Biosynthesis of Vitamin B_{12}: Regio-control in Peripheral *C*-Methylation of 20-Methylpyrrocorphins carrying Ester Side Chains

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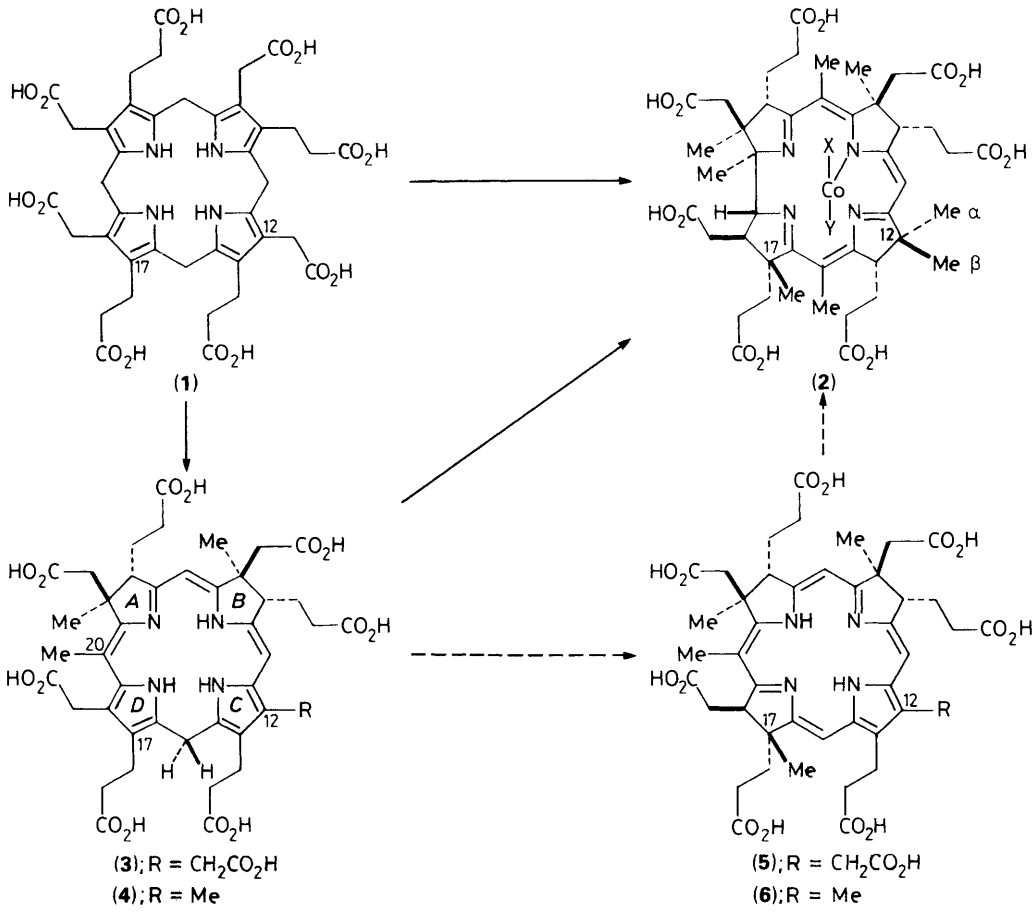
Mild methods are developed for the predominant generation of D-pyrrocorphins† from 20-methyldihydroisobacteriochlorins carrying ester side chains; the corresponding zinc(II) 20-methyl-D-pyrrocorphinates undergo methylation preferentially at C-17.

The biosynthesis of vitamin B_{12} proceeds *via* cobyrinic acid (**2**), which is generated from uroporphyrinogen III (**1**) by a multi-step sequence including eight *C*-methylations;[1] the order in which the first three methyl groups are introduced to form precorrin-3 (**3**) was known.[1] Pulse labelling experiments[2] then showed that the fourth *C*-methyl group is placed at C-17 which, taken with information about the oxidation level of

† A D-pyrrocorphin has ring-D 'pyrrolic' whereas a C-pyrrocorphin has a 'pyrrolic' ring-C.



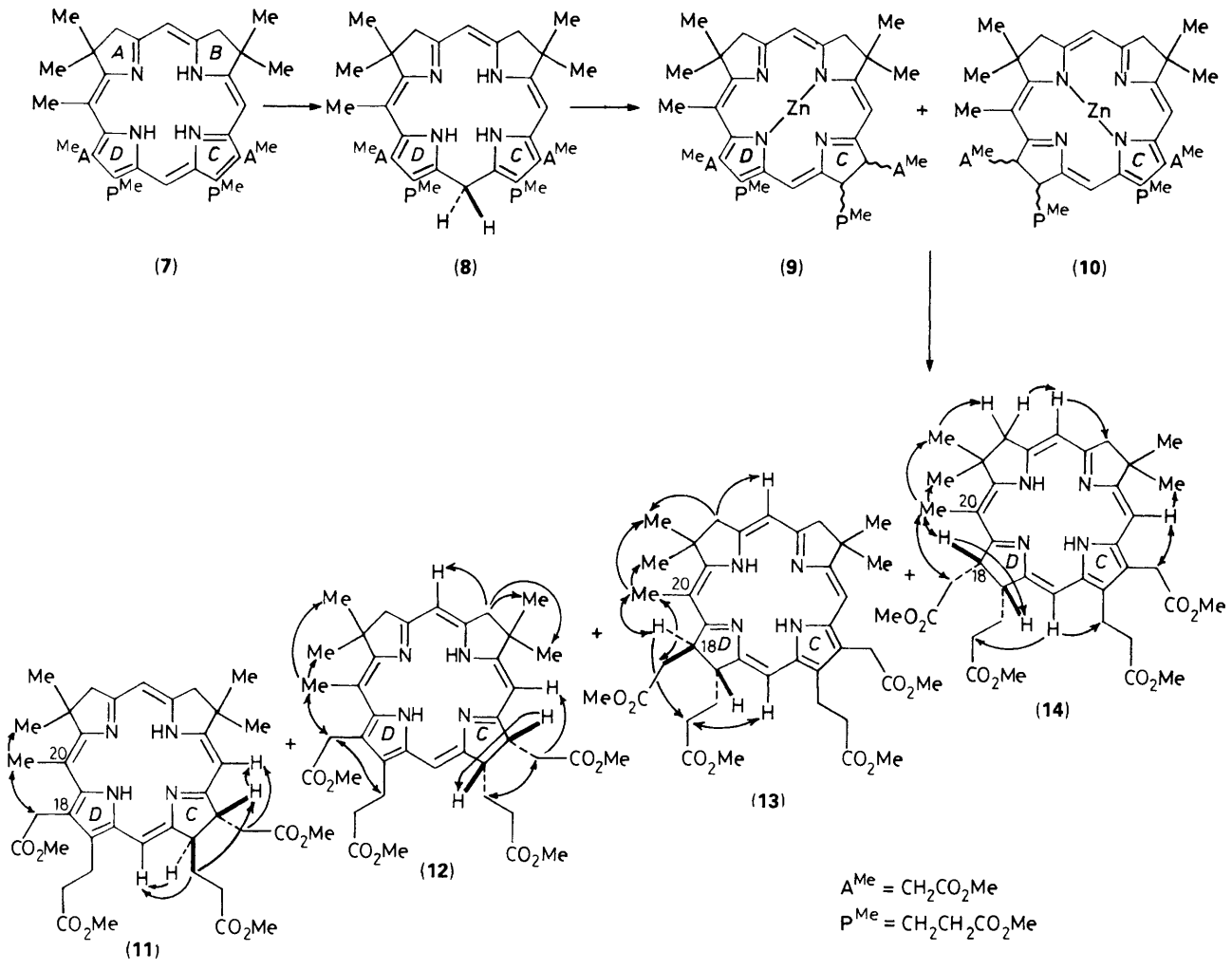
Scheme 1

earlier intermediates on the pathway,[3,4] pinpointed the pyrrocorphin (**5**) as a likely structure for the first tetramethylated intermediate (Scheme 1). Extension of the pulse labelling method[5—7] has confirmed this earlier conclusion. The likelihood of structure (**5**) depends additionally on the results from recent experiments[8] which indicated that decarboxylation of the 12-acetate residue [see (**1**)], which must happen at some stage to generate the 12-β-methyl group of cobyrinic acid (**2**), probably occurs after the pyrrocorphin system has been generated. Thus, the 12-methylpyrrocorphin (**6**) also becomes of interest as a possible second tetramethylated biosynthetic intermediate.

We aim to prepare the octamethyl esters of these pyrrocorphins (**5**) and (**6**) respectively from the octamethyl esters of the dihydroisobacteriochlorins (**3**) and (**4**) by peripheral *C*-methylation, building on knowledge from the important studies of Eschenmoser's group on the synthesis and chemistry of pyrrocorphins.[9,10] The aromatised isobacteriochlorins corresponding to structures (**3**) and (**4**) have now been constructed as their octamethyl esters by total synthesis.[11,12]

Our recent Communication[13] described the methods by which a symmetrical dihydroisobacteriochlorin carrying reactive ester side chains was peripherally *C*-methylated to yield pyrrocorphins of the desired structures. However, the systems (**3**) and (**4**) are not symmetrical and they also carry the important feature of a C-20 methyl group. Since both starting materials (**3**) and (**4**) are precious, methods must be devised to achieve (a) the maximum proportion of *C*-methylation at C-17 and (b) the highest amount of product with *trans*-ester side chains on ring-D.

The 20-methylisobacteriochlorin (**7**) was prepared to act as a close model by modification of the published methods.[14] It was reduced catalytically to afford the oxygen sensitive 20-methyldihydroisobacteriochlorin (**8**) (Scheme 2), 80%, which showed *m/z* 702.3670 (M^+ for $C_{39}H_{50}N_4O_8$ 702.3629). This reduction and all subsequent transformations were carried out in a glove box at <5 v.p.m. O_2. Tautomerization of (**8**) with triazabicyclo[4.4.0]dec-5-ene (TBD)[10] and zinc(II) iodide in tetrahydrofuran(THF)[13] at 80 °C followed by brief treatment with acetic acid afforded a pyrrocorphin fraction, total 56% together with recovered dihydroisobacteriochlorin (**8**), 16%. Separation of the pyrrocorphins by h.p.l.c. gave the *cis*-C-pyrrocorphin (**14**), 25% overall, and the *cis*-D-pyrrocorphin (**12**), 11% overall, which showed *m/z* 702.3636 and 702.3629, respectively (M^+ for $C_{39}H_{50}N_4O_8$ 702.3629). In addition, the *trans*-D-pyrrocorphin (**11**), 9%, and the *trans*-C-pyrrocorphin (**13**), 1%, were obtained as a mixture which showed *m/z* 702.3643. The structural assignments for (**11**), (**12**), and (**14**) were based on their compositions (accurate mass) and u.v.-visible spectra; 20-methyl-D-pyrrocorphins and 20-methyl-C-pyrrocorphins differ spectroscopically.[10] Furthermore, their 400 MHz ^1H n.m.r. spectra together with extensive decoupling and nuclear Overhauser enhancement (n.O.e.) difference spectroscopy gave unequivocal confirmation of their structure; only the essential connectivities are illustrated. It should be noted that the methylene protons, *e.g.* on the C-18 acetate residue of (**14**), are diastereotopic; the observed n.O.e. to the C-20 methyl group was from one of



Scheme 2

these two protons and the same holds for several other cases. These details have not been illustrated to avoid over-complication of the diagrams.

In contrast, tautomerization of the dihydro compound (**8**) as above followed by treatment of (**9**) and (**10**) with acetic acid for 20 h afforded in 59% yield a mixture of *trans*-C-pyrrocorphin (**13**) and *trans*-D-pyrrocorphin (**11**), in the ratio (**13**):(**11**) of 74:26 by n.m.r. analysis, *m/z* 702.3643. The structure of the major pyrrocorphin (**13**) follows from the illustrated connectivities. This essentially exclusive production of (**13**) and (**11**) under these conditions results from equilibration to form the thermodynamically favoured *trans*-isomers.[10,13] The predominance of the C-pyrrocorphin (**13**) may be due to relief of steric compression between the C-20 methyl and C-18 acetate groups; this unfavourable interaction is certainly evident[15] in the *X*-ray structure of the macrocycle (**7**).

The desired favouring of the Zn^{II}-D-pyrrocorphinate structure (**9**) was achieved by carrying out the tautomerisation of (**8**) using pyridine as solvent together with TBD and ZnI_2 at 80 °C followed by brief demetallation with acetic acid (*i.e.*, minimum equilibration). This afforded the pyrrocorphins (**11**)—(**14**) in 50% combined yield, shown by n.m.r. to be mainly the *trans*-D-pyrrocorphin (**11**), 48% of the mixture. The remainder was the *cis*-C-pyrrocorphin (**14**), 29%, the *cis*-D-isomer (**12**), 15%, and the *trans*-C-isomer (**13**), 8%. Thus the ratio of D-pyrrocorphins (**11**) and (**12**) to C-pyrrocorphins (**13**) and (**14**) was 63:37, which indicated that at least the same ratio held for (**9**):(**10**). Note that both *trans*- and *cis*-Zn^{II}-D-pyrrocorphinates (**9**) are valuable for the subsequent *C*-methylation step.

The mixture of (**9**) and (**10**), 57% yield, prepared as above using pyridine, was methylated at 80 °C with methyl iodide in THF (1:3) containing ZnI_2 to give the Zn^{II} corphinates (**15**) and (**16**), 61%, Scheme 3. Tautomerisation of this mixture using TBD followed by demetallation with acetic acid (2 h) gave four identified pyrrocorphins in 60% combined yield. H.p.l.c. separation afforded the *trans*-17-methyl-C-pyrrocorphin (**17**), 11%, the *cis*-17-methyl-C-pyrrocorphin (**18**), 17%, the *trans*-13-methyl-D-pyrrocorphin (**19**), 10%, and the *cis*-13-methyl-D-pyrrocorphin (**20**), 7%. They showed *m/z* 716.3740, 716.3831, 716.3824, and 716.3768, respectively (M^+ for $C_{40}H_{52}N_4O_8$ 716.3785.) Their structures were deduced as for the cases (**11**)—(**14**) above, the demonstrated connectivities being illustrated on structures (**17**)—(**20**).

The sequence (**7**) → (**8**) → (**9**) + (**10**) → (**15**) + (**16**) → (**17**, plus separable isomers) shows that methods are now available for conversion of the octamethyl ester of (**3**) into the corresponding ester of (**5**); this work is in progress.

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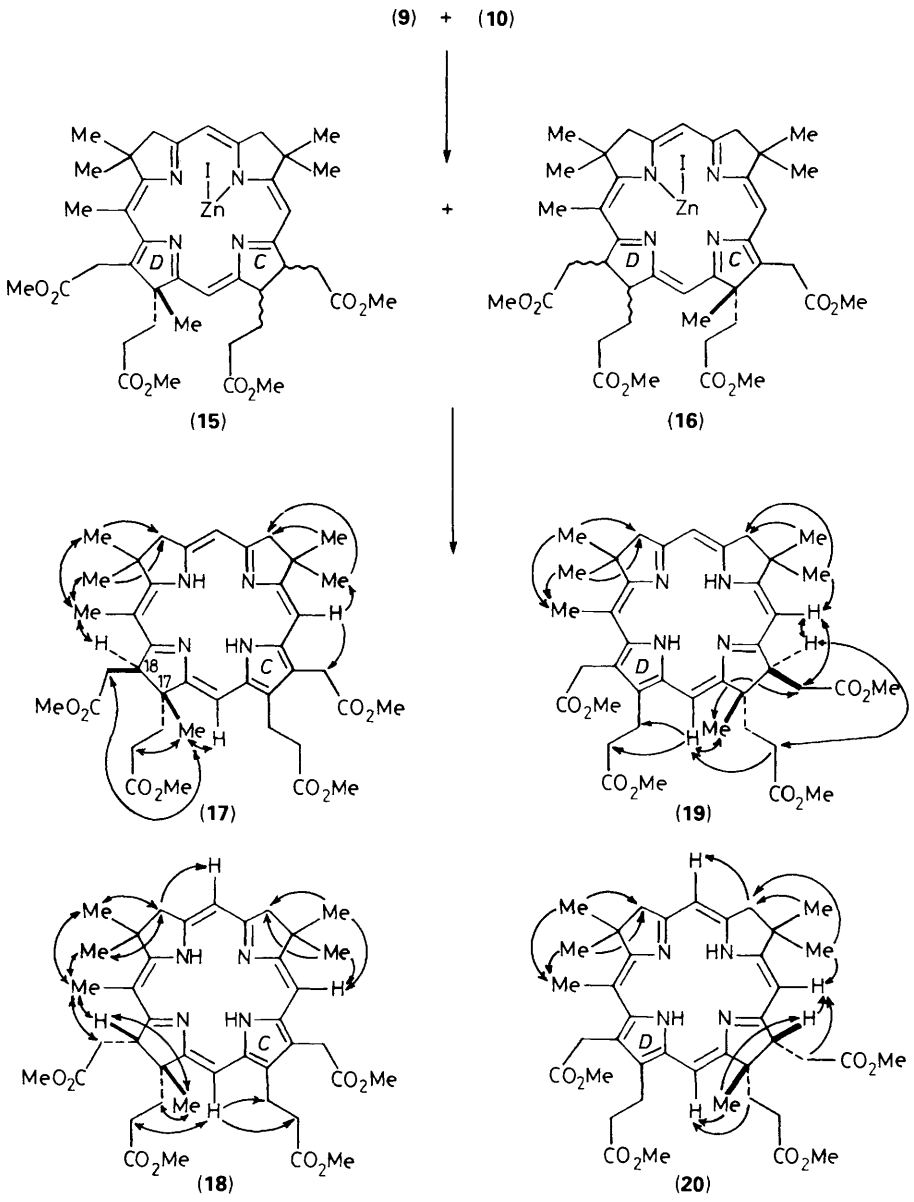

Scheme 3

experimental information, Dr. F. J. Leeper for his advice on n.m.r. spectroscopy and Dr. S. Handa for providing starting materials for the synthesis of (7).

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