First Total Synthesis of Redox Coenzyme Factor 420

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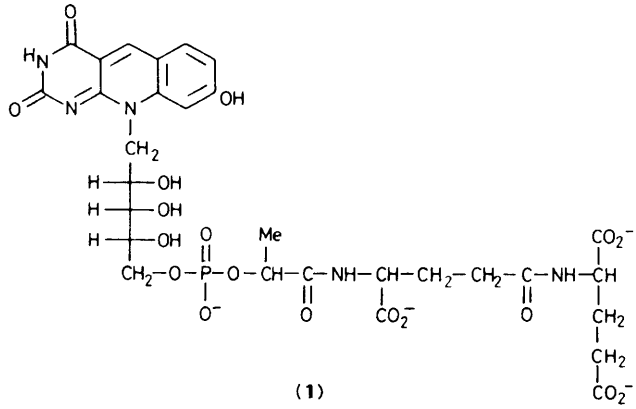
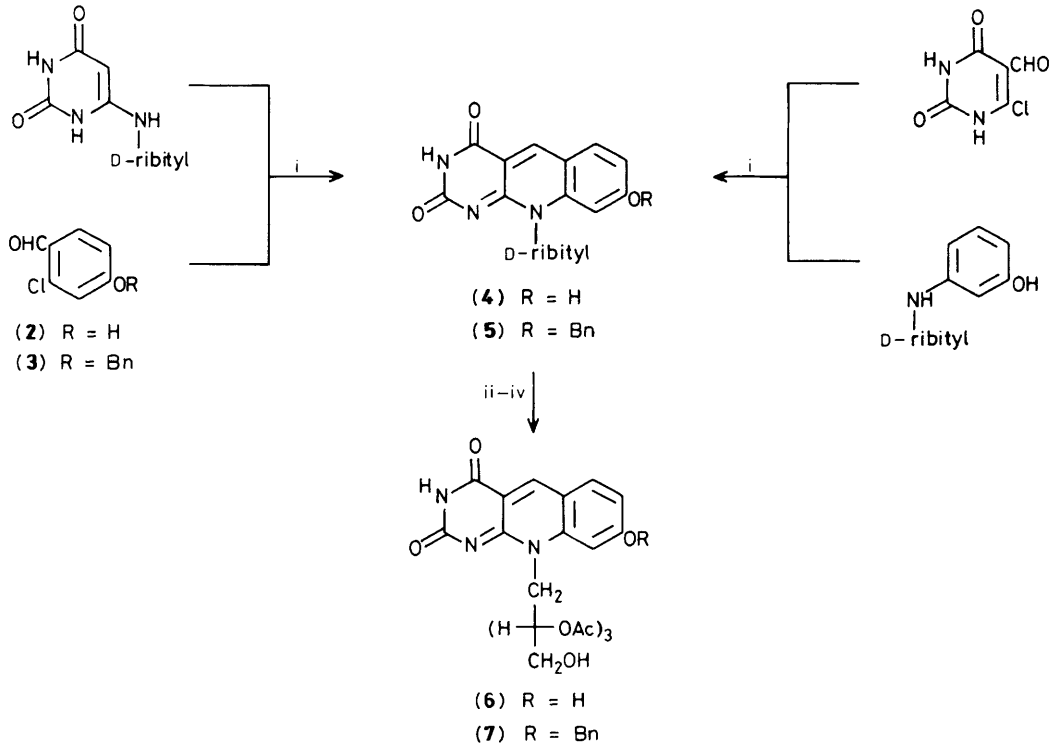
The first total synthesis of *Methanobacterium* redox coenzyme Factor 420 (F_{420}) has been achieved by the formation of a phosphotriester bond between a protected 10-D-ribityl-8-hydroxy-5-deazaisoalloxazine moiety and a peptide moiety, (L-lactate-γ-L-glutamyl)-L-glutamic acid tribenzyl ester, by the phosphite triester approach using β,β,β-trichloroethyl phosphorodichloridite, followed by successive deprotection.

Much of the renewed interest in 5-deazaflavins stems from the isolation of a unique coenzyme known as Factor 420 (F_{420}) from anaerobic methane-producing bacteria, *Methanobacterium* (strain M.o.H.).[1,2] Recently, closely related coenzymes[3] have also been isolated, all of which have been found to be multifunctional molecules[3,4] which play mainly redox roles in methanogens as low-potential electron carriers.[5] Coenzyme F_{420} was proposed to have structure (**1**), containing an 8-hydroxy-5-deazaisoalloxazine moiety, on the basis of chemical degradation and spectroscopic data,[2] but final confirmation of this structure by organic synthesis has been lacking. As part of our chemical investigation on 5-deazaflavin derivatives,[6] we report herein the first total synthesis of coenzyme F_{420}.

The synthetic plan was a convergent one, to connect the protected peptide moiety to the protected chromophore by a phosphate ester linkage, and on this basis we first synthesized the two components. The chromophoric moiety (**4**) of coenzyme F_{420}[7] was prepared by two routes without the need for protecting groups in 70% and 92% yields from 2-chloro-4-hydroxybenzaldehyde (**2**)[8] and 6-D-ribitylaminouracil[9] or 1-deoxy-1-[(3-hydroxyphenyl)amino]-D-ribitol[7] and 6-chloro-5-formyluracil,[10] respectively. Compound (**4**) was subse-

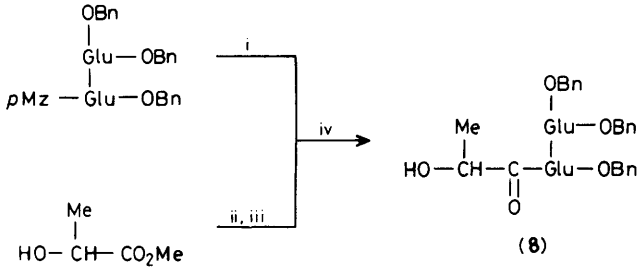


(**1**)

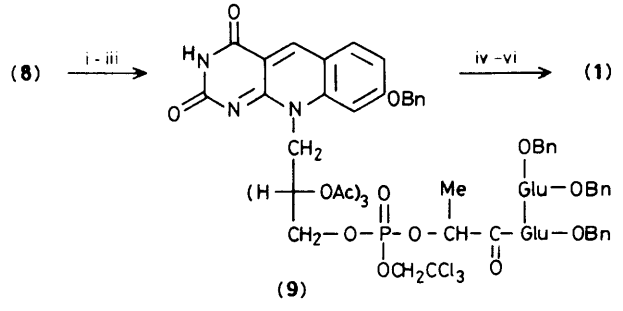


Scheme 1. *Reagents and conditions*: i, dimethylformamide (DMF), reflux, 3 h; ii, Ph_3CCl (2—3 equiv.), 0 °C → room temp., overnight; iii, Ac_2O, pyridine, overnight, room temp.; iv, $CHCl_3$, HCl (gas), 0 °C, 30 min.

quently transformed into (**6**) (m.p. 266—273 °C)†‡ with protected secondary hydroxyl groups by tritylation, acetylation, and detritylation (Scheme 1). (L-Lactate-γ-L-glutamyl)-L-glutamic acid tribenzyl ester (**8**), m.p. 98—101 °C $[\alpha]_D^{20}$ −23.39° (MeOH), another alcoholic part of the phosphodiester of F_{420}, was synthesized in 57% yield from a known compound, (*p*-methoxybenzyloxycarbonyl-γ-L-glutamyl)-L-glutamic acid tribenzyl ester,[11] which was condensed with L-lactic acid methyl ester by the azide using triethylamine as a base at 0 °C, after deprotection of the peptide N-terminus (Scheme 2). Owing to the very poor solubility of (**6**) in organic solvents and the presence of active hydrogens in both substrates, all attempts to connect these synthons by a phosphate triester linkage were unsatisfactory using several different approaches. This prompted us to employ a more lipophilic alcohol and a more forcing method for phosphate ester formation. Starting from 2-chloro-4-benzyloxybenzaldehyde (**3**), the 8-benzyloxy derivative (**7**), m.p. 133—135 °C, was obtained in a similar manner to (**6**) in 24% overall yield (Scheme 1) and was then combined with the peptide moiety (**8**) *via* the phosphite triester approach.[12] Thus, the reaction of β,β,β-trichloroethyl phosphorodichloridite with (**7**) and (**8**) in the presence of 2,6-lutidine in tetrahydrofuran (THF) at −70 to −20 °C (Scheme 3) furnished the F_{420} precursor (**9**) as a yellow fluorescent oil, after oxidative work-up with iodine, in 47% yield as an inseparable mixture of diastereoisomers at the phosphorus atom.



Scheme 2. *Reagents and conditions*: i, trifluoroacetic acid (TFA), anisole, 0 °C, 1 h; ii, H_2NNH_2, 0 °C, 24 h; iii, $NaNO_2$, HCl, 0 °C, 1 h; iv, DMF, Et_3N, 0 °C, 9 h.



Scheme 3. *Reagents and conditions*: i, $Cl_3CCH_2OPCl_2$, 2,6-lutidine, THF, −70 °C; ii, (**7**), THF, −70 → −20 °C, 5 min; iii, I_2, H_2O, THF, 2,6-lutidine, −20 → room temp., 30 min; iv, Zn/Cu couple, NH_4Cl, aq. EtOH, 55 °C, 3 h; v, H_2, 10% Pd/C, MeOH, overnight, room temp.; room temp.; vi, 14% aq. NH_4OH, in the dark, 0 °C, overnight.

† All new compounds were fully characterized by spectroscopic and combustion analyses.

‡ The 8-hydroxy group of the 5-deazaflavin remains intact on acylation, probably owing to paraquinoid–phenolic tautomerism.

The last sequence of the total synthesis was rather straightforward but low yield (Scheme 3). Successive deprotection by treatment with a Zn/Cu couple for the β,β,β-trichloroethyl ether group, catalytic hydrogenolysis on 10% palladized charcoal for the benzyl ester, ester, and the chromophoric moiety§ and finally hydrolysis with aqueous ammonia for the acetate groups, gave the crude product in 15.6% overall yield from (**9**). The sample produced was lyophilized and purified according to the literature method,[2] and it exhibited identical ^{1}H n.m.r. (D_2O) and secondary ion mass spectra and thin layer electrophoretic, paper chromatographic, and reverse phase t.l.c. behaviour to an authentic sample. We believe this synthesis offers final confirmation of the structure of F_{420}.

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§ Catalytic reduction of a 5-deazaflavin derivative usually affords the corresponding 1,5-dihydro compound, which is readily reoxidized by air on standing at ambient temperature.

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