Synthesis of a Proposed Isomer of F_{420} having α-Glutamyl Bonding.

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A proposed isomer of redox coenzyme F_{420}, having α-glutamyl bonding, has been synthesized from 8-benzyloxy-10-D-ribityl-5-deazaflavin and α-L-glutamyl-L-glutamic acid moiety, by the phosphite triester approach followed by deprotection procedures.

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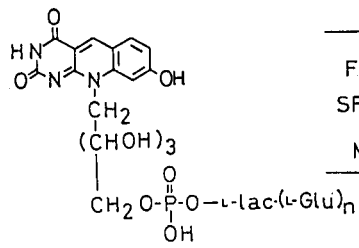
Several investigations about the functionalities of redox coenzyme F_{420} and the related compounds have been done for these few years in the field of biochemistry, organic chemistry, and photochemistry [1-5]. The coenzyme F_{420} isolated from anaerobic methane-producing bacteria plays important roles in the C-1 cycle involving eight-electrons reduction of CO_2 to CH_4 as a low potential electron carrier [6]. It is proposed that not only F_{420} but also closely related coenzymes SF_{420} (from *S. griceus*) [7] and M-1 (from *M. avium*) [8] can be substrates for the NADP-linked hydrogenase. Particularly SF_{420} is known to be the mixture of at least three molecules, which have the same 5-deazaflavin skeleton and different types of α-linked or γ-linked peptide components containing 2 to 4 L-glutamic acids (Figure 1). In the meantime, we have achieved the first total synthesis of F_{420} and finished the final identification with the neutral product [9].



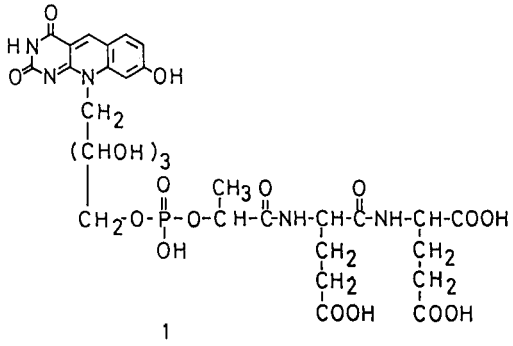
	n	Peptide bond
F420	2	γ
SF420	2~4	α or γ
M-I	1	—

Figure 1

In the present paper we describe the synthesis of a proposed isomer of F_{420}, having α-L-glutamyl bonding in its peptide sequences. The target for the synthesis this time was the F_{420} isomer **1** different from F_{420} only in α-bonding at the L-glutamyl-L-glutamic acid moiety, which was prepared by the same phosphite triester approach as reported already [9].

First we have developed the alternative convenient method to construct an 8-substituted 5-deazaflavin skeleton starting from β-resorcylaldehyde. Dibenzyl ether, dimethyl ether, and diacetate of β-resorcylaldehyde **3a-3c**

Structure of F_{420} Isomer



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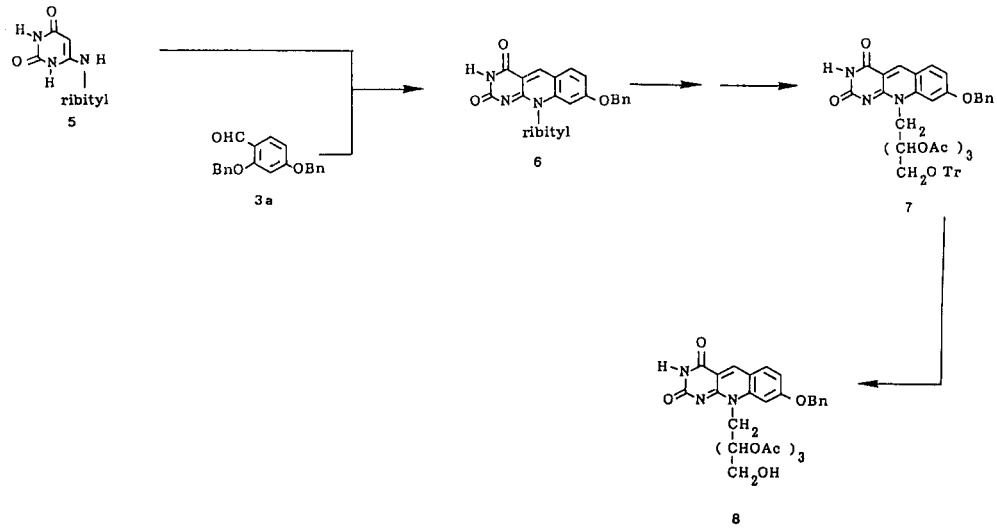
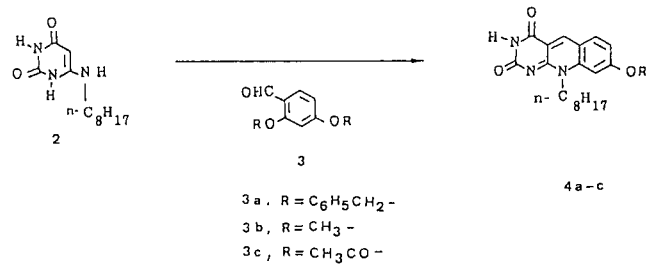


5 · 3a · 6 · 7 · 8

reacted smoothly with 6-*n*-octylaminouracil (**2**) to give 8-benzyloxy-, 8-methoxy-, and 8-acetoxy-5-deazaflavin **4a-4c** respectively. On the basis of these results, dibenzyl ether of β-resorcylaldehyde (**3a**) was heated with 6-D-ribityl-aminouracil (5) [10] in DMF to give 8-benzyl-oxy-10-D-ribityl-5-deazaflavin (6) in high yield (Figure 2).

Next, commercially available γ-benzyl-L-glutamate (9) was led to *N*-protected glutamic acid monobenzyl ester using *p*Mz group for amino function. This compound was condensed with L-glutamic acid dibenzyl ester using DCC as a condensing agent to give the corresponding dipeptide derivative **10** [11], which was condensed with L-methyl lac-



New Synthetic Pathway for Chromophoric Moiety

Figure 2

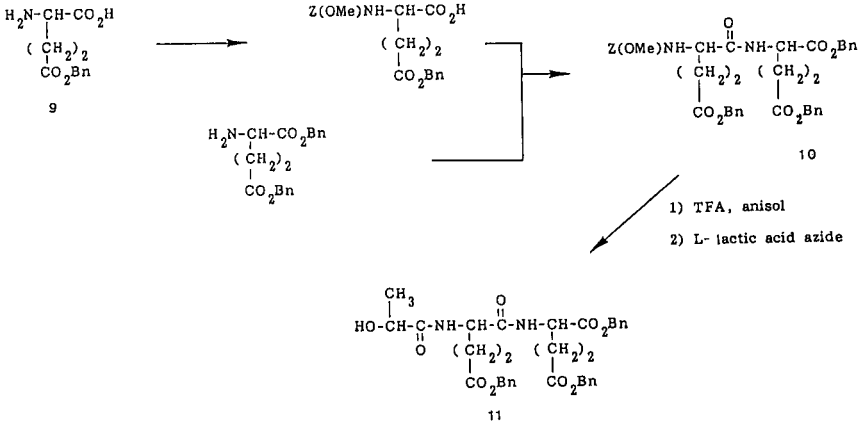


Fig. 3a Synthesis of the Peptide Moiety

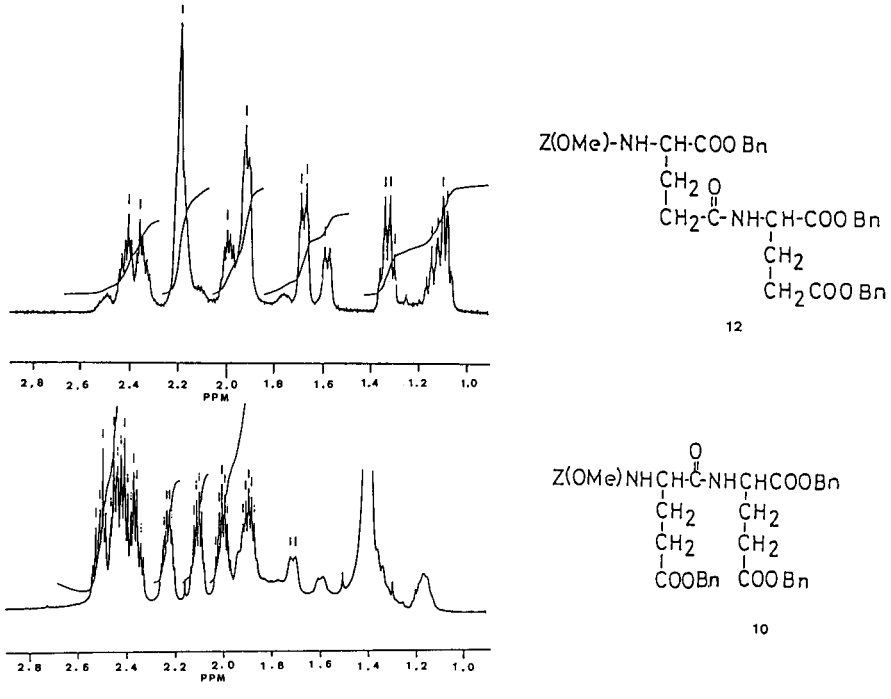


Fig. 3b 600 MHz NMR Spectral Difference between α-Dipeptide and γ-Dipeptide

Table 1

No.	R, R^1	Yield %	mp	MS m/z	IR ($CHCl_3$) cm^{-1}	NMR (deuteriochloroform) ppm	Formula	Analysis (%) Calcd./Found C	H	N
4a	$C_6H_5CH_2$–	61.1	>300	431	3240, 1590, 1450, 1250	8.81 (s, 1H), 8.70 (s, 1H), 7.82 (d, 1H, J = 8.8 Hz), 7.44 (m, 5H), 7.17 (dd, 1H, J = 8.8 Hz, J = 2.2 Hz), 7.03 (d, 1H, J = 2.2 Hz), 5.31 (s, 2H), 4.60 (s, 2H), 1.73 (m, 2H), 1.29 (m, 10H), 0.88 (m, 3H)	$C_{26}H_{29}N_3 \cdot H_2O$	69.48 69.32	6.90 6.55	9.35 9.43
4b	CH_3–	89.7	>300	355	3240, 1591, 1450, 1110	8.82 (s, 1H), 8.25 (s, 1H), 7.84 (d, 1H, J = 9.0 Hz), 7.11 (dd, 1H, J = 9.0 Hz, J = 2.2 Hz), 7.01 (d, 1H, J = 2.20 Hz), 4.69 (m, 2H), 4.03 (s, 3H), 1.61 (m, 8H), 1.28 (m, 4H), 0.88 (m, 3H)	$C_{20}H_{25}N_3O_3 \cdot 1/2H_2O$	65.93 66.04	7.14 6.97	11.54 11.58
4c	CH_3CO–	61.4	>300	383	3250, 1720, 1590, 1450	8.92 (s, 1H), 8.25 (s, 1H), 7.92 (d, 1H, J = 9.0 Hz), 7.22 (dd, 1H, J = 9.0 Hz, J = 2.2 Hz), 7.10 (d, 1H, J = 2.2 Hz), 4.65 (m, 2H), 2.38 (s, 3H), 1.60 (m, 8H), 1.30 (m, 4H), 0.94 (m, 3H)	$C_{21}H_{25}N_3O_4$	65.80 [a]	6.53	10.97

[a] Not analyzed because of the moisture absorption.

Table 2

Compound	Paper chromatography (Rf) [a]	Reverse phase tlc (Rf) [b]	Thin layer electrophoresis (cm) [c]
F_{420} authentic sample	0.32	0.59	9.2
F_{420} isomer (1)	0.30	0.55	8.9

[a] Developing solvent: *n*-BuOH:Py:H_2O = 1:1:1. [b] Developing solvent: CH_3CN:H_2O = 1:1. [c] Conditions: ; Buffer, AcOH:Py:H_2O = 16:8:976, (Merck cellulose 5577) 400V, 3 hours.

tate in the azide method to give the desired peptide fragment **11** (Figure 3a). As seen from the nmr spectra, there was a remarkable difference between α-L-glutamyl-L-glutamic acid **10** and γ-L-glutamyl-L-glutamic acid derivatives **12**, especially in their signal patterns of the methylene side chains (Figure 3b). This would be ascribed to the steric and electronic effects. Both fragments **8** and **11** were combined using 2,2,2-trichloroethyl phosphorodichloridite according to Letsinger's procedure [12] to give the phosphotriester **13** as a mixture of diastereomers at the phosphorous atom. The successive deprotection procedures which consist of treatment with a Zn/Cu complex for the 2,2,2-trichloroethyl group, catalytic hydrogenolysis on 10% palladium carbon for benzyl ester, ether and the chromophoric moiety, and final hydrolysis with aqueous ammonia for the acetate groups, gave the desired F_{420} isomer.

Fig. 4 Phosphorylation

The nmr spectral difference between F_{420} and the isomer diminished after the last successive deprotection. However, thin layer chromatographic behaviors showed different patterns compared with those of F_{420} (Table 2). The study whether this isomer is included in the natural SF_{420} or not is in progress.

EXPERIMENTAL

All materials not explicitly discussed were purchased from Wakenyaku Co., Nacalai Co. and Aldrich chemical Co. Proton NMR spectra were obtained with a JEOL-FX-200 and a Brucker-AM-600 Fourier Transform Spectrometer. Melting points were taken using a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotation were recorded on DIP 360 type (Japan Spectroscopic Co.) polarimeter at the sodium D-line and ambient temperature. Mass spectra were obtained with JEOL JMS 01SG-2.

Synthesis of 6-*n*-Octylaminouracil (**2**).

6-Chlorouracil (5.0 g, 34.1 mmoles) and *n*-octylamine (11.3 ml, 68.5 mmoles) in *n*-butanol (40 ml) were refluxed in argon atmosphere for 18 hours. *n*-Butanol was removed *in vacuo* and resulted crude precipitates were recrystallized from hot ethanol to give a desired (**2**) (4.77 g) in 59% yield, mp >300°; ms: m/z 239; ^{1}H-nmr (DMSO-d_6); 200 MHz δ 5.40 (s, 1H), 4.6 (m, 2H), 1.55 (m, 2H), 1.23 (m, 10H), 0.82 (m, 3H).

Anal. Calcd. for $C_{12}H_{20}N_3O_2 \cdot CH_3OH$: C, 57.56; H, 8.86; N, 15.50. Found: C, 57.68; H, 9.01; N, 15.87.

2,4-Dibenzyloxybenzaldehyde (**3a**).

A mixture of β-resorcylaldehyde (2,4-dihydroxybenzaldehyde) (14.0 g, 10.0 mmoles) and potassium carbonate (25.0 g) in dry acetone (200 ml) was cooled and benzyl chloride (27.8 ml, 1.2 equivalents) was added. The mixture was refluxed over night under an argon atmosphere. After the precipitate was filtered off, the solution was concentrated to a small volume, the residue was extracted with chloroform, and the chloroform extracts were evaporated to give the crude product. After the purification by silica gel column chromatography firstly with *n*-hexane, and then with chloroform, the product was crystallized from chloroform-ether (25.9 g, 81%), mp 87-88°; ir (chloroform): 2860, 1680, 1600, 1260, 1170, 1110 cm^{-1}; ^{1}H-nmr (deuteriochloroform): 200 MHz δ 10.35 (s, 1H), 7.80 (d, 1H, J = 8.0 Hz), 7.38 (m, 12H), 6.55 (s, 1H), 5.05 (s, 2H), 5.02 (s, 2H).

Anal. Calcd. for $C_{21}H_{18}O_3$: C, 79.22; H, 5.70. Found: C, 78.95; H, 5.51.

Synthesis of 2,4-Dimethoxybenzaldehyde (**3b**).

A mixture of β-resorcylaldehyde (1.4 g, 10.0 mmoles) and potassium carbonate (1.4 g) and methyl iodide (1.87 ml, 30.0 mmoles) in dry acetone (30 ml) was refluxed over night under an argon atmosphere. After potassium salts and other precipitates were filtered off, the solution was concentrated to a small volume, the desired product was crystallized from *n*-hexane (1.67 g, 99%), mp 68°; ir (chloroform): 2860, 1680, 1600, 1110 cm^{-1}; ^{1}H-nmr (deuteriochloroform): 200 MHz δ 10.29 (s, 1H), 7.81 (d, 1H, J = 8.8 Hz), 6.56 (dd, 1H, J = 8.8 Hz, J = 2.2 Hz), 6.45 (d, 1H, J = 2.2 Hz), 3.91 (s, 3H), 3.88 (s, 3H).

Anal. Calcd. for $C_9H_{10}O_3$: C, 65.05; H, 6.07. Found: C, 64.93; H, 6.10.

Synthesis of 2,4-Diacetoxybenzaldehyde (**3c**).

To the cold dry pyridine (8.0 ml), β-resorcylaldehyde (2.7 g, 20.0 mmoles) and acetic anhydride (8.0 ml) were added and stirred for 10 hours at room temperature. Pyridine and excess acetic anhydride were evaporated *in vacuo* and the product was crystallized from *n*-hexane (2.63 g, 60%), mp 65°; ir (chloroform): 2860, 1730, 1680, 1250 cm^{-1}. ^{1}H-nmr (deuteriochloroform): 200 MHz δ 10.87 (s, 1H), 7.90 (d, 1H, J = 8.4 Hz), 7.17 (dd, 1H, J = 8.4 Hz, J = 2.2 Hz), 7.05 (d, 1H, J = 2.2 Hz), 2.39 (s, 3H), 2.37 (s, 3H).

Anal. Calcd. for $C_{11}H_{10}O_5$: C, 59.46; H, 4.54. Found: C, 59.22; H, 4.40.

Synthesis of 8-*O*-Substituted-10-*n*-octyl-5-deazaflavins **4a-4c** General Procedure.

6-*n*-Octylaminouracil (**2**) (1.83 g, 7.7 mmoles) and 2,4-*O*-disubstituted benzaldehyde **3a-3c** (1.6-2 equivalents) were refluxed in DMF for 4-6 hours. The reaction mixture was removed from oil bath and the precipitate formed was filtered off and washed with ether several times. The crude products were recrystallized from chloroform-ether. (See the spectral data for each products in Table 1).

Synthesis of 8-Benzyloxy-10-D-ribityl-5-deazaflavin (**6**).

6-D-Ribitylaminouracil (**5**) (13.0 g, 50 mmoles) and **3a** (12.76 g, 40.0 mmoles) in DMF solution were refluxed for 4 hours. The reaction mixture was cooled slowly to room temperature and yellow precipitate was filtered off and washed with methanol. Yellow powder was recrystallized from DMF, yield 80%, mp 243-245°; ir (Nujol): 3250, 1590, 1450, 1260 cm^{-1}; ^{1}H -nmr (DMSO-d_6): 200 MHz δ 4.2-4.7 (m, 7H), 5.25 (s, 2H), 7.18 (d, 1H, J = 9.0 Hz), 7.25-7.60 (m, 5H), 7.68 (s, 1H), 8.05 (d, 1H, J = 9.0 Hz), 8.75 (s, 1H).

Anal. Calcd. for $C_{23}H_{23}N_3O_7 \cdot H_2O$: C, 58.58; H, 5.35; N, 8.91. Found: C, 58.20; H, 5.10; N, 8.75.

Synthesis of the 2′, 3′, 4′-Triacetyl-5′-trityl-5-deazaflavin (**7**).

The chromophore **6** (3.75 g, 15.5 mmoles) was dissolved in cold pyridine (100 ml) and freshly prepared trityl chloride (8.6 g, 18.6 mmoles) was added slowly to the solution. Then the solution was stirred for 2 hours at room temperature and further refluxed for 3 hours. After the completion of the reaction (which was checked by tlc), acetic anhydride (5.0 ml, 3.3 equivalents) was added dropwise to the cold reaction mixture. Excess acetic anhydride and pyridine were removed *in vacuo* and oily residue was extracted with chloroform. The chloroform extracts were washed with 5% hydrochloric acid and aqueous ammonium chloride, dried with magnesium sulfate, and concentrated to a small volume. Chromatography on silica gel with chloroform-methanol (10:1) afforded 6.16 g (50%) of the 5′-trityl ether, mp 207°; ir (chloroform): 1735, 1675, 1600, 1250 cm^{-1}; ^{1}H-nmr (deuteriochloroform): 200 MHz δ 8.87 (s, 1H), 8.13 (s, 1H), 7.78 (d, 1H, J = 8.3 Hz), 7.1-7.49 (m, 17H), 5.60-5.64 (m, 3H), 5.29-5.36 (m, 4H), 3.10-3.50 (m, 2H), 2.35 (s, 3H), 1.65 (s, 3H).

Anal. Calcd. for $C_{48}H_{43}N_3O_{10}$: C, 70.15; H, 5.25; N, 5.1. Found: C, 70.05; H, 5.2; N, 4.9.

Detritylation of Trityl Ether to Triacetate (**8**).

The above trityl ether **7** (6.0 g, 7.3 mmoles) was dissolved in chloroform (30 ml) and the solution was cooled at 0°, then chloroform (30 ml) containing hydrogen chloride gas was added. The solution was stirred at 0°C for 45-60 minutes. The reaction mixture was neutralized with aqueous sodium bicarbonate and extracted with chloroform. The chloroform layer was dried with magnesium sulfate and concentrated to a small voulme. The residue was purified by silica gel column chromatography with chloroform and the triacetate **8** was crystallized from chloroform-ether (1:3), yield 3.1 g (73%), mp 157°; ir (chloroform): 3200-3300, 1735, 1675, 1610, 1250 cm^{-1}; ^{1}H-nmr (deuteriochloroform): 200 MHz δ 8.86 (s, 1H), 8.51 (s, 1H), 7.84 (d, 1H, J = 8.3 Hz), 7.15-7.60 (m, 7H), 5.21-5.37 (m, 5H), 4.27-4.46 (m, 4H), 2.08 (s, 6H), 2.01 (s, 3H); $[\alpha]_D^{2^\circ}$ + 47.11 (c = 0.6134, chloroform).

Anal. Calcd. for $C_{29}H_{29}N_3O_{10} \cdot 1/2H_2O$: C, 59.18; H, 5.14; N, 7.14. Found: C, 59.48; H, 5.00; N, 6.95.

Removal of *p*Mz Group from Dipeptide **10**.

In the cold solution of TFA (20 ml) and anisol (4 ml) the dipeptide **10** [11] (5.4 g, 7.6 mmoles) was slowly added, and the solution was stirred for an hour. TFA and anisol were removed under reduced pressure. After adding a large amount of dry ether, freshly prepared hydrogen chloride gas was introduced to the ether solution. Ether was removed by decantation and the oily product formed was washed with dry ether several times. This oil was dried over phosphorus pentoxide *in vacuo* for 2 hours. THF (15 ml) solution of the dried product was cooled and triethylamine (2.0 ml, 1.2 equivalents) was added dropwise. The formed precipitate was filtered off and the filtrate was concentrated to a small volume to use in the next step.

Preparation of L-Lactic Acid Azide.

L-Methyl lactate (6.4 g, 60 mmoles) was added to the cold solu-

tion of hydrazine hydrate (100 ml). The solution was stirred for 2 hours at 0° and excess hydrazine was removed *in vacuo*. To the oily hydrazide thus obtained, ether (30 ml) *N* hydrochloric acid (100 ml) and sodium nitrite (4.0 g) were added. The reaction mixture, checked by potassium iodide starch paper (Wakenyaku Co. Ltd), was extracted with ether, the ether layer was dried with magnesium sulfate and concentrated to a small volume.

L-Lactyl-α-L-glutamyl-L-glutamic Acid Tribenzyl Ester (**11**).

Deprotected amine described above, which was dissolved in DMF (15 ml) containing triethylamine (0.1 ml) and the DMF solution of L-lactic acid azide containing triethylamine (0.2 ml) were mixed at 0° for 9 hours. DMF was removed under reduced pressure and ether was added to give the product as a white powder. It was filtered off and washed with ether several times (2.8 g, 4.53 mmoles, 73%), mp 89°; $[\alpha]_D^{20}$ −20.48 (methanol, c = 4.49%); ir (chloroform): 3380, 1730, 1668, 1500, 1250, 698 cm^{-1}; ^{1}H-nmr (deuteriochloroform): 200 MHz δ 8.0 (m, 1H), 7.6 (m, 1H), 7.31 (m, 15H), 7.1 (m, 1H), 5.14 (s, 2H), 5.10 (s, 2H), 5.07 (s, 2H), 4.75 (m, 1H), 4.2 (m, 2H), 1.9-2.5 (m, 8H), 1.37 (d, 3H, J = 6.8 Hz).

Anal. Calcd. for $C_{34}H_{38}N_2O_9$: C, 66.00; H, 6.19; N, 4.53. Found: C, 65.75; H, 6.30; N, 4.75.

Phosphorylation to the Protected F_{420} Isomer **13**.

To -78° solution of 2,6-lutidine (0.017 ml, 1.44 mmoles) and 2,2,2-trichloroethyl phosphorodichloridite (0.053 ml, 0.36 mmole) in THF (0.5 ml), THF solution of tripeptide **11** (247 mg, 0.4 mmoles) was added dropwise, then THF solution (1.0 ml) of the chromophoric triacetate **8** (202 mg, 0.35 mmole) was added slowly.

Subsequently dry ice acetone bath was removed to raise the reaction temperature to −20° in 2-3 minutes. The water-THF (1:1) solution (5.0 ml) of I_2 (100 mg) and 2,6-lutidine (0.1ml) was added to the reaction mixture, and then the aqueous $Na_2S_2O_3$ solution was added for excess iodine. The reaction mixture was extracted with chloroform, the chloroform layer was washed with 5% hydrochloric acid and aqueous sodium bicarbonate and was dried with magnesium sulfate. Chloroform was removed to a small volume, and the residue was purified by preparative tlc (*n*-hexane: ethyl acetate = 1:3) to give **13**. Yield 50 mg (20%); ir (chloroform): 1740, 1670, 1660, 1260 cm^{-1}; ^{1}H-nmr (deuteriochloroform): 200 MHz δ 8.80* (s, 1H), 8.77* (s, 1H), 7.2-7.5 (m, 22H), 4.5-5.5 (m, 7H), 1.65* (d, 3H, J = 7.0 Hz), 1.68* (d, 3H, J = 7.0 Hz), 1.6-2.5 (m, 8H). The marked asteric means the separated signals of diastereomers.

Anal. Calcd. for $C_{65}H_{67}N_5O_{21}PCl_3$: C, 56.10; H, 4.85; N, 5.03; P, 2.23. Found: C, 56.02; H, 4.85; N, 5.05; P, 1.95.

Successive Deprotections of the Protected F_{420} Isomer **13**.

Compound **13** (200 mg, 0.15 mmoles) was dissolved in ethanol (20 ml) and 50 mg of ammonium chloride was added to the solution. Activated Zn/Cu complex (200 mg) was added and the mixture was stirred at 55° for 2 hours. The solution was cooled down and acidified with 5% hydrochloric acid solution. The chloroform layer washed with aqueous sodium bicarbonate and with aqueous ammonium chloride was concentrated to a small volume to give the desired phosphodiester (170 mg). The phosphodiester (170 mg) and 10%-palladized charcoal (100 mg) in methanol (30 ml) were hydrogenated at room temperature in atmospheric pressure. The mixture was filtered off and the filtrate was evaporated *in vacuo* and the yellow fluorescent oil was then hydrolyzed without purification. The above oil (100 mg) was dissolved in methanol (10 ml, oxygen free), and the cold concentrated ammonium hydroxide (10 ml) was slowly added to the solution. Then the solution was stirred for 24-36 hours in the dark under an argon atmosphere to give the crude mixture including the desired product **1**.

Purification of the F_{420} Isomer **1**.

The mixture was neutralized with acetic acid and was diluted with water, and the yielding solution including ammonium salt was applied to a Sephadex G-15 column (100 x 2 cm), and the eluted fluorescent fraction was lyophilized; ^{1}H-nmr (deuterium oxide): 200 MHz δ 8.9 (s, 1H), 8.0 (d, 1H, J = 8.2 Hz), 7.35 (s, 1H), 7.18 (d, 1H, J = 8.2 Hz), 3.8-4.7 (m, 10H), 1.8-2.5 (m, 8H), 1.5 (d, 3H, J = 7.0 Hz). Other data are indicated in Table 2.

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