First Total Synthesis of Coenzyme Factor 420

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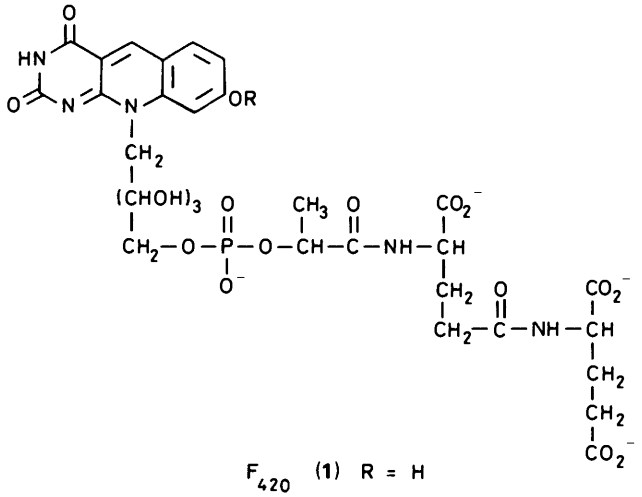
The first total synthesis of *Methanobacterium* redox coenzyme Factor 420 (F_{420}) has been achieved by the formation of a phosphotriester bond between a protected 8-hydroxy-10-D-ribityl-5-deazaisoalloxazine moiety and a peptide moiety, (L-lactoyl-γ-L-glutamyl)-L-glutamic acid tribenzyl ester, by the phosphite triester approach using 2,2,2-trichloroethyl phosphorodichloridite, followed by successive deprotection procedures.

Much recent interest in 5-deazaflavins stems from the isolation of a unique coenzyme known as Factor 420 (F_{420}) (**1**) from anaerobic methane-producing bacteria.[1] Coenzyme F_{420} has been found to be a multifunctional molecule which participates in both catabolic and anabolic redox process in methanogenesis as a low-potential electron carrier.[2] Recently, the photoreactivating enzyme cofactor, which is included in *Streptomyces griseus* and which catalyses the splitting of thymine dimer in the presence of visible light, was also found to be a 5-deazaflavin derivative similar to F_{420}.[3–5] Furthermore, closely related 5-deazaflavin derivatives with absorbance maxima at 390 nm, F_{390}-A (**2**) and F_{390}-G (**3**), have been isolated from oxidized cells of *Methanobacterium thermoautotrophicum*, and both compounds no longer act as hydrogenase substrates in methanogenesis.[6] 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,[1] but final confirmation of this structure by organic synthesis has been lacking. In the present paper we describe the full accounts of the total synthesis of coenzyme F_{420} by a convergent approach.[7]

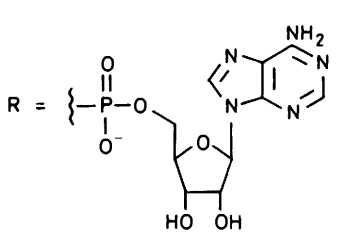
First of all, the chromophoric moiety of F_{420}[8] was synthesized by two routes without the need for protecting groups. One route afforded the product (**7**) from 2-chloro-4-hydroxybenzaldehyde (**5**)[9] and 6-D-ribitylaminouracil (**4**)[10] in 77% yield. The same product was obtained from 1-deoxy-1-[(3-hydroxyphenyl)amino]-D-ribitol (**10**)[8] and 6-chloro-5-formyluracil (**9**)[11] in 92% yield by another route. Compound (**7**) was transformed into the corresponding trityl ether (**11**) and then into the triacetate (**13**) with protected secondary hydroxy groups by tritylation, acetylation, and detritylation (Scheme 1). The hydroxy group at C-8 in the 5-deazaflavin seemed to have low reactivity for acylation, probably owing to paraquinoid–phenolic tautomerism.

Another alcoholic component of the phosphodiester of F_{420}, (L-lactoyl-γ-L-glutamyl)-L-glutamic acid tribenzyl ester (**15**) was synthesized from (*p*-methoxybenzyloxycarbonyl-γ-L-glutamyl)-L-glutamic acid tribenzyl ester (**16**),[12] which was condensed with L-methyl lactate (**17**) in the azide method with triethylamine as base at 0 °C, after deprotection of the peptide *N*-terminus (Scheme 2)).

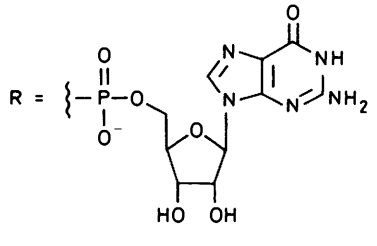
Owing to both the very poor solubility of triacetate (**13**) in organic solvents and the presence of active hydrogens in both substrates, all attempts to connect these components by a phosphate triester linkage were unsatisfactory using several different approaches. This prompted us to employ a more lipophilic alcohol and a more forcing method for phosphate ester formation. Thus the 8-benzyloxy derivative (**14**) of the chromophore was obtained *via* compounds (**8**) and (**12**) in a similar manner to compound (**13**), starting from 4-benzyloxy-2-chlorobenzaldehyde (**6**), in 24% overall yield. The combination of the chromophoric moiety (**14**) with the peptide moiety (**15**) was carried out according to the phosphite triester method.[13] The reaction of 2,2,2-trichloroethyl phosphorodichloridite with compounds (**14**) and (**15**) in the presence of 2,6-lutidine (2,6-dimethylpyridine) in tetrahydrofuran (THF) at −70 °C to −20 °C afforded the F_{420} precursor (**18**) as yellow fluorescent



F_{420} (**1**) R = H

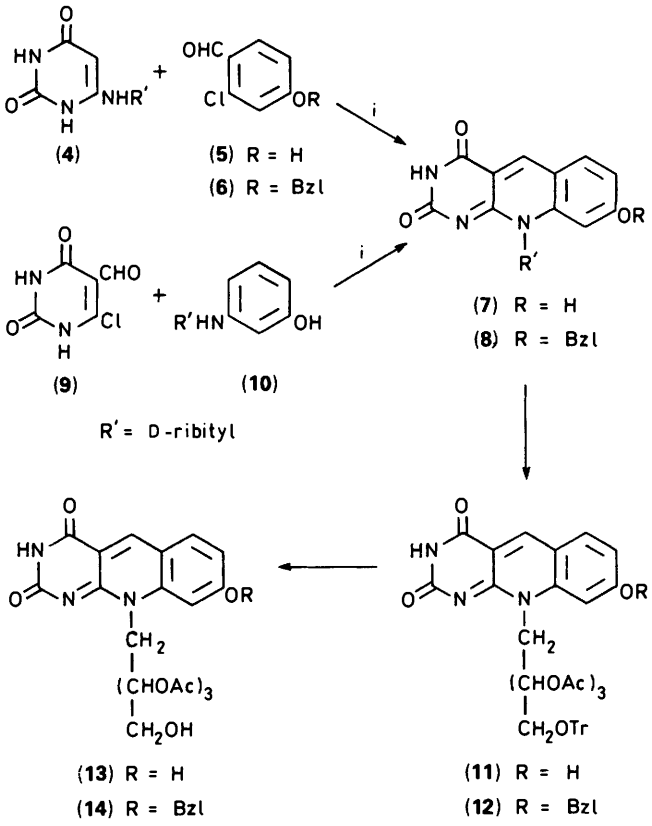


F_{390}-A (**2**)

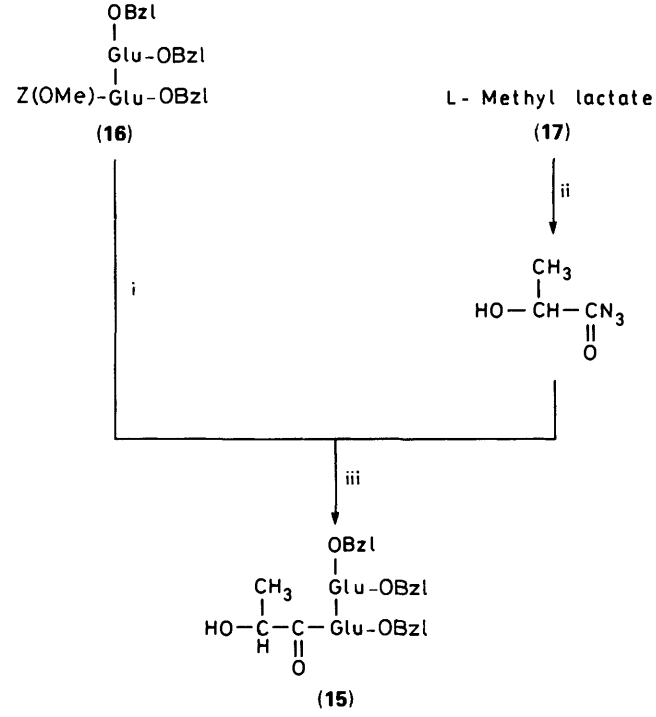


F_{390}-G (**3**)

Structures of compounds F_{420} and F_{390}



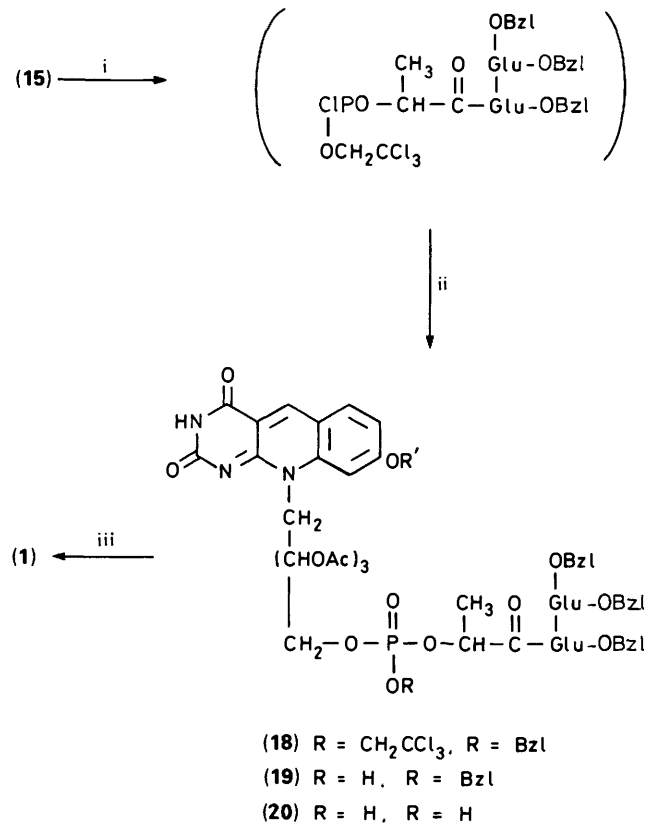
Scheme 1. Synthesis of the chromophoric moiety. *Reagents and conditions:* i, DMF, reflux.



Scheme 2. Synthesis of the peptide moiety. *Reagents and conditions:* i, TFA, anisole, 0 °C; ii, NH_2NH_2; then $[HNO_2]$; iii, Et_3N, DMF, 0 °C.

oil, after oxidative work-up with iodine and water, in 60% yield as an inseparable mixture of diastereoisomers at the phosphorus atom (Scheme 3).

The successive deprotection procedures as the last sequence



Scheme 3. Phosphite triester approach. *Reagents and conditions:* i, $CCl_3CH_2OPCl_2$, 2,6-lutidine; ii, (**14**), then I_2, water; iii, Zn/Cu, aq. NH_4Cl; H_2, 10% Pd–C; then NH_4OH

of the total synthesis were as follows. Treatment with a Zn/Cu couple for the 2,2,2-trichloroethyl group, catalytic hydrogenolysis on 10% palladized charcoal for the benzyl ester, the ether, and the chromophoric moiety, and final hydrolysis with aqueous ammonia for the acetate groups, gave a reaction mixture containing the product. In the above catalytic reduction, the chromophore afforded the corresponding 1,5-dihydro compound, which was readily reoxidized on exposure to air at ambient temperature.

The mixture was neutralized with acetic acid and purified according to the literature method.[1] Thus the solution was applied to a DEAE Sephadex A-25 column which was washed with water. The desired fraction was eluted with a linear gradient of ammonium hydrogen carbonate. The ammonium salt of F_{420} was applied to a Sephadex G-15 column for the preparation of a salt-free sample and the eluted fraction was lyophilized. The sample produced exhibited identical data and behaviour with an authentic sample on ^1H n.m.r. (D_2O) spectroscopy, secondary-ion mass spectroscopy, thin-layer electrophoresis, paper chromatography, and reverse-phase t.l.c. (Table). Thus the first total synthesis of coenzyme F_{420} has been completed and we believe this synthesis offers final confirmation for the structure of factor F_{420}.

Experimental

All materials not explicitly discussed were purchased from Wakenyaku Co., Nacalai tesque Co., and Aldrich chemical Co. ^1H NMR spectra were obtained with a JEOL JNM-FX-200 Fourier transform spectrometer. IR spectra were measured with a Shimdazu IR-400 spectrometer. M.p.s were taken using a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotation were recorded on a DIP 360

Table.

Sample	R_F values of paper chromatography[a]	Movement on thin-layer electrophoresis[b]	Secondary-ion mass spectra (MH^+)	$\delta_H(D_2O)$
F_{420} (authentic)	0.321	6.6 cm	774	8.90 (s, 1 H), 8.05 (d, *J* 8.1 Hz, 1 H), 7.40 (s, 1 H), 7.20 (d, *J* 8.0 Hz, 1 H), 3.80–4.70 (m, 10 H), 1.93—2.53 (m, 8 H), and 1.57 (d, *J* 7.0 Hz, 3 H)
(synthetic)	0.325	6.7 cm	774	8.85 (s, 1 H), 7.95 (d, *J* 8.3 Hz, 1 H), 7.37 (s, 1 H), 7.16 (d, *J* 8.0 Hz, 1 H), 3.80–4.70 (m, 10 H), 1.80–2.40 (m, 8 H), and 1.55 (d, *J* 7.0 Hz, 3 H)

[a] Developing solvent: Bu^{n}OH–pyridine–water (1:1:1). [b] Conditions: buffer AcOH–pyridine–water (16:8:976); thin layer (Merck cellulose 5577); 400 V; 2 h.

(Japan Spectroscopic Co.) polarimeter at the sodium D-line and ambient temperature. Secondary-ion mass spectra were obtained with a Hitachi MS-80 MS (8 kV; Xe was used to provide parimary beams of atoms).

4-Benzyloxy-2-chlorobenzaldehyde (**6**).—A mixture of 2-chloro-4-hydroxybenzaldehyde (**5**) (11.7 g, 74.8 mmol) and K_2CO_3 (10.0 g) in dry acetone (260 ml) was cooled and benzyl bromide (10.7 ml, 1.2 eq.) was added dropwise. Then the mixture was refluxed for 2 h under argon. The reaction mixture was concentrated to a small volume, the residue was extracted with chloroform, and the extract was evaporated to give the crude product. After purification by silica gel chromatography with chloroform, the *title product* was crystallized from $CHCl_3$–Et_2O, yield 80%, m.p. 68–69 °C; $\nu_{max}(CHCl_3)$ 1 700, 1 685, and 1 590 cm^{-1}; δ_H(200 MHz; $CDCl_3$) 10.30 (s, 1 H), 7.87 (d, *J* 8.3 Hz, 1 H), 7.40 (s, 5 H), 7.00 (d, *J* 2.44 Hz, 1 H), 6.94 (dd, *J* 8.3 Hz, 2.44 Hz, 1 H) and 5.11 (s, 2 H) (Found: C, 68.15; H, 4.35; Cl, 14.45. $C_{14}H_{11}ClO_2$ requires C, 68.15; H, 4.5; Cl, 14.4%).

8-Benzyloxy-7,8-didemethyl-5-deazariboflavin (**8**).—A solution of 6-D-ribitylaminouracil (**4**) (7.5 g, 28.7 mmol) and 4-benzyloxy-2-chlorobenzaldehyde (**6**) (7.78 g, 31.5 mmol) in dimethylformamide (DMF) (100 ml) was refluxed for 4 h. The reaction mixture was cooled slowly to room temperature and yellow precipitate was filtered off and washed with methanol. The yellow powder was recrystallized from DMF to give the title compound (6.85 g, 52.6%), m.p. 243—245 °C; ν_{max}(Nujol) 3 250, 1 590, 1 450, 1 200—1 250 cm^{-1}; δ_H[200 MHz; $(CD_3)_2SO$] 8.75 (s, 1 H), 8.05 (d, *J* 9 Hz, 1 H), 7.68 (s, 1 H), 7.25–7.60 (m, 5 H), 7.18 (d, *J* 9 Hz, 2 H), 5.25 (s, 2 H), and 4.2– 4.7 (m, 7 H) (Found: C, 57.2; H, 4.9; N, 8.4. $C_{23}H_{23}N_3O_7 \cdot H_2O$ requires C, 58.6; H, 5.35; H, 8.9%).

8-Benzyloxy-7,8-didemethyl-5-deazariboflavin 2′,3′,4′-Triacetate 5′-Trityl Ether (**12**).—The chromophore (**8**) (3.7 g, 15.5 mmol) was dissolved in cold pyridine (50 ml) and freshly prepared trityl chloride (8.6 g, 18.6 mmol) was added slowly to the solution. Then the solution was stirred for 2 h at room temperature and then refluxed for a further 3 h. After completion of the reaction (TLC), acetic anhydride (5 ml, 3.3 mol equiv.) was added dropwise to the cold reaction mixture without the isolation of the tritylated product. The yellow solution gradually changed to clear red and it was then stirred for 12 h. Excess of acetic anhydride and pyridine were removed under reduced pressure and the oily residue was extracted with chloroform and washed successively with 5% HCl and aq. NH_4Cl. The chloroform layer was dried with $MgSO_4$ and concentrated to a small volume. Chromatography of the residue on silica gel with $CHCl_3$–MeOH (10:1) afforded *compound* (**12**) (6.0 g, 47%), m.p. 207 °C; $\nu_{max}(CHCl_3)$ 1 735, 1 600, 1 675, and 1 200–1 250 cm^{-1}; δ_H(200 MHz; $CDCl_3$) 8.87 (s, 1 H), 8.13 (br s, 1 H), 7.78 (d, *J* 8.3 Hz, 1 H), 7.1—7.49 (m, 22 H), 5.60—5.64 (m, 3 H), 5.29–5.36 (m, 4 H), 4.10–4.50 (m, 2 H), 2.08 (br s, 3 H), and 2.01 (s, 6 H) (Found: C, 70.05; H, 5.2; N, 4.9. $C_{48}H_{43}N_3O_{10}$ requires C, 70.15; H, 5.25; N, 5.1%).

Detritylation of Trityl Ether (**12**) *to Triacetate* (**14**).—Compound (**12**) (6.0 g, 7.3 mmol) was dissolved in chloroform (30 ml) and the solution was cooled at 0 °C, then chloroform (30 ml) containing HCl gas was added. The solution was stirred at 0 °C for 45–60 min. The reaction mixture was neutralized with aq. $NaHCO_3$ and extracted with chloroform. The extract was dried with $MgSO_4$ and concentrated to a small volume. The residue was purified by silica gel column chromatography with chloroform and the triacetate (**14**) was crystallized from chloroform–ether (1:3) (3.1 g, 73.3%), m.p. 157 °C; $\nu_{max}(CHCl_3)$ 3 200–3 300, 1 735, 1 675, 1 610, and 1 200–1 250 cm^{-1}; δ_H(200 MHz; $CDCl_3$) 8.86 (s, 1 H), 8.51 (br s, 1 H), 7.84 (d, *J* 8.3 Hz, 1 H), 7.15–7.60 (m, 7 H), 5.21—5.37 (m, 5 H), 4.27–4.46 (m, 4 H), 2.08 (s, 6 H), and 2.01 (s, 3 H) (Found: C, 59.5; H, 5.0; N, 6.95. $C_{29}H_{29}N_3O_{10} \cdot 0.5H_2O$ requires C, 59.15; H, 5.1; N, 7.15%).

Removal of pMz Group from Protected Dipeptide (**16**).—The protected dipeptide (**16**) (14.2 g, 20 mmol) was slowly added to a cold solution of trifluoroacetic acid (TFA) (30 ml) and anisole (6 ml), and the mixture was stirred for 1 h. When the reaction was over, TFA and anisole were removed under reduced pressure. After dilution of the residue with a large amount of dry ether, fresh HCl gas was introduced to the ether solution. An oily product was formed soon. Ether was removed by decantation and the residue was washed with dry ether several times. The gum was dried over P_2O_5 *in vacuo* for 2 h. The dried product was dissolved in THF (30 ml). The solution was cooled and Et_3N (4.0 ml, 1.1 mol equiv.) was added dropwise. After 20 min the formed precipitate was filtered off and the filtrate was evaporated to small volume.

Preparation of L-*Lactic Acid Azide*.—L-Methyl lactate (**17**) (10.4 g, 0.1 ml) was added to a cold solution of hydrazine hydrate (200 ml). The solution was stirred for 2 h at 0 °C, and excess of the hydrazine was removed under reduced pressure. To the oily hydrazide thus obtained were added ether (30–50 ml), 1M-HCl (200 ml), and $NaNO_2$ (5.0 g). The reaction mixture was checked by potassium iodide–starch paper (Wakenyaku Co. Ltd.). The reaction mixture was extracted with ether, and the extract was dried with $MgSO_4$ and concentrated to a small volume.

L-Lactoyl-γ-L-glutamyl-L-glutamic Acid Tribenzyl Ester (**15**).—The above deprotected amine from compound (**16**) was dissolved in DMF (15 ml) containing Et_3N (0.1 ml); to this a DMF (34 ml) solution of L-lactic acid azide containing Et_3N (0.2 ml) was added and the mixture was stirred for 9 h at 0 °C.

DMF was removed under reduced pressure and ether was added to the residue. The white powder was filtered off and washed with ether several times to afford the title compound (**15**) (7.63 g, 61.7%); ν_{max}($CHCl_3$) 3 380, 1 730, 1 668, 1 500, 1 200–1 250, and 698 cm^{-1}; δ_H(200 MHz; $CDCl_3$) 7.30–7.46 (m, 15 H), 6.6 (br s, 1 H), 5.10 (s, 4 H), 5.15 (s, 2 H), 4.54–4.58 (m, 2 H), 4.20 (q, *J* 6.8 Hz, 1 H), 3.20–3.30 (br s, 1 H), 1.97–2.40 (m, 8 H), and 1.37–1.41 (d, *J* 6.8 Hz, 3 H); $[\alpha]_D^{20}$ −23.39 (*c* 2.112, MeOH) (Found: C, 66.0; H, 6.3; N, 4.65. $C_{34}H_{38}N_2O_9$ requires C, 66.0; H, 6.2; N, 4.55%).

Phosphorylation to the Protected F_{420} (**18**).—To a solution of 2,6-lutidine (0.42 ml, 3.6 mmol) and 2,2,2-trichloroethyl phosphorodichloridite (225.5 mg, 0.9 mmol) in THF (3 ml) at −78 °C was added dropwise a THF solution (1 ml) of the lactate (**15**) (618 mg, 1 mmol), then a THF solution (3 ml) of the triacetate (**14**) (290 mg, 0.5 mmol) was added slowly. Subsequently, the 'dry ice'–acetone bath was removed and the reaction temperature was raised to −20 °C in 2–3 min. A water–THF (1:1) solution of I_2 (228 mg) and 2,6-lutidine (0.1 ml) was added to the reaction mixture, and then aq. $Na_2S_2O_3$ was added. The reaction mixture was extracted with chloroform, and the extract was washed successively with 5% HCl and aq. $NaHCO_3$ and was dried with $MgSO_4$. Chloroform was removed under reduced pressure to give a small volume of residue, and the resulting gum was purified by preparative t.l.c. (n-hexane–AcOEt 1:3) to give oily compound (**18**) (325 mg; 60.0%); ν_{max}($CHCl_3$) 1 740, 1 670, 1 600, and 1 200–1 250 cm^{-1}; δ_H(200 MHz; $CDCl_3$) *8.79 and *8.80 (s 1 H), 7.83 (d, 1 H, *J* 9.5 Hz), 7.2–7.5 (m, 22 H), 4.5–5.7 (m, 7 H), *2.07, *2.11, *2.16, and *2.17 (each s, total 9 H), 1.8–2.5 (m, 8 H), and *1.65 and *1.68 (each d, *J* 7.0 Hz, 3 H). Asterisks indicate separate signals of diastereoisomers; secondary ion m.s.; (MH^+) (*m/z* 1 392), 1 391 (M^+).

Successive Deprotections of the Protected F_{420} (**18**).—Compound (**18**) (82 mg, 0.06 mmol) was dissolved in EtOH (10 ml), and water (10 ml) and excess of NH_4Cl were added to the solution. Activated Zn/Cu complex (80 mg) was added and the mixture was stirred at 55 °C for 2 h, monitoring by t.l.c. (AcOEt–MeOH 5:1). The solution was cooled, diluted with water, and 5% HCl was added for acidification. The reaction mixture was extracted with chloroform, and the extract was washed successively with aq. $NaHCO_3$ and aq. NH_4Cl, and concentrated to a small volume to give the diester (**19**). The next hydrogenolysis was performed without purification at this stage.

A mixture of compound (**19**) (170 mg) and 10%-palladized charcoal (100 mg) in methanol (30 ml) was hydrogenated at room temperature under atmospheric pressure. Hydrogen absorption ceased after the uptake of 5 equivalents of hydrogen. The mixture was filtered, and the filtrate was evaporated under reduced pressure and the yellow fluorescent oil (**20**) was then used in the next hydrolysis step.

The oil (**20**) (100 mg) was dissolved in methanol (10 ml; oxygen free), and then cold 28% aq. NH_4OH (10 ml) was slowly added to the solution. The solution was stirred for 24–36 h in the dark to give a reaction mixture including F_{420} (**1**).

Purification of Factor F_{420} (**1**).—The above reaction mixture was neutralized with acetic acid, diluted with water, and applied to a DEAE Sephadex A-25 column (20 × 1.5 cm; HCO_3^--form). The desired fraction was eluted with a linear gradient of 0–1M-ammonium hydrogen carbonate (500 ml). The resulting solution, including the ammonium salt of F_{420} (**1**), was applied to a Sephadex G-15 column (100 × 2 cm), and the eluted fluorescent fraction was lyophilized (22 mg, 25.6%). Analytical and spectroscopic data of compound F_{420} (**1**) are shown in the Table and are compared with those of an authentic sample.

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