Syntheses Relevant to Vitamin B_{12} Biosynthesis: the Malate Route to (−)-Ring-B Imide and Synthesis of the 2,7,20-Trimethylisobacteriochlorin

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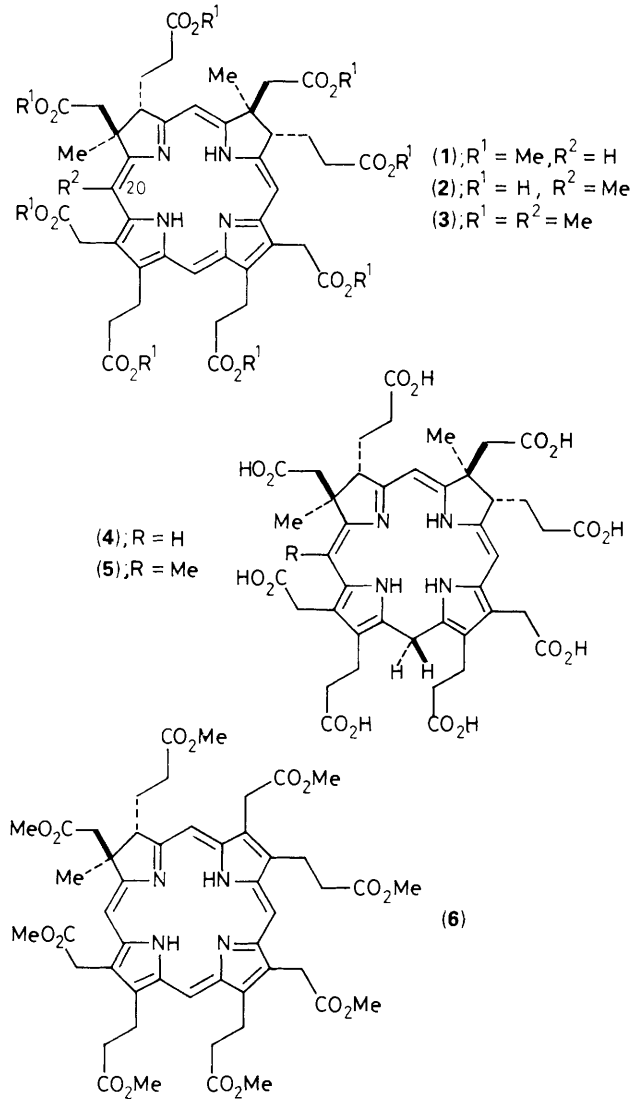
An efficient enantioselective synthesis of (−)-ring-B imide (**19**) from *R*-malic acid is outlined and this product is used for synthesis, by a photochemical route, of the 2,7,20-trimethylisobacteriochlorin (**3**), of importance for biosynthetic research on vitamin B_{12}.

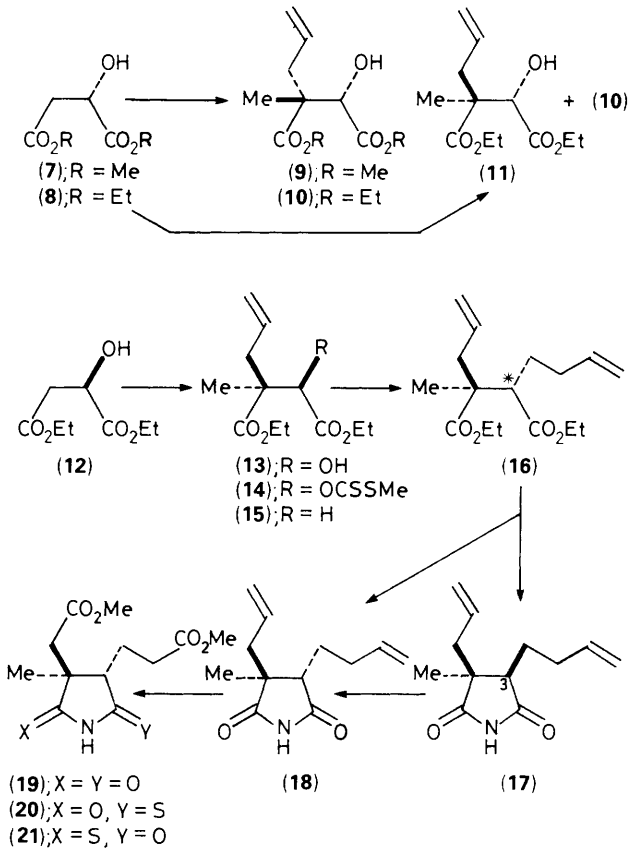
The trimethylisobacteriochlorin (**2**) was first isolated[1,2] from *Propionibacterium shermanii*, which biosynthesises vitamin B_{12} and its structure was elucidated[3] by a combination of spectroscopic and biosynthetic methods. But (**2**) is not a biosynthetic precursor of vitamin B_{12}, rather it is essentially certain that the dihydro form (**5**) is the true intermediate, precorrin-3,[4] which undergoes air oxidation to the aromatised macrocycle (**2**) during isolation. This view is based on direct isolation[5] from *P. shermanii* of the dimethylated intermediate, precorrin-2, which was shown to be the dihydro system (**4**); this acts[5] as a biosynthetic precursor of B_{12}. The fact that the monomethylated intermediate, precorrin-1, has also been shown to be generated at this same oxidation level[6] (8 double bonds) adds further support. Originally, the mono-,[7] di-,[8] and tri-methylated[1,2] macrocycles were isolated, after aromatisation by air oxidation, as their octamethyl esters (**6**), (**1**), and (**3**), respectively.

These pigments (**6**), (**1**), and (**3**) are important for future biosynthetic studies on vitamin B_{12} but are available only in small amounts from the natural source. Accordingly, a major effort has been made in Cambridge to develop synthetic routes to all three substances, with success for the monomethylated[9] (**6**) and dimethylated[10] (**1**) macrocycles. We now report the synthesis of the trimethylated isobacteriochlorin (**3**). The structures in the following Communication are numbered to continue the sequence used in this Communication; the two contributions should thus be read in conjunction.

Our earlier strategy[9,10] used the so-called ring-B imide[11] (**19**) to provide all the rings in (**6**) and (**1**) which carry chiral centres and the same plan was to be followed to synthesise (**3**). For the previous work, the imide (**19**) was either synthesised as a racemate[12] or produced in optically active form (expensively!) by degradation[11] of vitamin B_{12}. The first task was thus to develop an efficient synthesis of the required (−)-enantiomer of (**19**) as in Scheme 1; this imide has been synthesised previously on a small scale for stereochemical correlations.[13]

Alkylations of dianions derived from β-hydroxycarboxylic esters are diastereoselective[14,15] and methylation of dimethyl *S*-malate (**7**) followed by allylation had yielded[15,16] (**9**) with excellent stereochemical control but having the *opposite* configuration to that we required, see (**19**). But allylation followed by methylation (*i.e.* reversed order) of diethyl *S*-malate (**8**) gave only a 3 : 2 ratio of desired (**11**) to undesired (**10**) products. Accordingly, diethyl *R*-malate (**12**) was used





Scheme 1

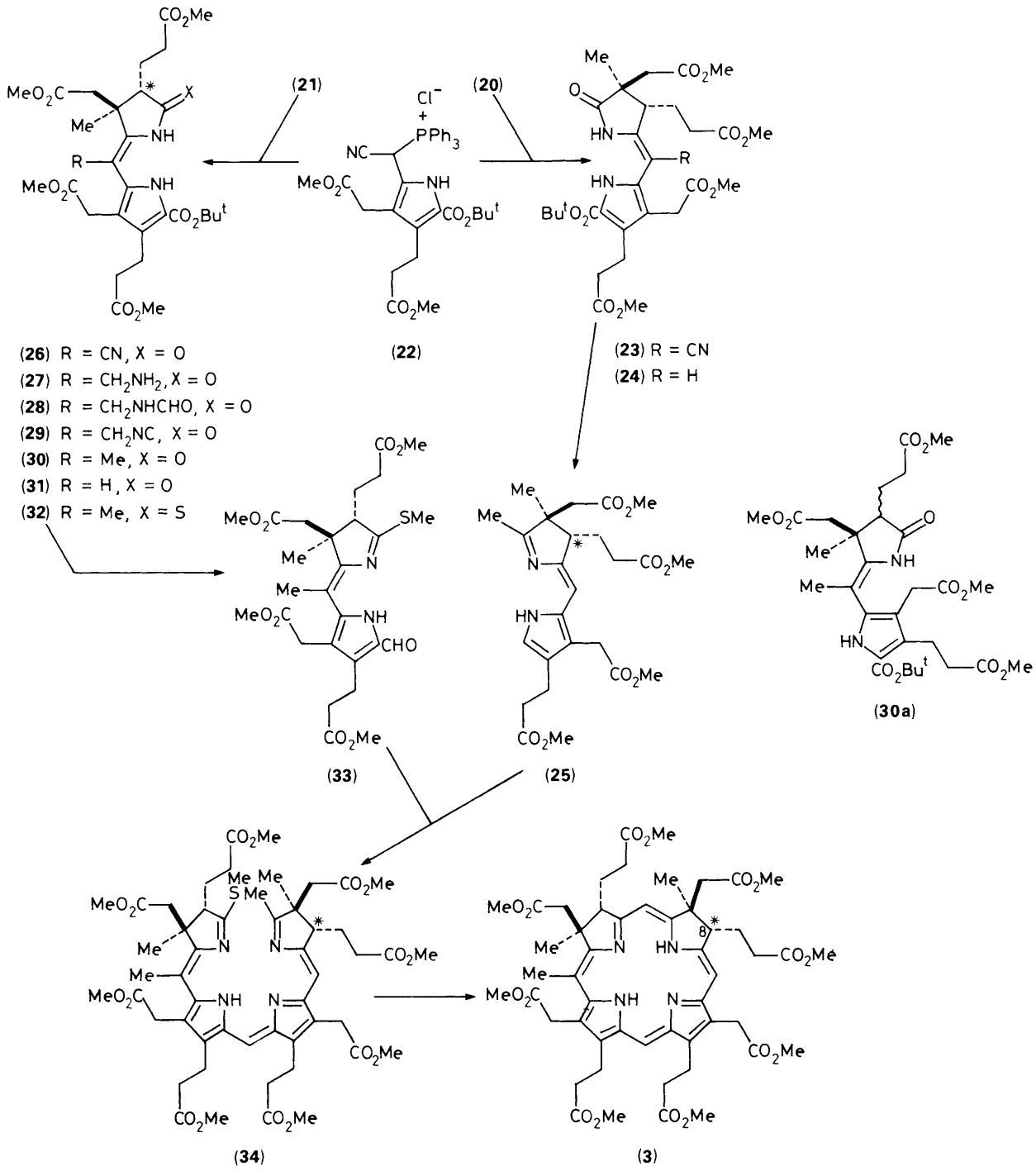
for methylation followed by allylation to yield (**13**) containing only 4% of the unwanted diastereoisomer. Removal of the hydroxy group from (**13**) *via* the xanthate ester[17] (**14**) to give (**15**) followed by alkylation with but-3-enyl bromide using 1,3-dimethyl-3,4,5,6-tetrahydropyrimidin-2(1*H*)-one as co-solvent yielded (**16**) as the major product in admixture with the epimer at the *-centre.

The ester (**16**) and its diastereoisomer, without separation, were hydrolysed with alkali and the resultant mixture of mono- and di-carboxylic acids was heated with urea[18] to yield the imides (**17**) and (**18**), ratio 1:3, respectively. The latter was isolated pure by crystallisation and the mother liquor material, enriched in (**17**), was epimerised at C-3 by KOBu^t in Bu^{t}OH to allow crystallisation of more of the desired *trans* product, (**18**). Oxidation of (**18**) with ruthenium tetroxide–sodium periodate and conversion of the product into its dimethyl ester yielded (−)-ring-B imide (**19**) as crystals for the first time, m.p. 60.5—61.5°C, $[\alpha]_D^{24}$ −79.6° (*c*, 2.5 in $CHCl_3$). Structure (**19**) was confirmed by *X*-ray analysis.[19] A single run by the foregoing sequence has yielded >10 g of crystalline (−)-ring-B imide (**19**).

The imide (**19**) was converted into the two monothioimides (**20**) and (**21**), and reaction of the former with the phosphonium salt (**22**) gave[10] the nitrile (**23**). This was converted *via* the essentially single *Z*-isomer of (**24**) into the eastern building block (**25**) largely by the reported steps[9,10] but with yield enhancing improvements to be described in our full paper. Some equilibration of the double bond stereochemistry and the *-centre occurred during these transformations and the asterisk on other structures in Scheme 2 similarly indicates the presence of both epimers. Pure isomers of key intermediates were isolated throughout for full characterisation but this was unnecessary for preparative runs because subsequent isomerisation could occur.

The isomeric monothioimide (**21**) was converted essentially as previously[10] into the required *E*-nitrile (**26**); only traces of the separable *Z*-isomer were formed. The nitrile residue of (**26**) was to serve as a source of the methyl group at C-20 of the final product (**3**). Three successful sequences were developed for the transformation –CN→–CH_3, the best being as follows.

Reduction of the nitrile (**26**) with borohydride catalysed[20] by Co^{II} gave the *Z*-amine (**27**), which, without isolation, was formylated using formic pivalic anhydride. The resultant formamide (**28**) was dehydrated by $POCl_3$ and 1,8-bisdimethylaminonaphthalene to afford the isonitrile (**29**) and this function was removed[21] to leave the required *C*-methyl group by reaction with tributyltin hydride initiated by azoisobutyronitrile. The product was the *Z*-lactam (**30**) containing variable amounts of the *E*-isomer, ranging from negligible to 50% in different runs. The separated *E*-isomer on heating with 1% HOAc in toluene was equilibrated to a 1:1.2 mixture of *Z*:*E* isomers; by this means, almost all of the product could be obtained as the *Z*-form (**30**). Both epimers at the *-centre of (**30**) were isolated and nuclear Overhauser effect (n.O.e.) difference n.m.r. spectroscopy showed the preferred conformation of both to be as (**30a**). In support, the u.v. spectrum of both epimers, as (**30a**), was unaffected by $Zn(OAc)_2$ (no Zn^{II}



Scheme 2

complex formed involving the two N centres) unlike the corresponding system[10] (**31**) lacking the *meso* *C*-methyl group. This resistance to adopting conformation (**30**) is considered again later.

The thiolactam (**32**) was prepared together with its *E*-isomer by heating (**30**) with Lawesson's reagent. These thiolactams reacted with trimethylorthoformate–TFA to give the western block (**33**) as a mixture of *Z*- and *E*-forms but with the welcome simplification that each was shown (by separation and n.m.r.) to be essentially one diastereoisomer (*trans* acetate–propionate). This mixed product was condensed with the eastern block (**25**) using methanolic hydrogen chloride to afford the *seco*-system (**34**), which on irradiation cyclised by an antarafacial 18π-electrocyclic process[22] to yield the trimethylisobacteriochlorin (**3**) in admixture with the C-8 epimer which had been isolated earlier[23] from the natural source. Such epimers are known [24] to be epimerised under basic conditions to the more stable *trans* arrangement and so represent valuable material. The desired product (**3**) was isolated by h.p.l.c. and shown by t.l.c., h.p.l.c., ^{1}H n.m.r.,

u.v.–visible and field desorption mass spectroscopy to be identical with the ester of the natural material isolated from *P. shermanii*.

Thus the structure of (**3**), previously based on a combination of spectroscopic and biosynthetic evidence, is confirmed. Over 75% of the synthetic steps used to build the western (**33**) and eastern (**25**) blocks were achieved in good-to-high yields (70—95%) and the rest in acceptable yields (50—70%). Current research is focussed on improving the yields (20—25%) of the final two steps to preparative levels, *e.g.* by overcoming the conformational problem outlined above with metal ion templates at the *seco*-stage (**34**).

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