PREPARATIVE METHOD OF OBTAINING GUANOSINE 5'-TRIPHOSPHATE

T. S. Lomakina and N. I. Grineva

Khimiya Prirodnykh Soedinenii, Vol. 1, No. 4, pp. 275-283, 1965

It is known that GTP*participates in the biosynthesis of RNA and protein. To investigate these important processes pure and satisfactorily available GTP is necessary. However, there is no convenient preparative method of obtaining GTP, although this problem has been solved for many nucleoside triphosphates.

Nucleoside triphosphates are formed from nucleoside 5'-monophosphates and orthophosphoric acid with the aid of dicyclohexylcarbodiimide in the presence of pyridinic and dimethylformamide [1, 2]; from amides of nucleoside 5'-monophosphates and salts of pyrophosphoric acid in pyridine, o-chlorophenol [3], tricresol, and acetonitrile [4]; and from the morpholides in anhydrous pyridine [2] and dimethyl sulfoxide [5].

The methods listed above lead to a mixture of substances: the starting materials, and the nucleoside 5'-mono-, di-, tri-, and polyphosphates. The ratio of these substances is determined by the individual properties of the nucleoside triphosphates, which have different tendencies to undergo disproportionation reactions [6].

The reaction of GMP and a ten-fold excess of tributylammonium orthophosphate with 500 times the amount of dicyclohexylcarbodiimide in pyridine for 48 hr leads to a mixture containing 5% of GMP, 15% of GDP, 71% of GTP, and 9% of GPP. The mixture is separated by ion-exchange chromatography on "Dowex" 2 with a total yield of nucleotide material of less than 50% [1]. On the preparative scale, ATP was obtained by this method with a yield of 36%. After

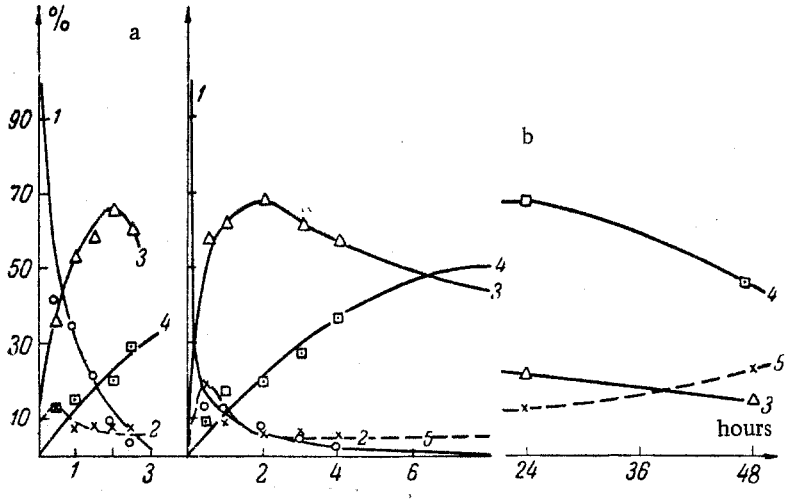


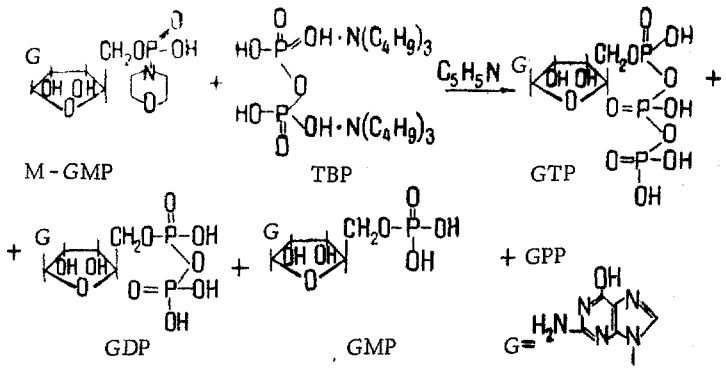
Fig. 1. Kinetics of the reaction of the morpholide of guanosine 5'-monophosphate with bis-(tributylammonium) pyrophosphate in pyridine at 27°: 1) M-GMP; 2) and 5) GMP; 3) GTP; 4) GDP; a) from the results of chromatography on DEAE-cellulose (HCO_3^-) in a linear gradient of $(C_2H_5)_3N \cdot H_2CO_3$, pH 7.6; b) from the results of chromatography on "Dowex" 1 × 4 ($HCOO^-$) in a linear gradient of $HCOONH_4$ in 0.5 N HCOOH.

3.5 hr, a mixture of GMP amide and pyrophosphate in o-chlorophenol gave 32% of GTP in the mixture [3]. Under these conditions, about 78% of ATP is formed. AMP morpholide with bis-(tributylammonium) pyrophosphate gives 57% of ATP in pyridine [2] and 54% in dimethylsulfoxide [5]. In dimethylsulfoxide dried with Linde molecular sieves, the yields of ATP, CTP, and deoxyribonucleoside triphosphates amount to 81-84% [5].

To develop a convenient preparative method for producing GTP, we have investigated the reaction of the 4-morpholino-N, N'-dicyclohexylformamidinium salt of GMP morpholide (M-GMP) with bis-(tributylammonium) pyrophos-

*The following abbreviations are used in this paper: GMP – guanosine 5'-monophosphate, GDP – guanosine 5'-diphosphate, GTP – guanosine 5'-triphosphate, GPP – guanosine 5'-polyphosphate, AMP – adenosine 5'-monophosphate, ADP – adenosine 5'-diphosphate, ATP – adenosine 5'-triphosphate, UTP – uridine 5'-triphosphate, CTP – cytidine 5'-triphosphate.

phate (TBP) in anhydrous pyridine. This reaction leads to the formation of GMP, GDP, GTP, and GPP:



The ratio of these substances in the mixture is governed mainly by the duration of the reaction.

In order to ascertain the optimum conditions for the formation of GTP, the kinetics of the process in pyridine at 27° without the access of moisture was studied. Samples of the reaction mixture were taken after predetermined intervals of time.

The reaction in the sample of the mixture was stopped by driving off the pyridine and the benzene in vacuum. The content of substances in the samples was determined by ion-exchange chromatography on "Dowex" 1 × 4 ($HCOO^-$) in a linear gradient of ammonium formate in formic acid (Fig. 1, b) and on DEAE-cellulose (HCO_3^-) in a linear gradient of triethylammonium bicarbonate (Fig. 1, a). Figure 2 shows the chromatographic profiles of the mixture after 2 and 24 hr.

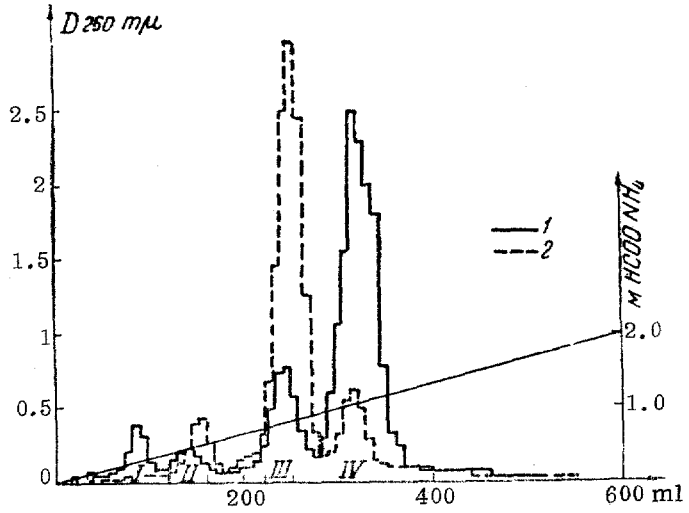


Fig. 2. Chromatograms of the reaction mixture on "Dowex" 1 × 4 ($HCOO^-$) in a linear gradient of $HCOONH_4$ in 0.5 N HCOOH: 1) After 2 hr; 2) after 24 hr; I) M-GMP; II) GMP; III) GDP; and IV) GTP (mixer) 300 ml of 0.5 N HCOOH, reservoir 300 ml of 2 M $HCOONH_4$ in 0.5 N HCOOH. Rate of elution 25 ml/hr; yield from the column 81%).

Under the conditions investigated, the amount of GTP formed (Fig. 1, curve 3) reached a maximum (65-67%) after 2 hr, and then fell to 21% after 24 hr. As the GTP disappeared, GDP accumulated (curve 4), from 14% after 30 min to 67% after 24 hr, and then its amount began to fall.

The initial M-GMP (Fig. 1, b, curve 1) falls sharply, not only because of the reaction considered but also because of hydrolysis of the M-GMP in the acid medium during chromatography. Under these conditions, the hydrolysis amounts to 43%, which corresponds to data on the hydrolysis of AMP morpholide in acid media [2]. The kinetic curve of M-GMP obtained on chromatography on DEAE-cellulose (Fig. 1, a, curve 1) shows that M-GMP disappears from the reaction mixture less rapidly. But after 2.5 hr, the M-GMP has reacted to the extent of 96%, giving 60% of GTP, 19% of GDP, and 7% of GMP.

We have also ascertained the influence of an excess of pyrophosphate on the yield of GTP. The contents of M-GMP and GTP were determined by thin-layer chromatography on cellulose in system A. It was established that when the excess of pyrophosphate was increased from two-fold to 170-fold, the yield of GTP rose from 49 to 79%:

Excess of bis-(tributylammonium) pyrophosphate, mmoles per mmole of GMP morpholide	Content of GTP in the reaction mixture, %
2	49
4	58
6	65
8	67
34	70
170	79

These results indicate that the optimum conditions for the formation of GTP from M-GMP and TBP in pyridine on the analytical scale of carrying out the reaction are a six- to eight-fold excess of pyrophosphate and a reaction time of 2 hr.

When the synthesis was carried out on the preparative scale (0.5 mmole of M-GMP), 65-67% of GTP was again formed in the reaction mixture. Chromatography of DEAE-cellulose (HCO_3^-) in a linear gradient of triethylammonium bicarbonate (Fig. 3) permitted the GTP to be obtained from the mixture in quantitative yield.

After evaporation of the aqueous solutions, the GTP fraction gave GTP with a yield of 91-94% by the precipitation of the tetrasodium salt with sodium iodide in acetone from a methanolic solution of the triethylammonium salt.

The GTP obtained, was, according to the results of ion-exchange chromatography on DEAE cellulose and thin-layer chromatography in systems A and B, a pure substance, the content of nucleotide material in which was 96%.

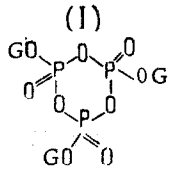
The proposed procedure is relatively simple and gives a high reproducibility of the results and a high yield of the sodium salt of pure GTP without the use of special solvents and drying agents. The initial M-GMP can be obtained fairly readily in high yield (up to ~70%) from guanosine by known methods [11, 12].

In the reaction of M-GMP with pyrophosphate, the complete kinetic relationships in GMP promotion should be noted, indicating the occurrence of two different reactions for its formation (Fig. 1, curves 2 and 5). Thus, at the beginning of the reaction, 12% of GMP was isolated, after which its content fell to 4-7% (Fig. 1, a, curve 2), rising again after 48 hr to 22% (Fig. 1, b, curve 5). The initial section of the kinetic curve 2 for GMP (Fig. 1, a) apparently expresses the first reaction of the process under study, and curve 5 (Fig. 1, b) the second one.

Curve 2 (Fig. 1, a) cannot be the result of the hydrolysis of M-GMP by traces of water in the reaction mixture, since M-GMP is stable in neutral and alkaline aqueous solutions for a long time, or the consequence of a disproportionation of the GDP, which is, to a smaller extent, the third successive reaction of the process studied. The content of GMP in connection with this reaction should not fall with time but rise (Fig. 1, b, curve 5).

The process of the formation of GMP expressed by curve 2 (Fig. 1, a) possibly represents a secondary reaction of the hydrolysis of an intermediate active phosphorylating compound when the reaction mixture is dissolved in water for analysis. It may be assumed that the intermediate substance is obtained from M-GMP at a high velocity and rapidly phosphorylates the pyrophosphate and that curve 2 (Fig. 1, a) is the over-all curve of its formation and consumption. The structure of the trimetaphosphate (I) may be proposed for the intermediate; its formation by an intermolecular nucleophilic attack of the M-GMP is likely:

(I)



G-5'-guanosyl

The intermediate formation of trimetaphosphate has previously been postulated by Khorana [1] in the reactions of AMP and H_3PO_4 and dicyclohexylcarbodiimide, and also by Todd and Michelson [15] in a series of phosphorylation reactions.

As is known, the velocity of the reaction of inorganic metaphosphates with water is considerably higher than the velocity of the reaction with H_3PO_4 [16]. Thus, their reaction with water is complete in half an hour, and that with

orthophosphates after 2 hr. This fact explains the negative influence of water on the formation of GTP from M-GMP. To obtain good results by the method mentioned, the absence of GMP from the M-GMP and of orthophosphate from the pyrophosphate is also essential.

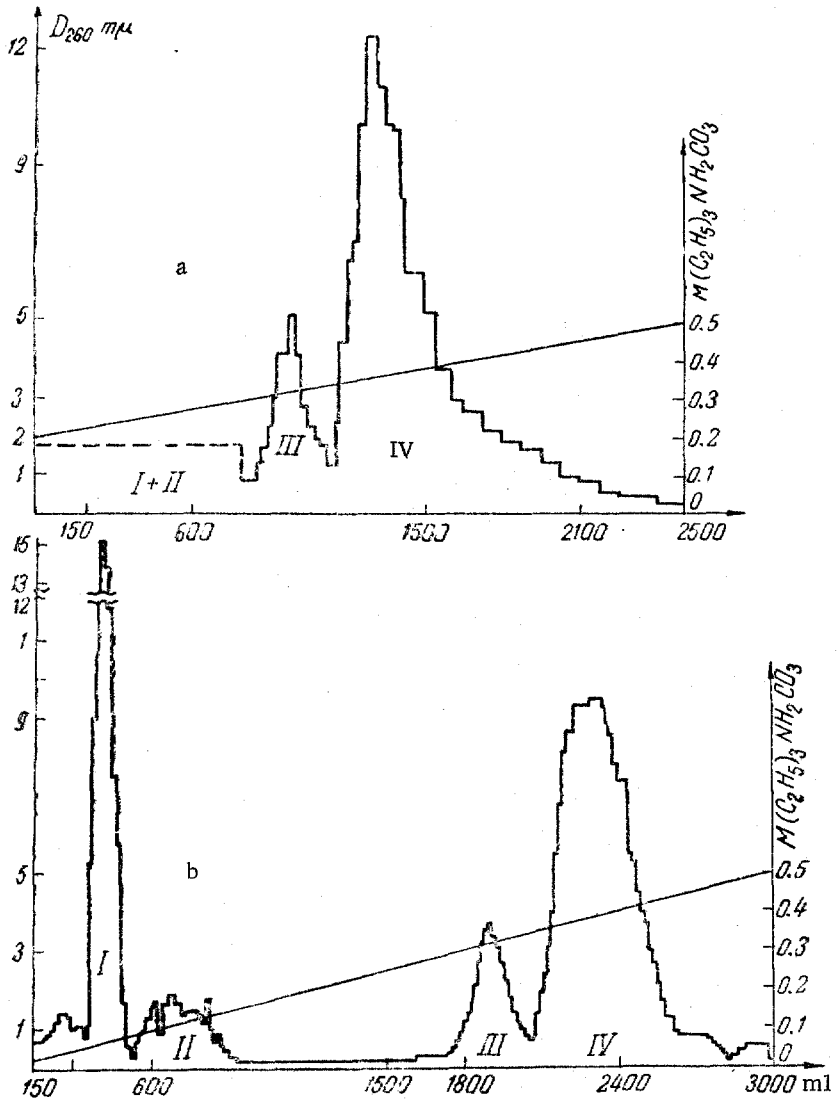


Fig. 3. Chromatography of a reaction mixture on DEAE cellulose (HCO_3^-) in a linear gradient of $(C_2H_5)_3N \cdot H_2CO_3$, pH 7.5. 1) M-GMP; II) GMP; III) GDP; IV) GTP; a) partial separation of a reaction mixture from 0.585 mmole of M-GMP (mixer – 1.25 liter of 0.2 M $(C_2H_5)_3N \cdot H_2CO_3$, reservoir – 1.25 liter of 0.5 M $(C_2H_5)_3N \cdot H_2CO_3$); b) complete separation of a reaction mixture from 0.5 mmole of M-GMP (mixer – 1.5 liter of 0.01 M $(C_2H_5)_3N \cdot H_2CO_3$, reservoir – 1.5 liter of 0.5 M $(C_2H_5)_3N \cdot H_2CO_3$.

The results of the kinetic investigation (Fig. 1) indicate that the interaction of M-GMP with TBP in pyridine for 48 hr includes four successive reactions:

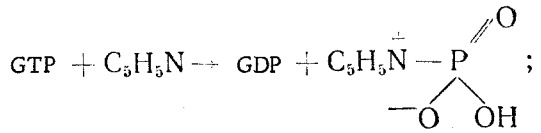
1) The formation of an intermediate compound, possibly I (Fig. 1, a, curve 2 – over-all curve of the formation and consumption of I):

$$3M\text{-}GMP \xrightarrow{\text{pyridine}} I;$$

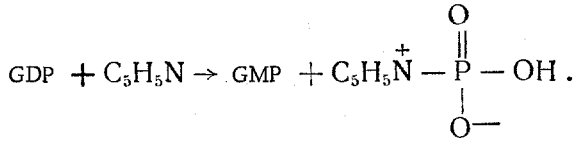
2) Phosphorylation of the pyrophosphate of I (Fig. 1, curve 3):

$$I + 3H_4P_2O_7 \longrightarrow 3\ GTP;$$

3) Disproportionation of GTP under the influence of pyridine, apparently by a mechanism analogous to the disproportionation of ATP [6] (Fig. 1, curve 4):



4) Disproportionation of GDP (Fig. 1, curve 5):



The formation of only a small amount of GPP (about 19% after 48 hr) is due to the excess of pyrophosphate in the reaction mixture, suppressing the formation of GPP.

Experimental

Thin-layer chromatography on cellulose (TLC). The TLC was carried out [17] with the following system of solvents: A) n-propanol – ammonia (sp gr. 0.90) – water (6 : 3 : 1); B) isobutyric acid-1 M ammonia-0.1 M solution of the disodium salt of ethylenediaminetetra-acetic acid (100: 60 : 1.6); C) isopropanol-ammonia (sp. gr. 0.90)-water (7 : 1 : 2); D) ethanol-1 M ammonium acetate (5 : 2), pH 7.5; and E) isobutyric acid-ammonia (sp.gr. 0.90)--water (57 : 4 : 39), pH 4.3. The solvents for the system were redistilled, and the aqueous ammonia was prepared by saturating distilled water with gaseous ammonia to sp. gr. 0.90.

Starting materials. The morpholine and tributylamine were dried with caustic potash and redistilled. "Pure for analysis" grade pyridine was boiled for 1 hr with barium oxide, stored over barium oxide, and was used after being freshly distilled over barium oxide, with careful protection from atmospheric moisture during distillation and during all manipulations. The content of water in the pyridine [10] was 6.5 μmole/liter.

Tetrasodium pyrophosphate was obtained by calcining 36.2 g of "pure for analysis" disodium orthophosphate at 500° for 5 hr [8]. Yield 19 g. According to the results of ascending chromatography on paper in system C [7], the pyrophosphate did not contain orthophosphate. The pyrophosphate spot remained at the start.

The evaporation of aqueous and pyridine solutions was carried out in a rotary evaporator at a vacuum of 5-10 mm Hg at a bath temperature not exceeding 30°.

The solution of triethylammonium bicarbonate was obtained by saturating a mixture of redistilled triethylamine and water with carbon dioxide to pH 7.5 with cooling. The ion-exchange chromatography was carried out at 4-6° on columns containing "Dowex" 1 × 4 (HCO^-) [13] and DEAE-cellulose (HCO_3^-) [14].

The GMP was synthesized by phosphorylating 8 mmoles of 2', 3'-O-isopropylideneguanosine (obtained from guanosine [11] with a yield of 94%) with 2-cyanoethyl phosphate in the presence of dicyclohexylcarbodiimide by Tener's method [12]. 5.68 g of the barium salt of GMP was precipitated [12] and was then dissolved in 200 ml of water; 10 g of "Dowex" 50W × 2 (H^+) was added to the solution and it was then passed through 23.0 g of the same resin, the free GMP being eluted with 1500 ml of water and freeze-dried. Yield 70%. R_f in system E 0.54, in system A 0.28-0.31.

Found $D_{260,\ pH\ 1}^{1mg/ml}$: 26.3, 26.6. Calculated for $C_{10}H_{14}O_8P \cdot 4H_2O$, $D_{260,\ pH\ 1}^{1mg/ml}$: 26.7.

The morpholide of GMP was obtained [2] in the form of the 4-morpholino-N, N'-dicyclohexylformamidinium salt from 1.67 mmole of GMP and 6.68 mmole of morpholine with the aid of 6.68 mmole of dicyclohexylcarbodiimide. Yield 96%. The substance was homogeneous to TLC in systems A, B, C, and D; R_f 0.45, 0.66, 0.39, and 0.46, respectively. The chromatography of 46.8 optical units of M-GMP (in water at 260 mμ) on DEAE cellulose (HCO_3^-) in a linear gradient of 0.01 → 0.5 M $(C_2H_5)_3N \cdot H_2CO_3$ (pH 7.5, 300 ml each in the mixer and the reservoir) gave a single sharp peak of M-GMP (D_{260} = 46.7) at a concentration of the eluting solution of 0.06-0.11 M.

Analysis of the M-GMP. A solution of 1.43 g of M-GMP in 0.5 ml of water was treated with 0.5 ml of 0.2 N sulfuric acid and was then left for 1 hr at room temperature and passed through 1 ml of "Dowex" 50 W × 2 (H^+) to free it from bases. The GMP formed was eluted with water, giving 19 ml of a solution with a total optical density at 260 mμ of 21.7.

Found, μmole of M-GMP in 1 mg: 1.31. Calculated for $C_{31}H_{52}N_9O_9P \cdot 4H_2O$, μmole in 1 mg: 1.26.

After the storage of the M-GMP in a closed vessel for a week, the number of micromoles in 1 mg was 1.02.

Reaction of M-GMP with bis-(tributylammonium) pyrophosphate. A solution of 1.67 mmole of $Na_4P_2O_7$ in 20 ml

of water was passed through 18 ml of "Dowex" 50 W × 2 in the pyridinium form [5] and was eluted with 30 ml of water, and the total eluate was concentrated to a volume of ~10 ml. 30 ml of pyridine and 4.2 mmoles of tributylamine were added to the solution, and it was evaporated to form a syrup and dehydrated by distillation with 10-ml portions of pyridine repeated four times.

0.2-mmole portions of the 4-morpholino-N, N'-dicyclohexylformamidinium salt of M-GMP were dried individually by twice-repeated evaporation with 5-ml portions of pyridine. Then a solution of the pyrophosphate in 2 ml of pyridine was added and the vessel was washed out additionally with 2-ml and 1-ml portions of pyridine. The homogeneous solution obtained was evaporated in vacuum, and the residue was dissolved in 6 ml of dry pyridine and kept at 27°. 0.75-ml samples were taken from the reaction mixture after 0.5, 1, 2, 3, 4, 24, and 48 hr. The moment of addition of the pyrophosphate to the M-GMP was taken as the beginning of the reaction. The reaction in the samples was stopped by distilling off first pyridine and then benzene (two 2-ml portions).

Analysis of the reaction mixture. After the elimination of the pyridine, the sample was dissolved in 5 ml of water, and 3.5 ml of the solution was deposited on 5-6 ml of "Dowex" 1 × 4 ($HCOO^-$) 200-400 mesh, 6 × 1.3 cm, washed with water until the optical density had fallen to zero, and chromatographed in a linear gradient of ammonium formate in 0.5 M formic acid. The reservoir contained 300 ml of a 2 M solution of $HCOONH_4$ in 0.5 M HCOOH, and the mixer 300 ml of 0.5 M HCOOH. The rate of elution was 25 ml/hr. The volume of the fractions was 7-8 ml. The yield from the column was 81%. The composition of the mixture was determined as a percentage ratio of the optical density (260 mμ) of the peak to the total density of nucleotide material eluted from the column. The kinetic curves are given in Fig. 1, b, and the profiles of the chromatography of the samples of reaction mixture after 2 and 24 hr in Fig. 2.

Chromatography on DEAE-cellulose (HCO_3^-) (40 × 1.2 cm) was carried out after the transfer of a solution of the sample in water in a linear gradient of triethylammonium bicarbonate, pH 7.5. The mixer contained 300 ml of 0.01 M triethylammonium bicarbonate, and the reservoir 300 ml of a 0.5 M solution of the same salt. The rate of elution was 25 ml/hr. The volume of the fractions was 10 ml. The yield from the column was 95%. The kinetic curves are shown in Fig. 1, a.

Influence of an excess of pyrophosphate. The reaction of 25 μmoles in each case of M-GMP with a 2-, 4-, 6-, 8-, 34-, or 170-fold excess of pyrophosphate was carried out as described above. After 2 hr, the pyridine was eliminated and the content of GTP was determined by TLC (results given above).

Chromatography of M-GMP on "Dowex" 1 × 4 ($HCOO^-$). 8.7 mg of M-GMP was dissolved in 10 ml of water (D_{260} 10.2), and 9.65 ml of the solution was chromatographed on 6 ml of "Dowex" 1 × 4 ($HCOO^-$) in a linear gradient of 0.5 N HCOOH → 2 M $HCOONH_4$ in 0.5 N HCOOH with 300 ml each in the mixer and the reservoir. An M-GMP peak (R_f in system A, 0.46) with 48.2 D_{260} (57.4%) and a GMP peak (R_f 0.30 in the same system) with 36.3 D_{260} (42.6%) were obtained.

Synthesis of GTP. A solution of 3.94 mmole of $Na_4P_2O_7$ in 20 ml of water was passed through 42 ml of "Dowex" 50 W × 2 in the pyridinium form (25 × 1.8 cm) and was eluted with 50 ml of water, the eluate being concentrated to a volume of ~25 ml. The residue was treated with 75 ml of pyridine and then with 7.9-8.5 mmole of tributylamine and the solution was evaporated. After the removal of the pyridine and the water, the residue was evaporated four times with 25-ml portions of dry pyridine. The bis-(tributylammonium) pyrophosphate obtained was transferred with three portions of pyridine (5, 3, and 3 ml) on to 0.585 mmole of M-GMP that had been dried by being heated with 10 ml of pyridine twice. After mixing, the reaction mixture was evaporated rapidly in vacuum, the residue, a bright yellow oil, was dissolved in 12 ml of dry pyridine with shaking, and the solution was left for 2 hr at 27° counting from the moment of mixing. The pyridine was distilled off in vacuum and 5 ml of dry benzene was added and evaporated, the evaporation with benzene being repeated twice. The residue was dissolved in 70 ml of water and the solution was transferred to a column of DEAE-cellulose (HCO_3^-), 50 × 4 cm, and was chromatographed in a linear gradient of triethylammonium bicarbonate (pH 7.5) at a temperature of 4-6°. The mixer contained 1250 ml of 0.2 M $(C_2H_5)_3N \cdot H_2CO_3$ and the reservoir 1250 ml of 0.5 M $(C_2H_5)_3N \cdot H_2CO_3$. The rate of elution was 25 ml/hr, and the volume of the fractions 25 ml.

The first 800 ml of eluate contained a mixture of 4-morpholino-N, N'-dicyclohexylformamidine, M-GMP, and GMP. GDP was collected in the 33rd to 45th fractions (11.5%), and GTP in the 46th to 98th fractions (67%) (a profile of the chromatography is shown in Fig. 3, a). The contents of M-GMP and GPP according to TLC in systems A were 28 and 66%, respectively.

The triphosphate fraction was evaporated to dryness at 26° and 5 mm Hg. The triethylammonium bicarbonate was eliminated by four-fold evaporation with 50-ml portions of methanol. The residue was dissolved in 10 ml of methanol, 2.36 ml of a 1 M solution of sodium iodide in dry acetone was added, and the mixture was diluted with 140 ml of acetone. The precipitate was centrifuged off, washed with acetone (3 × 40 ml), and dried in vacuum over phosphorus pentoxide at room temperature. Weight 243 mg, 61.2%. Colorless water-soluble powder, chromatographically homo-

geneous, R_f in systems A and B 0.2 and 0.34, respectively. Content of GTP in the sample 96%.

Found $D^{1mg/ml}_{260,\ pH\ 7}$: 16.5, 16.1: total phosphorus/guanosine ratio, P/G [9]: 2.95, 3.03. Calculated for $C_{10}H_{12}N_5O_{14}P_3Na_4 \cdot 4H_2O$, $D^{1mg/ml}_{260,\ pH\ 7}$: 17.1; P/G: 3.0.

The chromatography of a reaction mixture from 0.5 mmole of M-GMP on DEAE-cellulose (HCO_3^-) in a linear gradient of triethylammonium bicarbonate, pH 7.5, with 1500 ml of a 0.01 M solution of this salt in the mixer and 1500 ml of a 0.05 M solution in the reservoir (rate 25 ml/hr, volume of the fractions 15 ml, column 50 × 4 cm) gave 19.6% of M-GMP (30th-54th fractions), 7.8% of GMP (57th-75th fractions), 12.0% of GDP (136th-158th fractions), and 63.5% of GPP (159th-209th fractions). According to TLC in system A, the amount of GTP in the reaction mixture was 65.2%, the amount of M-GMP 19% (a profile of the chromatography is shown in Fig. 3, b). The yield of $Na_4GTP \cdot 4H_2O$ from the fraction was 91-94%.

Summary

1. The kinetics of the formation of guanosine 5'-triphosphate, 5'-diphosphate, and 5'-monophosphate in the reaction of the morpholide of guanosine 5'-monophosphate with bis-(tributylammonium) pyrophosphate in pyridine at 27° has been recorded.

2. In the reaction of the morpholide of guanosine 5'-monophosphate with bis-(tributylammonium) pyrophosphate, four successive reactions take place: those of the formation of an intermediate compound, of GTP, of GDP, and of GMP. The maximum formation of GTP (67%) occurs after 2 hours and of GDP (67%) after 24 hours.

3. A preparative method for obtaining pure tetrasodium guanosine 5'-triphosphate from the morpholide of guanosine 5'-monophosphate with its isolation from the mixture by chromatography on DEAE-cellulose in a linear gradient of triethylammonium bicarbonate has been developed. The yield calculated on the basis of the morpholide, is 61-65%.

REFERENCES

1. M. Smith and H. G. Khorana, J. Am. Chem. Soc., 80, 1141, 1958; R. W. Chambers and H. G. Khorana, J. Am. Chem. Soc., 79, 3752, 1957.
2. J. G. Moffatt and H. G. Khorana, J. Am. Chem. Soc., 83, 649, 1961.
3. D. Kessler, B. Moss, and R. W. Chambers, Biochim. et Biophys. Acta, 38, 549, 1960.
4. K. T. Tanaka, M. Honjo, J. Sanno, and M. Moriyama, Chem. Pharm. Bull., 8, 749, 1960.
5. J. G. Moffatt, Can. J. Chem., 42, 599, 1964.
6. W. E. Wehrli, D. L. M. Verheyden, and J. G. Moffatt, J. Am. Chem. Soc., 86, 1254, 1964.
7. C. S. Hanes and F. A. Isherwood, Nat., 164, 1107, 1949.
8. Sint. neorg. prep., Moscow, 3, 96, 1952.
9. C. H. Fiske and Y. Subbarow, J. Biol. Chem., 66, 375, 1925; L. F. Leloir and C. E. Cardini, Methods in Enzymol., 3, 840, N.Y., 1957.
10. F. Drahowzal and H. Konig, Osterr, Chem. Ztg., 55, 189, 1954; RZhKhim, 9439, 1955.
11. S. Chladek and J. Smrt, Collection, 28, 1306, 1963.
12. G. M. Tener, J. Am. Chem. Soc., 83, 159, 1961.
13. W. Cohn and J. X. Khym, Biochem. Preparations, 5, 40, N. Y., 1957.
14. R. K. Ralph, R. J. Young, and H. G. Khorana, J. Am. Chem. Soc., 85, 2008, 1963.
15. A. Todd, Proc. Acad. Sci, USA, 45, 1389, 1959; A. M. Michelson, The Chemistry of Nucleosides and Nucleotides, L., 134, 1963; S. M. Zhenodarova, Usp. khim., 34, no. 1, 95, 1965.
16. W. Polmann and G. Schramm, Biochim. et Biophys. Acta, 80, 1, 1964.
17. T. S. Lomakina, L. I. Gus'kova, and N. I. Grineva, to be published in KhPS [Chemistry of Natural Compounds], no. 5, 1965.

8 March 1965

Novosibirsk Institute of Organic Chemistry
Siberian Division AS USSR