data, mp, λ_{max}, and ϵ for **6** except for **6e** were reported previously.[1]

1-(*tert*-**Butylamino)-2,4-dinitronaphthalene (6e)**: mp 115–115.5 °C; λ_{max} 408 nm (ϵ 11600 [MeOH]); ^{1}H NMR (DMSO-d_6) δ 1.33 [s, 9 H, C(CH$_3$)$_3$], 7.97 (br s, 1 H, NH, overlapped with H^{6,7}), 8.03 (qui, 2 H, H^{6,7}, partially overlapped with NH), 8.70 (m, 2 H, H^{5,8}, overlapped with each other), 9.03 (s, 1 H, H^3), in which the suffix on hydrogen represents the positional number of a naphthalene moiety.

Determination of Products. The typical procedure for determination of the reaction products is described for the reaction of **6d** with methylamine. The 10-mL DMSO solution containing 5 mmol (0.137 g) of **6d** and 3 equiv of methylamine (40% solution) was stirred for the prescribed time at the prescribed temperature, and then the mixture was poured into 200 mL of water, acidified with the equiv of HCl based on the methylamine added, extracted with 200 mL of benzene three times, and dried over anhydrous MgSO$_4$. After the mixture was filtered, the benzene layer was subjected to HPLC (Shimazu LC-6A, silica gel, hexane–2-propanol (20:1 v/v).

Registry No. **6a**, 39139-78-1; **6b**, 27210-67-9; **6c**, 124855-05-6; **6d**, 118209-15-7; **6f**, 116062-03-4; **6g**, 124855-07-8; **6h**, 92859-08-0; **6i**, 68105-52-2; **9** (R = Pr), 124855-08-9; MeNH$_2$, 74-89-5; EtNH$_2$, 75-04-7; PrNH$_2$, 107-10-8; *i*-PrNH$_2$, 75-31-0; t-BuNH$_2$, 75-64-9; BuNH$_2$, 109-73-9; *p*-MeC$_6$H$_4$NH$_2$, 106-49-0; *p*-NO$_2$C$_6$H$_4$NH$_2$, 100-01-6; DMSO, 67-68-5.

Convenient Syntheses of Cytidine 5′-Triphosphate, Guanosine 5′-Triphosphate, and Uridine 5′-Triphosphate and Their Use in the Preparation of UDP-glucose, UDP-glucuronic Acid, and GDP-mannose

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Received September 20, 1989

This paper compares enzymatic and chemical methods for the synthesis of cytidine 5′-triphosphate, guanosine 5′-triphosphate, and uridine 5′-triphosphate from the corresponding nucleoside monophosphates on scales of ~10 g. These nucleoside triphosphates are important as intermediates in Leloir pathway biosyntheses of complex carbohydrates; the nucleoside monophosphates are readily available commercially. The best route to CTP is based on phosphorylation of CMP using adenylate kinase (EC 2.7.4.3); the route to GTP involves phosphorylation of GMP using guanylate kinase (EC 2.7.4.8); chemical deamination of CTP (prepared enzymatically from CMP) is the best synthesis of UTP. For the 10–200-mmol-scale reactions described in this paper, it is more convenient to prepare phosphoenolpyruvate (PEP), used in the enzymatic preparations, from D-(−)-3-phosphoglyceric acid (3-PGA) in the reaction mixture rather than to synthesize PEP in a separate chemical step. The in situ conversion of 3-PGA to PEP requires the coupled action of phosphoglycerate mutase (EC 2.7.5.3) and enolase (EC 4.2.1.11). The enzyme-catalyzed syntheses of uridine 5′-diphosphoglucose (UDP-Glc), uridine 5′-diphosphoglucuronic acid (UDP-GlcUA), and guanosine 5′-diphosphomannose (GDP-Man) illustrate the use of the nucleoside triphosphates.

Introduction

As part of a broad program[3] to develop synthetic techniques based on glycosyl transferases for the preparation of glycoproteins, glycolipids, and proteoglycans,[4] we wished to develop convenient routes to cytidine 5′-triphosphate (CTP), guanosine 5′-triphosphate (GTP), and uridine 5′-triphosphate (UTP). Enzyme-catalyzed reactions of these three compounds with monosaccharides are central reactions in the biosynthesis of the nucleoside phosphate sugars required by glycosyl transferases in mammalian biochemistry (CMP-NeuAc, GDP-Fuc, GDP-Man, UDP-Gal, UDP-GalNAc, UDP-Glc, UDP-GlcNAc, UDP-GlcUA, and UDP-Xyl).[3]

An important issue in planning synthetic tactics concerns the method of synthesizing the NTPs and nucleoside phosphate sugars for use in enzyme-catalyzed reactions: should they be synthesized independently and used as stoichiometric reagents (in which case chemical, enzymatic or fermentation syntheses would all, in principle, be acceptable) or should they be generated and used in situ (in which case only enzymatic syntheses would be acceptable)? We have decided initially to develop synthetic methods that generate the NTPs and nucleoside phosphate sugars as stoichiometric reagents, rather than relying on their generation in situ, for five reasons. First, this type of approach is the most practical. Developing complex systems of coupled enzymes is difficult. If the syntheses of the NTPs and nucleoside phosphate sugars can be developed and optimized separately, the final systems are simpler. Second, this approach has greater generality. If convenient routes to all of the NTPs and nucleoside phosphate sugars can be developed, these compounds are then available for the full range of oligo- and polysaccharide syntheses. Third, this approach is the most flexible. By conducting syntheses of these compounds separately, it is possible to use whatever synthetic method works best for each, without concern for the compatibility of these methods. Fourth, separating syntheses of the nucleoside phosphate sugars from the steps involving use of these compounds in forming glycosidic bonds permits the latter reactions to be conducted in a way that optimizes the use of the glycosyl transferases (normally the most difficult enzymes to obtain and use).[3] Finally, this approach is more likely to be successful for the synthesis of unnatural compounds, where analogues of the natural reactants may have to be synthesized chemically.

CTP, GTP, and UTP are all available from commercial sources, but their cost precludes their use in multigram-scale reactions. We do not discuss in detail the synthesis of adenosine 5′-triphosphate (ATP) here because it is relatively inexpensive compared with other NTPs,[5] and

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Table I. Scale and Yields for Enzymatic Synthesis of Nucleoside Triphosphates

NTP	enzyme[a]	phosphoryl donor[b]	amount of NTP, g (yield, %)	reaction time (days)
CTP	AdK	3-PGA	145 (92)	3
GTP	GK	3-PGA	12 (82)	3
	GK	PEP	12 (86)	3
	AdK (Mn^{2+})	PEP		3, no reactn
	AdK (Mn^{2+}/Mg^{2+})	PEP		3, no reactn
UTP	AdK	3-PGA	12 (92)	5
	NMPK	3-PGA	10 (58)	10; incomplete
	NMPK	PEP	6 (92)	1
	AdK (Mn^{2+})	PEP	0.92[c]	3
	AdK	PEP	1.16[c]	3
	AdK (Mn^{2+}/Mg^{2+})	PEP	0.92[c]	3
ATP	AdK	3-PGA	9 (91)	1

[a] Magnesium(II) was present in all reactions unless noted otherwise. AdK = adenylate kinase (EC 2.7.4.3); GK = guanylate kinase (EC 2.7.4.8); NMPK = nucleosidemonophosphate kinase (EC 2.7.4.4). [b] 3-PGA = D-(−)-3-phosphoglyceric acid; PEP = phosphoenolpyruvate. [c] Product was not assayed.

because it has already been synthesized enzymatically on a 50-mmol scale.[6]

Objective. Our objective in this work was to develop convenient syntheses on ~10-g scale of CTP, GTP, and UTP of sufficient purity for use in enzyme-catalyzed reactions. Four strategies can be used to produce NTPs: (1) enzymatic synthesis (using cell-free enzymes), (2) chemical synthesis, (3) fermentation, and (4) isolation from natural sources. We considered the latter two methods to be too unfamiliar to be useful in classical synthetic organic chemistry laboratories and did not investigate their merits. We conclude that enzymatic methods provide the most convenient routes to CTP and GTP. Chemical deamination of CTP (produced enzymatically) is the best route to UTP.

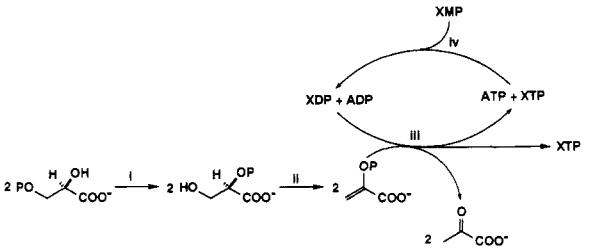
Methods of Enzymatic Synthesis. Enzymatic conversion of a NMP to a NTP requires two kinases: one for NMP and one for NDP (eq 1). The synthesis of NTPs

$$\text{NMP} \xrightarrow{\text{kinase 1}} \text{NDP} \xrightarrow{\text{kinase 2}} \text{NTP} \qquad (1)$$

from NDPs is straightforward. Three kinases are available that convert all four of the NDPs (ADP, CDP, GDP, and UDP) to the corresponding NTPs: pyruvate kinase[7] (PK, EC 2.7.1.40) uses phosphoenolpyruvate (PEP) as a phosphoryl donor, acetate kinase[7] (EC 2.7.2.1) uses acetyl phosphate, and nucleosidediphosphate kinase[7] (EC 2.7.4.6) uses ATP. We chose pyruvate kinase as kinase 2 because PEP is more stable than acetyl phosphate and pyruvate kinase is less expensive than nucleoside diphosphate kinase.[5]

The preparation of NDPs from NMPs is more difficult than the preparation of NTPs from NDPs. No one, stable, inexpensive enzyme is known that converts all of the NMPs to NDPs. We examined three commercially available kinases: adenylate kinase[8] (AdK, EC 2.7.4.3), guanylate kinase[7] (GK, EC 2.7.4.8), and nucleosidemonophosphate kinase[7] (NMPK, EC 2.7.4.4). In vivo, adenylate kinase phosphorylates AMP and guanylate kinase[7] phosphorylates GMP. Adenylate kinase also phosphorylates CMP at synthetically useful rates.[9,10] Two other specific

Scheme I.[a] Enzymatic Synthesis of Nucleoside Triphosphates



[a] (i) Phosphoglycerate mutase (EC, 2.7.5.3); (ii) enolase (EC 4.2.1.11); (iii) pyruvate kinase (EC 2.7.1.40); (iv) adenylate kinase (EC 2.7.4.3, X = A, C, U), guanylate kinase (EC 2.7.4.8, X = G) or nucleoside monophosphate kinase (EC 2.7.4.4, X = U). P = phosphate. Table I lists scales and yields.

kinases, cytidyl kinase (EC 2.7.4.14) and uridyl kinase[7] (EC 2.7.1.48) are not commercial products. Nucleosidemonophosphate kinase uses ATP to phosphorylate AMP, CMP, GMP, and UMP.[11]

A serious drawback to the use of NMPK is its instability and cost. Furthermore, preparations of NMPK are not homogeneous, and a mixture of kinases may actually be present. Because adenylate kinase is the least expensive and most stable of these three kinases, we tried to use it to phosphorylate UMP and GMP. We were able to convert UMP to UDP using adenylate kinase, but not GMP to GDP.

Many kinases use ATP as a phosphorylating agent. ATP usually is recycled from ADP by using pyruvate kinase and PEP[12,13] or acetate kinase and acetyl phosphate.[14] A recent review summarizes the relative merits of these two methods to regenerate ATP in organic synthesis.[5] PEP is more stable in solution than is acetyl phosphate and is thermodynamically a stronger phosphoryl donor.[5] Commercial PEP is, however, too expensive ($4800/mol) to use in reactions on a preparative scale (>50 mmol of PEP is required for the larger reactions described in this paper), so it must be synthesized in a separate step. For this work, we developed a convenient method (the PGA method, Scheme I) for the enzymatic synthesis of PEP from the relatively inexpensive D-(−)-3-phosphoglyceric acid (3-PGA, $250/mol).[15]

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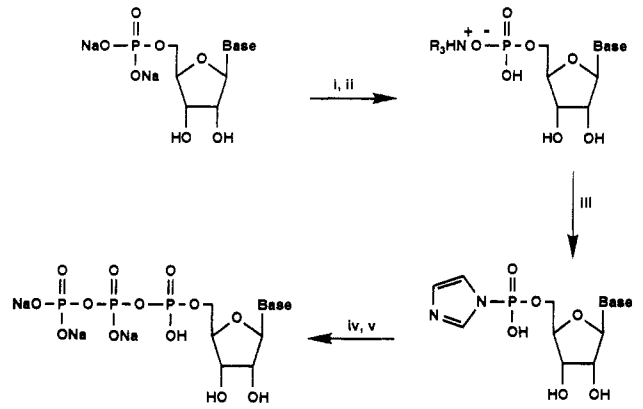
Scheme II.[a] Chemical Synthesis of Nucleoside Triphosphates Using the Carbonyldiimidazole Method



[a] (i) Dowex H^+; (ii) R_3N (R = $CH_3(CH_2)_7$ or $CH_3(CH_2)_3$), MeOH/EtOH; (iii) carbonyldiimidazole, CH_3CN, 25 °C, 1 day; (iv) $[CH_3(CH_2)_3]_3N^+H_4P_2O_7^-$, CH_3CN, 1 day; (v) $NaClO_4$, $(CH_3)_2CO$. Base = adenosine, cytidine, guanosine, or uridine. Table II lists scales and yields.

Methods of Chemical Synthesis. A large repertoire of chemical methods for the preparation of NTPs from NMPs is available.[16-20] We chose to activate the NMPs as nucleoside imidazolates[21-24] (using the carbonyldiimidazole method, Scheme II) because the reaction of NMPs with 1,1′-carbonyldiimidazole occurs under relatively mild conditions compared with other methods and does not require a purification step. Other methods of chemical synthesis may be equally satisfactory.

Results and Discussion

Enzymatic Synthesis. Table I summarizes the scales and yields for enzymatic syntheses.

CTP. Adenylate Kinase/Pyruvate Kinase Method. The AdK/PK method consistently produced ~25 mmol of CTP starting from 10 g of CMP, 2 equiv of PEP, and a catalytic amount of ATP.[9] For larger scale reactions (200 mmol), we found it more convenient, albeit more expensive,[25] to generate PEP in situ using the PGA method than to synthesize PEP in a separate chemical step.

The consumption of HCl during the course of the reaction provided a convenient way to monitor the progress of the reaction.[26] Analysis by ^{31}P NMR and ^1H NMR spectroscopy also allowed the conversion of CMP to CTP to be followed (Figure 1, C and D). Simple precipitation with ethanol (1:1, v/v) provides CTP (and the other NTPs) of sufficient purity for use in enzyme-catalyzed synthesis. Analysis by ^{31}P and ^1H NMR spectroscopy indicated that ~1% each of ATP, dipyruvate, 3-PGA, and ethanol were also present (Figure 1). If pure material were required, many purification methods based on ion-exchange chromatography exist (for examples, see the part of the Experimental Section describing chemical preparations of nucleoside triphosphates). Preparation of ATP in a similar manner using PEP generated in the reaction mixture from 3-PGA was also successful.

Table II. Scale and Yields for Chemical Synthesis of Nucleoside Triphosphates Using the Carbonyldiimidazole Method

NTP	amount, g (yield, %)[a]	NTP	amount, g (yield, %)[a]
ATP	4.1 (80)[b]	CTP	0.59 (73)[c]
GTP	4.2 (78)[b]	UTP	0.62 (76)[c]

[a] After ion-exchange chromatography. Purity >95% according to analysis by ^1H and ^{31}P NMR spectroscopy. [b] Tri-*n*-butylammonium salt. [c] Tri-*n*-octylammonium salt.

A catalytic amount of ATP or CTP was needed to initiate the reaction catalyzed by adenylate kinase (Scheme I). The subsequent reaction catalyzed by pyruvate kinase forms 1 equiv of CTP and regenerates either triphosphate. In the synthesis of CTP, we used a catalytic amount of ATP rather than CTP because ATP is less expensive than CTP and because the value of $k_{cat.}/K_m$ with pyruvate kinase for ADP (~1.4×10^6, pH 7.5) is greater than the corresponding value for CDP (~1.2×10^4, pH 7.5).[27] In practice, the reaction is indeed qualitatively faster when ATP rather than CTP initiates the reaction.

Two additional operational details are worth noting. First, we did not use the MEEC[28] technique because, on a 10-g scale, transport of the nucleoside phosphates across the regenerated cellulose acetate membrane proceeded at an inconveniently slow rate. Second, in early experiments, we added bovine serum albumin (BSA, 1–10 mg/mL) to stabilize the soluble enzymes,[29] but, based on qualitative observations, the presence of BSA is not necessary.

UTP. Nucleoside Monophosphate Kinase/Pyruvate Kinase Method. We made UTP using the NMPK/PK method, but NMPK deactivated rapidly. Others have noted the instability of NMPK and have increased its stability by immobilizing the enzyme.[30,31] The AdK/PK method yielded UTP in the presence of Mg^{2+} in 1-g scale experiments, but the rate of reaction was too slow to be useful on a 10-g scale unless large amounts of enzyme were used. We tried replacing Mg^{2+} with Mn^{2+} and mixtures of Mg^{2+} and Mn^{2+} but we did not observe a useful change in the rate of the reaction.

GTP. Guanylate Kinase/Pyruvate Kinase Method. We made GTP using either PEP or 3-PGA as the ultimate phosphoryl donor using guanylate kinase. We used less Mg^{2+} (~0.5 equiv) than in other experiments because GMP precipitated when more Mg^{2+} was added. The major impurity in the GTP produced enzymatically is GDP. GTP is unstable,[32] and we did observe decomposition of

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(25) Estimated cost (based on research-scale quantities of reagents from US Biochemical or Aldrich) for the chemical synthesis[13] of 1 mol of PEP from 2 mol of pyruvate, 2 mol of bromine, and 1.9 mol of trimethyl phosphite, $44; from 1 mol of 3-PGA ($250) using 5000 U each of phosphoglycerate mutase, enolase, and pyruvate kinase, $295. In the synthesis of CTP described in this paper, the amounts of enzymes were not optimized to minimize the cost; adenylate kinase is the limiting reagent so one would not need twice as much of the other enzymes to make 1 mol of PEP.
(26) The net change in charge of the species present explains the need for HCl: $ROPO_2^{2-} + 2CH_2{=}C(OPO_2^{2-})CO_2^- + 2H^+ \rightarrow ROPO_2OPO_2OPO_2^{4-} + 2CH_3C(O)CO_2^-$. This expression is only approximate because of the different values of pK_a of the reactants and products and because the reaction solutions usually are buffered.
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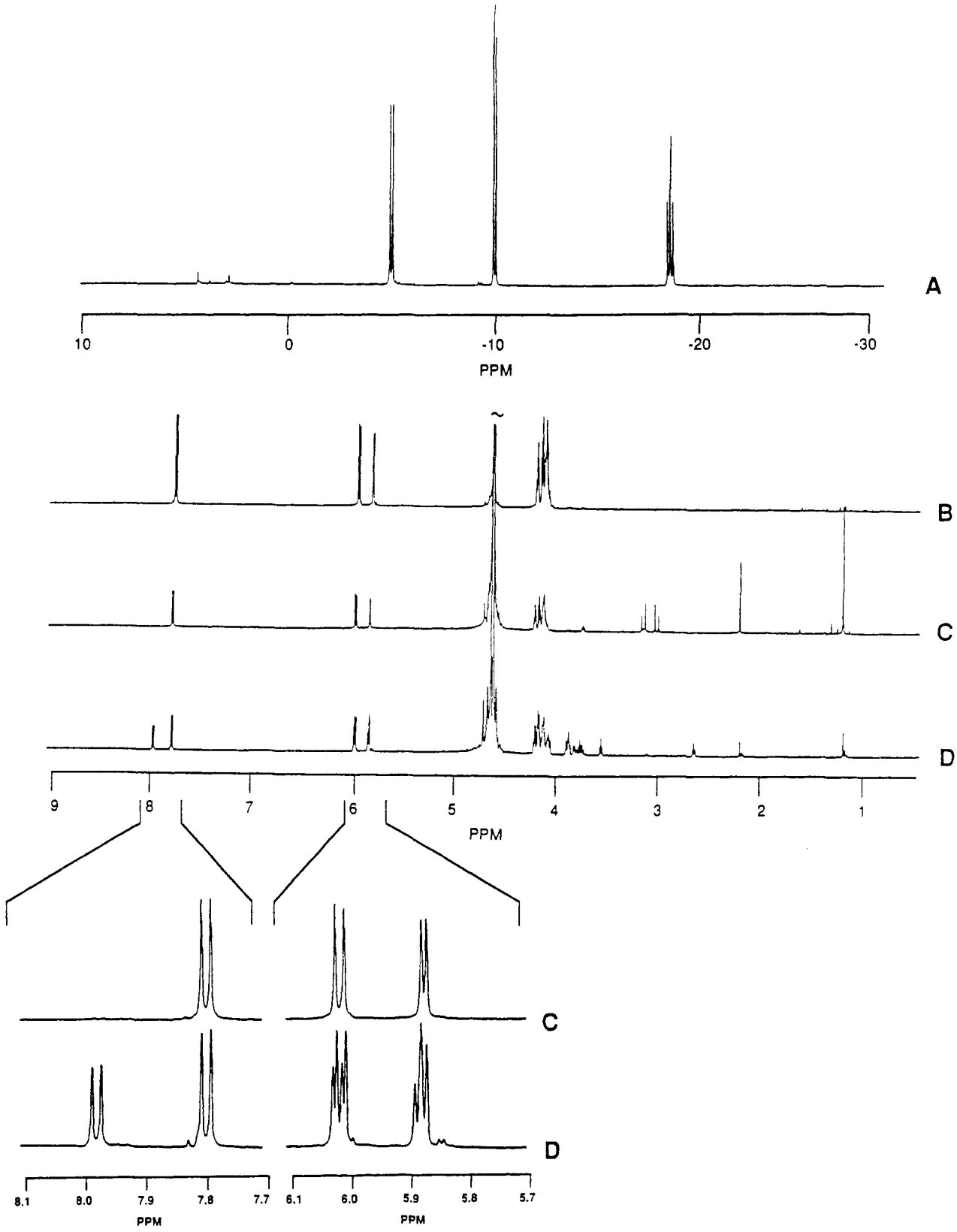

Figure 1. Reaction progress as determined by NMR spectroscopy for the synthesis of 0.2 mol of CTP from CMP and 3-PGA according to Scheme I. The solvent was D_2O; the large peak at 4.67 ppm in the ^1H NMR spectra (500 MHz) was due to DOH. (A) Decoupled ^{31}P NMR spectrum of product CTP after precipitation with EtOH/H_2O (1:1, v/v). (B) ^1H NMR spectrum of product CTP after precipitation with EtOH/H_2O (1:1, v/v). Most of the pyruvate, dipyruvate, and triethanolamine buffer present in the reaction mixture were removed. (C) ^1H NMR spectrum (and expansion) of the reaction mixture before precipitation of CTP with EtOH. Most of the CMP and 3-PGA originally present was converted to CTP and pyruvate (s, 2.2 ppm). Some dipyruvate (s, 1.2 ppm and 2 d, ~3 ppm) also formed. (D) ^1H NMR spectrum (and expansion) of the reaction mixture after 18 h at 58% conversion of CMP to CTP.

GTP to GDP (according to analysis by ^{31}P NMR) during workup. Further purification of GTP before use in enzyme-catalyzed synthesis is not necessary: we used GTP prepared from GMP to synthesize GDP-mannose (GDP-Man) in a reaction catalyzed by GDP-mannose pyrophosphorylase (GDP-Man PPase, EC 2.7.7.13) isolated

$$\text{GTP} + \alpha\text{-Man-1-P} \xrightarrow{\text{GDP-Man PPase}} \text{GDP-Man} + 2P_i \quad (2)$$

from brewers' yeast (eq 2).[33]

An effort to replace guanylate kinase with the less expensive adenylate kinase was not successful. We observed no production of GTP from GMP and ATP using the AdK/PK/PEP system in the presence of either Mg^{2+} or Mn^{2+}.

Chemical Synthesis. Carbonyldiimidazole Method. Following published procedures (Scheme II),[21-24] we prepared ATP, CTP, UTP, and GTP from the corresponding NMPs as tri-*n*-alkylammonium salts in quantities of 1–5 g (~1–10 mmol, ~75% yield) after ion-exchange chromatography[34] on DEAE-cellulose (Table II). Chromatography may not be necessary in certain applications: for example, unpurified UTP (containing pyrophosphate salts as the major contaminant) was used in the synthesis of UDP-glucose (UDP-Glc) in the presence of α-D-glucose 1-phosphate (Glc-1-P), uridine-5′-diphosphoglucose pyrophosphorylase (UDP-Glc PPase, EC 2.7.7.9), and inorganic pyrophosphatase (PPase, EC 3.6.1.1) (eq 3).[35] UDP-Glc dehydrogenase (UDP-Glc DH, EC 1.1.1.22) catalyzed the NAD^+-dependent oxidation of UDP-Glc to UDP-glucuronic acid (UDP-GlcUA);[36] a coupled reaction recycled NAD^+ using pyruvate and L-lactate dehydrogenase (LDH, EC 1.1.1.27) (eq 4).[5]

$$\text{UTP} + \alpha\text{-Glc-1-P} \xrightarrow[\text{PPase}]{\text{UDP-Glc PPase}} \text{UDP-Glc} + 2P_i \quad (3)$$

$$\text{UDP-Glc} \xrightarrow{\text{UDP-Glc DH}} \text{UDP-GlcUA} \quad (4)$$

$$\text{lactate} \xrightarrow[\text{NAD}^+]{\text{LDH}} \text{pyruvate}$$

Yields of the NTPs were approximately the same when either the tri-*n*-butylammonium salt or the tri-*n*-octylammonium salt of the NMPs were used. We used the tri-*n*-butylammonium salts for larger scale reactions because they are less expensive to prepare than the tri-*n*-octylammonium salts.

Several modifications of the published procedures improved the preparation of NTPs using the carbonyldiimidazole method. The use of acetonitrile rather than dimethylformamide as the solvent simplified the workup, because acetonitrile is the more easily removed by evaporation. We found that only 4 molar equiv of 1,1′-carbonyldiimidazole was required per equiv of NMP, rather than the reported five;[24] using fewer than 4 equiv resulted in lower yields. Expensive, crystallized tri-*n*-butylammonium pyrophosphate is not required; we prepared a 1 M solution of this reagent *without* using ion-exchange chromatography simply by dissolving anhydrous pyrophosphoric acid and tri-*n*-butylamine in acetonitrile.

UTP. Deamination of CTP. Chemical deamination of CTP to UTP at 4 °C using sodium nitrite in aqueous acetic acid converted CTP to UTP (eq 5).[37,38] We noted some decomposition of UTP to UDP and UMP according to analysis by thin-layer chromatography when the deamination was performed at room temperature.

$$\text{CTP} \xrightarrow[\text{AcOH, 4 °C}]{\text{NaNO}_2} \text{UTP} \quad (5)$$

This method is more convenient than the enzymatic synthesis of UTP from UMP. UTP obtained by this deamination route was used to produce UDP-glucose using UDP-Glc PPase (eq 3). The successful synthesis of UDP-Glc using UTP prepared by this procedure establishes that any NO_2^- carried through the purification does not deactivate UDP-Glc PPase (or, we presume, other enzymes).

Because NMPK is expensive and CTP easily obtained, we also examined the enzymatic deamination of CTP to UTP.[39] In model systems, deamination of CMP to UMP with adenosine deaminase (EC 3.5.4.4) or with 5′-adenylic acid deaminase (EC 3.5.4.6) was not successful. We did not try to deaminate CTP using these enzymes.

Techniques for Monitoring Reactions. Several techniques are useful for monitoring the synthesis of NTPs. Thin-layer chromatography on PEI-cellulose is a particularly convenient analytical method.[31,32] The characteristic chemical shifts and coupling patterns in the phosphorus and proton NMR spectra also allow quantitative analysis of the course of the reactions. Several methods using HPLC have also been described.[6,31,40]

Purification. Many reports describe the purification of NTPs by ion-exchange chromatography.[34,41] We did not require analytically pure material and simply precipitated the NTPs by adding ethanol (1:1, v/v) following the enzyme-catalyzed reactions. Pyruvate and contaminating inorganic and buffer salts do not precipitate to a significant extent under these conditions.[42] The NTPs obtained in this way can be used in enzyme-catalyzed reactions without further purification. If pure material is desired, this initial precipitation step simplifies purification by ion-exchange chromatography.

Manipulation of Enzymes. The intent of this study was to develop methods to synthesize the NTPs that could be performed conveniently in organic chemistry laboratories. The enzymes were treated as off-the-shelf reagents and were neither assayed nor immobilized (although all of the enzymes used have been immobilized in other work).[30,31,43] We note that soluble enzymes can be recovered by ultrafiltration.[44] We did add an antioxidant (2-mercaptoethanol or dithiothreitol) and performed the reactions in an atmosphere of nitrogen because most of the enzymes used have air-sensitive thiol groups. We prefered to use enzymes obtained as lyophilized powders to avoid

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the precipitation of magnesium ammonium phosphate salts that often occurs when suspensions in ammonium sulfate are used.

Conclusion

Methods primarily based on enzymatic synthesis rather than on chemical synthesis are most convenient for the synthesis of CTP and GTP (eq 6 and eq 7).[45] UTP is best

$$\text{CMP} \xrightarrow[\text{PEP}]{\text{AdK}} \text{CTP} \xrightarrow[\text{AcOH}]{\text{NaNO}_2} \text{UTP} \quad (6)$$

$$\text{GMP} \xrightarrow[\text{PEP}]{\text{GK}} \text{GTP} \quad (7)$$

synthesized from CMP by using a two-step procedure involving both enzymatic and nonenzymatic steps (eq 6). The most convenient preparation of PEP for these reactions is that based on in situ conversion of 3-PGA. Simple precipitation of the NTPs with ethanol yields material of sufficient purity for use in enzyme-catalyzed synthesis. A summary of the best method to make each NTP is thus: CTP, adenylate kinase/PGA method; GTP, guanylate kinase/PGA method; UTP, deamination of CTP with $NaNO_2$/AcOH.

Experimental Section

Materials and Methods. Adenosine deaminase (from calf intestinal mucosa, EC 3.5.4.4), adenylate kinase (from chicken muscle, EC 2.7.4.3), 5'-adenylic acid deaminase (from *Aspergillus sp.*, EC 3.5.4.6), enolase (from bakers' yeast, EC 4.2.1.11), guanylate kinase (from bovine brain, EC 2.7.4.8), inorganic pyrophosphatase (from bakers' yeast, EC 3.6.1.1), nucleosidemonophosphate kinase (from bovine liver EC 2.7.4.4), pyruvate kinase (from rabbit muscle, EC 2.7.1.40), and uridine-5'-diphosphoglucose pyrophosphorylase (from bakers' yeast, EC 2.7.7.9) were lyophilized powders from Sigma. Alkaline phosphatase (from *Escherichia coli*, EC 3.1.3.1), phosphoglycerate mutase (from rabbit muscle, EC 2.7.5.3), and L-lactate dehydrogenase (from rabbit muscle, EC 1.1.1.27) were crystalline suspensions in solutions of ammonium sulfate from Sigma. GDP-mannose pyrophosphorylase (EC 2.7.7.13) was isolated from brewers' yeast (US Biochemical)[33] and UDP-glucuronic pyrophosphorylase (EC 1.1.1.22)[36] was isolated from calf liver acetone powder (Sigma). Commercial enzymes were not assayed; the activities stated by the manufacturer are reported here (1 unit (U) converts 1 μmol of substrate to products per minute under assay conditions). Ion-exchange resin (Dowex 50W-X8, H^+ form, 20–50 mesh, unless noted otherwise) was from Bio-Rad. Chemicals and solvents were reagent grade and were used without further purification, unless noted. Water was distilled from glass in a Corning AG-1b still. CMP (free acid) and GMP (sodium salt) were each obtained in 1-kg quantity from Miwon Foods Co., Ltd., Seoul, Korea. The sodium salt of AMP was obtained in 1-kg quantity from Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan. D-(−)-3-Phosphoglyceric acid (3-PGA) was purchased as the barium salt from either US Biochemical Co., ICN Biochemicals, or Sigma. The potassium salt of PEP was synthesized from pyruvic acid as described.[13] The ^1H and ^{31}P NMR spectra of the nucleoside triphosphates and nucleoside diphosphate sugars (UDP-Glc, UDP-GlcUA, GDP-Man) were in accord with those of commercial samples; the products coeluted with authentic compounds when analyzed by thin-layer chromatography. The yields of nucleoside triphosphates were determined by enzymatic assay.[46] Reactions were conducted at room temperature (~22–24 °C) unless noted otherwise. Addition of solutions of HCl (contained in a buret) by a peristaltic pump driven by a pH controller maintained the pH of reaction mixtures in the ranges stated. Polyethylenimine-cellulose plates for thin-layer chromatography were from Aldrich.

Cytidine 5'-Triphosphate (AdK/PK/PGA Method). A suspension of 173 g of 3-PGA (barium salt, dihydrate, ~95%, 461 mmol) in 500 mL of water was vigorously stirred with ~600 mL of ion-exchange resin (H^+ form) for 30 min. The resin was removed by filtration and washed four times with 100-mL portions of water. The combined, clear, pale-yellow filtrates were neutralized with solid KOH and used directly in the next step.

CMP (free acid, 71 g, 220 mmol), ATP·Na_2·$3H_2O$ (1.33 g, 2.20 mmol), $MgCl_2$·$6H_2O$ (51 g, 250 mmol), and triethanolamine (1.9 g, 10 mmol) were added to the solution of 3-PGA and the pH was adjusted to pH 7.6 by addition of 5 M KOH. The solution (total volume of 1 L) was deaerated for 30 min with nitrogen; 2-mercaptoethanol (0.25 mL, 3 mmol) was added. Adenylate kinase (10 000 U), pyruvate kinase (5000 U), enolase (4000 U), and phosphoglycerate mutase (5000 U) were then added and the solution was stirred under a positive pressure of nitrogen. Addition of 3 M HCl maintained the pH at 7.5–7.8 during the course of the reaction. After 48 h, consumption of HCl had ceased but analysis by ^1H NMR spectroscopy indicated that the reaction was not complete. An additional 32 g of 3-PGA (barium salt, 84 mmol) was stirred with 120 mL of ion-exchange resin (H^+ form), filtered, and washed, and the pH of the solution was adjusted to pH 7.6 as described above. This solution of 3-PGA was deaerated and added to the reaction mixture.[47] After stirring for an additional 24 h, analysis by ^1H NMR spectroscopy indicated that the reaction was complete. A total of 130 mL of 3 M HCl had been consumed. The total amount of 3-PGA (barium salt) added was 205 g (544 mmol).

For isolation of CTP, ~150-mL portions of the clear, pale-yellow reaction mixture were transferred into twelve 500-mL polypropylene centrifuge tubes and 150 mL of absolute ethanol was added to each tube. A white precipitate formed immediately. The tubes were cooled in an ice bath for 15 min (final temperature of the ethanol–water solutions was 6 °C) and then centrifuged (4 °C, 10000*g*, 10 min). The supernatants were decanted and the combined, sticky pellets were dissolved in a total of 600 mL of water. The resulting solution was divided equally among six 250-mL centrifuge tubes and 100 mL of absolute ethanol was added to each tube. The tubes were cooled and centrifuged as above. Lyophilization of the combined pellets provided 145 g of an off-white powder containing 202 mmol of CTP (92% yield based on CMP; 90% purity for CTP·K_3); the water content (7.14%) was determined by the Karl Fischer method. Thin-layer chromatography (polyethylenimine-cellulose; eluant,[34] 2.0 M HCOOH/2.0 M LiCl, 1:1, v/v) indicated that ATP was present in addition to CTP; neither CMP nor CDP were detected. Analysis by ^1H and ^{31}P NMR spectroscopy indicated that ~1% each of ATP, dipyruvate, 3-PGA, inorganic phosphate, and ethanol were present.

Guanosine 5'-Triphosphate (GK/PK/PGA Method). A suspension of 17 g of 3-PGA (barium salt, dihydrate, ~95%, 52 mmol) in 100 mL of water was stirred with ~100 mL of ion-exchange resin (Na^+ form) for 40 min. Most of the material dissolved, but a fine, silky, white suspension remained. The resin and suspension were removed by filtration and washed twice with 50-mL portions of water. The combined, clear filtrates were used directly in the next step.

GMP·Na_2·$3H_2O$ (10 g, 22 mmol), ATP·Na_2·$3H_2O$ (250 mg, 0.4 mmol), $MgCl_2$·$6H_2O$ (1.5 g, 7.4 mmol), KCl (1.6 g, 22 mmol), and 50 mL of a 0.1 M solution of Tris buffer (pH 7.6) were added to the solution of 3-PGA. A white precipitate formed when magnesium ions were added, but it did not interfere with the reaction. The pH of the solution was adjusted to pH 7.6 by addition of 5 M NaOH, the volume was adjusted to 300 mL with water, and

(45) The estimated costs (based on research-scale quantities from US Biochemical or Aldrich) of the phosphorylating reagents required to convert 1 mol of a nucleoside monophosphate to the triphosphate according to the methods presented are comparable: based on 2.5 mol of 3-PGA, $738 (see footnote 25); based on 4 mol of carbonyl diimidazole ($700) and 4 mol of pyrophosphoric acid ($64), $764. This comparison does not account for costs of solvents and their disposal. In practice, if economic factors rather than convenience were the most important consideration, the phosphorylating reagents would be synthesized from inexpensive precursors in each case.

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the solution was deaerated for 30 min with nitrogen. Guanylate kinase (10 U), pyruvate kinase (1000 U), enolase (500 U), and phosphoglycerate mutase (1000 U) were then added and the solution was stirred under a positive pressure of nitrogen. Addition of 1 M HCl maintained the pH at 7.5–7.7 during the course of the reaction. After 3 days, 48.6 mL of HCl had been consumed and analysis by thin-layer chromatography (polyethyleniminecellulose; eluant, 1.0 M LiCl/0.5 M $(NH_4)_2SO_4$, 1:1, v/v) and ^{31}P NMR spectroscopy indicated that the reaction was >95% complete.

For isolation of GTP, 350 mL of absolute ethanol was added to the solution (350 mL). The resulting precipitate was collected by centrifugation (10000*g*, 10 min) and was dissolved in 300 mL of water. Additional ethanol (300 mL) was added and the centrifugation step was repeated. Lyophilization of the pellet provided 12 g of a white powder containing 18 mmol of GTP (82% yield) according to enzymatic analysis (88% purity for GTP·Na_3). According to analysis by ^{31}P NMR spectroscopy, some GDP formed during workup.

Guanosine 5′-Triphosphate (GK/PK/PEP Method). The execution of this reaction was similar to the previous one. Pyruvate kinase (1000 U) and guanylate kinase (10 U) were added to a solution of GMP·Na_2·$3H_2O$ (10 g, 22 mmol), PEP (5.4 g, 26 mmol), ATP·Na_2·$3H_2O$ (130 mg, 0.2 mmol), $MgCl_2$·$6H_2O$ (1.0 g, 4.9 mmol), and KCl (1.64 g, 22 mmol) in 300 mL of a 0.1 M solution of Tris buffer (pH 7.5). After 1 day an additional 5.4 g of PEP was added and after 2 days more $MgCl_2$·$6H_2O$ (1.0 g) was added. After 3 days, 25.7 mL of HCl had been consumed and analysis by ^1H NMR spectroscopy indicated that the conversion of GMP to GTP was complete.

Isolation of GTP by precipitation with ethanol as described above provided 12 g of a white powder containing 19 mmol of GTP (86% yield) according to enzymatic analysis (93% purity for GTP·Na_3).

Guanosine 5′-Triphosphate (AdK/PK/PEP/Mn^{2+} Attempts). Adenylate kinase (1000 U) and pyruvate kinase (1000 U) were added to a solution of 1.00 g of GMP·Na_2·$3H_2O$ (2.17 mmol), 100 mg of ATP·Na_2·$3H_2O$ (0.2 mmol), 1.1 g of PEP·K^+ (5.2 mmol), 250 mg of $MnCl_2$·$4H_2O$ (1.3 mmol), and 23 mg of dithiothreitol in 50 mL of 0.1 M solution of Tris buffer (pH 7.7). Analysis by thin-layer chromatography (polyethylenimine-cellulose; eluant,[34] 2.0 M HCOOH–2.0 M LiCl, 1:1, v/v) indicated no conversion of GMP to GTP within 3 days.

Guanosine 5′-Diphosphate (AdK/ATP/Mn^{2+}–Mg^{2+} Attempts). A solution of 100 mg of GMP·Na_2·$3H_2O$ (0.22 mmol), 100 mg of ATP·Na_2·$3H_2O$ (0.17 mmol), 50 mg of $MnCl_2$·$4H_2O$ (0.25 mmol), 50 mg of $MgCl_2$·$6H_2O$ (0.25 mmol), and 1 mg of dithiothreitol in 15 mL of a 0.1 M solution of Tris buffer (adjusted to pH 8) was deaerated with nitrogen and adenylate kinase (100 U) was added. Analysis by thin-layer chromatography as in the preceding experiment indicated no formation of GDP within 3 days.

Uridine 5′-Triphosphate (NMPK/PK/PGA Method). The reaction was performed as described for GTP using the GK/PK/PGA method. The initial solution contained UMP·Na_2·$2.5H_2O$ (10.0 g, 24 mmol), 3-PGA (19 g, 58 mmol, of the barium salt was converted to the sodium form), ATP·Na_2·$3H_2O$ (150 mg, 0.24 mmol), $MgCl_2$·$6H_2O$ (5.1 g, 25 mmol), KCl (1.9 g, 25 mmol), Tris-HCl (3.15 g, 20 mmol), 2-mercaptoethanol (0.1 mL), nucleosidemonophosphate kinase (8 U), pyruvate kinase (1000 U), enolase (500 U), and phosphoglycerate mutase (1000 U) in a total volume of 200 mL of water (pH 7.6). Additional nucleosidemonophosphate kinase (8 U) was added after 5 days and after 6 days additional pyruvate kinase (1000 U), enolase (500 U), and phosphoglycerate mutase (1000 U) were added. After 8 days, 29.3 mL of 1 M HCl had been consumed and the reaction was stopped even though it was not complete. Precipitation of UTP with ethanol as described above provided 10.5 g of a white powder containing 14 mmol of UTP (58% yield) according to enzymatic analysis (73% purity for UTP·Na_3).

Uridine 5′-Triphosphate (NMPK/PK/PEP Method). The reaction was performed as described for GTP using the GK/PK/PEP method. The reaction solution contained UMP·Na_2·$2.5H_2O$ (5.0 g, 12 mmol), PEP (6.0 g, 29 mmol), ATP·Na_2·$3H_2O$ (73 mg, 0.12 mmol), $MgCl_2$·$6H_2O$ (2.5 g, 12 mmol), dithiothreitol (46 mg), nucleosidemonophosphate kinase (8 U), and pyruvate kinase (1000 U) in a total volume of 100 mL of 0.1 M solution of Tris buffer (pH 7.6). After 24 h, 21.4 mL of 1 M HCl had been consumed and analysis by ^{31}P NMR indicated that the reaction was complete. Precipitation of UTP with ethanol as described above provided 6.3 g of a white powder containing 11 mmol of UTP (92% yield) according to enzymatic analysis (>95% purity for UTP·Na_3).

Uridine 5′-Triphosphate (AdK/PK/PGA Method). The reaction was performed as described for GTP using the GK/PK/PGA method. The initial solution contained UMP·Na_2·$2.5H_2O$ (10.0 g, 24 mmol), 3-PGA (19 g, 58 mmol, of the barium salt was converted to the sodium form), ATP·Na_2·$3H_2O$ (150 mg, 0.24 mmol), $MgCl_2$·$6H_2O$ (5.1 g, 25 mmol), KCl (1.9 g, 25 mmol), Tris-HCl (3.15 g, 20 mmol), dithiothreitol (100 mg), adenylate kinase (10 000 U), pyruvate kinase (1000 U), enolase (500 U), and phosphoglycerate mutase (1000 U) in a total volume of 200 mL of water (pH 7.7). After 5 days, 36.0 mL of 1 M HCl had been consumed. Precipitation of UTP with ethanol as described above provided 12 g of a white powder containing 22 mmol of UTP (92% yield, >95% purity for UTP·Na_3).

Adenosine 5′-Triphosphate (AdK/PK/PGA Method). The reaction was performed as described for GTP using the GK/PK/PGA method. The initial solution contained AMP·Na_2·H_2O (8.2 g, 20 mmol), 3-PGA (16 g, 44 mmol, of the barium salt was converted to the sodium form), ATP·Na_2·$3H_2O$ (100 mg, 0.17 mmol), $MgCl_2$·$6H_2O$ (4.0 g, 20 mmol), KCl (1.5 g, 20 mmol), Tris-HCl (400 mg), dithiothreitol (100 mg), adenylate kinase (1000 U), pyruvate kinase (1000 U), enolase (500 U), and phosphoglycerate mutase (1000 U) in a total volume of 100 mL of water (pH 7.7). After 1 day, 29.6 mL of 1 M HCl had been consumed. Precipitation of ATP with ethanol as described above provided 9 g of a white powder containing 16 mmol of ATP (80% yield) according to enzymatic analysis (>95% purity for ATP·Na_3).

Chemical Syntheses; General Procedures. Free Acids of Nucleoside Monophosphates. Each nucleoside monophosphate disodium salt (1.0 mmol) was dissolved in 25 mL of water and stirred with 5 mL of ion-exchange resin (Dowex 50W-X8, H^+ form, 50–100 mesh) for 1 h. The solution was decanted and the resin was washed 5 times with 50-mL portions of water. Rotary evaporation of the combined aqueous solutions at reduced pressure provided the free acids as amorphous powders.

Tri-*n*-butylammonium Salts of Nucleoside Monophosphates. The free acid of a nucleoside monophosphate (1.0 mmol) was suspended in a mixture of 10 mL of MeOH and 10 mL of EtOH, tri-*n*-butylamine (185 mg, 1.0 mmol) was added, and the reaction mixture was refluxed until the solid dissolved (~1 h). The solution was cooled and evaporated. The residue was dried by repeated addition and evaporation of 10 mL of dioxane. The salt was obtained in quantitative yield after further drying at ~0.1 Torr over $CaSO_4$.

Standard Solution of Tri-*n*-butylammonium Pyrophosphate. A suspension of anhydrous pyrophosphoric acid (17.8 g, 0.10 mol) in 60 mL of acetonitrile in a 100-mL volumetric flask was cooled in an ice bath and tri-*n*-butylamine (18.5 g, 0.10 mmol) was added. Once the solid dissolved (~1 h), the solution was allowed to warm to room temperature. Addition of acetonitrile to a final volume of 100 mL provided a 1.0 M standard solution of tri-*n*-butylammonium pyrophosphate.

Preparation of Nucleoside Triphosphates. The following reaction was performed under an atmosphere of argon. The nucleoside monophosphate tri-*n*-butylammonium salt (1 mmol) and carbonyldiimidazole (4 mmol) were placed in a flame-dried flask sealed with a silicone septum. Acetonitrile (20 mL) was added and the reaction mixture was stirred for 1 day. MeOH (3 mmol) was then added. After 30 min, an aliquot of the standard pyrophosphate solution (4 mL, 4 mmol) was added. After 1 day, the solvent was removed by rotary evaporation at reduced pressure and the residue treated with 20 mL of MeOH. The resulting precipitate was removed by filtration and washed with ~10 mL of MeOH. The combined solutions were concentrated to ~25 mL and a saturated solution of $NaClO_4$ in acetone was added (~20 mL) followed by diethyl ether (5 mL). The resulting precipitate contains the sodium salts of the nucleoside triphosphate and pyrophosphoric acid. In the case of UTP, this mixture was used directly in the synthesis of UDP-Glc. The nucleoside triphosphates were purified by anion-exchange chromatography

(DEAE cellulose; eluant, a gradient of triethylammonium bicarbonate, 0.1 to 0.4 M in 3 L, pH 7.5). Triethylammonium bicarbonate was removed by repeated addition and evaporation of ethanol. The triethylammonium salts were dissolved in a small amount of methanol (~20 mL) and 10 mL of a saturated solution of $NaClO_4$ in acetone was added to obtain the sodium salt. The precipitated sodium salt was collected by filtration and dried in vacuo over $CaSO_4$. Table II records the yields obtained for ATP, CTP, UTP, and GTP.

Uridine 5'-Triphosphate (Chemical Deamination Method). Solutions in this experiments were made by using cold (4 °C) water. The reaction was performed at 4 °C. To a solution of CTP (10 g, 14 mmol, prepared using adenylate kinase as described above) in 50 mL of water, was added a solution of $NaNO_2$ (15 g, 217 mmol) in 40 mL of cold water. A solution of 1:1 (v/v) acetic acid/water (30 mL) was added and the solution was stirred. After 48 h, 150 mL of ethanol was added and the resulting precipitate was collected by centrifugation (5000*g*, 10 min) and redissolved in 100 mL of water. The precipitation step with ethanol (100 mL) was repeated, the precipitate obtained after centrifugation was dissolved in 25 mL of water, and the pH of the solution was adjusted to pH 7.5 with 5 M NaOH. Lyophilization of this solution provided 7.8 g of a white powder containing 14 mmol of UTP (>95% yield) according to enzymatic analysis (>95% purity for $UTP{\cdot}Na_3$).

UDP-glucose. UTP synthesized by chemical deamination of CTP and by chemical synthesis (before purification by ion-exchange chromatography) was transformed into UDP-glucose according to the following general procedure. A solution of UTP (100 mg) and α-D-glucose 1-phosphate (100 mg, disodium salt, excess) in 5 mL of a 0.1 M solution of Tris buffer (pH 7.6, 4 mmol of $MgCl_2$) was placed in a 10-mm NMR tube containing 1 mL of D_2O and the solution was deaerated with argon for at least 10 min. A ^{31}P NMR spectrum was recorded, and 10–50 U of uridine-5'-diphosphoglucose pyrophosphorylase and 10–50 U of inorganic pyrophosphatase were added. Analysis by ^{31}P NMR spectroscopy indicated the formation of UDP-Glc (confirmed by comparison with an authentic sample) and inorganic phosphate and the disappearance of UTP and some of the Glc-1-P.

UDP-glucuronic Acid. UDP-glucose dehydrogenase was isolated from calf liver acetone powder according to the first five steps of the procedure described by Strominger et al.[36,48] A mixture of UDP-Glc DH (10 U), UDP-Glc (60 mg, 0.1 mmol), NAD^+ (15 mg, 0.02 mmol), pyruvate (15 mg, 0.15 mmol), and L-LDH (100 U) was dissolved in 4 mL of a 0.05 M solution of Tris buffer (pH 7.5). The solution was adjusted to pH 8, flushed with argon, and stirred at room temperature. Addition of 0.5 M NaOH maintained the pH at 7.5 to 8.2 during the course of the reaction. The progress of the reaction was monitored by thin-layer chromatography (polyethylenimine-cellulose; eluant,[34] 1 M AcOH/4 M LiCl, 8:2, v/v). After 1 day, most of the UDP-Glc had been converted to UDP-GlcUA. The reaction mixture was applied to a column of ion-exchange resin (AG 1-X2, bicarbonate form, 200–400 mesh, 1.5 cm × 4 cm). The column was washed with 25 mL of water and 25 mL of 0.25 M NH_4HCO_3 and then eluted with a 0.5 to 1 M gradient of NH_4HCO_3. The fractions containing UDP-GlcUA were pooled and concentrated to give a white powder. Excess ammonium bicarbonate was removed by dissolving the powder in 5 mL of water and adding ion-exchange resin (Dowex 50W-X8, 20–50 mesh, H^+ form) to the stirred solution until the pH was 7. The solution was filtered and lyophilized to give 41 mg of UDP-GlcUA (68% yield).

GDP-mannose. An extract of GDP-mannose pyrophosphorylase was prepared from 40 g of brewers' yeast according to the method of Munch-Peterson[33] and dissolved in 5 mL of a 0.05 M solution of Tris buffer (pH 7.5). To this solution were added 0.05 mL of a 1 M solution of sodium fluoride, 0.45 mL of a 0.1 M solution of EDTA, 92 mg of $MgCl_2{\cdot}6H_2O$ (0.45 mmol), followed by a solution of 51 mg of α-mannose 1-phosphate (dicyclohexylammonium salt, 0.10 mmol),[49] and 140 mg of GTP (0.24 mmol) in 1 mL of a 0.05 M solution of Tris buffer (pH 7.5). The reaction mixture was stirred at room temperature and monitored by thin-layer chromatography (polyethylenimine-cellulose; eluant,[34] 1 M AcOH/3 M LiCl, 9:1, v/v). After 24 h, the reaction flask was placed in a bath of boiling water for 1.5 min and the resulting precipitate was removed by centrifugation (7000*g*, 10 min). The supernatant was stirred with 150 U of alkaline phosphatase at 37 °C for 90 min[50,51] and then applied to a column of ion-exchange resin (Dowex 1X2-400, formate form, 200–400 mesh, 1 cm × 25 cm). The column was washed with 150 mL of water and 500 mL of 2 M formic acid. The eluants were discarded. Elution of the column with 4 M formic acid (450–500 mL) and lyophilization of the eluant gave a residue containing GDP-Man. The residue was dissolved in 10 mL of water and stirred for 10 min with ~1 g of ion-exchange resin (Dowex 50W-X8, H^+ form). The solution was decanted and the resin was washed three times with 10-mL portions of water. The pH of the combined solutions was adjusted to pH 8.0 with a 1 M solution of NaOH. Lyophilization of the resulting solution gave 36 mg of GDP-Man (0.055 mmol, 54% yield).

Acknowledgment. This work was supported by NIH Grant GM 30367. Dr. David Anton (E. I. DuPont de Nemours and Co., Experimental Station) suggested investigating procedures for the chemical deamination of CTP to UTP. Mr. M. J. Kim (Chief of Trade Section, Miwon Foods Co., Ltd., Seoul, Korea) kindly supplied CMP and GMP.

Registry No. 3-PGA·Ba·$2H_2O$, 86879-11-0; CMP, 63-37-6; $CMP{\cdot}Bu_3N$, 51450-21-6; GMP, 85-32-5; GMP-2Na, 5550-12-9; $GMP{\cdot}Bu_3N$, 75252-11-8; UMP, 58-97-9; UMP·2Na, 3387-36-8; $UMP{\cdot}Bu_3N$, 87713-40-4; AMP, 61-19-8; AMP·2Na, 4578-31-8; $AMP{\cdot}Bu_3N$, 59618-80-3; $ATP{\cdot}2Na{\cdot}3H_2O$, 51963-61-2; ATP·3Na, 20978-32-9; CTP·3K, 124992-46-7; CTP·3Na, 54619-78-2; GTP·3Na, 36051-31-7; UTP·3Na, 19817-92-6; PEP, 138-08-9; UDP-Glc, 133-89-1; UDP-GlcUA, 2616-64-0; GDP-Man, 3123-67-9; Glc-1-P·2Na, 56401-20-8; $Man\text{-}1\text{-}P{\cdot}(C_6H_{11})_2NH$, 72946-45-3; tributylammonium pyrophosphate, 50859-18-2.

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