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A facile synthesis of cyclic bis(3′→5′)diguanylic acid

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Abstract—This paper describes a new method for synthesizing biologically important cyclic bis(3′→5′)diguanylic acid (cGpGp) in a higher yield than that of the existing synthetic method. In the new synthesis, the following two means, in place of those used in the existing synthesis are employed as main strategies to cause the increase in product yield. One of these distinctive strategies in the new synthesis is that the phosphoramidite method is used for the preparation of a key synthetic intermediate of a linear guanylyl(3′→5′)guanylic acid derivative. This method allowed higher-yield formation of the intermediate than that by the triester method used in the existing synthesis. The second distinctive strategy used in the new synthesis is that allyloxycarbonyl and allyl groups are used for the protection of two guanine bases and two internucleotide bonds, respectively. These four allylic protectors can be removed all at once by the organopalladium-catalyzed reaction under neutral conditions. Thus, deprotection of the protected cGpGp precursor was achieved in the present synthesis in a shorter step and under milder conditions than the deprotection achieved in the existing synthesis, which uses diphenylacetyl and *o*-chlorophenyl groups as protectors for two guanine bases and two internucleotide bonds, respectively, whose full removal requires two different procedures including rather harsh basic treatment. As a result, technical loss and decomposition of the target product in the new synthesis is remarkably reduced.

1. Introduction

Cyclic bis(3′→5′)diguanylic acid (cGpGp) (**5**) is a naturally-occurring substance with biologically important properties such as (i) regulation of cellulose synthesis in the bacterium Acetobacter xylinum[1,2] and (ii) elevation of an expression of the CD4 receptor and cell cycle arrest in Jurkat cells.[3] Accordingly, studies aimed at the effective use of cGpGp are actively being carried out in various research fields such as biology, pharmacy, and clinical research. In order to perform these studies, a sufficient supply of cGpGp and its artificial analogs is crucial. Therefore, development of chemical methods capable of synthesizing both cGpGp and its artificial analogs is strongly demanded. Thus far, van Boom and his co-workers has reported one example of such a chemical synthesis.[1,2] However, this synthesis seems to have some problems with regard to the product yield. For example, the reported yield of a linear guanylyl(3′→5′)-guanylic acid derivative (an important key intermediate), which is prepared by means of the triester method, is not satisfactorily high. Further, there is some apprehension that removal of the diphenylacetyl protectors from guanine bases, which is achieved by heating (50°C) with conc. aqueous ammonia, causes decomposition of the target compound and consequently decreases the product yield.[4] This paper reports a new method for the synthesis of cGpGp, by which these drawbacks in the existing synthesis are resolved.[5]

2. Results and discussion

Scheme 1 outlines the synthesis of cGpGp achieved in our laboratory. First, the building block **2** was prepared in a 95% overall yield through (1) the imidazolium perchlorate (IMP)-promoted reaction[6] of the nucleoside phosphoramidite **1**[7] and 2-cyanoethanol in acetonitrile in the presence of molecular sieves (MS) 3A (30 min),[8] (2) oxidation using a 2-butanone peroxide (BPO)/toluene solution (5 min),[9] and (3) detritylation with dichloroacetic acid in dichloromethane (5 min). Subsequently, the two building blocks **1** and **2** (using 1 equiv. each) were condensed by the assistance of imidazolium perchlorate in acetonitrile with MS 3A, and the resulting product was subjected to oxidation with 2-butanone peroxide followed by detritylation with dichloroacetic acid to provide the guanylyl-(3′→5′)guanosine 3′-phosphate derivative **3** in a 94% isolated yield. The structure of **3** was confirmed by the $^{1}H-^{1}H$ COSY NMR spectrum, the $^{31}P-^{1}H$ COSY NMR spectrum, and the MALDI-TOF high resolution mass spectrum.

The compound **3** was exposed to a conc. aqueous NH_3/

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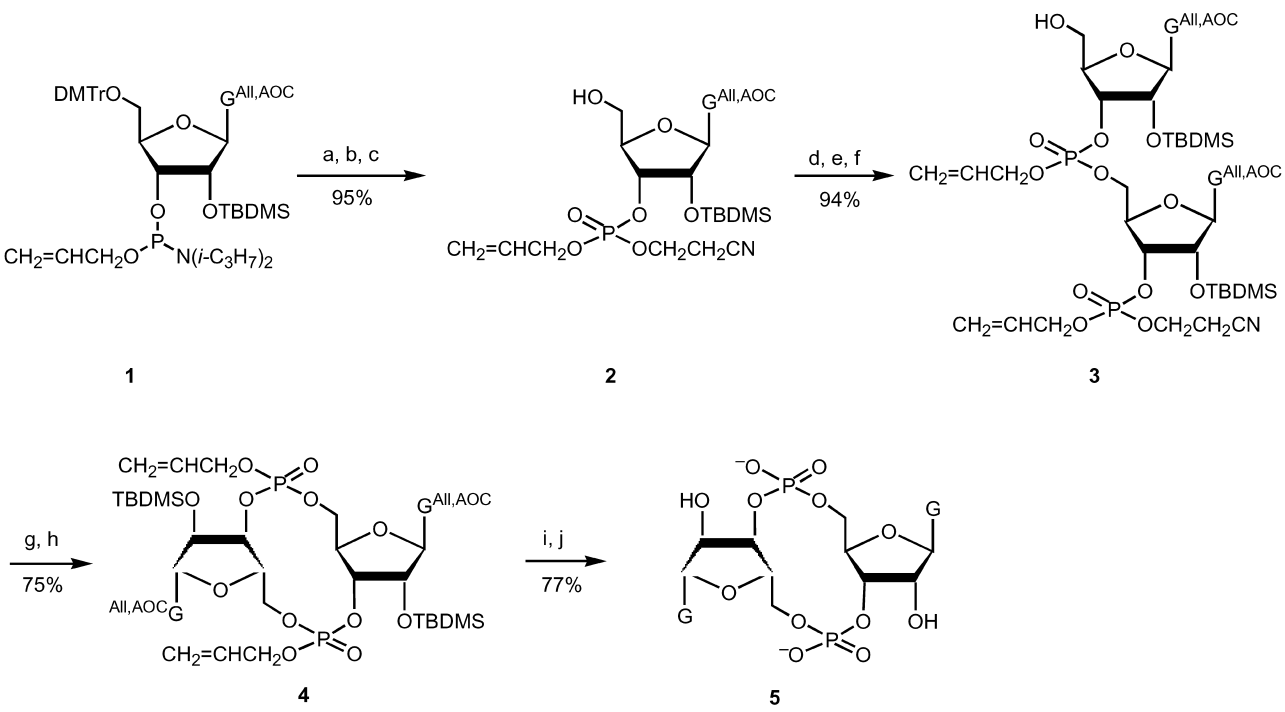

$G^{All,AOC}$ = N^2-(allyloxycarbonyl)-O^6-(allyl)guanyl

Scheme 1. Synthesis of cGpGp: (a) $HOCH_2CH_2CN$, IMP, MS 3A, CH_3CN, 30 min; (b) a 6.7% BPO/toluene solution, 5 min; (c) $Cl_2CHCOOH$, CH_2Cl_2, 0°C, 5 min; (d) the phosphoramidite **1**, IMP, MS 3A, CH_3CN, 30 min; (e) a 6.7% BPO/toluene solution, 5 min; (f) $Cl_2CHCOOH$, CH_2Cl_2, 0°C, 5 min; (g) conc. aq. NH_3–CH_3OH (1:10 v/v), 60 min; (h) TPSCl, *N*-methylimidazole, THF, 12 h; (i) $Pd_2(dba)_3{\cdot}CHCl_3$, $P(C_6H_5)_3$, n-$C_4H_9NH_3^+HCOO^-$, THF, 10 min; (j) $(C_2H_5)_3N{\cdot}3HF$, 12 h.

methanol (1:10 v/v) solution (60 min) to remove the cyanoethyl group, and the resulting product was converted to the cyclic compound **4** by treatment with a mixture of triisopropylbenzenesulfonyl chloride (TPSCl) and *N*-methylimidazole[10] in a 0.05 M THF solution (20 h). According to chromatographic analysis, this cyclization afforded two major products, **4a** and **4b**. These two products were isolated by chromatography and subjected to structure determination by spectral analyses including the ^1H–^1H COSY NMR spectrum, the ^{31}P–^1H COSY NMR spectrum, and the MALDI-TOF high resolution mass spectrum. Figure 1 shows ^{31}P–^1H COSY NMR spectra of **4a** and **4b**. The ^{31}P signals were observed at δ −2.31 and 1.91 ppm in the spectrum of **4a** and at δ 1.50 ppm in the spectrum of **4b**. In the ^1H NMR spectrum of **4a**, signals due to C(5′)CH_2OH and C(3′)*CH*OH observed around δ 3.6–3.9 and 4.2–4.3 ppm, respectively, in the spectrum of **3** disappeared. Instead, signals for four protons due to two C(5′)H_2OP(O)O and those for two protons due to two C(3′)*H*OP(O)O were observed around δ 4.6–4.7 and 5.3–5.4 ppm, respectively; these ^1H signals correlate with the ^{31}P signals. Similar phenomena were observed in the ^{31}P–^1H COSY NMR spectrum of **4b**. These observations supported that **4a** and **4b** are stereoisomers having the cyclic bis(3′→5′)diguanylic acid structure. The ESI-TOF high resolution mass spectra (positive mode) of **4a** and **4b**, showing molecular ion peaks at *m/z* 1247.4496 for **4a** and 1247.4426 for **4b** [calcd for **4** ($C_{52}H_{77}N_{10}O_{18}P_2Si_2^+$) ($M+H^+$) 1247.4435], also supported this structure. Thus, the yield of the cyclic product **4**, as a mixture of **4a** and **4b**, was 75%.

The fully protected compound **4** (a mixture of **4a** and **4b**)[11] was deblocked by treatment with a catalytic amount of $Pd_2[(C_6H_5CH{=}CH)_2CO]_3{\cdot}CHCl_3$ in the presence of triphenylphosphine and butylammonium formate in THF[12] (10 min) (removal of all allylic protectors on guanine bases and phosphate moieties), followed by exposure to $(C_2H_5)_3N{\cdot}3HF$[13] (12 h) (removal of 2′-*O*-*tert*-butyldimethylsilyl protecting groups). The ^{31}P NMR analysis of the resulting product indicated that this two-step deprotection formed cGpGp (**5**) in a 77% overall yield. Reverse-phase chromatography afforded a pure sample of the diammonium salt of **5** (see, Fig. 2). The structure of this product was confirmed by the following spectral analyses. The ESI-TOF high resolution mass spectrum (negative mode) (Fig. 3) showed a molecular ion peak at *m/z* 689.0853, which is consistent with the molecular weight of 689.0876 calculated for **5** [($C_{20}H_{23}N_{10}O_{14}P_2^-$) ($M-H^-$)]. The ^1H NMR spectrum measured at 50°C[14] in D_2O was identical with that of cGpGp illustrated in the literature.[1] In the ^{31}P NMR spectrum, a sole signal was observed at δ −0.86 ppm, which is also consistent with that previously reported (δ −0.62 ppm).[1] The UV spectrum showed an absorption at λ_{max}=254 nm (ε 23,700), identical with that previously reported (λ_{max}=252 nm; ε 24,700).[1] Further, the ^{13}C NMR spectrum indicated only five signals at δ 62.8, 70.9, 73.7, 80.8, and 90.6 ppm as those due to ribose

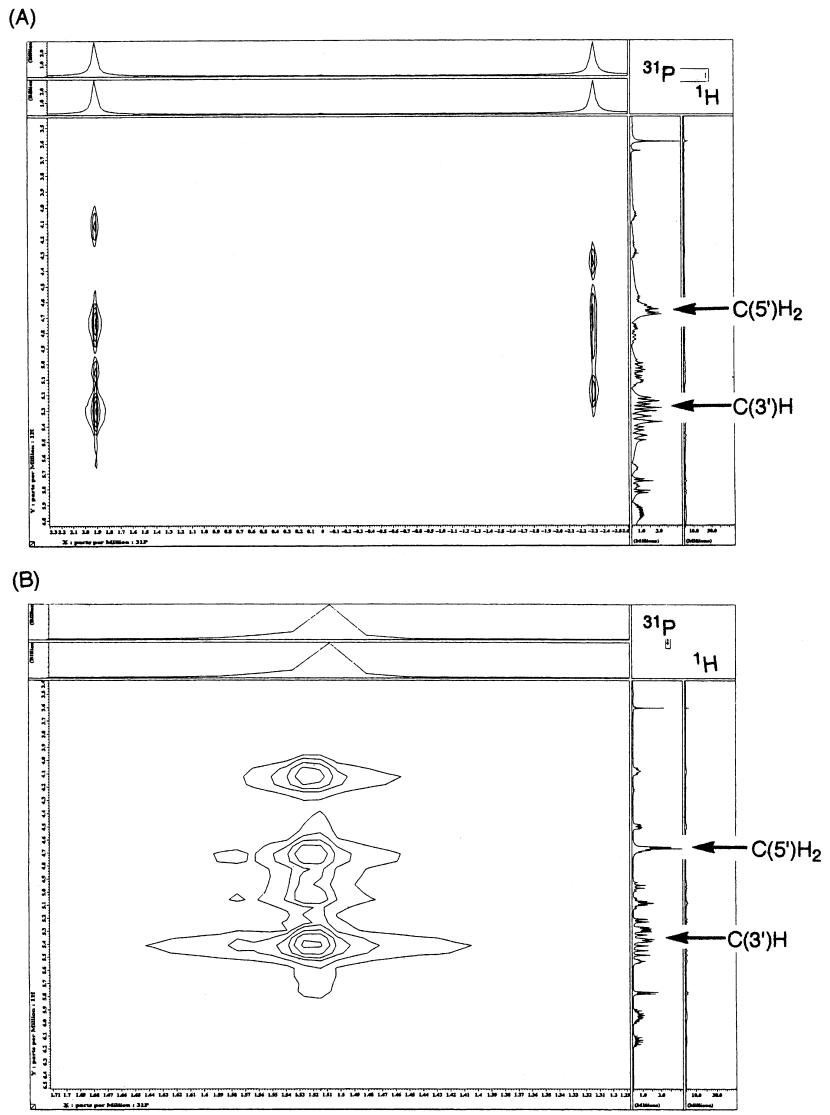

Figure 1. The $^{31}P-^{1}H$ COSY NMR spectra of **4a** (A) and **4b** (B).

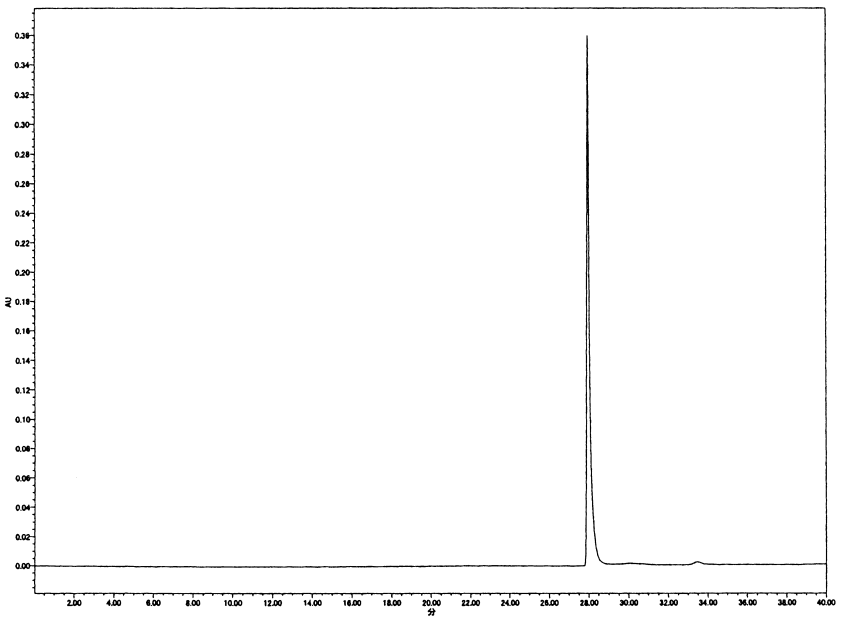

Figure 2. HPLC profile of purified **5**. Conditions: COSMOSIL $5C_{18}$-AR-300 column (25×200 mm); buffer A, 1 mM ammonium acetate; buffer B, 80% acetonitrile–1 mM ammonium acetate; gradient, A:B=100:0 to A:B=40:60 in 60 min; detection 254 nm, flow rate, 5.0 mL/min, temperature 40°C.

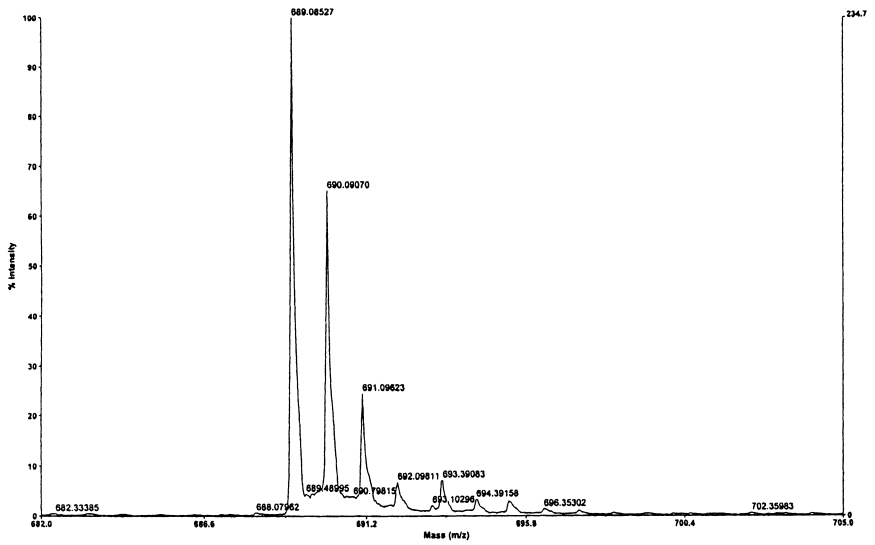

Figure 3. The ESI-TOF mass spectrum (negative mode) of **5** [calcd for $C_{20}H_{23}N_{10}O_{14}P_2^-$ ($M-H^-$) 689.0876].

moieties and this observation supported the symmetric structure of the product.

3. Conclusion

We have developed a new method for synthesis of cGpGp, which has several advantages over the existing one.[1,2] In the existing synthesis, the yield of a linear guanylyl(3′→5′)-guanylic acid intermediate, which is prepared by the triester method using one building block slightly excessive to another building block, is not quite satisfactorily high. By contrast, in the present synthesis, the linear diguanylic acid intermediate **3** is prepared in an excellent yield via the MS 3A-presence phosphoramidite method using the two building blocks **1** and **2** in stoichiometric amounts; the yield of **3** is ca. 20% higher than that of a similar intermediate in the existing synthesis. Further, in the existing synthesis, because diphenylacetyl groups are employed as the protectors of guanine bases, whose subsequent deprotection requires treatment with hot concentrated ammonia, there is the risk that the deprotection will cause decomposition of the target product.[4] By contrast, the present synthesis does not include such risk, because it uses allyloxycarbonyl groups for the protection of guanine bases, and these groups can be removed by treatment with an organopalladium catalyst under mild, almost neutral conditions. Furthermore, the existing synthesis uses *o*-chlorophenyl protectors for the phosphate moieties, which are not deprotected under the conditions for removal of the *N*-diphenylacetyl protectors. Therefore, two steps of procedure are required for the removal of these protectors from a fully protected cGpGp derivative. On the other hand, because the present synthesis uses the allyl groups for the protection of internucleotide bonds, and these groups are removable together with the *N*-allyloxycarbonyl protectors by the organopalladium reaction, we can deblock the four allylic protecting groups from the fully protected cGpGp **4** in one step. This improvement may diminish loss of the desired product in the deprotection process. In addition, the present synthesis constructs the cyclic diguanylic acid structure by the phosphoramidite method, and thus has the capability for use in preparation of artificial analogs of cGpGp with modified internucleotide linkages such as phosphorothioates,[15] phosphoroselenoates,[16] and boranophosphates.[17]

4. Experimental

4.1. General

A UV spectrum was taken on a JASCO V-500 spectrometer. NMR spectra were obtained on a JEOL JNM-α400 or ECA-500 instrument. The ^1H, ^{13}C, and ^{31}P NMR chemical shifts are described as δ values in ppm relative to $(CH_3)_4Si$ (for ^1H and ^{13}C) and 85% H_3PO_4, respectively. Measurement of MALDI-TOF HRMS and ESI-TOF HRMS spectra was carried out on Applied Biosystems Voyager MDE and Mariner spectrometers, respectively. HPLC analysis was carried out using a COSMOSIL $5C_{18}$-MS column (Nacalai Tesque, ODS-5 μm, 4.6×250 mm) on a Waters 2695 Separations Module chromatograph with a Waters 2996 Photodiode Array detector. Preparative HPLC using a COSMOSIL $5C_{18}$-AR-300 column (Nacalai Tesque, 25×200 mm) was achieved with an ÄKTA explorer (Amersham Biosciences). Nacalai Tesque silica gel 60 (neutrality, 75 μm) was used for column chromatography. Unless otherwise stated, reactions were carried out at ambient temperature. Reactions requiring anhydrous conditions were carried out under an argon atmosphere in flasks dried by heating at 400°C under reduced pressure (1.0–3.0 mm Hg) or by treatment with 5% dichlorodimethylsilane/dichloromethane solution followed by washing with anhydrous dichloromethane and then heating at 100°C.

4.2. Material and solvents

The protected guanosine 3′-phosphoramidite **1**,[7] imidazolium perchlorate,[6] and tris(dibenzylideneacetone)dipalladium(0)-chloroform complex $[Pd_2(dba)_3{\cdot}CHCl_3]$[18] were prepared by the reported methods. A 6.7% 2-butanone peroxide/dimethyl phthalate–toluene solution was prepared

by dilution of a commercially supplied 55% 2-butanone peroxide/dimethyl phthalate solution (Kishida) with toluene. Acetonitrile, DMF, and dichloromethane were used distilled from CaH_2. Diethyl ether, THF, and toluene were used after drying by reflux over sodium-benzophenone ketyl. Other organic solvents were used as commercially supplied without any purification. Nacalai tesque silica gel 60 (neutrality, 75 μm) was used for column chromatography. Solid and amorphous organic substances were used dried over P_2O_5 at 50–60°C for 8–12 h under reduced pressure (1.0–3.0 mm Hg). Powdery MS 3A and 4A were used after drying the commercially supplied one (Nacalai tesque) at 200°C for 12 h under reduced pressure (1.0–3.0 mm Hg).

4.2.1. N^2-(Allyloxycarbonyl)-O^6-(allyl)-2′-*O*-(*tert*-butyldimethylsilyl)guanosine 3′-(allyl 2-cyanoethyl)phosphate (2). A heterogeneous mixture of the phosphoramidite **1** (2.0 g, 2.0 mmol), 2-cyanoethanol (0.16 mL, 170 mg, 2.4 mmol), and powdery MS 3A (200 mg) in acetonitrile (20 mL) was stirred at 25°C for 30 min. To this was added imidazolium perchlorate (670 mg, 4.0 mmol) and stirring was continued for additional 30 min. To the resulting mixture was added a 6.7% 2-butanone peroxide/dimethyl phthalate–toluene solution (4.0 mL). The mixture was stirred for 5 min. MS 3A was removed by passage through a Celite 545 pad. The filtrate was diluted with ethyl acetate (100 mL) and washed with an aqueous $NaHCO_3$-saturated solution followed by brine. The organic layer was separated, dried over sodium sulfate, and concentrated to give a residual material. This material was dissolved in dichloromethane (20 mL) and cooled at 0°C. To the solution was slowly added dichloroacetic acid (3.3 mL, 5.2 g, 40 mmol) and then the mixture was stirred at the same temperature for 5 min. The reaction mixture was poured to an aqueous $NaHCO_3$-saturated solution (100 mL) and the organic layer was separated. The aqueous layer was extracted with dichloromethane (100 mL, 50 mL×2). The combined organic solutions were dried and concentrated to give a residual product. This crude material was subjected to column chromatography on silica gel (40 g) eluted with a 1:1 mixture of ethyl acetate and hexane followed by a 1:10:10 mixture of methanol, ethyl acetate, and hexane to afford **2** (1.38 g, 95% yield; a mixture of two diastereomers) as a colorless amorphous solid: IR (CH_2Cl_2) 3420, 3048, 2305, 1757, 1607, 1524, 1462, 1412, 1294, 1190, 1038, 756 cm^{-1}; ^1H NMR (CD_3OD) −0.27 (s, 3H), −0.03 (s, 3H), 0.74 (s, 9H), 2.94 (t, *J*=6.0 Hz, 2H), 3.85–3.97 (m, 2H), 4.30–4.43 (m, 3H), 4.70 (m, 4H), 5.00 (m, 1H), 5.08–5.10 (m, 2H), 5.24–5.52 (m, 7H), 6.00–6.20 (m, 4H), 8.39 (s, 1H); ^{31}P NMR (CD_3OD) −4.69, −4.54.

4.2.2. The guanylyl(3′→5′)guanosine 3′-phosphate 3. A mixture of the phosphoramidite **1** (1.6 g, 1.6 mmol) and the 5′-*O*-free nucleoside phosphate **2** (1.1 g, 1.6 mmol) in the presence of powdery MS 3A (200 mg) in acetonitrile (15 mL) was stirred at 25°C for 30 min. The mixture was added imidazolium perchlorate (540 mg, 3.2 mmol) and stirred for additional 30 min. To this was added a 6.7% 2-butanone peroxide/dimethyl phthalate–toluene solution (3.2 mL). After 5 min, the reaction mixture was passed through a Celite 545 pad to remove MS 3A. The filtrate was concentrated to afford a viscous oil. This material was dissolved in dichloromethane (20 mL). To this solution was added dichloroacetic acid (3.3 mL, 5.2 g, 40 mmol) at 0°C. After stirring for 5 min, the reaction mixture was poured to an aqueous $NaHCO_3$-saturated solution (100 mL) and the organic layer was separated. The aqueous layer was extracted with dichloromethane (100 mL, 50 mL×2). The combined organic solutions were dried and concentrated. The resulting material was chromatographed on a column of silica gel (40 g) using a 1:1 ethyl acetate–hexane mixture and then a 1:20:20 methanol–ethyl acetate–hexane mixture as eluent to afford **3** (1.95 g, 94% yield; a mixture of two diastereomers) as a colorless amorphous solid: ^1H NMR ($CDCl_3$) −0.36–0.03 (m, 12H), −0.68–0.77 (m, 18H), 1.78 (br s, 1H), 2.79–2.81 (m, 2H), 3.79–3.96 (m, 4H), 4.30–4.36 (m, 4H), 4.48–4.71 (m, 14H), 4.99–5.50 (m, 14H), 5.75–6.19 (m, 8H), 7.26–8.67 (m, 4H); ^{31}P NMR ($CDCl_3$) −1.32, −1.24, −1.11, −1.05, −0.88, −0.81; HRMS (MALDI$^+$) calcd for $C_{55}H_{83}N_{11}O_{19}P_2Si_2^+$ ($M+H^+$) 1318.4797, found 1318.5267.

4.2.3. Intramolecular cyclization of 3 giving the protected cyclic bis(3′→5′)diguanylic acid 4. To a solution of **3** (660 mg, 0.5 mmol) in methanol (10 mL) was added at 25°C a concentrated aqueous ammonia solution (1.0 mL) and the resulting solution was stirred for 60 min. The reaction mixture was concentrated in vacuo. The resulting residue was dissolved in toluene (20 mL) and the solvent was evaporated. This treatment was carried out twice more to give a colorless amorphous solid. This material was dissolved in THF (100 mL). Powdery MS 4A was added to the solution and the resulting mixture was stirred at 25°C for 30 min to remove methanol, ammonia, and water. The MS 4A was removed by filtration. To the filtrate were successively added *N*-methylimidazole (0.08 mL, 82 mg, 1.0 mmol) and 2,4,6-triisopropylbenzenesulfonyl chloride (300 mg, 1.0 mmol). The resulting solution was stirred at 25°C for 20 h. An aliquot of the reaction mixture was subjected to TLC analysis using a 1:10:10 mixture of methanol, ethyl acetate, and hexane as an eluent to indicate that **4a** (R_f=0.40) and **4b** (R_f=0.49) was formed as major products. The whole reaction mixture was directly concentrated. The resulting residual material was chromatographed on a silica gel (40 g) column using a 1:2 mixture of ethyl acetate and hexane and then a 1:30:30 mixture of methanol, ethyl acetate, and hexane to afford **4a** (270 mg) and **4b** (200 mg) as both amorphous solids. The total yield of **4** was 75%.

Compound **4a**: ^1H NMR ($CDCl_3$) −0.31, −0.21, −0.03, 0.04 (4 s, 12H), 0.76 (s, 18H), 4.03–4.09 (m, 2H), 4.26–4.34 (m, 2H), 4.54–4.69 (m, 8H), 4.74–4.86 (m, 4H), 4.97–5.15 (m, 6H), 5.21–5.52 (m, 12H), 5.64–5.68 (m, 1H), 5.74–5.82 (m, 3H), 5.94–6.01 (m, 4H), 6.08–6.18 (m, 2H), 7.53 (s, 1H), 7.78 (s, 1H), 7.81 (s, 1H), 8.03 (s, 1H); ^{31}P NMR ($CDCl_3$) −2.31, 1.91; HRMS (ESI$^+$) calcd for $C_{52}H_{77}N_{10}O_{18}P_2Si_2^+$ ($M+H^+$) 1247.4426, found 1247.4496.

Compound **4b**: ^1H NMR ($CDCl_3$) −0.22, −0.08 (2 s, 12H), 0.74 (s, 18H), 4.07 (m, 2H), 4.48–4.51 (m, 2H), 4.65–4.68 (m, 8H), 4.96 (q, *J*=11 Hz, 2H), 5.03–5.13 (m, 4H), 5.19–5.58 (m, 16H), 5.77 (d, *J*=7.0 Hz, 1H), 5.93–5.97 (m, 4H), 6.11–6.17 (m, 2H), 7.78 (s, 2H), 7.85 (s, 2H); ^{31}P NMR

($CDCl_3$) 1.50; HRMS (ESI^+) calcd for $C_{52}H_{77}N_{10}O_{18}P_2Si_2^+$ ($M+H^+$) 1247.4426, found 1247.4435.

4.2.4. Full deprotection of 4 giving cGpGp 5. To a solution of **4** (a mixture of **4a** and **4b**) (50 mg, 0.04 mmol) in THF (1.6 mL) were added triphenylphosphine (16 mg, 0.06 mmol), *n*-butylamine (49 μL, 36 mg, 0.48 mmol), formic acid (18 μL, 22 mg, 0.48 mmol), and $Pd_2(dba)_3 \cdot CHCl_3$ (12 mg, 0.012 mmol). The resulting mixture was stirred at 25°C for 30 min. During this period, white solid precipitated. To this mixture was added ethyl acetate (10 mL). The precipitate was collected by filtration and dried in vacuo. This solid material was mixed with $(C_2H_5)_3N \cdot 3HF$ (0.3 mL) and the mixture was stirred at 25°C for 12 h. An aliquot (30 μL) of the reaction mixture was taken out and subjected to the following analysis. The aliquot was dissolved in a mixture of a 0.1 M Na_2HPO_4 aqueous solution (40 μL) and D_2O (500 μL). The resulting solution was subjected to the ^{31}P NMR measurement; the yield of the desired product **5** was estimated by comparison of intensity of the signal due to **5** (δ −0.94 ppm) and that due to Na_2HPO_4 (0.78 ppm). The yield of **5** indicated by this analysis was 77%. The whole aliquot taken out was mixed again with the reaction mixture. To the reaction mixture was added a 1 mM ammonium formate buffer solution (1 mL). The resulting mixture was vigorously stirred under heating at 30–40°C to precipitate a pale yellow solid. After removal of the resulting precipitate, the aqueous solution was subjected to preparative HPLC using a COSMOSIL $5C_{18}$-AR-300 column [25 (diameter)×200 (height) mm]. Elution was carried out by the following conditions [A=a 1.0 mM ammonium acetate buffer solution, B=a 0.2 mM ammonium acetate solution in a 20:80 mixture of H_2O and acetonitrile; gradient: 0–8 min A 100%, 8–55 min linear gradient A 100% to A 40%/B 60%, 55–63 min B 100%; detection 254 nm; flow rate 10 mL/min] to give the diammonium salt of **5** (12 mg, 40% yield). UV (a 50 mM solution of NH_4OAc in H_2O) λ_{max} 254 nm (ε 23,700); ^1H NMR (D_2O) 4.04–4.06 (m, 2H), 4.38–4.44 (m, 4H), 5.08 (s, 2H), 5.33 (s, 2H), 6.12 (s, 2H), 8.25 (s, 2H); ^{13}C NMR (D_2O) 62.8, 70.9, 73.7, 80.8, 90.6, 116.4, 136.9 (weak), 150.4 (weak), 154.1, 157.8; ^{31}P NMR (D_2O) −0.86; HRMS (ESI^-) calcd for $C_{20}H_{23}N_{10}O_{18}P_2^-$ ($M-H^-$) 689.0876, found 689.0853.

In a similar manner, a pure sample of **4a** or **4b** was fully deprotected to give **5**.

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4. In Refs. 1,2, there is no information about the yield of the desired compound in the deprotection of the *N*-diphenylacetyl protectors. Thus, we cannot know whether the deprotection causes decomposition of the desired product in the existing synthesis. However, according to experiments carried out by us, cGpGp was decomposed to no small extent by treatment with hot conc. aqueous ammonia to give undesired products. The structure of the products was not determined because analytical samples of them were not obtained though chromatographic purification was attempted under several conditions; we supposed that the products are those resulting from cleavage of internucleotide linkage.
5. This paper eliminates another defect of the existing synthesis. In the field of chemical synthesis, detailed experimental protocol is very important for reexamination of the synthesis. However, Refs. 1,2 do not describe in detail the protocol; this is troublesome for further investigations. By contrast, this paper provides detailed experimental procedures for all reactions.
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