monoacids **2b**, **d**–**f** was confirmed with the help of PFG-HMBC data. There, cross-peaks of the carbonyl region and of the carbonyl signals at 172.05, 171.12, and 171.87 ppm of **2b** correlate both with $COOCH_3$ and with CH_2 signals at 2.26/2.59, 2.39/2.69 (*AB* systems), and 2.62 ppm (*m*), assigned to $CH_2(2^1)$, $CH_2(7^1)$, and $CH_2(18^1)$, respectively. With the same strategy, the more difficult task of the identification of the (free) propionic-acid side chain of **2b** was achieved as follows: Of the remaining four carbonyl signals, the three at 173.0, 173.6, and 173.9 ppm exhibit heteronuclear long-range correlations both to CH_3O signals (at 3.70, 3.68, and 3.63 ppm) and to CH_2 signals

Table 1. *^{13}C-NMR Chemical-Shift Values for the Cobester Monoacids* **2b**, **d**–**f** *and Signal Assignments Based on 2D-NMR Spectra*[a])

	2b	**2d**	**2e**	**2f**		**2b**	**2d**	**2e**	**2f**
C(15^1)	15.28	15.28	15.34	15.22	MeO	51.82	51.82	51.88	51.79
C(5^1)	15.93	15.87	15.84	15.90	C(18^4)(MeO)	52.36	52.36	52.33	52.36
C(2A)	16.98	16.95	16.92	17.01	C(13)	53.85	53.85	53.94	53.91
C(17B)	18.48	18.45	18.42	18.60	C(8)	54.36	54.75	54.42	54.39
C(7A)	19.29	19.20	19.29	19.35	C(3)	56.88	56.79	56.79	56.82
C(12A)	19.86	19.77	19.74	19.83	C(17)	58.47	58.53	58.59	58.47
C(20)	22.02	21.96	21.90	22.19	C(19)	75.00	74.94	74.97	75.03
C(3^1)	24.83	25.10	25.07	25.13	C(1)	82.73	82.70	82.73	82.82
C/13^1)	25.79	25.52	25.37	25.73	C(10)	91.24	91.39	91.27	91.24
C(8^1)	26.54	26.69	26.63	26.60	C(15)	102.26	102.44	102.77	102.83
C(17^2)	29.83	29.80	29.71	29.86	C(5)	104.11	103.79	103.85	103.67
C(13^2)	30.85	30.64	30.94	30.88	CN(β)[c])	130.59	129.39	129.33	129.87
C(8^2)	31.21	31.21	31.12	31.18	CN(α)[c])	131.22	131.46	133.38	131.43
C(12B)	31.21	31.36	31.12	31.30	C(14)	163.45	163.63	163.42	163.39
C(18^1)	31.87	31.81	31.78	32.08	C(6)	163.63	163.81	163.75	163.78
C(17^1)	32.74	32.71	32.65	32.98	C(7^2)	171.12	171.03	170.94	171.09
C(3^2)	33.52	33.85	33.76	33.88	C(9)	171.66	171.93	171.78	171.60
C(18)	39.45	39.45	39.42	39.54	C(18^2)	171.87	171.83	171.78	171.78
C(2^1)	41.39	41.33	41.27	41.30	C(2^2)	172.05	171.99	171.96	172.08
C(7^1)	42.44	42.47	42.41	42.44	(C17^3)	173.01	172.92	172.86	174.41
C(2)	45.92	45.89	45.89	45.95	C(8^3)	173.60	174.53	173.48	173.51
C(12)	47.09	47.15	46.94	47.14	C(13^3)	173.90	173.93	174.44	173.96
C(7)	48.70	48.73	48.73	48.79	C(3^3)	174.41	172.74	172.68	172.77
MeO	51.61[b])	51.61[b])	51.67	51.58[b])	C(4)	175.70	175.64	175.73	175.55
MeO	51.61	51.82	51.73	51.64	C(16)	175.79	175.94	176.06	176.15
MeO	51.61	51.82	51.79	51.79	C(11)	176.30	176.66	176.69	176.48
MeO	51.73	51.82	51.79	51.79					

[a]) The δ [ppm] values were measured at 75.47 MHz in $CDCl_3$ solution (c = 0.22M). For numbering, see *Fig. 1*.

[b]) Assigned to C(13^5).

[c]) CN(α) = C1LA, CN(β) = C1LB (see *Fig. 1*); tentative assignment based on comparison of chemical-shift values.

[10]) Heteronuclear single-quantum coherences are to be preferred over multiple-quantum coherences [29] because of their narrow lineshapes, especially beneficial for overcrowded spectra.

Table 2. *^{1}H-NMR Chemical-Shift Values and Signal Assignments Based on 2D-NMR Spectra for the Cobester Monoacids* **2b**, **d–f** (500 MHz, $CDCl_3$). For numbering, see *Fig. 1*.

	2b	**2d**	**2e**	**2f**
Me(12B)	1.20	1.20	1.22	1.22
Me(17B)	1.26	1.27	1.27	1.27
Me(2A)	1.34	1.36	1.35	1.35
Mc(12A)	1.37	1.37	1.34	1.35
Mc(20)	1.51	1.45	1.46	1.47
Me(7A)	1.57	1.57	1.58	1.58
$CH_2(17^1)$	1.72 2.42	1.73, 2.42	1.73, 2.43	1.73, 2.38
$CH_2(8^1)$	1.74, 2.12	1.95, 2.10	1.77, 2.13	1.72, 2.12
$CH_2(13^1)$	1.83, 2.07	1.84, 2.09	2.18	1.84, 2.05
$CH_2(3^1)$	2.07, 2.16	2.08, 2.20	1.94, 2.05	2.04, 2.21
$Me(5^1)$	2.18	2.19	2.19	2.19
$Me(15^1)$	2.23	2.24	2.23	2.23
$CH_2(17^2)$	2.18, 2.57	2.14, 2.55	2.13, 2.56	2.23, 2.50
$CH_2(13^2)$	2.26, 2.51	2.23, 2.53	2.21, 2.55	2.22, 2.53
$CH_2(2^1)$	2.26, 2.59	2.27, 2.59	2.27, 2.59	2.26, 2.58
$CH_2(8^2)$	2.37, 2.45	2.28, 2.37	2.36	2.39
$CH_2(7^1)$	2.39, 2.69	2.38, 2.70	2.38, 2.71	2.39, 2.70
$CH_2(3^2)$	2.47, 2.59	2.55, 2.65	2.54, 2.65	2.54, 2.65
$CH_2(18^1)$	2.62	2.62	2.62	2.61
H–C(18)	2.82	2.81	2.83	2.83
H–C(13)	3.03	3.05	3.10	3.02
H–C(8)	3.45	3.50	3.49	3.44
$Me(13^5)O$	3.63	3.62	–	3.62
$Me(2^4)O$	3.67	3.69	3.69	3.68
$Me(8^5)O$	3.68	–	3.66	3.67
$Me(7^4)O$	3.69	3.68	3.69	3.68
$Me(17^5)O$	3.70	3.72	3.70	–
$Me(3^5)O$	–	3.70	3.72	3.72
$Me(18^4)$	3.76	3.76	3.76	3.74
H–C(19)	3.80	3.83	3.82	3.83
H–C(3)	3.82	3.84	3.84	3.82
H–C(10)	5.58	5.60	5.60	5.58

assigned to the *f*-, *d*-, and *e*-side chains, respectively. From the fourth carbonyl signal at 174.41 ppm, heteronuclear correlations are observed only to a CH_2 *m* at 2.47/2.59 ppm, due to $CH_2(3^2)$. The structures of **2d**, **e–f**, could by analyzed by the same strategy, exploring heteronuclear long-range correlation with the carbonyl C-atoms[11]).

A crystal of the cobester monoacid **2b**, grown from acetone/hexane, was subjected to an X-ray single-crystal structure analysis. The monoacid **2b** crystallizes in the orthorhombic space group $P2_12_12_1$ with 4 molecules per unit cell, plus a considerable amount of disordered solvent (at least 14 solvent atoms per molecule of **2b**). The extended region of disordered solvent density severely limited the accuracy of the crystal structure analysis, which refined to a residual of $R = 0.116$ for 2371 significant reflections. *Fig. 1* shows a projection of the molecule into the N_4 plane, together with the atom numbering used.

[11]) Spectral overlap and/or low intensity of the cross-peaks did not allow the unambiguous localization of the long-range correlations to all propionyl side chain CH_2 groups.

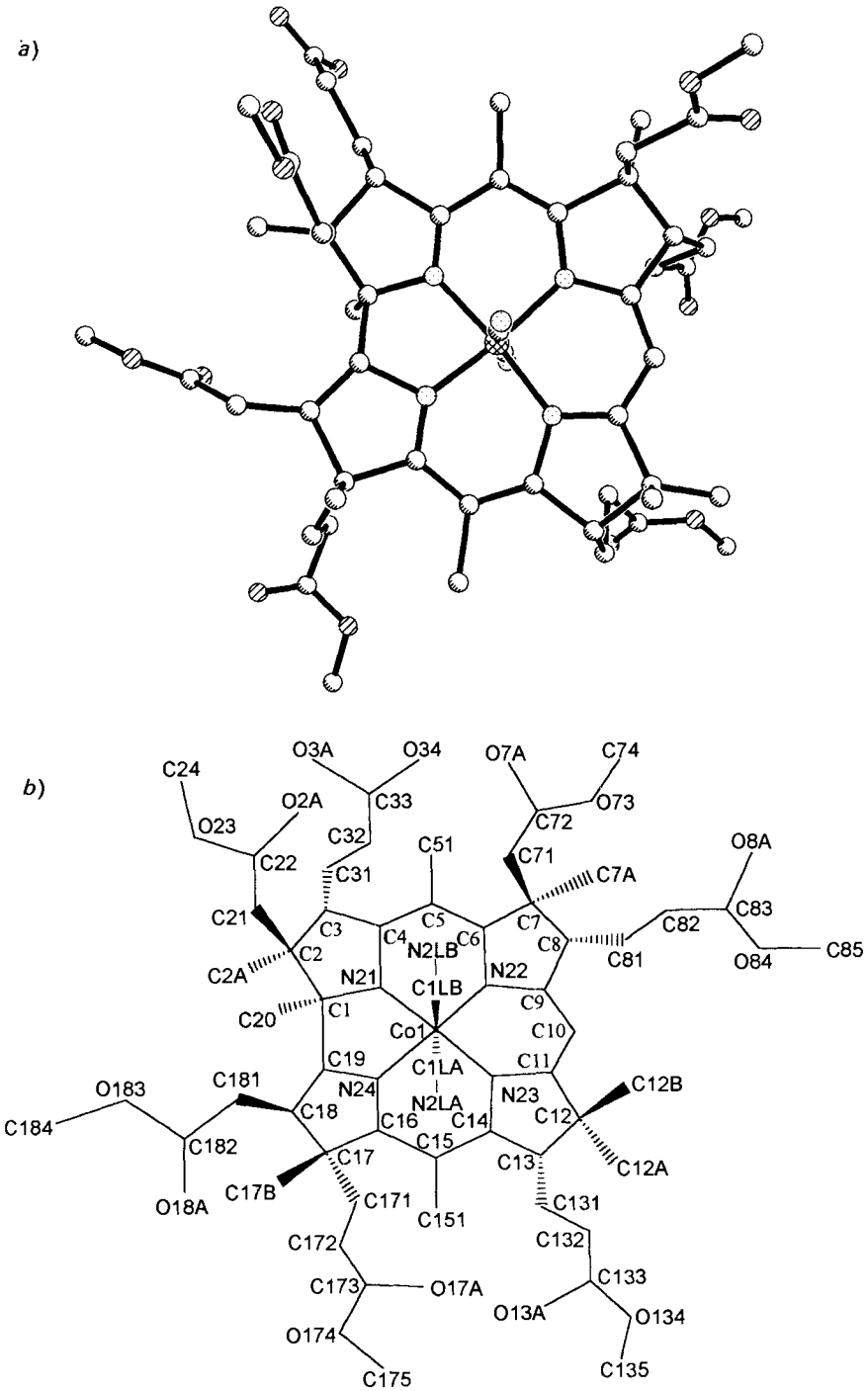


Fig. 1. *X-Ray structural analysis of cobester* b-*monoacid* **2b**. *a*) Ball-and-stick representation of projection vertical to corrin-ring plane; *b*) numbering scheme for **2b**. See *Exper. Part* for details.

Clearly, in **2b** all C_2- and C_3-side chains, except the one attached at C(3), carry a methyl-ester function, confirming the spectroscopically derived structural assignment as a cobester *b*-monoacid. The crystal packing is characterized by an intermolecular H-bond between the COOH function of the *b*-chain and the N-atom of one of the Co-coordinated CN groups of a symmetry-equivalent monoacid molecule. In addition, the propionyl side chains of **2b** are indicated to exhibit the conformational properties characteristic of cobyrinic-acid derivatives [15a][12]): axial orientation (with respect to the corrin core) and synclinal conformation of the *d*- and *e*-propionate substituents (at C(8) and C(13)), equatorial orientation and antiperiplanar conformation of the *f*-propionate side chain (at C(17)), and axial orientation with antiperiplanar conformation of the *b*-propionic-acid chain (at C(3)). The degree of non-planarity of the corrin ring, as expressed by the 'fold angle'[13]) is 7.3°, comparable to the values observed for other cobester derivatives (7.5° [31a], 4.3° [31b], 5.2° [31c]).

Discussion and Outlook. – We prepared the four hexamethyl hydrogen *Coα,Coβ*-dicyanocobyrinates **2b,d–f**, with a free *b*-, *d*-, *e*-, and *f*-propionic-acid function, respectively, by partial hydrolysis of the heptamethyl cobyrinate (**1**) [9] [10]. A thorough NMR-spectroscopic analysis of **2b,d–f** confirmed their earlier (tentative) structural assignment [10] as well as that of the corresponding monoamides **3b,d–f** [13] [19] [22] [23]. Accordingly, under the investigated conditions, the acid-catalyzed hydrolysis of **1** occurred with considerable selectivity (> 20:1; see also *Footnote 6*) at the propionate rather than at the acetate side chains, but with little selectivity among the former (**2b**/**2d**/**2e**/**2f** 1.11:1.0:1.07:1.01). These results are qualitatively in line with the earlier experience concerning the acid-catalyzed hydrolysis of the amide functions of vitamin B_{12} and of some of its derivatives [8], as well as the acid-catalyzed methanolysis of vitamin B_{12} [11]: the terminal functions of the propionyl side chains (on the α-side) of the cobyrinic-acid derivatives react considerably faster in solvolytic reactions than those of the acetyl side chains (on the β-face).

In contrast to the selectivity of acid-catalyzed reactions, in cyanide-containing aqueous MeOH, *i.e.* under basic conditions, **2b**/**2d**/**2e**/**2f** were obtained from **1**, in a ratio of 4.9:1.3:1.0:2.1, indicating preferential attack at the *b*-propionate function. This indeed parallels the outcome of the partial ammonolysis of **1** which selectively produced the monoamides **3b**/**3d**/**3e**/**3f** in a ratio of 5.3:1.4:1.0:2.3 [13].

The previously noted selectivity pattern for the hydrolysis of carboxamide functions of cobamides was interpreted on the basis of steric effects, the carbonyl C-atom of the acetamide groups being considerably less accessible than those of the propionamide groups [7] [8]. However, a more detailed parallel is noted here between the relative rates of the nucleophilic, solvolytic attack on the propionate substituents in **1** and the X-ray crystallographic information concerning the typical orientation of the propionyl substituents in crystalline vitamin-B_{12} derivatives [1], in **1** [15a], and in **2b** (see above) in

[12]) The *Cambridge Structural Data Base* (version January 1994) contains coordinate data for the following four cobyrinic-acid derivatives: heptamethyl dicyanocobyrinate (cobester; refcode BIRCOU [31a]); cobyric acid undecahydrate (COBYIC [31b]); heptamethyl dicyanocobyrinate 2-propanol solvate (CODZAW10 [31c]); vitamin-B_{12} hexacarboxylic-acid degradation product (VITCAC [31d]).

[13]) The 'fold angle' of the corrin ligand is defined as the angle between least-squares plane through the atoms N(21), C(4), C(5), C(6), N(22), C(9), C(10) and C(10), C(11), N(23), C(14), C(15), C(16), N(24) (for atom numbering, see *Fig. 1*).

particular: The orientation of the propionyl substituents with respect to the corrin core is axial for the *e*- and *d*-side chains, but equatorial for the *f*-chain and considerably more in plane (although pseudoaxial) also for the *b*-substituent [1] [15a]. Accordingly, the more axial propionate functions of **1** are more slowly attacked under the conditions of ammonolysis [13], and their base-catalyzed hydrolysis presumably does reflect the order of their steric accessibility. The same order of steric access of (polar) propionyl substituents is also indicated by the chromatographic behavior of the monoacids **2b, d–f** (as well as of the monoamides **3b, d–f**) on silica gel: they consistently elute in the order *e*-isomer first, then *d*-isomer, *f*-isomer, and finally *b*-isomer, suggesting the equatorial (polar) group to interact better with the solid support than a corresponding axial one.

The relative rates of acid-catalyzed hydrolysis of the propionate functions of **1**, in contrast, do not strictly give the same order of selectivity. Depending upon the experimental conditions, the propionate group of the *b*-side chain is not found to be the most reactive. Possibly the corrin-bound Co-center and its lower axial ligand can activate the acid-catalyzed hydrolysis in an intramolecular fashion, imposing in part an order of reactivity of the four propionate functions that tends to be opposite to that characteristic of the intermolecular attack by external nucleophiles. Accordingly, the reactivities of the (peripheral) ester functions are controlled largely by their degree of substitution, *i.e.* propionate *vs.* acetate substituent, but are influenced also by more subtle structural factors, such as the orientation of energetically favorable conformations of the particular side chain with respect to the corrin core.

As noted already earlier [21], the chemical shifts of the ^{13}C-NMR signals in the spectra prove particularly sensitive to local changes and are valuable for the purpose of (tentatively) localizing the modified side-chain function: when compared to **1** [21], the hexamethyl hydrogen cobyrinates **2b, d–f** (and the amides **3b, d–f** [23]) show significant differences of the ^{13}C-NMR chemical shifts of methylene C-atoms of one propionyl group. These shifts can only in part be traced back to a local substituent effect [25], but presumably result predominantly from conformational differences of the side chains. Interestingly, the carbonyl signals themselves of the modified substituents are shifted only little (up to *ca.* 2 ppm). However, the signal of one of the CN ligands (the α-CN ligand?) is affected considerably (its chemical shift changes by up to *ca.* 3 ppm). This is observed in a strikingly parallel fashion for the two series of the cobester monoacids **2b, d–f** and of the cobester monoamides **3b, d–f** [23]. The affected signal of the (α?) CN ligand is considerably displaced for the *e*-isomers in particular (with the *d*-isomers in second place), while less so in the *b*- and *f*-isomers. This down-field shift occurs in an unexpected parallel with the X-ray crystallographic observation discussed above, concerning the orientation and steric accessibility of the four propionyl substituents in **1** [15a] [31a] [31c], in **2b** (see *Fig. 1*), and in other vitamin-B_{12} derivatives [1]. Accordingly, the termini of the *e*- and *d*-side chains are placed closer to the α-CN ligand than those of the *b*- and *f*-side chains. The downfield displacement of one CN ^{13}C-signal presumably is caused by intramolecular $CN_x \cdots H{-}O$ H-bonding interactions, which are most relevant, potentially, for the *e*-isomer **2e**, while least so for the isomer **2b**[14]).

The structural analysis outlined in the *Results*' section allowed the complete and unambiguous '*de-novo*' delineation of the constitution of each one of the four monoacids

[14]) Other (intermolecular!) examples for such interactions are found for **2b** (see above) and for the propan-2-ol solvate of **1** (see [31c]) in the solid state.

2b, d–f, confirming the earlier analysis. As an alternative strategy for the purpose of a structural characterization of constitutionally intact, functionalized cobyrinic-acid derivatives, we would like to propose a recipe for signal assignment that makes use of the topologically unique attachment of each individual side chain. The basic principles are reminiscent of pattern recognition procedures, which have found widespread application in NMR spectroscopy (*i.e.*, in procedures for automated assignments [32]). Indeed, multidimensional spectroscopy provides information about spins and their mutual couplings. The spin system can, therefore, be referred to as a *coupling network*, a graph where the spins are represented by *nodes* and the couplings by *edges*. A coupling network is even a chromatic graph, when the chemical shift (carried by nodes) and the magnitude and sign of the couplings (carried by edges) are included [33a]. A family of experiments were designed to determine fragments or *subgraphs* within a molecule, be it with topological filtration techniques or spin-topology-selective excitation [33].

Fig. 2 shows, for the four cobester monoacids **2b, e–f**, the sketches of topological graphs of the four propionic-acid side chains and their bonded neighborhood (ring segment with directly bonded centers and propionic-acid side chain). In the graphs, the H-bearing C-atoms (CH, CH_2, and CH_3) are symbolized by squares, quaternary C-atoms by circles. Accordingly, the fragments within the cobester monoacids can be represented by 'black-and-white' graphs. Clearly, each propionyl side chain can be characterized by a constitutionally different neighborhood and accordingly by a (topologically) different (C–H)-bonding network. The latter information is available exactly from HSQC (or HMQC) and HMBC experiments. The expected complete sets of cross-peaks from HMBC experiments for the different propionyl side chains differ characteristically and accordingly allow for a straightforward differentiation between the four side chains. The corresponding two-dimensional graphs show the complete set of the expected cross-peaks from heteronuclear long-range correlations between quaternary C-atoms (vertical axis) and C-bound H-atoms (horizontal axis; *Fig. 2*). *Fig. 3* shows a comparison between the theoretically derived patterns of long-range cross-peaks (as given in *Fig. 2*) and the experimentally observed patterns for the cobester *b*-monoacid **2b**. There is a high degree of coincidence between the theoretical and the experimentally observed patterns. Deviations are found for the CH_2 region, as well as for ring D, presumably due to small ^3J(C,H) couplings.

Accordingly, a relatively simple pattern-recognition strategy can be used to identify different fragments within a vitamin-B_{12}-derived molecule. Based on the information available from HMBC and HSQC experiments, the heteronuclear coupling network can be established and associated with a graph. This graph is unique for a given within the cobyrinate structure (*e.g.* a given side chain in a cobyrinic-acid derivative). This assignment strategy can be an attractive and time-saving way to identify the location of functional peripheral groups in complex molecules, such as corrinoids and other complex tetrapyrrolic compounds. Apparently, the heteronuclear long-range coupling networks have not been used so far for automated assignment procedures, but it can be anticipated that topological arguments will develop to be a good starting point for this kind of analysis.

In the light of our results, it appears appropriate to comment on a series of communications by *Murakami*, *Hisaeda*, and coworkers concerning synthesis and analysis of reactivities of 'hydrophobic vitamin-B_{12} complexes' [20], obtained from cobester (**1**) [9]

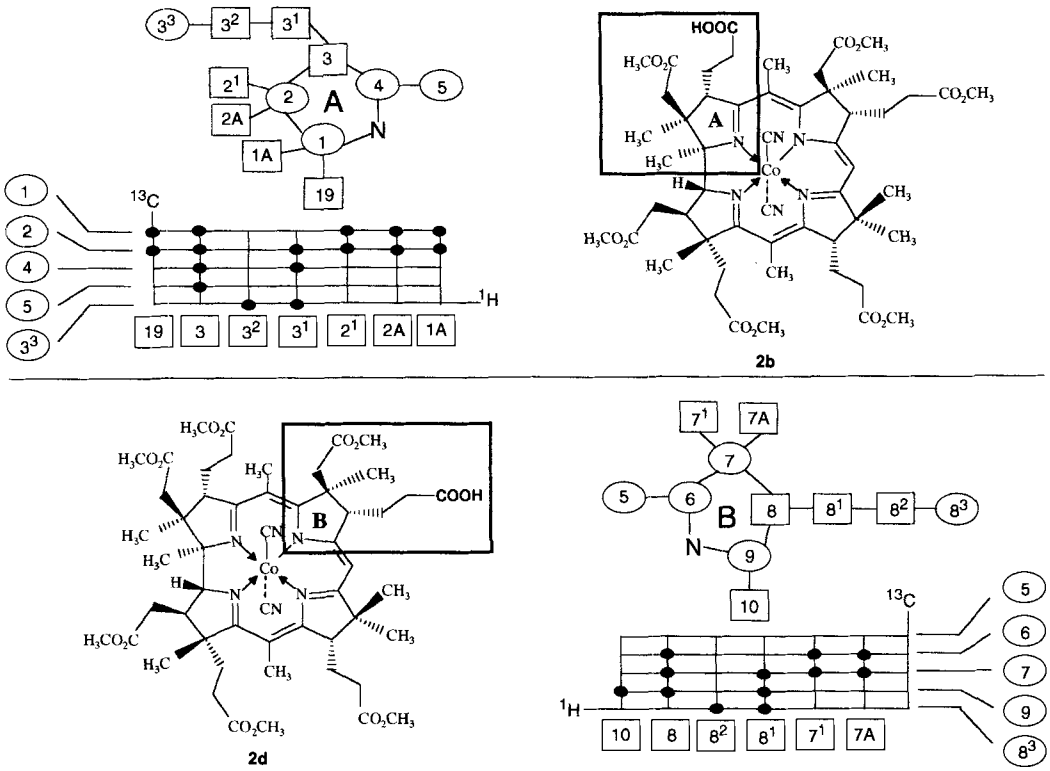
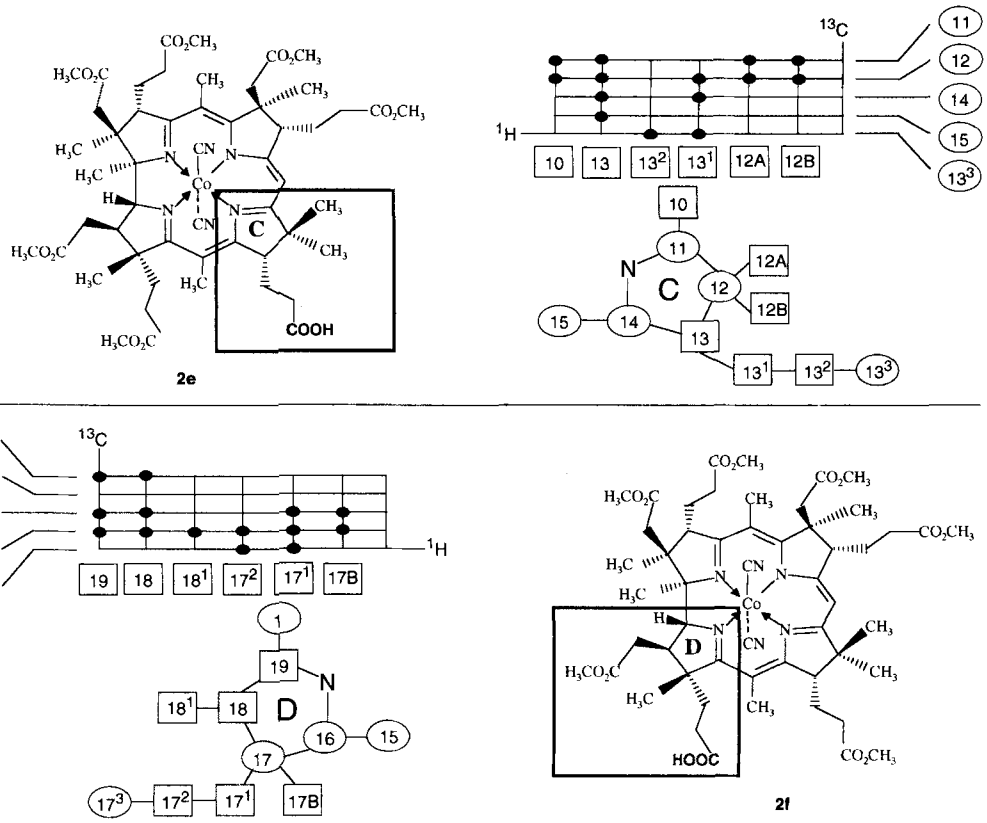


Fig. 2. *Sketch of topological graphs of ring segments with propionic-acid function of the four cobester monoacids* **2b**, **e–f** *and comparison with expected patterns of cross-peaks for heteronuclear long-range correlations.* Vertical axes: quaternary C-atoms; horizontal axes: C-bound H-atoms. For atom numbering, see *Fig. 1.*

[10]. These authors reported on the partial hydrolysis of **1** in an aqueous MeOH solution containing KCN [20a]. Accordingly, gel filtration chromatography on *Sephadex LH-20* of the crude hydrolysis mixture from **1** yielded a potassium hexamethyl cobyrinate in 28% yield to which the structure (of the potassium salt) of **2f** was attributed, apparently without consideration of the regioisomers **2b**, **2d**, and **2e** [20a]. In our hands, the hydrolysis of **1** under the conditions reported [20a] led to a mixture of the potassium salts of the four hexamethyl hydrogen cobyrinates **2b**, **d–f**, among which *ca.* 50% were due to the *b*-isomer **2b** (see above and *Exper. Part*). The structures of the functionalized hexa- and pentamethyl cobyrinates [20] prepared from the alleged potassium salt of **2f** [20a], therefore, should be reconsidered.

Obviously, with the hexamethyl hydrogen cobyrinates **2b**, **d–f** straightforwardly accessible by partial hydrolysis of the heptamethyl cobyrinate **1**, a set of 'building blocks' is now at hand for the specific build-up of lipophilic vitamin-B_{12} derivatives in which any particular one of the four propionyl side chains on the α-side carries a new functional group. Previously, only the *c*-acetyl side chain on the β-face offered a similar opportunity by way of the hexamethyl hydrogen cobyrinate **2c**. The *c*-acetic-acid group of **2c** was used



as a simple anchor point on the β-face for a uniform attachment of functional groups, so far without intention to specifically influence the coordination properties of the corrin-bound Co-center [17] [18][15]). However, as examplified by vitamin B_{12} itself and by the spectrum of corrinoids containing a complete nucleotide function [35], the organometallic and redox chemistry of the Co-ion in vitamin-B_{12} derivatives can be 'tuned' by the (intramolecular and) axial coordination of a base to the corrin-bound metal center [14] [36]. For such an influence to be most effective on the organometallic chemistry of the Co-center, axial coordination on the α-face should most profitably be controlled. For this purpose, the hexamethyl hydrogen cobyrinates **2b**, **d**–**f** provide an excellent starting point. Accordingly, they were used meanwhile for the specific build-up of lipophilic vitmain-B_{12} analogues with an intramolecularly coordinating base, such as the natural nucleotide in 'B_{12}-hexamethyl esters' [4] [37], and with much simpler imidazole appendages [38]. On the other extreme, the inhibition of the tendency of potential ligands to

[15]) Lipophilic *c*-esters derived from **2c** [17] were most recently used by *Keese* and coworkers [34] to study model reactions for the coenzyme-B_{12}-catalyzed methylmalonyl-succinyl rearrangement.

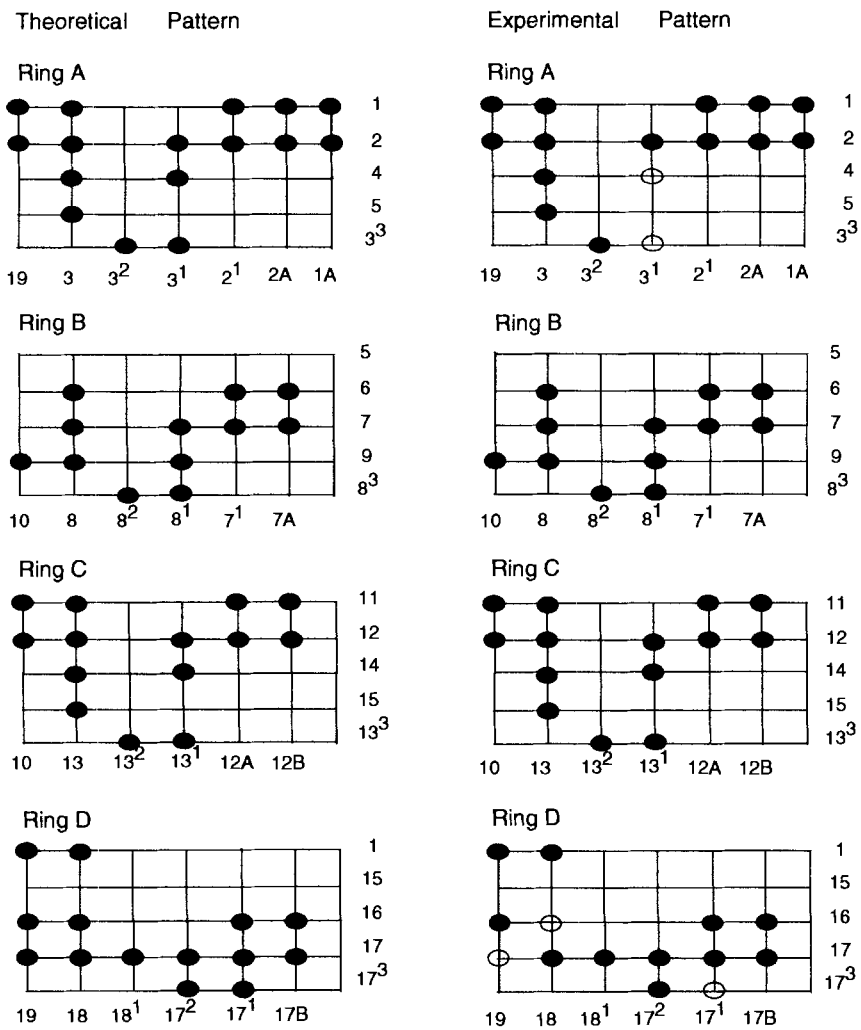


Fig. 3. *Pattern analysis of cross-peaks due to heteronuclear long-range couplings* ($^{2,3}J$(C,H)) *in the spectrum of* **2b**. Vertical axes: quaternary C-atoms; horizontal axes: C-bound H-atoms. Left: theoretically expected pattern; right: experimentally observed pattern in the HMBC spectrum of **2b** (open circles denote expected cross-peaks, where unambiguous observation is missing; see *Exper. Part* for details).

coordinate to the α-side of the Co-center can also be addressed with covalently attached blocking groups [10] [39].

Accordingly, the systematic preparative access to the lipophilic vitamin-B_{12} building blocks **2b**, **d**–**f**, by partial hydrolysis of cobester (**1**), has opened the door to further synthetic and structural exploration of more complex vitamin-B_{12}-derived structures.

We would like to thank Prof. *A. Eschenmoser* for his interest and his support of the work carried out at the ETH. We are grateful to PD Dr. *B. Jaun* and Ms. *B. Brandenberg* (ETH) for recording NMR spectra, *R. Dohner* and *H. Hediger* (ETH) for UV/VIS and CD spectra, and Dr. *W. Amrein* and *R. Häfliger* (ETH) for mass spectra. This work was supported by the *Swiss National Science Foundation* (project Nos. 2.447–0.84, 2.206–0.86, and 25.253.88) and by the *Austrian Science Foundation FWF* (project Nos. P-9334 and P-8371).

Experimental Part

1. *General. Solvents, Reagents, and Spectroscopy*: Heptamethyl *Coα,Coβ*-dicyanocobyrinate (cobester; **1**) [9]; MeOH, toluene, hexane, acetone, acetone cyanohydrine, all *Fluka puriss., p.a.*; MeOH (1% HCN) [10]; CH_2Cl_2, redistilled; H_2SO_4, KCN, *Merck*; $NaHCO_3$, *Fluka purum, p.a.*; cotton wool (dried); CC: silica gel *60* (*Merck*). TLC: silica gel plates (*Merck* No. *5721*). UV/VIS (MeOH (0.1% HCN)): *Perkin-Elmer PE 555*. CD (MeOH (0.1% HCN)): *Jobin-Yvon Mark III*. IR ($CHCl_3$, 4%): *Perkin-Elmer PE-283* and *PE-938G*; in cm^{-1}. ^{1}H-NMR ($CDCl_3$, *ca.* 15 mM): *Bruker WM-300*; 300.14 MHz; $SiMe_4$ as internal reference (= 0 ppm). ^{13}C-NMR ($CDCl_3$, *ca.* 220 mM): *Bruker WM-300*; 75.47 MHz; $SiMe_4$ as internal reference (=0 ppm); multiplicities from off-resonance-decoupled spectrum. FAB-MS: *Kratos AEI MS-50* fitted with *M*-scan FAB system; matrix 3-nitrobenzyl alcohol; xenon, 8.3 cV.

2D-NMR Experiments: *Varian-UNITYplus* 500-MHz spectrometer. Pulsed field gradient enhanced double-quantum filtered correlation spectroscopy (PFG-DQF-COSY): N-type coherence pathways; spectra in absolute-value mode; 256 single-scan FID's of 1*K* complex points; applied pulsed-field gradients (PFG's): durations of 2.5 ms 9.9 G/cm and 19.8 G/cm (double-quantum filtered COSY); *Gaussian* line broadening (t_1 and t_2); zero filling in t_1, 512 × 1*K* complex points (base-line-correction routines were not applied).

Pulsed field gradient enhanced heteronuclear single-quantum coherence experiment (PFG-HSQC): Two sets of spectra were recorded and processed according to the recipe of *States et. al.* [40] to yield a pure absorption spectrum with quadrature in *F*1. The one-bond ^{1}H,^{13}C shift correlation spectra typically resulted from a 2 × 256 × 1024 data matrix size, 4 scans per t_1 value, delay time between scans of 1 s. The first gradient was applied with a strength of 19.75 G/cm and a 2 ms duration, while the second gradient pulse of 19.48 G/cm was applied for 0.5 ms. Both gradients were rectangular and were applied along the *z*-axis. Decoupling (during acquisition) was achieved with the use of the GARP decoupling sequence [41], using a 3.8 kHz radio-frequency field. Shifted square sine-bell windows were used both in t_1 and t_2.

Pulsed field gradient enhanced ^{1}H-detected multiple-bond heteronuclear multiple-quantum coherence (PFG-HMBC): Magnitude-mode spectra were obtained using the standard enhanced HMQC pulse sequence with an additional delay for the evolution of the long-range heteronuclear coupling. The multiple-bond ^{1}H,^{13}C shift correlation spectra typically resulted from a 512 × 1024 data matrix size, with 16 scans per t_1 value and a delay time between scans of 1 s. The first two gradients were applied with the strengths of 9.87 G/cm each, while the third gradient pulse had a strength of 4.98 G/cm. The durations of the three gradient pulses were 2 ms. All gradients were rectangular and were applied along the *z*-axis. *Gaussian* line broadening were used prior to *Fourier* transformation both in t_1 and t_2.

2. *Preparative Experiments*. 2.1. *Hexamethyl Hydrogen Cobyrinates* **2b, d–f** *by Partial Hydrolysis of Cobester* (**1**). From a soln. of **1** (2.2 g, 2.02 mmol) in MeOH (40 ml), *ca.* 20 ml of the solvent were evaporated, and the remaining soln. of **1** was cooled in ice. Then cold 20% (*v*/*v*) H_2SO_4/H_2O (100 ml) was added and the flask evacuated (*via* a cold trap) with a water aspirator. The hydrolysis was continued at 0° for 3 h 40 min, during which time 18 ml of solvent were collected in the trap. Then the mixture was neutralized by addition of $NaHCO_3$ (64 g, 760 mmol) and subsequently poured into a 1-l separating funnel, containing 300 ml each of CH_2Cl_2 and of 1M aq. phosphate buffer pH 3. The mixture was cautiously shaken (with release of pressure due to evolution of gas), and, in a fume hood, KCN (500 mg, 7.6 mmol) was added. The mixture was vigorously shaken and the violet org. phase separated. The aq. phase was completely decolorized by washing with CH_2Cl_2 (2 × 100 ml). The combined org. phase was filtered (dried cotton wool plug) and evaporated. The violet residue was dissolved in a minimal amount of CH_2Cl_2 and transferred into a separating funnel, containing 350 ml each of toluene and 0.1M aq. $NaHCO_3$. After vigorous shaking, the org. layer containing unreacted **1** was separated, and from the remaining aq. phase the monoacids **2b, d–f** were extracted first by 350 ml (and then 100 ml) of CH_2Cl_2. To isolate the hydrolysis products still remaining in the colored aq. phase, the latter was acidified to pH 3 by addition of 1M aq. H_3PO_4, followed by addition of KCN (*ca.* 100 mg, 1.5 mmol), and extracted with CH_2Cl_2, as before.

The three org. extracts were worked up as follows: The toluene extract was dried by filtration through dried cotton wool and evaporated and the uniform residue dried for 3 h at r.t./high vacuum: 1.14 g (52%) of **1**. The CH_2Cl_2 extract was washed with 0.1M aq. phosphate buffer pH 3 (200 ml) to which KCN (100 mg, 1.5 mmol) had been added, filtered through dried cotton wool, and dried: 840 mg (39%) of **2b**/**2d**/**2e**/**2f** *ca.* 1:1:1:1. The monoacids **2b,d–f** were then isolated as described below. The CH_2Cl_2 extract containing the more polar fraction was dried analogously to give 210 mg (9.5%) of hydrolysate as a purple solid.

The raw mixture of the monoacids **2b, d–f** was separated by column chromatography at atmospheric pressure (750 g of silica gel, CH_2Cl_2/MeOH (containing 1% HCN)/H_2O 95.5:4:0.5 (*v*/*v*); 4 purple bands). The chemically uniform fractions were combined, dried at the rotatory evaporator, taken up in CH_2Cl_2 (100 ml), and washed with

KCN-containing aq. phosphate buffer pH 3 (*100 ml*). Filtration through dried cotton wool and drying (rotatory evaporator, then 1 h at r.t./high vacuum) yielded (in the order of their elution): 211 mg (9.8%) of **2e**, 197 mg (9.2%) of **2d**, 202 mg (9.4%) of **2f**, and 217 mg (10.2%) of **2b**. Each of these raw monoacid samples was dissolved in acetone (50 ml) to which hexane (*ca.* 70 ml) was added, followed by a seeding crystal. The open crystallization flasks were then placed into a desiccator, furnished with a crystallizing dish that contained a mixture of acetone cyanohydrin (16 ml) and hexane (84 ml). The loaded desiccator, to which a slight vacuum was applied, was left to stand in the dark for 20 days. The mother liquors were discarded and the crystalline samples dried for 16 h at r.t./high vacuum: 200 mg (18.1%) of **2b**, 182 mg (17.6%) of **2d**, 192 mg (17.8%) of **2e**, and 186 mg (19.0%) of **2f**. These crystalline samples were identified by ^{1}H-NMR and TLC comparison with analogous preparations from another batch which was used for the characterization of **2b, d–f**, as described further below.

2.2. *Control Experiments. Partial Hydrolysis of* **1** *in CF_3COOH/H_2O-Sat. Toluene.* Crystalline **1** (16 mg, 15 μmol) was dissolved in H_2O-sat. toluene (20 ml), and CF_3COOH (0.5 ml) was added. The mixture was stored at r.t. for 14 days, diluted with CH_2Cl_2 (40 ml), and washed with 0.1M aq. phosphate buffer pH 4 (40 ml) in which KCN (*ca.* 300 mg) had been dissolved. The org. phase was filtered through dried cotton wool and evaporated and the residue separated by prep. TLC (3 silica gel plates, CH_2Cl_2/MeOH (0.5% HCN) 9:1). Six chromatographically well separated fractions were eluted from the adsorbent with CH_2Cl_2/MeOH (0.5% HCN) 4:1. The eluants were taken up in CH_2Cl_2 (50 ml) and washed with KCN-containing 0.1M aq. phosphate buffer pH 4 (50 ml). The org. phases were evaporated and dried to give 9.9 mg (9.1 μmol) of unreacted **1**, 1.2 mg (1.1 μmol) of **2b**, 0.9 mg (0.8 μmol) of **2d**, 0.9 mg (0.8 μmol) of **2e**, and 2.4 mg (2.25 μmol) of **2f**, besides *ca.* 0.9 μmol of more polar hydrolysis products. The sample of **2f** obtained this way was crystallized from acetone/hexane and identified by ^{1}H-NMR and TLC. The other fractions were identified by TLC.

Partial Hydrolysis of **1** *in KCN-Containing Aq. MeOH.* To a soln. of **1** (150 mg, 140 μmol) in MeOH (1.5 ml), H_2O (60 μl) and KCN (16 mg, 0.2 mmol) were added. The violet soln. was magnetically stirred and kept at 30° for 2 h. Then the solvents were evaporated at 30°, and the residue was taken up in CH_2Cl_2 (100 ml) and washed with 0.1M aq. phosphate buffer pH 5 (100 ml). The org. phase was filtered through dried cotton wool and evaporated, the residue taken up in toluene (25 ml) and washed with KCN-containing 0.1M carbonate/hydrogencarbonate buffer pH 10 (25 ml), and the toluene phase evaporated: 123 mg (115 μmol) of unreacted **1**. The aq. phase was extracted with CH_2Cl_2 (2 × 25 ml), the org. extract washed with KCN-containing aq. phosphate buffer pH 5 (25 ml) and evaporated, and the residue (*ca.* 24 mg) separated by prep. TLC (2 silica gel plates) and worked up, as described above. The 4 monoacid fractions were precipitated from CH_2Cl_2 soln. by addition of hexane, the supernatants were removed and the precipitates dried for 2 h at r.t./high vacuum. They were identified by TLC and ^{1}H-NMR: 10.5 mg (9.8 μmol) of **2b**, 2.8 mg (2.6 μmol) of **2d**, 2.1 mg (2.0 μmol) of **2e**, and 4.5 mg (4.3 μmol) of **2f**, *i.e.* **2b**/**2d**/**2e**/**2f** *ca.* 4.9:1.4:1.0:2.15.

3. *Spectral Characterization of* **2b, d–f**. a,c,d,e,f,g-*Hexamethyl* b-*Hydrogen* Coα,Coβ-*Dicyanocobyrinate* (*Cobester* b-*Acid*; **2b**): TLC (CH_2Cl_2/MeOH (1% HCN) 19:1): R_f 0.21. M.p. 203–208° (dec.). UV/VIS ($c = 3.16 \cdot 10^{-5}$ M): 277 (4.04), 310 (br., 3.98), 352 (sh, 4.18), 368 (4.50), 418 (3.38), 514 (sh, 3.78), 543 (3.95), 582 (4.04). CD ($c = 3.16 \cdot 10^{-5}$ M): 252 (−12.6), 276 (1.26), 308 (−9.64), 327 (sh, −6.96), 347 (−12.2), 366 (−8.38), 396 (21.0), 426 (12.8), 504 (−1.9), 541 (−3.0), 582 (−4.9); λ_0 at 268, 289, 374, 461. IR: 3500–2500*w*, 2995*w*, 2955*w*, 2120*w*, 1735*s*, 1580*s*, 1500*s*, 1440*s*, 1400*m*, 1370*m*, 1105*m*, 1005*m*. ^{1}H-NMR: *Table 2*; 1.20, 1.26, 1.34, 1.38, 1.51, 1.57 (6*s*, 18 H, 6 Me); 1.60–1.90 (*m*, 3 H); 1.90–2.72 (*m*), superimposed by 2.18, 2.23 (2*s*, 6 H, 2 Me), in total *ca.* 25 H; 2.82 (*dt*, of *ABMX*, J = 5.8, 5.8, 10.6, 1 H); 3.02 (*dd*, X' of $A'B'X'$, J = 4.4, 6.1, 1 H); 3.45 (*dd*, X'' of $A''B''X''$, J = 4.4, 8.0, 1 H); 3.63, 3.67, 3.68, 3.69, 3.70, 3.76 (6*s*, 18 H, 6 MeO); 3.80 (*d*, M of *ABMX*, J = 10.6, 1 H), superimposed by 3.83 ('*d*', $J \approx 8$, 1 H); 5.58 (*s*, 1 H). ^{13}C-NMR; *Table 1*. FAB-MS: 1076 (8), 1075 (18), 1074 (27, M^+); 1051 (8), 1050 (22), 1049 (56), 1048 (100, $[M - CN]^+$); 1023 (21), 1022 (31, $[M - 2\,CN]^+$); 1004 (4), 1003 (4); 989 (6); 974 (6, $[M - CN - C_3H_6O_2]$); 949 (4), 948 (5, $[M - 2\,CN - C_3H_6O_2)^+$).

a,b,c,e,f,g-*Hexamethyl* d-*Hydrogen* Coα,Coβ-*Dicyanocobyrinate* (*Cobester* d-*Acid*; **2d**): TLC (CH_2Cl_2/MeOH (1% HCN) 19:1): R_f 0.31 M.p. 166–168° (dec.). UV/VIS ($c = 3.39 \cdot 10^{-5}$ M): 277 (4.03), 310 (br., 3.96), 352 (sh, 4.16), 369 (4.47), 418 (3.36), 514 (sh, 3.76), 543 (3.92), 582 (4.02). CD ($c = 3.39 \cdot 10^{-5}$ M): 253 (−12.1), 282 (0.59), 309 (−9.74). 327 (−6.94), 348 (−10.5), 368 (−6.20), 397 (19.9), 427 (11.8), 505 (−1.8), 542 (−3.1), 584 (−5.0); λ_0 at 273, 289, 374, 463. IR: 3680*w*, 3500–2500*w*, 2995*w*, 2955*m*, 2120*w*, 1735*s*, 1580*s*, 1500*s*, 1440*s*, 1400*m*, 1370*m*, 1105*m*, 1005*m*. ^{1}H-NMR: *Table 2*; 1.20, 1.26, 1.35, 1.36, 1.45, 1.56 (6*s*, 18 H, 6 Me); 1.68–2.72 (*m*), superimposed by 2.19, 2.24 (2*s*, 6 H, 2 Me), in total *ca.* 28 H; 2.80 (*dt*, X of *ABMX*, J = 5.3, 5.3, 10.6, 1 H); 3.05 (*dd*, X' of $A'B'X'$, J = 3.8, 4.5, 1 H); 3.50 (*t*, X'' of $A''B''X''$, J = 6, 1 H); 3.62, 3.68, 3.69, 3.70, 3.72, 3.76 (6*s*, 18 H, 6 MeO); 3.83 (*d*, M of *ABMX*, J = 10.6, 1 H), superimposed by 3.83 ('*d*', $J \approx 8$, 1 H); 5.60 (*s*, 1 H). ^{13}C-NMR: *Table 1*. FAB-MS: 1076 (6), 1075 (16), 1074 (25, M^+); 1051 (5), 1050 (20), 1049 (56), 1048 (100, $[M - CN]^+$); 1023 (21), 1022 (30, $[M - 2$

$CN]^+$); 1004 (4), 1003 (4), 1002 (4); 990 (6), 989 (6), 988 (7); 974 (6, $[M - CN - C_3H_6O_2]^+$); 962 (6); 949 (5), 948 (5, $[M - 2\ CN - C_3H_6O_2]^+$).

a,b,c,d,f,g-*Hexamethyl* e-*Hydrogen* Coα,Coβ-*Dicyanocobyrinate* (*Cobester* e-*Acid*; **2e**): TLC (CH_2Cl_2/MeOH (1% HCN) 19:1): R_f 0.33. M.p. 176° (dec.). UV/VIS ($c = 3.44 \cdot 10^{-5}$ M): 277 (4.04), 310 (br., 3.98), 353 (sh, 4.17), 369 (4.49), 419 (3.38), 514 (sh, 3.76), 544 (3.93), 583 (4.03). CD ($c = 3.44 \cdot 10^{-5}$ M): 252 (−12.2), 276 (1.82), 308 (−9.74), 327 (−6.83), 348 (−11.9), 368 (−8.86), 396 (20.9), 427 (12.6), 505 (−2.2), 542 (−3.2), 584 (−4.9); λ_0 at 268, 290, 375, 461. IR: 3680*w*, 3500–2500*w* (br.), 2995*w*, 2955*m*, 2120*w*, 1735*s*, 1580*s*, 1500*s*, 1440*s*, 1400*m*, 1370*m*, 1105*m*, 1005*m*. ^{1}H-NMR: *Table 2*; 1.22, 1.27, 1.34, 1.35, 1.46, 1.58 (6*s*, 18 H, 6 Me); 1.60–2.73 (*m*), superimposed by 2.19, 2.23 (2*s*, 6 H, 2 Me), in total *ca.* 28 H; 2.82 (*dt*, *X* of *ABMX*, $J = 6, 6, 10.6$, 1 H); 3.10 (*dd*, *X'* of *A'B'X'*, $J = 4.2, 6.2$, 1 H); 3.49 (*dd*, *X''* of *A''B''X''*, $J = 4.6, 7.7$, 1 H); 3.66, 3.69 (double int.), 3.70, 3.72, 3.76 (5*s*, 18 H, 6 MeO); 3.83 (*d*, *M* of *ABMX*, $J = 10.6$, 1 H), superimposed by 3.84 ('*d*', $J \approx 7$, 1 H); 5.60 (*s*, 1 H). ^{13}C-NMR: *Table 1*. FAB-MS: 1076 (8), 1075 (18), 1074 (27, M^+); 1051 (8), 1050 (22), 1049 (56), 1048 (100, $[M - CN]^+$); 1023 (21), 1022 (31, $[M - 2\ CN]^+$); 1006 (4), 1004 (4); 990 (6), 989 (6); 974 (6, $[M - CN - C_3H_6O_2]^+$); 962 (8), 961 (7), 960 (8); 949 (4), 948 (5, $[M - 2\ CN - C_3H_6O_2]^+$).

a,b,c,d,e,g-*Hexamethyl* f-*Hydrogen* Coα,Coβ-*Dicyanocobyrinate* (*Cobester* f-*Acid*; **2f**; see also [12] [13]): TLC (CH_2Cl_2/MeOH (1% HCN) 19:1): R_f 0.27. M.p. 198–202° (dec.) UV/VIS ($c = 3.35 \cdot 10^{-5}$ M): 277 (4.03), 310 (br., 3.97), 353 (sh, 4.17), 368 (4.49), 419 (3.37), 514 (sh, 3.77), 543 (3.94), 582 (4.03). CD ($c = 3.35 \cdot 10^{-5}$ M): 253 (−12.2), 280 (0.75), 309 (−10.2), 327 (sh, −6.42), 347 (−12.1), 367 (−8.36), 396 (21.1), 426 (13.0); 503 (−1.8), 541 (−2.4), 583 (−3.9); λ_0 at 270, 289, 374, 461. IR: 3500–2500*w* (br.), 2995*w*, 2955*m*, 2120*w*, 1735*s*, 1580*s*, 1500*s*, 1440*s*, 1400*m*, 1370*m*, 1105*m*, 1005*m*. ^{1}H-NMR; *Table 2*; 1.20, 1.27, 1.36, 1.37, 1.52, 1.56 (6*s*, 18 H, 6 Me); 1.60–1.90 (*m*, 3 H); 1.90–2.75 (*m*), superimposed by 2.18, 2.24 (2*s*, 6 H, 2 Me), in total *ca.* 25 H, 2.82 (*dt*, *X* of *ABMX*, $J = 5.6, 5.6, 10$, 1 H); 3.02 (*t*, *X'* of *A'B'X'*, $J = 4.5$, 1 H); 3.44 (*dd*, *X''* of *A''B''X''*, $J = 5.0, 7.8$, 1 H); 3.62, 3.67, 3.68, 3.69, 3.72, 3.74 (6*s*, 18 H, 6 MeO); 3.79 (*d*, of *ABMX*, $J = 10$, 1 H), superimposed by 3.81 ('*d*', $J \approx 8$, 1 H); 5.58 (*s*, 1 H). ^{13}C-NMR: *Table 1*. FAB-MS: 1076 (8), 1075 (15), 1074 (22, M^+); 1050 (22), 1049 (56), 1048 (100, $[M - CN]^+$); 1023 (21), 1022 (43, $[M - 2\ CN]^+$); 1008 (5), 1006 (4), 1004 (4), 990 (6), 989 (7), 988 (6); 974 (6, $[M - CN - C_3H_6O_2]^+$); 963 (6), 961 (6), 949 (5), 948 (7 $[M - 2\ CN - C_3H_6O_2]^+$).

4. *X-Ray Single-Crystal Structure Analysis of* **2b**. Crystals were grown at r.t. from a soln. of **2b** in acetone/hexane as described in *Section 2.1*. A crystal of approximate dimensions 0.2 × 0.3 × 0.3 mm was removed from its mother liquor, immersed in a drop of hydrocarbon oil, and shock-cooled in the coldstream of the goniometer-cryostat.

Diffraction data were collected on a modified *Stoe* diffractometer equipped with a locally constructed N_2-cold-stream low-temperature device using graphite monochromated MoK_α radiation ($\lambda = 0.71069$ Å). Unit-cell parameters were obtained by least-squares refinement against the setting angles of 42 reflections with $7° \leq 2\vartheta \leq 21°$. Crystals are orthorhombic, space group $P2_12_12_1$, with 4 formula units ($C_{53}H_{71}N_6O_{14}Co$, formula weight 1075.1, excluding solvent molecules) in the unit cell: $a = 17.679$ (11), Å, $b = 23.279$ (13) Å, $c = 16.227$ (16) Å, $V = 6678$ (2) Å^3, $d_{calc} = 1.069$ g/cm^3 (excluding solvent).

Intensity data were collected at 92 (2) K using an ω-step scan technique with a fixed scan range of $\Delta\omega = 2.4°$. The scan speed was variable and depended on the results of a fast prescan. Backgrounds were estimated from the intensity readings at the two ends of the scan profile. Three standard reflections from different regions of reciprocal space were periodically monitored (every 100 reflections). Their intensities showed no systematic trends during data collection.

Net intensities I and their estimated errors $\sigma(I)$ were calculated using standard procedures. *Lorentz* and polarization corrections were applied to I and $\sigma(I)$, but no absorption correction ($\mu = 0.31$ mm^{-1}). All reflections for one octant of reciprocal space ($0 \leq h \leq 19$, $0 \leq k \leq 25$, $0 \leq l \leq 17$) with $(\sin\vartheta)/\lambda \leq 0.54$ Å^{-1} were collected. Leading to 4083 unique and 2371 significant ($I/\sigma(I) > 2$) reflections.

Structure solution of **2b** involved locating the Co-atom from a *Patterson* map and then determining the positions of the lighter atoms from subsequent electron-density maps. Atomic coordinates, isotropic and anisotropic (see below) atomic displacement parameters (a.d.p.'s) were refined with a full-matrix least-squares program [42] which minimized the quantity $\Sigma w(F_o^2 - F_c^2)^2$ with $w = 1/[\sigma^2(F_o^2) + (14 \times P)^2 + 195 \times P]$, $P = (\max(F_o^2, 0) + 2 \times F_c^2)/3$, using all reflections. Scattering factors were taken from [43].

In view of the low observable-to-parameter ratio, a variety of restraints was applied during least-squares refinement: Chemically equivalent bond lengths and 1,3-distances of the ester side chains were restrained to be equal, the atoms of the ester groups were restrained to be coplanar. Anisotropic a.d.p.'s were refined for all non-H-atoms with a 'rigid-bond' restraint [44], *i.e.* the components of the anisotropic displacement parameters on the direction of the bond were restrained to be equal within an effective standard deviation of 0.01 Å^2. The same restraint was applied to 1,3-distances. Moreover, the u_{ij} components were restrained to approximate isotropic

behavior and to be similar for neighboring atoms. H-Atom positions were calculated and refined as 'riding' on their respective non-H-atom. Methyl torsion angles were chosen to maximize the electron density at the three calculated H-atom positions and allowed to refine. The (isotropic) a.d.p.'s for the H-atoms were set to 1.5 times the equivalent isotropic a.d.p. of the adjacent non-H-atom. Disordered solvent electron density was observed at several locations within the asymmetric unit. Attempts to interpret it in terms of defined solvent molecules failed, and the solvent density was eventually accounted for by including 10 isotropic C-atoms.

Refinement of 737 parameters against 4083 intensity data and 1248 restraints converged at the following values for the reliability indices: $wR_2 = [\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]]^{1/2} = 0.38$ (for all 4083 reflections), $R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o| = 0.116$ for 2371 reflections with $F_o > 4\sigma(F_o)$ and 0.21 for all 4083 data, goodness of fit $S = [\Sigma[w(F_o^2 - F_c^2)^2]/[n - p]]^{1/2} = 1.07$ ($n = 4083$, number of observations; $p = 737$, number of parameters). The highest peak in a final difference electron density map was 0.85 e/Å^3.

The atomic numbering used for the description of the structure is defined in *Fig. 1*. Detailed crystal-structure data may be obtained from the Director of the *Cambridge Crystallographic Data Centre*, 12 Union Road, Cambridge CB2 1EZ, UK.

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