# Biosynthesis of Vitamin $B_{12}$: Formation of Pyrrocorphins by Peripheral *C*-Methylation of Precorrin-3 Octamethyl Ester

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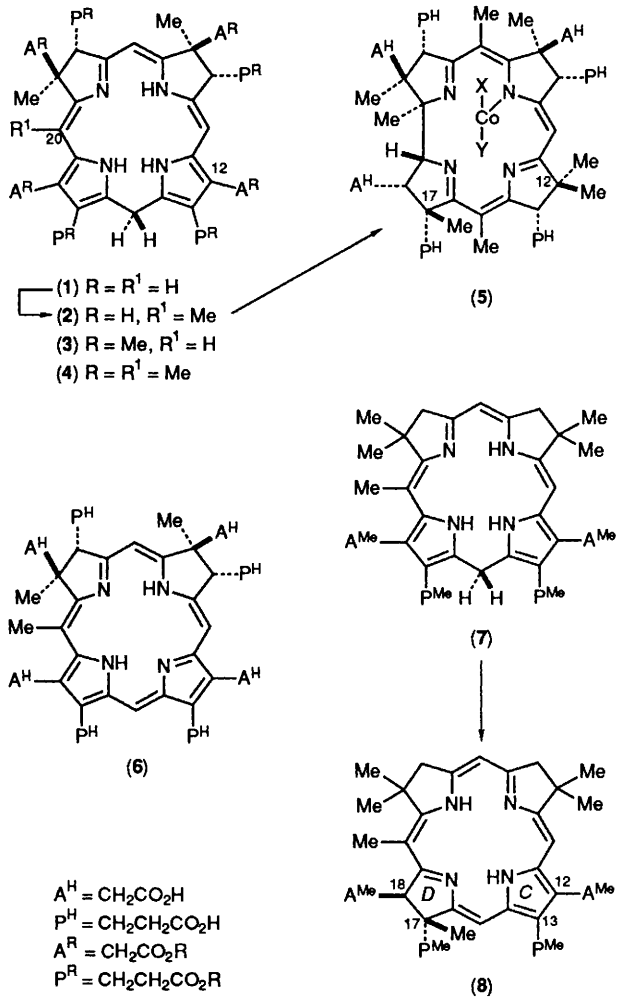
The ester of precorrin-3 (**4**) is converted by metallation–tautomerisation followed by *C*-methylation and tautomerisation–demetallation into a family of isomers from which are isolated *trans*-, and *cis*-*C*-pyrrocorphins for biosynthetic studies on vitamin $B_{12}$.

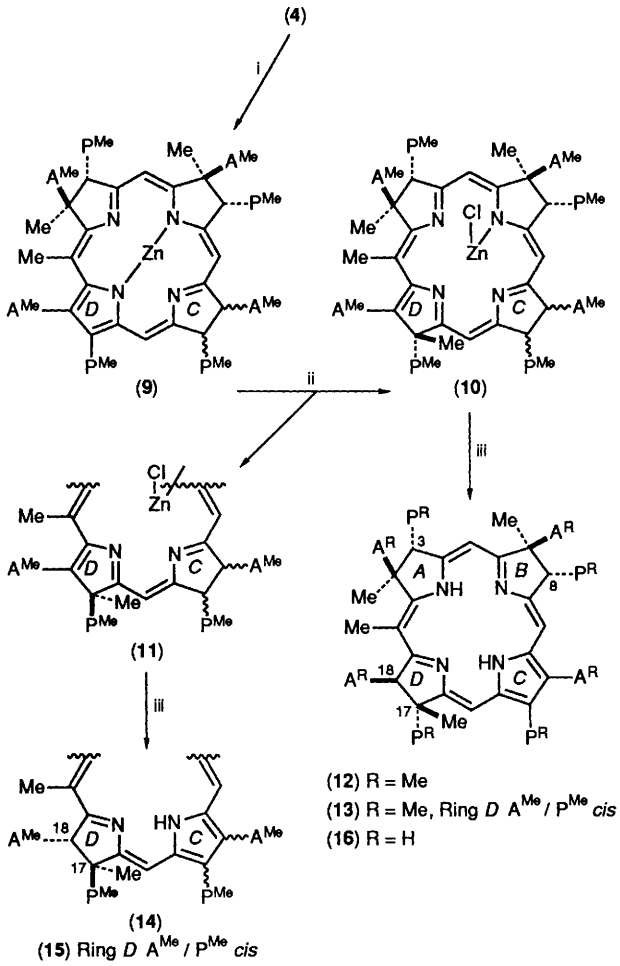
Cobyrinic acid (**5**), a late biosynthetic precursor of vitamin $B_{12}$, possesses seven *C*-methyl groups which, together with an eighth methyl which is later eliminated in acetic acid,[1] are introduced sequentially by methylase enzymes, Scheme 1. The dimethylated intermediate (**1**) was isolated[2] as its ester (**3**) and it is essentially certain that the trimethylated intermediate is (**2**) since the structure of its aromatised form was shown[3] to be (**6**). Pulse-labelling experiments proved[4] that the fourth *C*-methyl group is introduced at C-17 which pointed to the *C*-pyrrocorphin† (**16**) as the tetramethylated intermediate, Scheme 2. Experiments exploring the timing of decarboxylation of the C-12 acetate residue[5] emphasised the interest of (**16**) as a likely biosynthetic intermediate. Subsequent pulse-labelling studies[6,7] gave information about the order of the last four *C*-methylations.

The aim of the present work is the preparation of (**16**) as its ester (**12**) from (**4**). That this should, in principle, be possible, was established by the successful preparation[8] of the *trans*-*C*-pyrrocorphin (**8**) from (**7**) together with the *cis*-isomer and the *trans*-, and *cis*-isomers of the analogous 13-methylated *D*-pyrrocorphins. No significant quantities of pyrrocorphins arising from methylation at C-18 were detected; this finding has importance for experiments described below. These pilot experiments were based on the pioneering studies of Eschenmoser's group[9] of tautomerisations and *C*-methylations using macrocyles carrying peripheral methyl groups.

† A *D*-pyrrocorphin has ring-D 'pyrrolic' and similarly for a *C*-pyrrocorphin.




Scheme 1




Scheme 2. *Reagents*: i, triazabicyclo[4.4.0]dec-5-ene (TBD); $ZnI_2$, pyridine, 80 °C; ii, MeI, $ZnI_2$, tetrahydrofuran, then NaCl, $H_2O$; iii, TBD then HOAc. All reactions at <5 vpm $O_2$.

The desired sequence for the preparation of pyrrocorphin (**12**) is (**4**) → (**9**) → (**10**) → (**12**) but clearly there are major regio- and stereo-chemical problems. Conditions favouring the formation of $Zn^{II}$ *D*-pyrrocorphinates analogous to (**9**) over the undesired $Zn^{II}$ *C*-pyrrocorphinates had been devised in the model experiments.[8] Accordingly, these conditions (see Scheme 2) were used to produce the $Zn^{II}$ *D*-pyrrocorphinates (**9**), together with the ring-C isomers; yield 63% from the dihydroisobacteriochlorin (**4**) prepared from natural octa-acid (**6**). The mixture of pyrrocorphinates was *C*-methylated with either [$^{13}$C]- or [$^{12}$C]-methyl iodide to yield $Zn^{II}$ corphinates (55%) containing (see later) the C-17 methylated products (**10**) and (**11**). Tautomerisation of this mixture followed by demetallation gave a family of isomeric pyrrocorphins (34%) which were fractionated by HPLC. All the intermediates in this work and also the final pyrrocorphins (*e.g.* **12**) are extremely labile and must be handled with utmost care at <5 vpm $O_2$. Indeed, the work is close to the limit of the possible; repetitive fractionation of the final pyrrocorphins leads to their destruction. Nevertheless, the major products were isolated in remarkably good state (see later) for spectroscopic study.

Six larger peaks from HPLC contained the favoured products (*ca.* 75% of total), three smaller peaks the less favoured ones (*ca.* 18% of total), and there were three small peaks (*ca.* 7% of total) which have not been examined. The samples from the six larger peaks all showed the characteristic UV-VIS spectrum for the pyrrocorphin chromophore,[9] typically a broad band (354 nm) with shoulders and a side peak (375 nm) and weaker long wavelength bands (496, 527, 572 nm); relative intensities, respectively, 1.0, 0.7, 0.23, 0.21, 0.19. Furthermore, field-desorption mass spectroscopy (FD MS) for these six samples in the $^{13}$C-series showed in each case the required mass of 1005 for pyrrocorphin (**12**) and its isomers.

20-Methyl *C*-pyrrocorphins (*e.g.* **8**) can be distinguished from *D*-pyrrocorphins by $^1$H NMR spectroscopy; the signal from the C-20 methyl group appears at δ 2.27—2.37 in the *C*-isomers and at δ 2.78—2.81 in the *D*-isomers[8,9] whilst H-5, H-10, and H-15 appear at δ 5.8—5.94, 7.1—7.2, and 7.08—7.22, respectively for the *C*-isomers but at 5.65—5.73, 5.69—5.74, and 6.86—7.01 for the *D*-isomers.[8—10] The $^1$H NMR spectra of the above six samples allowed selection of four fractions which contained the desired *C*-pyrrocorphins‡ (*e.g.* **12** and **13**). Then $^{13}$C NMR spectra of these four fractions from the $^{13}$C series allowed discrimination of two containing the *trans*-isomers (high field $^{13}$C signals δ 19.28, 19.52 due to γ-effect) from two containing the *cis*-isomers (low field signals

‡ NMR and FD MS data are available for the *D*-pyrrocorphins present in the other two main peaks.

δ 27.54, 27.74). In addition, the $^{13}C$ spectra allowed determination of what proportion the major pyrrocorphin represented of the total *C*-pyrrocorphins present in that fraction. The values for the two *cis*-fractions were 100 and 93% and for the two *trans*-fractions 82 and 65%. The remaining 35% in the last fraction consisted of two isomers of which the largest was 20% of the whole. These minor closely related isomers probably arise by inversion at C-3 or C-8 to the less favoured *cis*-configurations on ring A or ring B, a known process under basic conditions in similar systems.[11]

*C*-Pyrrocorphins can only arise by methylation at C-17 or C-18 of (**9**). However, the absence of appreciable methylation at C-18 in experiments leading to the closely related 20-methyl model system[8] (see **8**) supports the view that the foregoing four favoured *C*-pyrrocorphins are all C-17 methylated. On this basis, they are the *trans*-isomers (**12**) and (**14**) and the *cis*-isomers (**13**) and (**15**); the first of this set is the desired one, of great biosynthetic interest.

The chiral centre at C-17 in such isomers (**12**), (**13**), (**14**), and (**15**) cannot be correlated by NMR with those on rings A and B. Discrimination of (**12**) from (**14**) and (**13**) from (**15**) and final structure proof will be by degradation to materials of known absolute configuration being prepared by asymmetric synthesis.

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