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Synthesis of Vitamin B_{12} Derivatives Incorporating Peripheral Cytosine and N-Acetylcytosine

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Abstract: The synthesis and structural characterisation of vitamin B_{12} derivatives incorporating a cytosine and a N^4-acetyl cytosine in the side chain of the corrin and having the cobalt in different oxidation states are described. $NaBH_4$ reduction led to reduction of Co(III) to Co(I) but also to reduction and deacetylation of the cytosine moiety. Reaction conditions were found where only Co reduction was achieved.

Introduction

The mechanism of the B_{12} dependent methylmalonyl-succinyl rearrangement continues to be a challenging problem since the electronic nature of the thioester 1,2-migration has not yet been elucidated. The participation of Co in the rearrangement has been evoked and refused in different models. The rearrangement in the several models developed could occur via an organometallic complex, by electron transfer from Co to the initially formed radical to generate an anionic species or via free radicals.[1-8]

In order to corroborate the Co participation in the rearrangement, we have developed models that introduce molecular recognition and binding of the B_{12} derivatives and the corresponding substrates through hydrophobic interactions or hydrogen bonds. Our models have shown that such interactions increase the amount of rearrangement by keeping the originally formed radical close to the Co corrinoid for a prolonged period of time.[9-13]

We have already shown that adenine and thymine (A-T) base pairing is able to induce the rearrangement and we have now developed a new model incorporating guanine and cytosine (G-C) base paring (Fig. 1). Considering that the association constant K_{ass} for A-T is 10^2 M^{-1}, whereas K_{ass} for G-C is 10^4 M^{-1} in $CDCl_3$,[14, 15] the G-C model will complement the A-T model already published and provide further information about the effect of peripheral hydrogen bonds in the migration of thioester in the methylmalonyl-succinyl rearrangement. Furthermore, the stronger G-C interaction should favour the rearrangement more efficiently than the A-T bonding. The strong noncovalent binding of the substrate and the B_{12} derivative should better prevent the

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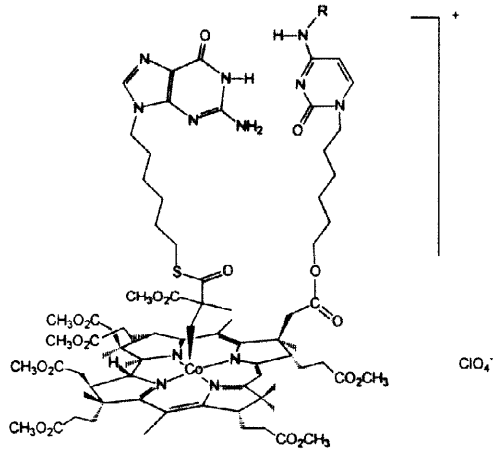
radicals from diffusing away from the corrin macrocycle and therefore provide the environment for enantioselective rearrangement and enantioselective radical reactions.



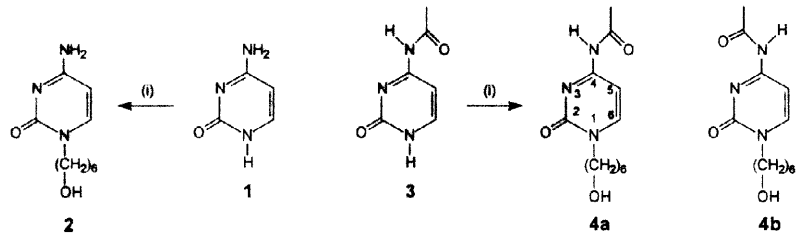
R = H, acetyl

Fig. 1

As a first step toward our model with guanine-cytosine, we describe here the synthesis and the reactivity of vitamin B_{12} derivatives with cytosine and N^4-acetyl-cytosine incorporated into the side chain.

Results and Discussion

The alcohols 1-(6-hydroxy-hexyl)-cytosine (**2**) and 1-(6-hydroxy-hexyl)-N^4-acetyl-cytosine (**4**) were prepared by alkylation of cytosine **1** and N^4-acetyl cytosine **3** with 1-bromo-hexanol as indicated in Scheme 1.



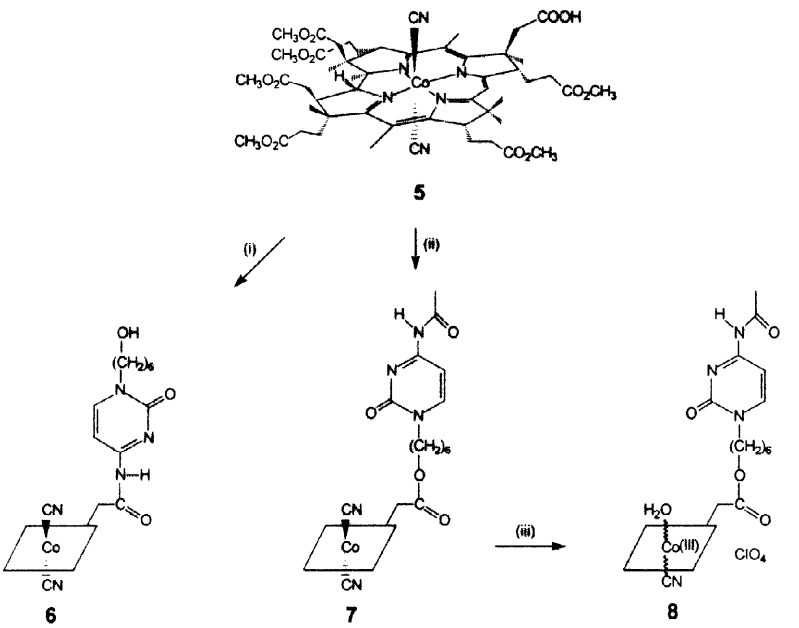
(i) NaH, HO-$(CH_2)_6$-Br, DMF, rt, 24 h

Scheme 1

It should be noted that due to the restricted rotation about the exocyclic C-N bond,[16] the N^4-acetyl group should be proximal to C5, as indicated in **4a**, and should not block Watson-Crick base pairing.[17] On the other hand if the acetyl group were rotated into proximal orientation with respect to N3 (**4b**), we would expect N3···O (from the acetyl group) repulsion destabilising this conformation.[18] Therefore, Watson-Crick type hydrogen bonding is feasible in **4**. Our ^1H NMR studies indicated that the proton C5-H which has a δ value of 5.84 ppm in **2** moved downfield to 7.34 ppm in the acetylated **4**, whereas the shifts for C6-H were 7.58 ppm and 7.97 ppm in CD_3OD, respectively (the numbers in the cytosine ring are indicated in **4** in Scheme 1). This corresponds to a

change of 1.5 ppm for C5-H and 0.39 ppm for C6-H. Similar values were found for cytosine in D_2O (the C5-H proton which usually has a δ value of 6.04 for cytidine moved downfield to 7.25 for acCy[17]).

Cobester-c-acid (cob(III)yrinic acid a,b,d,e,f,g-hexamethyl ester) **5** was activated with EDC·HCl (N-[3-(dimethylamino)propyl]-N'-ethyl-carbodiimide hydrochloride) and esterified with 1-(6-hydroxy-hexyl)-N^4-acetyl-cytosine **4** to give the dicyano cobester **7** in 73% isolated yield. Reaction of cobester c-acid **5** with 1-(6-hydroxy-hexyl)-cytosine **2** in the presence of EDC·HCl did not give the ester **11**. From a mixture of products, one complex could be isolated in 30% yield having ^1H and ^{13}C NMR and MS consistent with structure **6** (Scheme 2). Treatment of the dicyano complex **7** with 30% $HClO_4$ in CH_2Cl_2, followed by washing with phosphate buffer (pH 7 with 1% $NaClO_4$) led to the corresponding aqua-cyano complex **8** in 98% yield as a mixture of Coα/Coβ isomers (Scheme 2).



(i) **2**, EDC·HCl, DMAP, 0 °C to rt, CH_2Cl_2/DMF.

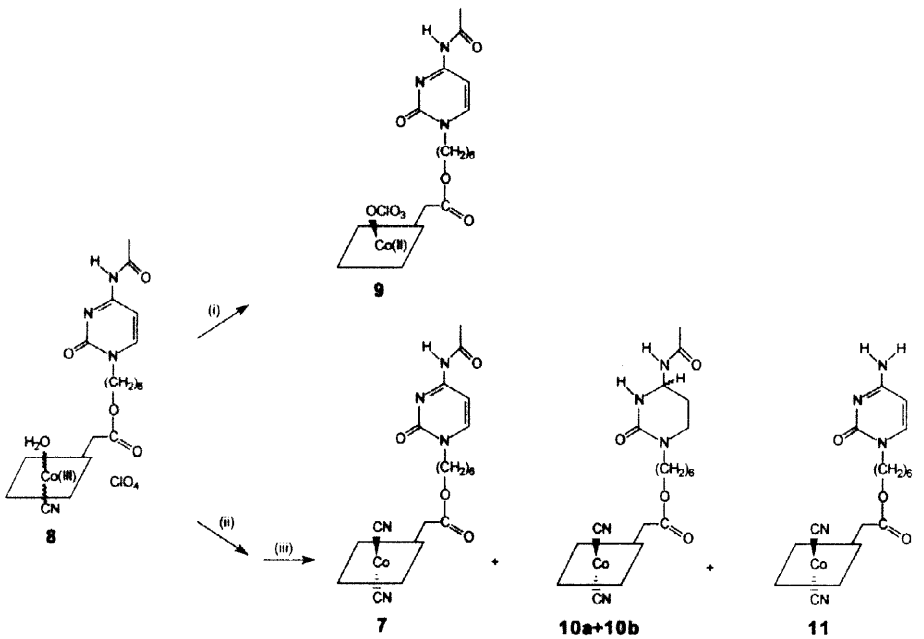
(ii) **4**, EDC·HCl, DMAP, 0 °C to rt, CH_2Cl_2/DMF. (iii) 30% $HClO_4$, CH_2Cl_2.

Scheme 2

The structures of **7** and **8** were studied by COSY and NOE experiments. The chemical shifts for C5-H and C6-H in the cytosine ring in **7** are 7.35 ppm and 7.58 ppm respectively in $CDCl_3$. The C5-H shifted upfield to 6.71 ppm and the C6-H downfield to 8.36 ppm in **8** (two signals are observed for each hydrogen corresponding to the two α/β co-ordination isomers) with respect to their positions in **7**. Since the side chain does not change in **8**, the upfield or downfield shifts could be attributed to a co-ordination of the base with the Co after ligand exchange. Nevertheless a possible interaction between the cytosine moiety in the side chain and the corrin ring in **8** could not be detected by NOE. The conformation of the N^4 acetyl group was confirmed by NOE experiment. Interaction between NH of the N^4-acetyl and C5-H was not found, whereas the NOE effects between C5-H and C6-H; NH and the methyl group of acetyl, as well as C6-H and the methylene group of N1-CH_2 were observed.

Reduction of ***8*** *with* $NaBH_4$

Treatment of **8** with excess of $NaBH_4$ (75 molequiv.), followed by acid oxidation to the Co(II) perchlorate, gave a mixture of three compounds, which were treated with oxygenated aqueous KCN to give the corresponding dicyano Co(III) complexes; N^4-acetyl-3,4,5,6-tetrahydrocytosine complex **10** was formed in 46% yield, besides the acetylcytosine **7** (5%) and the deacetylated cytosine **11** (9%) derivatives (Scheme 3).



(i) a) 10 equiv. $NaBH_4$ in MeOH, 0 °C, 1 min. b) 30% $HClO_4$.

(ii) a) 75 equiv. $NaBH_4$ in MeOH, rt, 15 min. b) 30% $HClO_4$. (iii) 0.1 M aqueous KCN, CH_2Cl_2

Scheme 3

The 3 components of the reduction-reoxidation reaction were separated by column chromatography. Subsequently the two diastereoisomers **10a** and **10b**, which are epimeric at 4-position of the reduced ring, were separated by column chromatography and analysed separately. The structure of the reduction product was derived from the ^1H, ^{13}C NMR and ESI-MS spectrum. The ^1H NMR spectrum of **10a** (or **10b**) shows a multiplet at 5.28 (or 5.29) ppm (1H) that was attributed to the proton at C4; as well as a multiplet from 3.2 ppm to 3.4 ppm (2H) for the methylene protons at C-6.[19]

The Co(II)perchlorate **9** could, however, be prepared, without further reduction or deacetylation, when **8** was reduced with 10 molequiv. of $NaBH_4$ at 0°C for just 1 min. In this case **9** was obtained in 90% yield.

Conclusions

Derivatives of vitamin B_{12} with peripheral N^4-acetyl cytosine group were prepared. Reduction of the aqua-cyano Co(III) complex with $NaBH_4$ led to the Co(I) derivative but also to reduction and deacetylation of the cytosine moiety. However, by controlling the conditions, only cobalt reduction could be achieved. Excess

reducing agent and longer reaction time favoured the formation of the 3,4,5,6-tetrahydro-cytosine derivative. The Co(II) complexes were transformed into the corresponding dicyano Co(III) derivatives for structure elucidation; ^{1}H, ^{13}C NMR, UV and ESI-MS confirmed the structure of the reduced products. The synthesis and the structure elucidation of the products from the $NaBH_4$ reduction have now led to the synthesis of the complexes shown in Fig 1. The synthesis of the guanine containing substrate, the synthesis of the alkylated complexes and their photolysis will be described soon.

Experimental

General. Reagents were purchased from Fluka Chemie AG. Solvents for chemical reactions and chromatography were distilled prior to use. DMF: abs. puriss. Columm chromatography (CC): silica gel 60 (40-60 μm) from *Baker* (analysed reagents). TLC: reactions monitored on *Alugram® Sil G/UV$_{254}$* from *Macherey-Nagel*, detection with a *Camag-53000* UV lamp (λ 254 nm) or an aq. $KMnO_4$ soln. UV/VIS: *Hewlett-Packard-8451-A* diode-array spectrophotometer; λ_{max} (log ε) in nm. IR: *Perkin-Elmer 1600 FTIR*; KBr discs or $CHCl_3$ soln. in 0.2-mm path NaCl cells; in cm^{-1}. NMR: *Bruker-AC-300* (^{1}H, 300 MHz; ^{13}C, 75 MHz) and *Bruker-AC-500* (^{1}H, 500 MHz; ^{13}C, 125 MHz); δ in ppm rel. to $CDCl_3$ (δ(H) 7.27, δ(C) 77.00) or CD_3OD (δ(H) 4.84, δ(C) 51.53) in Hz; ^{13}C multiplicities from DEPT spectra. Mass spectra: EI-MS: *Varian MAT-CH-7A*, 70 eV; in *m/z* (%). LSI-MS: *Fisions Instruments VG AutoSpec*, acceleration voltage 8 kV, ionisation Cs^{+} (32 keV); matrix: 1,3-dithiothreitol (DTT)/1,3-dithioerythrol (DTE) 5:1; in *m/z* (%). ESI-MS: *Fisions Instrument VG Platform II*; positive-ion measurements (3.5 kV); in *m/z* (%); solvents: MeCN/H_2O 1:1. *Cyclic Voltammertry*: Potentiostat *AMEL 553*; software CACYCO 3.0; mess cell *Metrohm 6.1415.110*; reference electrode *Metrohm 6.0726.100* (Ag/AgCl), electrolyte bridge *Metrohm 6.1231.000* and *6.1227.000*; working electrode *Metrohm 6.0804.010* (glassy carbon electrode, pre-treated by mechanical polishing with Al_2O_3 *Metrohm 6.2802.000*); auxiliary electrode was a Pt wire; scan rates 100 mVs^{-1}; *E* in V. Deoxygenation achieved by passing a stream of Ar through the solutions.

1-(6-Hydroxy-hexyl)-cytosine (**2**). NaH (9.6 mg, 0.40 mmol) was added portionwise to cytosine **1** (44.4 mg, 0.40 mmol) suspended in 2 ml DMF. After stirring at room temperature for 0.5 h, 1-bromo-hexanol (36.2 mg, 0.20 mmol) was added dropwise to the clear solution. The mixture was stirred for 18 h. Methanol (0.14 ml) was then added and the solvents were evaporated. The solid obtained was dissolved in methanol and silica gel was added. The mixture was dried and submitted to CC (AcOEt/MeOH 2:1): 30.7 mg (73%) of **2**. White powder. R_f 0.36 (AcOEt/MeOH 2:1). m.p.: 132-135 °C. IR (KBr): 3399, 3112, 3054, 2930, 2863, 2776, 2374, 2336, 1674, 1612, 1545, 1497, 1439, 1382, 1363, 1214, 1128, 1066, 1042, 998, 797, 725 cm^{-1}. ^{1}H-NMR (300 MHz, CD_3OD): δ 1.33 (m, 4 H), 1.49 (m, 2 H), 1.65 (m, 2H), 3.49 (t, J = 6.4 Hz, 2 H), 3.73 (t, J = 7.4 Hz, 2 H), 5.84 (d, J = 7.4 Hz, 1 H), 7.58 (d, J = 7.4 Hz, 1 H). ^{13}C-NMR (75 MHz, CD_3OD): δ 29.36 (t), 30.15 (t), 32.92 (t), 36.26 (t), 53.73 (t), 65.60 (t), 98.43 (d), 150.73 (d), 170.04 (s). EI-MS: *m/z* 211 (51, M^{+}), 194 (11), 180 (36),

166 (45), 152 (44), 138 (49), 125 (92), 112 (75), 96 (24), 81 (100), 69 (34), 55 (27), 41 (33). HR-EI-MS Calcd for $C_{10}H_{17}N_3O_2$: 211.1321, Found: 211.1315.

Coα,Coβ-Di(cyano-kC)-N^c-[1-N^4-(6-hydroxyl-hexyl)-cytosyl] cob(III)yrinic Acid-c-amide a,b,d,e,f,g-*Hexamethyl Ester* (**6**). To the mixture of **2** (287.9 mg, 1.36 mmol), **5** [20] (332.7 mg, 0.31 mmol) and 4-(dimethylamino)pyridine (DMAP; 75.7 mg, 0.62 mmol) in 10 ml dry CH_2Cl_2 and 10 ml DMF, cooled under Ar to 0°C, N-[3-(dimethylamino)propyl]-N′-ethylcarbodimide hydrochloride (EDC·HCl; 178.3 mg, 0.93 mmol) was added. After removing the cooling bath, the reaction mixture was stirred at room temperature for 2 h. The solvents were evaporated. The residue was purified by CC (hexane/CH_2Cl_2/i-PrOH/MeOH (0.1% HCN) 7:2:1:2). 131.0 mg (33%) of violet **6**: R_f (hexane/CH_2Cl_2/i-PrOH/MeOH (0.1% HCN) 7:2:1:2): 0.38. UV (c = 3.94×10^{-5} M, CH_2Cl_2): $\lambda_{max}(\varepsilon)$ 236 (1.86), 280 (0.77), 316 (0.96), 372 (1.56), 422 (0.15), 548 (0.53), 586 (0.67). IR ($CHCl_3$): 3409, 3016, 1746, 1669, 1636, 1593, 1502, 1444, 1382, 1310, 1161, 1109, 1085, 1013, 941, 898, 869 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.16-1.90 (m, superimposed 1.20 (s), 1.26 (s), 1.29 (s), 1.36 (s), 1.50 (s), 1.72 (s), total 30 H), 1.90-2.75 (m, superimposed 2.08 (s), 2.23 (s), total 24 H), 2.75-2.88 (m, 1 H), 2.91-2.99 (m, 1 H), 3.00-3.09 (m, 1 H), 3.55-4.00 (m, superimposed 3.63 (s), 3.66 (s), 3.67 (s), 3.68 (s), 3.69 (s), 3.76 (s), total 24 H), 5.54 (s, 1 H), 7.31 (d, J = 7.4 Hz, 1 H), 7.53 (d, J = 7.0 Hz, 1 H), 9.50 (s, 1 H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 15.37 (q), 15.50 (q), 17.12 (q), 18.57 (q), 19.18 (q), 19.77 (q), 22.11 (q), 24.82 (t), 25.18 (t), 25.76 (t), 26.11 (t), 28.78 (t), 29.64 (t), 29.73 (t), 30.84 (t), 30.92 (t), 31.10 (q), 31.64 (t), 32.33 (t), 32.49 (t), 33.77 (t), 39.27 (d), 41.56 (t), 46.07 (s), 46.63 (t), 47.09 (s), 50.60 (t), 51.58 (q), 51.64 (q), 51.68 (q), 51.79 (q), 51.81 (q), 52.29 (q), 53.49 (d), 56.39 (d), 56.60 (d), 58.68 (s), 62.33 (t), 75.00 (d), 82.77 (s), 91.15 (d), 96.30 (d), 102. 66 (s), 105.49 (s), 148.36 (d), 155.93 (s), 161.21 (s), 161.85 (s), 163.17 (s), 170.48 (s), 171.99 (s), 172.52 (s), 172.63 (s), 172.89 (s), 173.54 (s), 173.83 (s), 175.40 (s), 175.84 (s), 176.15 (s). LSI-MS: *m/z* 1241 (100, $[M\text{-}CN\text{-}1]^+$), 1215 (94, $[M\text{-}2CN\text{-}1]^+$), 1004 (32), 962 (49), 155 (38).

1-(6-Hydroxy-hexyl)-N^4-acetyl-cytosine (**4**). NaH (81.6 mg, 3.4 mmol) was added portionwise to N^4-acetyl-cytosine [21] (**3**; 521.2mg, 3.4 mmol) suspended in 100 ml DMF. After stirring at room temperature for 0.5 h, 1-bromo-hexanol (814.5 mg, 4.5 mmol) was added dropwise to this clear solution. The mixture was stirred for 18 h. After addition of 0.14 ml methanol, the solvents were evaporated. The solid obtained was dissolved in methanol and silica gel was given. The mixture was dried and submitted to CC (AcOEt/MeOH 5:1): 784.8 mg (91%) of **4**. White powder. R_f 0.44 (AcOEt/MeOH 5:1). m.p: 149-151°C. IR (KBr): 3426, 3179, 3106, 3042, 2932, 2850, 1708, 1648, 1571, 1502, 1438, 1383, 1324, 1269, 1237, 1159, 1068, 1013, 986, 828, 789, 684 cm^{-1}. ^{1}H-NMR (300 MHz, CD_3OD): δ 1.36 (m, 4 H), 1.51 (m, 2 H), 1.72 (m, 2H), 2.14 (s, 3 H), 3.51 (t, J = 6.4 Hz, 2 H), 3.87 (t, J = 7.4 Hz, 2 H), 7.34 (d, J = 7.4 Hz, 1 H), 7.97 (d, J = 7.0 Hz, 1 H). ^{13}C-NMR (75 MHz, CD_3OD): δ 26.99 (q), 29.02 (t), 29.83 (t), 32.40 (t), 35.93 (t), 54.39 (t), 65.27 (t), 100.52 (d), 153.61 (d), 166.67 (s), 175.52 (s). EI-MS: *m/z* 253 (49, M^+),252 (61, $[M\text{-}1]^+$), 238 (29), 210 (64), 194 (100), 180 (40), 166 (52), 152 (49), 138 (51), 125 (80), 111 (64), 96 (11), 81 (47), 69 (11), 55 (10), 43 (20), 28 (10). HR-EI-MS Calcd for $C_{12}H_{19}N_3O_3$: 253.1426, Found: 253.1414. Anal. Calcd for $C_{12}H_{19}N_3O_3$: C 56.88, H 7.56, N 16.60; Found: C 56.59, H 7.51, N 16.30.

Co*α*,Co*β*-*Di(cyano-kC) cob(III)yrinic Acid* a,b,d,e,f,g-*Hexamethyl* c-*[6-(1-N⁴-acetyl)-cytosyl-hexyl]-ester* (**7**). To the mixture of **4** (512.5 mg, 2.03 mmol), **5** (435.4 mg, 0.405 mmol) and 4-(dimethylamino)pyridine (DMAP; 99.0 mg, 0.810 mmol) in 4 ml dry CH_2Cl_2 and 5 ml DMF, cooled under Ar to 0°C, N-[3-(dimethylamino)propyl]-N′-ethylcarbodimide hydrochloride (EDC·HCl; 232.9 mg, 1.22 mmol) was added. After stirring at room temperature for 1 h, the solvents were evaporated. The residue was purified by CC (CH_2Cl_2/MeOH (0.1% HCN) 20:1). 384.9 mg (73%) of violet **7**. R_f (CH_2Cl_2/MeOH (0.1% HCN) 20:1) 0.32. UV (c = 3.82 × 10^{-5} M, CH_2Cl_2): $\lambda_{max}(\varepsilon)$ 238 (2.07), 280 (0.91), 312 (1.06), 372 (1.89), 424 (0.19), 550 (0.60), 590 (0.78). IR ($CHCl_3$): 3690, 3438, 3024, 2954, 1732, 1662, 1582, 1502, 1438, 1368, 1266, 1200, 1154, 1104, 806 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.16-1.90 (m, superimposed 1.19 (s), 1.26(s), 1.34 (s), 1.36 (s), 1.50 (s), 1.53 (s), total 30 H), 1.98-2.72 (m, superimposed 2.18 (s), 2.22 (s), 2.25 (s), total 27 H), 2.78-2.83 (m, 1 H), 2.99-3.03 (m, 1 H), 3.43-3.48 (m, 1 H), 3.62, 3.65, 3.67, 3.69, 3.71, 3.75 (6s, 18 H), 3.75-3.79 (m, 1 H), 3.79-3.82 (m, 1 H), 3.82-3.89 (m, 2 H), 4.02-4.12 (m, 2 H), 5.58 (s, 1 H), 7.35 (d, *J* = 7.3 Hz, 1 H), 7.58 (d, *J* = 7.3 Hz, 1 H), 9.46 (s, 1 H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 15.22 (q), 15.92 (q), 16.90 (q), 18.43 (q), 19.11 (q), 19.76 (q), 22.00 (q), 24.80 (q), 24.90 (t), 25.54 (t), 25.64 (t), 26.06 (t), 26.47 (t), 28.33 (t), 28.79 (t), 29.68 (t), 30.68 (t), 31.02 (t), 31.09 (q), 31.76 (t), 32.52 (t), 33.69 (t), 39.21 (d), 41.08 (t), 42.24 (t), 45.58 (s), 46.99 (s), 48.57 (s), 50.78 (t), 51.56 (q), 51.58 (q), 51.75 (q), 51.80 (q), 52.34 (q), 53.57 (d), 54.02 (d), 56.57 (d), 58.28 (s), 64.43 (t), 74.74 (d), 82.52 (s), 91.15 (d), 96.61 (d), 102.14 (s), 103.54 (s), 148.59 (d), 155.77 (s), 162.69 (s), 163.40 (s), 163.63 (s), 170.61 (s), 171.43 (s), 171.73 (s), 171.92 (s), 172.74 (s), 172.92 (s), 173.54 (s), 173.86 (s), 175.25 (s), 175.59 (s), 176.19 (s). LSI-MS: *m/z* 1283 (100, $[M\text{-}CN\text{-}1]^+$), 1257 (76, $[M\text{-}2CN\text{-}1]^+$), 133 (57). ESI-MS: *m/z* 1284 (100, $[M\text{-}CN]^+$), 687 (12), 674 (19), 653 (37), 642 (22, $[M\text{-}CN]^{++}$). Anal. Calcd for $C_{65}H_{88}N_9O_{16}Co$: C 58.94, H 6.84, N 9.14; Found: C 59.58, H 6.77, N 9.62.

Co*α*(*or* Co*β*)-*Aqua*-Co*β*(*or* Co*α*)-*(cyano-kC) cob(III)yrinic Acid* a,b,d,e,f,g-*Hexamethyl* c-*[6-(1-N⁴-acetyl)-cytosyl-hexyl]ester Perchlorate* (**8**). **7** (324.5 mg, 0.25 mmol) in CH_2Cl_2 (28 ml) was treated with a 30% aqueous $HClO_4$ solution (22 ml) and sonicated for 15 min under periodic evacuation (2×) to eliminate HCN. The aqueous phase was extracted with CH_2Cl_2, the organic phase was washed with H_2O, 1 M phosphate buffer pH 7 (+ 1% $NaClO_4$), filtered through cotton and the solvent was evaporated. The residue was dissolved in CH_2Cl_2 (5 ml), precipitated with Et_2O/hexane 1:2 (250 ml) and dried under high vacuum to give 340.0 mg (98%) of **8** as an orange red solid. UV (c = 3.80 × 10^{-5} M, CH_2Cl_2): $\lambda_{max}(\varepsilon)$ 240 (2.66), 278 (1.71), 320 (2.66), 354 (2.28), 406 (0.73), 488 (0.93). IR ($CHCl_3$): 3022, 2954, 1732, 1610, 1578, 1500, 1438, 1352, 1266, 1232, 1200, 1106, 896 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.12-2.07 (m, superimposed 1.18 (s), 1.39 (s), 1.45 (s), 1.55 (s), 1.75 (s), total 30 H), 2.08-2.85 (m, superimposed 2.33 (s), 2.39 (s), total 27 H), 3.05 (m, 1 H), 3.30-3.43 (m, 1 H), 3.57-3.83 (m, superimposed 3.63 (s), 3.69 (s), 3.72 (s), 3.80 (s), total 18 H), 3.84-4.18 (m, 6 H), 4.32 (1 H), 6.31/6.44 (2s, total 1 H), 6.62/6.71 (2s, total 1 H), 8.29 (d, *J* = 7.4 Hz)/8.36 (d, *J* = 7.3 Hz) (total 1 H), 11.29/11.37 (2s, total 1 H). LSI-MS: *m/z* 1257 (100, $[M\text{-}ClO_4^-\text{-}H_2O\text{-}CN\text{-}1]^+$), 1215 (27, $[M\text{-}ClO_4^-\text{-}H_2O\text{-}CN\text{-}$

CH_3CO-1]$^+$. ESI-MS: *m/z* 1284 (31, [M-ClO_4^--H_2O]$^+$), 674 (42), 654 (49), 642 (100, [M-ClO_4^--H_2O]$^{++}$), 622 (17).

Co*β-(Perchlorato) cob(II)yrinic Acid* a,b,d,e,f,g-*Hexamethyl* c-*[6-(1-N^4-acetyl)-cytosyl-hexyl]ester* (**9**). A solution of **8** (97.7 mg, 0.069 mmol) in MeOH (25 ml) was deoxygenated for 15 min under Ar and, after cooling to 0°C, treated portionwise with $NaBH_4$ (26.3 mg, 0.69 mmol). Stirring for 1 min gave a green solution. After addition of 30% aqueous $HClO_4$ solution (15 ml, already deoxygenated for 15 min and cooled at 0°C), the colour turned immediately to orange. The mixture was extracted with CH_2Cl_2, the combined organic extracts were washed with 1 M phosphate buffer (pH 7, + 1% $NaClO_4$), dried (Na_2SO_4) and evaporated. The residue was dissolved in CH_2Cl_2 (1 ml) and precipitated from Et_2O/hexane 1:2 (50 ml): **9** (85.3 mg, 90%). Brown solid. UV (c = 3.40 × 10^{-5} M, CH_2Cl_2): $\lambda_{max}(\varepsilon)$ 232 (0.74), 266 (0.64), 314 (0.73), 472 (0.33). IR ($CHCl_3$): 3023, 2954, 1732, 1570, 1490, 1438, 1364, 1266, 1106, 896 cm^{-1}. LSI-MS: ***m/z*** 1257 (100, [M-ClO_4^--1]$^+$), 1215 (93, [M-ClO_4^--CH_3CO]$^+$). ESI-MS: *m/z* 1257 (4, [M-ClO_4^--1]$^+$), 673 (18), 629 (100, [M-ClO_4^--1]$^{++}$), 608 (82, [M-ClO_4^--CH_3CO]$^{++}$). Anal. Calcd for $C_{63}H_{88}N_7O_{20}ClCo$: C 54.42, H 6.46, N 6.86; Found: C 55.73, H 6.53, N 7.22. CV (reversible waves): in MeCN (0.1 M $LiClO_4$): E_p^{red} (Co^{II}/Co^{I}) = -0.58 V, E_p^{ox} (Co^{I}/Co^{II}) = - 0.49 V; in MeOH (0.1 M LiClO4): E_p^{red} (Co^{II}/Co^{I}) = - 0.59 V, E_p^{ox} (Co^{I}/Co^{II}) = - 0.52 V.

Co*α*,Co*β-Di(cyano-kC) cob(III)yrinic Acid* a,b,d,e,f,g-*Hexamethyl* c-*[6-(1-N^4-acetyl-3,4,5,6-tetrahydrocytosyl)hexyl]ester* (**10**) and Co*α*,Co*β-Di(cyano-kC) cob(III)yrinic Acid* a,b,d,e,f,g-*Hexamethyl* c-*[6-(1-cytosine)hexyl]ester* (**11**). As described for **9**, with **8** (98.0 mg, 0.07 mmol) and $NaBH_4$ (198.6 mg, 5.25 mmol) at room temperature for 15 min. The obtained Cob(II)yrinate mixture was dissolved in CH_2Cl_2 and 0.1 M aqueous KCN solution was added. The mixture was sonicated in ultrasoundbath for 30 min and then extracted with CH_2Cl_2. The organic phase was dried through cotton, the solvents were evaporated and the residue was submitted to CC (CH_2Cl_2/MeOH (0.1% HCN) 10:1): **7**, 4.6 mg (5%), R_f 0.45; **10**, 42.2 mg (46%), R_f 0.36; **11**, 8.3 mg (9%), R_f 0.31. R_f: CH_2Cl_2/MeOH (0.1% HCN) 10 : 1.

10 UV (c = 4.90 × 10^{-5} M, CH_2Cl_2): $\lambda_{max}(\varepsilon)$ 234 (1.95), 280 (0.77), 316 (0.64), 372 (1.84), 424 (0.20), 550 (0.59), 590 (0.74). IR ($CHCl_3$): 3690, 3438, 3022, 2956, 1732, 1654, 1582, 1502, 1438, 1402, 1372, 1264, 1202, 1104, 1016 cm^{-1}. LSI-MS: *m/z* 1287 (100, [M-CN-1]$^+$), 1261 (51, [M-2CN-1]$^+$). ESI-MS: *m/z* 1288 (12, [M-CN]$^+$), 645 (100, [M-CN+1]$^{++}$), 602 (96, [M-CN+1-CH_3CO]$^{++}$). The two diastereoismers of **10** were separated by a second chromatography (CH_2Cl_2/MeOH (0.1% HCN) 10 : 1) to give **10a** and **10b** (~ 1:4). **10a** ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.15-1.93 (m, superimposed 1.20 (s), 1.26 (s), 1.35 (s), 1.38 (s), 1.51 (s), 1.57 (s), total 32 H), 1.93-2.76 (m, superimposed 1.95 (s), 2.19 (s), 2.23 (s), total 27 H), 2.77-2.89 (m, 1 H), 2.98-3.09 (m, 1 H), 3.15-3.39 (m, 4 H), 3.40-3.54 (m, 1 H), 3.59-3.89 (m, superimposed 3.63 (s), 3.67 (s), 3.69 (s), 3.70 (s), 3.73 (s), 3.77 (s), total 20 H), 4.05-4.18 (m, 2 H), 5.23 (s, 1 H), 5.30 (m, 1 H), 5.59 (s, 1 H), 6.28-6.41 (m, 1 H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 15.25 (q), 15.97 (q), 16.95 (q), 18.46 (q), 19.14 (q), 19.81 (q), 22.05 (q), 23.17 (q), 24.91 (t), 25.55 (t), 25.68 (t), 26.07 (t), 26.50 (t), 27.03 (t), 27.44 (t), 28.29 (t), 29.68 (t), 30.70 (t), 30.92 (q), 31.06 (t), 31.79 (t), 32.54 (t), 33.71 (t), 39.21 (d), 41.08 (t), 41.38 (t), 42.28 (t), 45.58 (s), 47.01

(s), 47.14 (t), 48.60 (s), 51.61 (q), 51.64 (q), 51.83 (q), 51.85 (q), 52.40 (q), 53.58 (d), 54.07 (d), 56.58 (d), 56.94 (d), 58.30 (s), 64.55 (t), 74.75 (d), 82.53 (s), 91.20 (d), 102.19 (s), 103 59 (s), 154.68 (s), 163.46 (s), 163.65 (s), 163.69 (s), 169.88 (s), 170.68 (s), 171.45 (s), 171.71 (s), 171.93 (s), 172.76 (s), 172.92 (s), 173.60 (s), 173.88 (s), 175.26 (s), 175.61 (s), 176.24 (s). **10b** ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.12-1.90 (m, superimposed 1.18 (s), 1.25 (s), 1.34 (s), 1.36 (s), 1.50 (s), 1.56 (s), total 32 H), 1.90-2.76 (m, superimposed 1.94 (s), 2.18 (s), 2.22 (s), total 27 H), 2.76-2.89 (m, 1 H), 2.98-3.09 (m, 1 H), 3.13-3.39 (m, 4 H), 3.40-3.53 (m, 1 H), 3.58-3.89 (m, superimposed 3.62 (s), 3.66 (s), 3.68 (s), 3.69 (s), 3.71(s), 3.75 (s), total 20 H), 4.01-4.18 (m, 2 H), 5.22 (s, 1 H), 5.28 (m, 1 H), 5.58 (s, 1 H), 6.61 (m, 1 H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 15.25 (q), 15.97 (q), 16.95 (q), 18.46 (q), 19.14 (q), 19.81 (q), 22.06 (q), 23.12 (q), 24.93 (t), 25.57 (t), 25.68 (t), 26.12 (t), 26.51 (t), 27.06 (t), 27.47 (t), 28.32 (t), 29.70 (t), 30.70 (t), 31.05 (t), 31.11 (q), 31.78 (t), 32.54 (t), 33.70 (t), 39.22 (d), 41.08 (t), 41.40 (t), 42.29 (t), 45.58 (s), 46.56 (t), 47.00 (s), 48.60 (s), 51.61 (q), 51.64 (q), 51.83 (q), 51.85 (q), 52.40 (q), 53.57 (d), 54.07 (d), 56.59 (d), 56.85 (d), 58.30 (s), 64.56 (t), 74.75 (d), 82.53 (s), 91.20 (d), 102.19 (s), 103.58 (s), 154.71 (s), 163.46 (s), 163.66 (s), 163.69 (s), 170.64 (s), 171.45 (s), 171.71 (s), 171.92 (s), 172.76 (s), 172.92 (s), 173.58 (s), 173.88 (s), 175.27 (s), 175.62 (s), 176.25 (s).

11 UV ($c = 3.59 \times 10^{-5}$ M, CH_2Cl_2): $\lambda_{max}(\varepsilon)$ 234 (1.63), 280 (0.74), 316 (0.43), 372 (1.26), 424 (0.13), 550 (0.40), 590 (0.50). IR ($CHCl_3$): 3690, 3438, 3016, 2956, 1732, 1652, 1582, 1502, 1438, 1368, 1264, 1200, 1104, 806 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.15-1.94 (m, superimposed 1.21 (s), 1.27 (s), 1.36 (s), 1.38 (s), 1.52 (s), 1.56 (s), total 30 H), 1.95-2.75 (m, superimposed 2.20 (s), 2.24 (s), total 24 H), 2.76-2.90 (m, 1 H), 3.04 (m, 1 H), 3.49 (m, 1 H), 3.60-3.85 (m, superimposed 3.64 (s), 3.68 (s), 3.70 (s), 3.71 (s), 3.73 (s), 3.77 (s), total 22 H), 4.00-4.15 (m, 2 H), 5.48 (br s, 2 H), 5.61 (s, 1 H), 5.63 (d, J = 7.4 Hz, 1 H), 7.22 (d, J = 7.0 Hz, 1 H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 15.12 (q), 15.85 (q), 16.82 (q), 18.33 (q), 18.96 (q), 19.66 (q), 21.90 (q), 24.79 (t), 25.54 (t), 25.59 (t), 26.03 (t), 26.40 (t), 28.27 (t), 28.76 (t), 29.58 (t), 30.57 (t), 30.92 (t), 31.01 (q), 31.65 (t), 32.41 (t), 33.55 (t), 39.09 (d), 41.03 (t), 42.12 (t), 45.48 (s), 46.87 (s), 48.48 (s), 49.92 (t), 51.48 (q), 51.52 (q), 51.72 (q), 51.73 (q), 52.27 (q), 53.45 (d), 53.90 (d), 56.43 (d), 58.17 (s), 64.50 (t), 74.61 (d), 82.41 (s), 91.08 (d), 93.76 (d), 102.05 (s), 103.40 (s), 145.25 (d), 156.48 (s), 163.32 (s), 163.59 (s), 165.78 (s), 170.47 (s), 171.37 (s), 171.59 (s), 171.77 (s), 172.63 (s), 172.77 (s), 173.41 (s), 173.73 (s), 175.16 (s), 175.51 (s), 176.14 (s). LSI-MS: *m/z* 1241 (100, $[M\text{-}CN\text{-}1]^+$), 1215 (88, $[M\text{-}2CN\text{-}1]^+$). ESI-MS: *m/z* 1242 (2, $[M\text{-}CN]^+$), 621 (100, $[M\text{-}CN]^{++}$).

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