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Synthesis of Cytidine, Uridine, and Adenosine 5′-(2,6-Diamino-2,6-dideoxy-α-D-glucopyranosyl diphosphates) (Neosamine C-CDP, -UDP, and -ADP)

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Three potential biosynthetic intermediates for aminocyclitol antibiotics, neosamine C-CDP, -UDP, and -ADP, have been synthesized from D-glucosamine by chemical means. 2,6-Diamino-2,6-dideoxy-α-D-glucopyranosyl phosphate, derived from the corresponding glycosyl bromide, was condensed with three nucleosides (CMP, UMP and AMP) by a known procedure. The configurations of the neosamine C nucleoside diphosphates have been confirmed by ^{13}C NMR spectra.

In the last decade, the biosynthesis of aminocyclitol antibiotics (neomycin, gentamicin, spectinomycin, streptomycin, and related compounds) has been investigated actively in several laboratories.[1)] Of the subunits of the antibiotics, the amino sugars and aminocyclitols are constructed from rather simple sugars (usually, D-glucosamine and D-glucose) as primary metabolites. These results were obtained largely by incorporation experiments involving both radioisotope-labeled and stable isotope-labeled sugars. However, the reactions involved in bond-formation between the amino sugars and the aminocyclitols are far less understood. Thus, while it is well known that D-glucosamine is incorporated into neomycin, there are two possible pathways for this incorporation (Scheme 1). In one pathway neosamine C (2,6-diamino-2,6-dideoxy-D-glucose) is converted to a nucleoside diphosphate and attached directly to deoxystreptamine to give neamine. In the second pathway D-glucosamine is converted to a nucleoside diphosphate, then attached to deoxystreptamine to give paromamine, which is subsequently aminated at C-6″ to give neamine, In both cases, an amino sugar serves as a glycosyl donor from its nucleoside diphosphate for transfer to the aminocyclitol moiety.

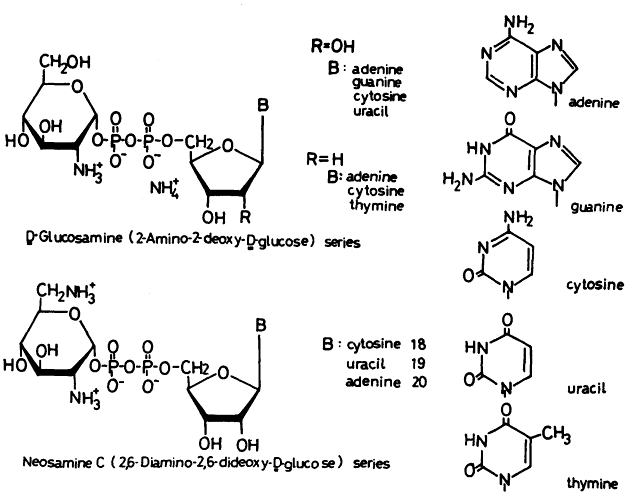
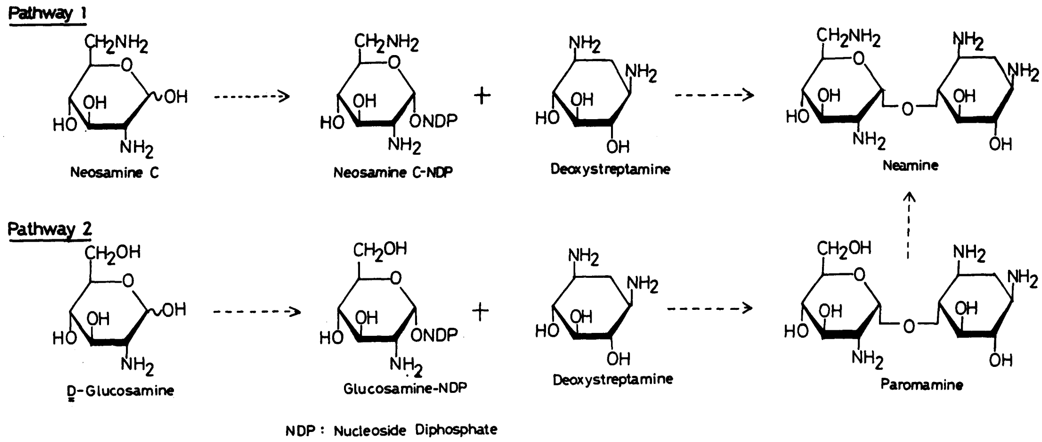


Fig. 1.

A large number of sugar nucleoside diphosphates have been identified in nature[2)] (in higher plants, microorganisms, *etc.*) and many of the naturally occurring sugar nucleoside diphosphates are thought to be glycosyl donors in the formation of oligo- and polysaccharides in living systems.[3)] A number of naturally occurring sugar nucleoside diphosphates possess *N*-acetyl-D-glucosamine as the amino sugar[4)] and nucleoside diphosphates of *N*-acetylglucosamine have been synthesized not only for biosynthetic research, but also for the elucidation of the reactivity of sugar phosphate esters. For example, Jeanloz *et al.*,[5)] recently synthesized uridine 5′-(2-acetamido-2-deoxy-α-D-mannopyranosyluronic acid di-



NDP : Nucleoside Diphosphate

Scheme 1.

phosphate), which is considered to be a glycosyl donor to lipids in *Micrococcus lysodeikticus*. To our best knowledge, however, only one report[6] has appeared on chemical (rather than enzymatic) synthesis of a nucleoside diphosphate containing free D-glucosamine (or other free amino sugar) as the sugar moiety.

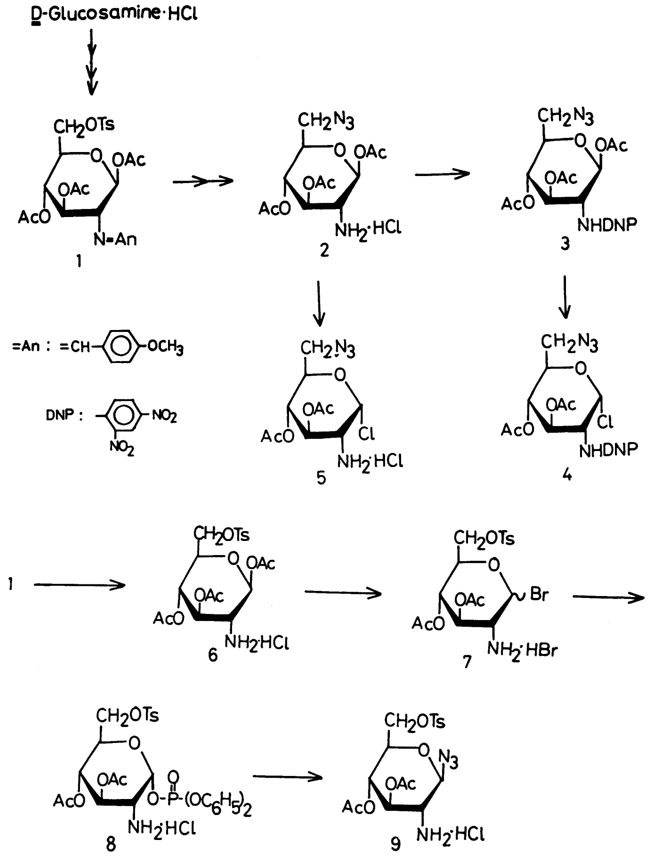
In order to investigate the second possibility, we recently synthesized[7] seven nucleoside diphosphates of glucosamine, namely, dTDP-, UDP-, CDP-, ADP-, GDP-, dCDP-, and dADP-glucosamines (Fig. 1) by chemical means and used the same procedure to synthesize several nucleoside diphosphates of D-[1-^{3}H]-glucosamine from the labeled D-glucosamine.

In the present communication, we describe the synthesis of three neosamine C nucleoside diphosphates, namely, cytidine-, uridine-, and adenosine-5′-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl diphosphates, **18**—**20**, respectively, compounds which can be employed in examining the first biosynthetic pathway of Scheme 1, *i.e.*, that neosamine is transferred directly to deoxystreptamine.

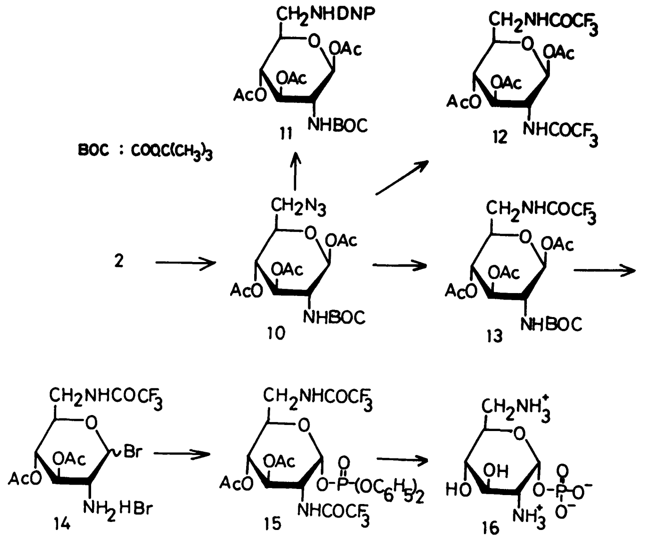
Initially, we examined the stability of a protected D-glucosaminyl phosphate (diphenyl 3,4,6-tri-*O*-acetyl-α-D-glucosaminyl phosphate hydrobromide) prepared previously by us[7] in basic media: (1) In pyridine at room temperature for 3 h, (2) in *N*,*N*-dimethylformamide containing sodium azide at 120 °C for 30 min, (3) in ammonia-saturated methanol at 5 °C for 23 h, and (4) in methanol containing aqueous sodium hydrogencarbonate at room temperature for 70 min. Under all conditions, phosphate bond cleavage (C–O–P) was observed, so a route to neosamine C nucleoside diphosphates involving introduction of an amino group (or its equivalent) at C-6 of the glucosaminyl phosphate was abandoned.

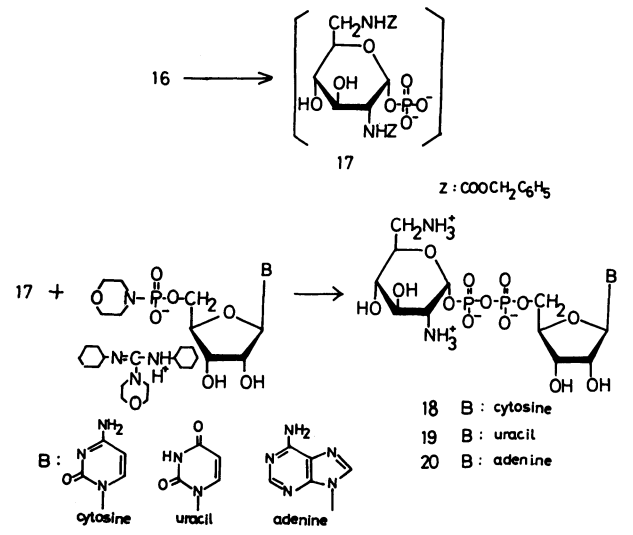
Next, introduction of an amino group at C-6 of D-glucosamine prior to formation of the sugar phosphate ester was investigated. D-Glucosamine hydrochloride was converted to the *N*,*O*-protected 6-*O*-tosylate (**1**) by a known procedure,[8] then to the known 6-azido derivative (**2**).[9] In order to introduce a phosphate ester and an α-linkage at the anomeric center, protection of the 2-amino group with a non-participating functionality is essential. For this purpose, **2** was converted to its *N*-(2,4-dinitrophenyl) derivative (**3**), then to the corresponding α-glucosaminyl chloride (**4**) by a known procedure.[9] In addition, **2** was converted to the glycosyl chloride (**5**) possessing a free amino group at C-2. Both of the α-glucosaminyl chlorides, **4** and **5**, were treated with diphenyl hydrogenphosphate in dry benzene in the presence of triethylamine (the same procedure employed for phosphorylation of D-glucosaminyl bromide).[7] At room temperature no phosphate was introduced in either case. At elevated temperature (45 to 80 °C for 4 to 5 h) a complex mixture was formed, as shown by TLC, and the mixture was not further investigated.

In the next attempted synthesis, a 6-*O*-tosylate possessing a free amino group at C-2 (**6**) was prepared by acid treatment of **1** and converted to the glycosyl bromide (**7**) (of undetermined anomeric cofiguration), since in general, glycosyl bromides are more reactive than the corresponding chlorides. Treatment of **7** with diphenyl hydrogenphosphate at room temperature for 18 h gave the desired α-linked phosphate (**8**) in 35% yield. The α-linkage was confirmed by the ^{1}H NMR spectrum (showing a triplet at δ 6.22, $J_{1,2}= J_{1,P}=4.5$ Hz, for H-1). It has been reported that ^{1}H-^{31}P coupling in an R_2CH–O–P system is 5 to 7 Hz.[10] Substitution of the 6-*O*-tosyl group by the azido group was tried (sodium azide in acetonitrile at 55 °C for 3 h). The predominant product, isolated as crystals was the glycosyl azide (**9**) instead of the expected 6-azido derivative. Disappearance of the phenoxy protons and presence of the tosylate protons were observed in the ^{1}H NMR spectrum of **9**. Furthermore, an azido group absorption (2090 cm^{-1}) appeared in the IR spectrum of **9** and elemental analyses also supported the glycosyl azide structure. Thus, introduction of the amino group at C-6 prior to phosphate formation, appears indispensible.



In the successful synthesis the free amino group in **2** was temporarily protected with a *t*-butoxycarbonyl group to afford **10**. The azido group of **10** was hydrogenated and the amino group formed was treated with 2,4-dinitrofluorobenzene to afford **11**. Compound **10** was also converted to **12** and **13** by hydrogenolysis, followed by trifluoroacetylation. When trifluoroacetylation of the 6-amino group was carried out in dichloromethane, cleavage of the 2-*N*-*t*-butoxycarbonyl group occurred, so that subsequent 2,6-di-*N*-trifluoroacetylation afforded **12** as the sole product in low yield (39%); the structure was confirmed by its ^{1}H NMR and IR spectra. On the other hand, when *N*-trifluoroacetylation





in dichloromethane was carried out in the presence of pyridine, only 6-*N*-acetylation was observed and **13** was isolated in 72% yield from **10**. Compound **13** was then converted in quantitative yield to the glycosyl bromide (**14**) by treatment of hydrobromic acid in acetic acid at room temperature for 16 h. During this strong acid treatment removal of the *N*-*t*-butoxycarbonyl group occurred simultaneously. Because of its low solubility in several organic solvents, the configuration of the anomeric center of **14** could not be determined.

Without further purification, **14** was treated with diphenyl hydrogenphosphate (1 molar equivalent) in dry benzene in the presence of triethylamine at room temperature for 1 h. The glycosyl phosphate formed (TLC: R_f 0.48, $PhCH_3 : EtOH=5:1$) was quite unstable on silicic acid, so purification at this stage was avoided and the free amino on C-2 was immediately protected with a trifluoroacetyl group to afford **15** in 86% yield form **14**. Since the yield during formation of glycosyl phosphates is, in general, moderate or low, the yield of **15** is remarkable. An α-configuration in **15** was confirmed by its ^{1}H NMR spectrum (H-1, δ 5.95, doublet of doublets, $J_{1,2}=4$ Hz, $J_{1,P}=6$ Hz).

Deprotection of **15** was achieved by hydrogenolysis (removing the phenyl group on the phosphate), followed by saponification. 2,6-Diamino-2,6-dideoxy-α-D-glucopyranosyl phosphate (**16**) was purified by ion exchange and its structure was confirmed by ^{1}H and ^{13}C NMR spectra. In the ^{1}H NMR spectrum of **16** a doublet of doublets (H-1, δ 5.66, $J_{1,2}=3.2$ Hz, $J_{1,P}=7.2$ Hz) revealed the existence of an α-glycosyl phosphate. The ^{13}C NMR spectrum of **16** resembled the spectrum of α-D-glucosaminyl phosphate,[7] except for the chemical shift of C-6 (at δ 41.4 for **16** and at δ 61.1 for the glucosaminyl phosphate), indicative of the replacement of a hydroxyl group by an amino group. The coupling constants between the phosphorus nucleus and C-1 and C-2 were approximately equal to those of the glucosaminyl phosphate ($J_{C,P}=4.3$ Hz for C-1, and $J_{C,P}=8.5$ Hz for C-2 in **16**; 4.9 Hz and 7.8 Hz, respectively, in the glucosaminyl phosphate). At this stage, we had in hand the desired neosamine C phosphate **16** in moderate overall yield from D-glucosamine, and the remaining problem to synthesis of the final amino sugar nucleoside diphosphate was the condensation of **16** with the nucleoside 5′-monophosphates. Several examples[11] are known of the condensation reaction and we intended to use a procedure slightly modified from that of Khorana and Moffatt[12] employing nucleoside 5′-phosphoromorpholidate as phosphate donors. However, **16** has an extremely low solubility in organic solvents for the condensation (including *N*,*N*-dimethylformamide, dimethyl sulfoxide, and hexamethylphosphoric triamide), even at elevated temperature. To convert **16** to a more soluble derivative, the 2- and 6-amino groups of **16** were converted to their benzyloxycarbonyl derivatives. The bis(*N*-benzyloxycarbonyl) derivative (**17**), without isolation, was dissolved in dry pyridine and condensed with nucleoside (cytidine, uridine, and adenosine) 5′-phosphoromorpholidate (as their *N*,*N*′-dicyclohexyl-4-morpholinecarboxamidine salts). The mixtures were stirred at room temperature for 5 to 12 d. After catalytic hydrogenolysis to remove the *N*-benzyloxycarbonyl groups, the reaction mixtures were purified on Dowex 1×8 (formate) column. The desired three neosamine C NDP's (**18**—**20**) were eluted with dilute formic acid, and their structures were established by ^{1}H and ^{13}C NMR spectra. Though the yields were unsatisfactory (20% for neosamine C-CDP, (**18**); 34% for neosamine C-UDP, (**19**); and 33% for neosamine C-ADP, (**20**)), **18**—**20** were predominant products in the reaction mixtures. All of the carbons in **18**—**20** were assigned in their ^{13}C NMR spectra from the chemical shifts of nucleoside 5′-monophosphates[13] and those of **16**. Four doublets are observed in the sugar skeleton carbon region, and those are attributed to C-4 ($J_{C,P}>8$ Hz) and C-5 ($J_{C,P}<6$ Hz) on D-ribose, and C-1 ($J_{C,P}\approx5$ Hz) and C-2 ($J_{C,P}>8$ Hz) on neosamine C. These patterns are typical for sugar nucleoside diphosphates. The three neosamine C NDP's were also characterized by their fast atom bombardment (FAB) mass spectra, which in each case gave the expected M+H peak in the positive ion mode: *m*/*z* 564 for neosamine C-CDP, 565 for -UDP, and 588 for -ADP.

In spite of rather low yields in the final condensation reaction, the synthesis developed in this communication is applicable for a general synthesis of free amino sugar nucleoside diphosphates.

Experimental

Melting points were determined with a Büchi apparatus (Model 510) and are uncorrected. Solutions were evaporated under diminished pressure at a bath temperature below 40 °C. Specific rotations were measured in a 1-dm tube with a JEOL DIP-4 polarimeter. TLC was performed on glass plates coated with Wako Gel B-5 F, and compounds were detected by UV light and by spraying with sulfuric acid followed by heating. Cellulose TLC was performed on plastic sheets coated withc ellulose (Eastman Chromatogram Sheet, 13254); 0.2% ninhydrin–pyridine solution was used for visualization of amino sugars. IR spectra were recorded with a Hitachi Model-225 spectrometer. ^{1}H NMR (90 MHz) spectra were recorded with a Varian EM-390 spectrometer. ^{1}H NMR (100 MHz) and ^{13}C NMR (25 MHz) spectra were recorded with a Varian XL-100 spectrometer. Chemical shifts for $CDCl_3$ solutions are reported from internal tetramethylsilane, and chemical shifts for D_2O solutions are reported from internal DSS. Mass spectra were taken on a Varian MAT-731 mass spectrometer, using Xenon as the reagent gas. The FAB gun was an Ion Tech FAB gun, attached to either an Ion Tech or Spellman high voltage power supply. Elemental analyses were performed by Mr. Saburo Nakada of this university, to whom our thanks are due.

*1,3,4-Tri-O-acetyl-2-deoxy-2-(*p*-methoxybenzylideneamino)-6-O-tosyl-β-D-glucopyranose (**1**).* This compound was prepared from 2-deoxy-2-(*p*-methoxybenzylideneamino)-D-glucose by the method of Morel;[8] mp 198—199 °C, $[\alpha]_D^{22}$ +98.2° (*c* 2.0, $CHCl_3$) [lit,[8] mp 203—204 °C, $[\alpha]_D^{20}$ +99.3° (*c* 2, $CHCl_3$)].

*1,3,4-Tri-O-acetyl-2-aminc-6-azido-2,6-dideoxy-β-D-glucopyranose Hydrochloride (**2**).* Compound **1** was converted to the 6-azide, then hydrolyzed to prepare the title compound by the known method;[9] mp 149 °C (decomp), $[\alpha]^{D}_{23}$ +53.1° (*c* 1, H_2O) [lit,[9] mp 147—154 °C (decomp), $[\alpha]_D^{25}$ +55.8° (*c* 1, H_2O)].

*1,3,4-Tri-O-acetyl-6-azido-2,6-deoxy-2-(2,4-dinitroanilino)-β-D-glucopyranose (**3**).* This compound was prepared from **2** by the known method;[9] mp 116—117 °C, $[\alpha]_D^{22.5}$ +99.0° (*c* 1, $CHCl_3$) [lit,[9] mp 115.5—116 °C, $[\alpha]_D^{29}$ +108° (*c* 1, $CHCl_3$)].

*3,4-Di-O-acetyl-6-azido-2,6-dideoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl Chloride (**4**).* This glucosaminyl chloride was prepared from **3** by a slight modification of the known procedure.[9] Hydrogen chloride-saturated ether (0 °C, 30 ml) and acetyl chloride (3 ml) were added to 800 mg (1.61 mmol) of **3**, and the solution was stirred at room temperature in the dark for 19 h. Then, an additional 20 ml of hydrogen chloride-saturated ether was added after 22 h, and stirring was continued for 20 h more; TLC revealed the disappearance of **3** (R_f 0.22; $PhCH_3$: AcOEt=6 : 1) and the formation of a single product (R_f 0.29). The precipitated crystals were collected by filtration, washed with dry ether, and dried to afford 372 mg (73%) of **4** which was used without further purification: mp 173 °C (decomp), $[\alpha]_D^{21.5}$ +150° (*c* 1.0, $CHCl_3$) [lit,[9] mp 170 °C (decomp.), $[\alpha]_D^{25}$ +167° (*c* 1, $CHCl_3$)]; ^{1}H NMR (DMSO-d_6, 90 MHz): δ 1.87 (s, 3H, OAc), 2.09 (s, 3H, OAc), 3.37 (dd, 1H, $J_{6a,6b}=13.8$ Hz, $J_{5,6a}=4.5$ Hz, H-6a), 3.58 (dd, 1H, $J_{6a,6b}=13.8$ Hz, $J_{5,6b}=3$ Hz, H-6b), 4.24—4.49(m, 2H,H-2 and H-5), 5.26(t, 1H, $J_{3,4}=J_{4,5}=9$ Hz, H-4), 5.55 (t, 1H, $J_{2,3}=J_{3,4}=9$ Hz, H-3), 6.26 (d, 1H, $J_{1,2}=3.6$ Hz, H-1), 7.13 (d, 1H, $J_{5',6'}=9.3$ Hz, H-6′ of DNP), 8.28 (dd, 1H, $J_{5',6'}=9.3$ Hz, $J_{3',5'}=2.7$ Hz, H-5′), 8.75 (d, 1H, $J_{NH,2}=9$ Hz, NH), 9.10 (d, 1H, $J_{3',5'}=2.7$ Hz, H-3′).

*3,4-Di-O-acetyl-2-amino-6-azido-2,6-dideoxy-α-D-glucopyranosyl Chloride Hydrochloride (**5**).* Dry hydrogen chloride was introduced with ice cooling to a glacial acetic acid solution (50 ml) of **2** (500 mg, 1.36 mmol) containing 2 ml of acetyl chloride until the solution was saturated. The solution was stirred at room temperature in the dark for 24 h, then evaporated. The residue was coevaporated with toluene and triturated with chloroform (30 ml), insolbuble material was removed by filtration, and the filtrate was concentrated to half volume. After addition of 30 ml of ether the solution was cooled at 5 °C overnight. The precipitated crystals were collected by filtration and dried to afford 339 mg, which consisted of **5** and **2** in a 4 : 1 ratio based on the ^{1}H NMR spectrum and elemental analyses: IR (KBr) 2080 (N_3), 1760 (C=O) and 765 cm^{-1} (Cl); ^{1}H NMR (DMSO-d_6, 90 MHz) δ 2.01 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.16 (s, 0.6H, OAc), 3.53 (m, 3H, H-6a, H-6b, and H-2 or H-5), 4.00—4.25 (m, 1H, H-2 or H-5), 5.01 (t, 1H, $J_{3,4}=J_{4,5}=9$ Hz, H-4), 5.24 (t, 1H, $J_{2,3}=J_{3,4}=9$ Hz, H-3), 5.92 (d, 0.2H, $J_{1,2}=8.7$ Hz, H-1 on **2**), 6.68 (d, 0.8H, $J_{1,2}=3.0$ Hz, H-1 on **5**), 9.02 (bs, 3H, NH_3^+). The mixture was used without further purification. Found: C, 36.05; H, 4.79; N, 15.86; Cl, 18.42%. Calcd for $C_{12}H_{18}N_4O_7\cdot HCl$ (20%)+$C_{10}H_{15}ClN_4O_7\cdot HCl$ (80%): C, 35.86; H, 4.80; N, 16.12; Cl, 18.46%.

*1,3,4-Tri-O-acetyl-2-amino-2-deoxy-6-O-tosyl-β-D-glucopyranose Hydrochloride (**6**).* To a vigorously stirred solution of **1** (1.28 g, 2.22 mmol) in acetone (15 ml), 2 ml of 5 M hydrochloric acid was added dropwise at 55 °C. The mixture was stored at 5 °C overnight, and the precipitated white crystals were collected by filtration, then dried. The crude crystals were recrystallized from methanol to give 568 mg (52%) of **6**, mp 206 °C (decomp), which was used in the next step without further purification.

*3,4-Di-O-acetyl-2-amino-2-deoxy-6-O-tosyl-D-glucopyranosyl Bromide Hydrobromide (**7**).* A solution of 30% hydrogen bromide in acetic acid (20 ml) was added to a glacial acetic acid (40 ml) solution of **6** (518 mg, 1.04 mmol) and the mixture was stirred at room temperature for 20 h, then evaporated with toluene. The residue was triturated with chloroform, and the insoluble crystals were collected by filtration, washed with chloroform, and dried to afford 559 mg (95%) of **7**, mp 159 °C (decomp), which was used without further purification. Found: C, 36.58; H, 4.10; N, 2.33%. Calcd for $C_{17}H_{23}Br_2NO_8S$: C, 36.38; H, 4.13; N, 2.50%.

*Diphenyl 3,4-Di-O-acetyl-2-amino-2-deoxy-6-O-tosyl-α-D-glucopyranosyl Phosphate Hydrochloride (**8**).* Diphenyl hydrogenphosphate (Aldrich, 0.736 g, 2.94 mmol) and triethylamine (0.42 ml, 3.01 mmol) were added to a suspension of **7** (1.65 g, 2.94 mmol) in dry benzene (45 ml) containing triethylamine (0.42 ml, 3.01 mmol). After the mixture was stirred at room temperature in the dark for 18 h, TLC revealed the presence of a single product (R_f 0.52, positive to UV and ninhydrin, $PhCH_3$: EtOH=5 : 1). Precipitated triethylammonium bromide was removed by filtration and washed with benzene. The combined filtrate and washings were evaporated to afford a pale yellow syrup which was triturated with 2 M hydrochloric acid (10 ml) to give crystals, which were filtered, washed with cold water, and dried. The crude **8** obtained (1.87 g) was recrystallized from methanol and ether to afford 697 mg (35%) of pure **8**: mp 140 °C (sintered), 146 °C (decomp); $[\alpha]_D^{22}$ +88.8° (*c* 0.98, DMSO); ^{1}H NMR (DMSO-d_6, 90 MHz). δ 1.90 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.40 (s, 3H, Ts–CH_3), 3.48 (sb, 3H, NH_3^+), 3.70—4.11 (m, 4H, H-2, 5, 6a, 6b), 4.98 (t, 1H, $J_{3,4}=J_{4,5}=9$ Hz, H-4), 5.25 (t, 1H, $J_{2,3}=J_{3,4}=9$ Hz, H-3), 6.22 (t, 1H, $J_{1,2}=J_{1,P}=4.5$ Hz, H-1), 7.28 [m, 10H

$OPO(OP\underline{h}_2)$], 7.41 and 7.66 (m, 4H, Ts–$C_6\underline{H}_4$). Found: C, 49.64; H, 4.90; N, 1.78%. Calcd for $C_{29}H_{33}ClNO_{12}PS \cdot H_2O$: C, 49.47; H, 5.01; N, 1.99%.

*3,4-Di-O-acetyl-2-amino-2-deoxy-6-O-tosyl-β-D-glucopyranosyl Azide Hydrochloride (**9**).* After a mixture of **8** (150 mg, 0.219 mmol), sodium azide (178 mg, 2.74 mmol) and triethylamine (0.31 ml, 0.22 mmol) in acetonitrile (10 ml) was stirred at 55 °C for 3 h, TLC revealed the disappearance of **8** (R_f 0.10, $PhCH_3$: MEK=4 : 1) and the formation of two products (R_f 0.20 and 0.00). Insoluble material was removed, the filtrate was evaporated, and the residue was purified on silica gel column (Wako gel C-300, 9.5 g, 12 mm × 145 mm, $PhCH_3$: EtOH=5 : 1). Fractions showing R_f 0.45 on TLC ($PhCH_3$: EtOH=5 : 1) were combined and evaporated to afford 51.1 mg of an oily residue, which was triturated with 2 M hydrochloric acid (3 ml). The precipitated crystals were collected and dried to afford 39.8 mg (38%) of **9**: mp 148 °C (decomp); $[\alpha]_D^{22.5}$ +11.6° (*c* 0.23, $CHCl_3$); IR (KBr) 2090 (N_3) and 1765 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$, 90 MHz) δ 1.96 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.43 (s, 3H, Ts-CH_3), 2.60—2.81 (m, 1H), 3.70—3.91 (m, 1H), 4.07 (d, 2H, J=4 Hz, H-6a and 6b), 4.41 (d, 1H, J=10 Hz, H-1), 4.82—4.92 (m, 2H), 7.29 and 7.73 (AB quartet, 4H, Ts-C_6H_4). Found: C, 42.36; H, 4.93; N, 11.39%. Calcd for $C_{17}H_{23}ClN_4O_8S$: C, 42.64; H, 4.84; N, 11.70%.

*1,3,4-Tri-O-acetyl-6-azido-2-(t-butoxycarbonylamino)-2,6-dideoxy-β-D-glucopyranose (**10**).* Di-*t*-butyl dicarbonate (4.97 g, 23.8 mmol) was added to a solution of **2** (6.58 g, 17.9 mmol) in a mixture of dioxane (60 ml) and water (30 ml) containing 1.71 g (20.3 mmol) of sodium hydrogencarbonate and the mixture was stirred at 0 °C for 2 h, then at room temperature for 16 h. Thin-layer chromatography revealed the disappearance of **2** (R_f 0.02; $PhCH_3$: MEK=5 : 1) and the formation of a sole product (R_f 0.35). The mixture was evaporated and the residue was extracted with ethyl acetate (150 ml). The organic extract was washed with water (150 ml; 75 ml × 2), dried over anhydrous sodium sulfate, and evaporated. The crude crystals were recrystallized from ethanol and methanol to afford 6.44 g (84%) of **10**: mp 172—173 °C; $[\alpha]_D^{23}$ +18.5° (*c* 2.1, $CHCl_3$); IR (KBr) 2120 (N_3), 1760 (C=O), 1700 (NC=O), and 1530 cm^{-1} (NH); ^{1}H NMR ($CDCl_3$, 90 90 MHz) δ 1.43 (s, 9H, $COOC(CH_3)_3$, 2.04 (s, 6H, OAc), 2.11 (s, 3H, OAc), 3.33 (d, 2H, J=4 Hz, H-6a and 6b), 3.63—3.83 (m, 2H, H-2 and H-5), 4.58 (d, 1H, $J_{NH,2}$=9 Hz, NH), 4.96 (t, 1H, $J_{3,4}=J_{4,5}$=9 Hz, H-4), 5.15 (1H, $J_{2,3}=J_{3,4}$=9 Hz, H-3), and 5.65 (d, 1H, $J_{1,2}$=9 Hz, H-1). Found: C, 47.30; H, 5.94; N, 12.77%. Calcd for $C_{17}H_{26}N_4O_9$: C, 47.44; H, 6.09; N, 13.02%.

*1,3,4-Tri-O-acetyl-2-(t-butoxycarbonylamino)-2,6-dideoxy-6-(2,-4-dinitroanilino)-β-D-glucopyranose (**11**).* A solution of **10** (696.3 mg, 1.62 mmol) in a mixture of ethyl acetate (5 ml) and ethanol (50 ml) containing 0.27 ml of 6 M hydrochloric acid was hydrogenated at room temperature in the presence of 10% palladium on charcoal (70 mg) under 345 kPa initial hydrogen atmosphere. After 2.5 h, TLC revealed the disappearance of **10** (R_f 0.36; $PhCH_3$: MEK=5 : 1) and the formation of a single ninhydrin-positive product (R_f 0.00). The catalyst was removed by filtration and the filtrate was evaporated to afford white crystals, which were dissolved in ethanol (7 ml). 2,4-Dinitrofluorobenzene (750 mg, 4.03 mmol) in acetone (28 ml) and sodium hydrogencarbonate (325.5 mg, 3.88 mmol) were added, and the mixture was stirred at room temperature in the dark for 24 h. Thin-layer chromatography revealed the formation of a single product (R_f 0.33, $PhCH_3$: MEK=4 : 1). After evaporation of the mixture, the residue was extracted with chloroform, insoluble material was removed by filtration, and the filtrate was evaporated. The residue was purified on silica gel (Wako gel C-300, 40 g, 23 mm × 220 mm, $PhCH_3$: MEK=4 : 1). Fractions showing R_f 0.33 were combined and evaporated to afford crude crystals which were recrystallized from chloroform and ethanol to yield 717 mg (78%) of **11**: mp 238 °C (decomp), $[\alpha]_D^{23}$ +38.3° (*c* 1.9, $CHCl_3$); IR (KBr) 3360 (NH), 1755 (C=O), 1695 (NC=O), 1620 (C=C), 1590 (NO_2), 1525 cm^{-1} ($\underline{N}$H); ^{1}H NMR ($CDCl_3$, 90 MHz) δ 1.42 [s, 9H, $COOC(C\underline{H}_3)_3$], 2.06 (s, 3H, OAc), 2.12 (s, 6H, 2 × OAc), 3.39—3.95 (m, 4H, H-2, H-5, H-6a, and H-6b), 4.61 (d, 1H, $J_{NH,2}$=10 Hz, H-2), 4.97 (t, 1H, $J_{3,4}=J_{4,5}$=9 Hz, H-4), 5.25 (t, 1H, $J_{2,3}=J_{3,4}$=9 Hz, H-3), 5.65 (d, 1H, $J_{1,2}$=9 Hz, H-1), 6.91 (d, 1H, $J_{5',6'}$=9 Hz, H-6′ of DNP group), 8.18 (dd, 1H, $J_{5',6'}$=9 Hz, $J_{3',5'}$=3 Hz, H-5′), 8.79 (t, 1H, $J_{NH,6}$=6 Hz, NH), 9.06 (d, 1H, $J_{3',5'}$= 3 Hz, H-3′). Found: C, 48.19; H, 5.27; N, 9.56%. Calcd for $C_{23}H_{30}N_4O_{13}$: C, 48.42; H, 5.30; N, 9.82%.

*1,3,4-Tri-O-acetyl-2,6-dideoxy-2,6-bis(trifluoroacetamido)-β-D-glucopyranose (**12**).* Compound **10** (568 mg, 1.32 mmol) was hydrogenated as described for the preparation of **11**, and the crude crystals were dissolved in 20 ml of dry dichloromethane and acylated by adding 1.0 ml (7.19 mmol) of trifluoroacetic anhydride dropwise with mechanical stirring under ice cooling. After the mixture had been stirred at room temperature for 16 h, TLC revealed a single product(R_f 0.40; $PhCH_3$: MEK=3 : 1). The mixture, diluted with 20 ml of dichloromethane, was washed with aqueous sodium hydrogencarbonate until neutral, then with water (20 ml; 10 ml × 2), then dried (Na_2SO_4). Evaporation of the extract gave crude crystals which were recrystallized from chloroform–ether to afford 220 mg (39%) of **12**: mp 220—221 °C (decomp); $[\alpha]_D^{23}$ +2.1° (*c* 0.98, MeOH); IR (KBr) 1760 (C=O), 1710 (NC=O), 1555 (NH), 1220 and 1180 (C–F) cm^{-1}; ^{1}H NMR (CD_3OD, 90 MHz) δ 1.95 (s, 3H, OAc), 2.01 (s, 3H, OAc), 2.04 (s, 3H, OAc), 3.43—4.39 (m, 4H, H-2, H-5, H-6a and H-6b), 4.67 (t, 1H, $J_{3,4}=J_{4,5}$=9 Hz, H-4), 5.27 (t, 1H, $J_{2,3}=J_{3,4}$=9 Hz, H-3), 5.75 (d, 1H, $J_{1,2}$=9 Hz, H-1). Found: C, 38.83; H, 3.78; N, 5.71%. Calcd for $C_{16}H_{18}F_6N_2O_9$: C, 38.72; H, 3.65; N, 5.64%.

*1,3,4-Tri-O-acetyl-2-(t-butoxycarbonylamino)-2,6-dideoxy-6-trifluoroacetamido-β-D-glucopyranose (**13**).* Compound **10** (991 mg, 2.30 mmol) was hydrogenated as described above and the crude 6-amino derivative in dry dichloromethane (30 ml) and dry pyridine (8 ml) was *N*-acylated directly by adding trifluoroacetic anhydride (0.55 ml, 3.95 mmol) under ice cooling. After the mixture had been stirred at room temperature for 30 min, TLC revealed a single product (R_f 0.51; $PhCH_3$: EtOH=5 : 1). The mixture was diluted with dichloromethane (40 ml) and the organic mixture was washed with diluted aqueous sodium hydrogencarbonate to neutrality, washed with water (80 ml; 40 ml × 2), dried, and evaporated to afford a crystalline residue which was recrystallized from chloroform–ether to give 828 mg (72%) of **13**: mp 214 °C (decomp); $[\alpha]_D^{22}$ +20.8° (*c* 2.1, $CHCl_3$); IR (KBr) 1755 (C=O), 1700 (NC=O), 1525 (NH), 1220 and 1170 cm^{-1} (C–F); ^{1}H NMR ($CDCl_3$, 90 MHz) δ 1.41 [s, 9H, $COOC(C\underline{H}_3)_3$], 2.02 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.09 (s, 3H, OAc), 3.21—3.95 (m, 4H, H-2, H-5, H-6a, and H-6b), 4.80 (d, 1H, $J_{NH,2}$= 9 Hz, 2-NH), 4.85 (t, 1H, $J_{3,4}=J_{4,5}$=9 Hz, H-4), 5.15 (t, 1H, $J_{2,3}=J_{3,4}$=9 Hz, H-3), 5.60 (d, 1H, $J_{1,2}$=9 Hz, H-1), 6.89 (t, 1H, $J_{NH,6}$=6 Hz, 6-NH). Found: C, 45.74; H, 5.35: N, 5.34%. Calcd for $C_{19}H_{27}F_3N_2O_{10}$: C, 45.60; H, 5.44; N, 5.60%.

*3,4-Di-O-acetyl-2-amino-2,6-dideoxy-6-trifluoroacetamido-D-glucopyranosyl Bromide Hydrobromide (**14**).* To a solution of **13** (305 mg, 0.609 mmol) in dry dichloromethane (3 ml), 25% hydrogen bromide in acetic acid (2.0 ml) was added dropwise under ice cooling. The stirred mixture gradually warmed to room temperature, then was stirred 16 h longer, and finally was evaporated with toluene to afford 306 mg (quantitative) of crude bromide (**14**), mp 174—178 °C (decomp), which was used without further purification. Found: C, 28.42; H, 3.68; N, 5.77%. Calcd for $C_{12}H_{17}Br_2F_3N_2O_6$: C, 28.71; H, 3.41; N, 5.58%.

Diphenyl 3,4-Di-O-acetyl-2,6-dideoxy-2,6-bis(trifluoroacetamido)-α-D-glucopyranosyl Phosphate (15). Diphenyl hydrogenphosphate (Aldrich, 503 mg, 2.01 mmol) and triethylamine (0.60 ml, 4.30 mmol) were added to a suspension of **14** (1.01 g, 2.01 mmol) in dry benzene (distilled over sodium). The mixture was stirred at room temperature in the dark for 1 h, when TLC revealed formation of a UV-absorbing product (R_f 0.48; $PhCH_3$: EtOH=5 : 1). Triethylamine (1.20 ml, 8.60 mmol) and trifluoroacetic anhydride (0.60 ml, 4.31 mmol) were added to the mixture, under ice cooling, and the mixture was stirred at 0 °C for 30 min, when TLC revealed a single product with UV-absorption (R_f 0.60; $PhCH_3$: EtOH=5 : 1). The mixture was washed with water (80 ml; 20 ml × 4), then dried (Na_2SO_4). After removal of the organic solvent, the residue was crystallized from methanol to yield 1.18 g (86%) of **15**: mp 128—132 °C (decomp); $[\alpha]_D^{27.5}$ +59.3° (*c* 0.72, $CHCl_3$); IR (KBr) 1750 (C=O), 1710 (NC=O), 1210, 1180 (C–F), and 970 cm^{-1} (P=O); ^{1}H NMR ($CDCl_3$, 90 MHz) δ 1.94 (s, 3H, OAc), 2.02 (s, 3H, OAc), 3.21—3.65, 4.06—4.52 (m,4H, H-2, H-5, H-6a and H-6b), 4.93 (t, 1H, $J_{3,4}=J_{4,5}=9$ Hz,H-4), 5.29 (t, 1H, $J_{2,3}=J_{3,4}=9$ Hz, H-3), 5.95 (dd, 1H, $J_{1,2}=4$ Hz, $J_{1,2}=4$Hz, $J_{1,P}=6$ Hz, H-1), 6.99—7.34 (m, 12H, phenyl, 2-NH and 6-NH). Found: C, 45.60; H, 3.84; N, 3.88%. Calcd for $C_{26}H_{25}F_6N_2O_{11}P$: C, 45.49; H, 3.67; N, 4.08%.

2,6-Diamino-2,6-dideoxy-α-D-glucopyranosyl Phosphate (16). A solution of **15** (120 mg, 0.174 mmol) in glacial acetic acid (5 ml) was hydrogenated in the presence of platinum oxide (30 mg) under 390 kPa initial hydrogen atmosphere at room temperature for 47 h. The catalyst was removed by filtration and the filtrate was evaporated to afford 93 mg of semicrystalline residue. The residue was dissolved in 2.6 ml of methanol and 1.12 ml of 1.25 M potassium methoxide in methanol was added. The mixture was stored at 5 °C for 96 h, an additional 0.56 ml of the base was added, and the mixture stood at room temperature for 20 h longer, when TLC (cellulose, *n*-BuOH : AcOH : H_2O=5 : 2 : 3) revealed two ninhydrin-positive products (R_f 0.36 and 0.27). The reaction mixture was applied to a Dowex 50W×8 column (pyridinium form, 7 ml wet volume, 9 mm × 100 mm), which was eluted with water (40 ml), 0.01 M aqueous ammonia (24 ml) and 0.1 M aqueous ammonia (30 ml) successively (6-ml fractions). A concentrate of fractions 1 to 3 consisted of two products [R_f 0.36 and 0.27, BAW 523, IR (KBr) 1670 cm^{-1} (NC=O)], while a concentrate of fractions 13 to 15 (7.4 mg) consisted of pure **16** (R_f 0.27). The concentrate of fractions 1 to 3 (73 mg) was hydrolyzed under reflux for 2 h with 100 mg of sodium hydroxide in water (2 ml), when TLC revealed a single compound, **16**, (R_f 0.27), which was purified on an ion exchange column (Dowex 50W × 8, pyridinium form, 2.5 ml wet volume, 6.5 mm × 70 mm). The column was eluted with water (8 ml) and 0.1 M aqueous ammonia (10 ml) successively (2-ml fractions). Fractions 6 to 9 gave 14.9 mg (total 22.3 mg, 50%) of pure **16** mp 205 °C (decomp); $[\alpha]_D^{18.5}$ +33.1° (*c* 2.14, H_2O); IR (KBr) 3100 (NH), 1640 (NH), 1540 (NH), 1120 (P=O), 960 cm^{-1} (PO); ^{1}H NMR (D_2O, 100 MHz) δ 3.00—3.58 ,3.86—4.37 (m, 6H, H-2, H-3, H-4, H-5, H-6a, and H-6b), 5.66 (dd, 1H, $J_{1,2}=3.2$ Hz, $J_{1,P}=7.2$ Hz, H-1); ^{13}C NMR (D_2O, 25 MHz, TMS as internal standard) δ 41.4 (s, C-6), 55.4 (d, $J_{C,P}=8.5$ Hz, C-2), 69.6 (s, C-4), 70.6 (s, C-3), 72.5 (s, C-5), 92.0 (d, $J_{C,P}=4.3$ Hz, C-1). Found: C, 26.94; H, 5.75; N, 10.27%. Calcd for $C_6H_{15}N_2O_7 \cdot \frac{1}{2}H_2O$: C, 27.02; H, 5.86; N, 10.50%.

Cytidine 5′-(2,6-Diamino-2,6-dideoxy-α-D-glucopyranosyl Diphosphate) (CDP-neosamine C) (18). *N*-(Benzyloxycarbonyloxy)succinimide (68 mg, 0.27 mmol) in ethyl acetate (2 ml) was added to an aqueous solution (10 ml) of **16** (70.4 mg, 0.27 mmol) containing 23 mg (0.27 mmol) of sodium hydrogencarbonate. The mixture was stirred vigorously at 0 °C for 2 h, then at room temperature for 17 h. Additional *N*-benzyloxycarbonylating reagent (68 mg, 0.27 mmol) and sodium hydrogencarbonate (23 mg) were added, and stirring continued 15 h longer, when TLC (cellulose, *n*-BuOH : AcOH : H_2O=5 : 2 : 3) showed formation of the bis(benzyloxycarbonylamino) derivative (**17**) (R_f 0.70, UV absorbing). After removal of solvents by evaporation, the residue was dissolved in 50% aqueous ethanol in minimum volume, then applied on Dowex 50W × 8 (pyridinium form, 6 ml wet volume). The column was eluted with 50% aqueous ethanol (50 ml), trioctylamine (0.14 ml, 0.32 mmol) was added to the eluate, and the aqueous solution was evaporated. The residue was coevaporated with dry pyridine (32 ml; 8 ml × 4), finally dissolved in dry pyridine (10 ml), and cytidine 5′-phosphoromorpholidate *N*,*N*′-dicyclohexyl-4-morpholinecarboxamidine salt (Sigma, 187 mg, 0.27 mmol, dried over phosphorus pentaoxide *in vacuo* at 80 °C for 28 h) was added to the mixture, which was stirred at room temperature for 5 d, then evaporated. The residue was dissolved in 50% aqueous ethanol (7 ml) and hydrogenated in the presence of 10% palladium on charcoal (30 mg) under 390 kPa initial hydrogen atmosphere at room temperature for 48 h, when TLC (cellulose) revealed a mixture of six compounds (*n*-BuOH : AcOH : H_2O=5 : 2 : 3; R_f 0.70, 0.66, 0.54, 0.29, 0.27, and 0.15 with the latter compound predominant, each compound ninhydrin and/or UV-positive). The mixture was applied to a Dowex 1×8 column (formate form, 90 ml wet volume, 22 mm × 260 mm), which was washed with water (200 ml), then gradient eluted with aqueous formic acid (0.0 M to 0.1 M, 4.5 ml fractions). Fractions showing R_f 0.15 were combined and evaporated with water to afford **18** (31.1 mg, 20% pure by TLC) as a colorless glass: $[\alpha]_D^{23}$ +36.5° (*c* 1.0, H_2O); ^{1}H NMR (D_2O, 90 MHz) δ 2.67—4.36 (m, 11H, H-2′, H-3′, H-4′, H-5′ of D-ribose, H-2″, H-3″, H-4″, H-5″, H-6″ of neosamine C), 5.67—5.90 (m, 3H, H-5 of cytosine, H-1′ and H-1″), 7.78 (d, 1H, $J_{5,6}=7.5$ Hz, H-6); ^{13}C NMR (D_2O, 25 MHz, TMS) δ 41.4 (s, C-6″), 54.9 (d, $J_{C,P}=8.8$ Hz, C-2″), 66.8 (d, $J_{C,P}=6.1$ Hz, C-5′), 70.4, 70.6, 71.2 (s's, C-3″, C-4″, and C-2′), 72.2 (s, C-5″), 75.1 (s, C-3′), 83.2 (d, $J_{C,P}=8.7$ Hz, C-4′), 90.6 (s, C-1′), 93.7 (d, $J_{C,P}=5.4$ Hz, C-1″), 97.5 (s, C-5), 142.3 (s, C-6), 155.9 (s, C-2), 174.6 (s, C-4); FABMS *m*/*z* 564 (M+H).

Uridine 5′-(2,6-Diamino-2,6-dideoxy-α-D-glucopyranosyl Diphosphate)(UDP-neosamine C) (19). Compound **16** (63.6 mg, 0.25 mmol) was converted to its bis(benzyloxycarbonyl) derivative, **17**, as described in the preparation of **18**. To 50 ml of the 50% aqueous ethanol eluate from the Dowex column, trioctylamine (0.14 ml, 0.32 mmol) was added. Evaporation was followed by evaporation with dry pyridine (32 ml, 8 ml × 4) and finally, solution in dry pyridine (10 ml). Uridine 5′-phosphoromorpholidate *N*,*N*′-dicyclohexyl-4-morpholinecarboxamidine salt (Sigma, 198 mg, 0.29 mmol, dried prior to use) was added to the solution, and the mixture was stirred at room temperature for 12 d. After evaporation of the mixture, the residue was dissolved in 7 ml of 50% aqueous ethanol and hydrogenated for 69 h at room temperature in the presence of 10% palladium on charcoal (30 mg) under 390 kPa initial hydrogen atmosphere. Cellulose TLC (*n*-BuOH : AcOH: H_2O : 5 : 2 : 3) revealed five compounds (R_f 0.71, 0.44, 0.34, 0.28 and 0.16, all UV and/or ninhydrin positive with the latter compound predominant). The reaction mixture was applied to a Dowex 1×8 column (formate form, 100 ml wet volume, 22 mm×310 mm), which was washed with water (80 ml) and gradient eluted with aqueous formic acid (0.0 M to 0.1 M, 4.5 ml fractions). Fractions showing R_f 0.16 (fractions 29 to 31) were combined and evaporated and finally coevaporated with water (12 ml, 6 ml×2) to afford 46.7 mg (34%) of TLC-homogeneous **19** as a colorless glass: $[\alpha]_{\mathrm{D}}^{23}$ +35.2° (*c* 1.25, H_2O); ^1H NMR (D_2O, 100 MHz, TMS) δ 2.66—4.39 (m, 11H, H-2′, H-3′, H-4′, H-5′ on D-ribose and H-2″, H-3″, H-4″, H-5″, H-6″ on neosamine C), 5.79—5.96 (m, 3H, H-1′, H-1″, and H-5 on uracil), 7.88 (d, 1H, $J_{5,6}$=8.0 Hz, H-6); ^{13}C NMR (D_2O, 25 MHz, TMS) δ 41.4 (s, C-6″), 54.8 (d, $J_{C,P}$=9.4 Hz, C-2″), 66.8 (d, $J_{C,P}$=6.0 Hz, C-5′), 70.2, 70.4, 71.2 (each s, C-2′, C-3″, and C-4″), 72.1 (s, C-5″), 74.7 (s, C-3′), 83.2 (d, $J_{C,P}$=9.4 Hz, C-4′), 89.6 (s, C-1′), 93.2 (d, $J_{C,P}$=5.4 Hz, C-1″), 103.4 (s, C-5), 142.4 (s, C-6), 155.4 (s, C-2), 174.6 (s, C-4); FABMS *m/z* 565 (M+H).

*Adenosine 5′-(2,6-Diamino-2,6-dideoxy-α-D-glucopyranosyl Diphosphate) (ADP-neosamine C) (**20**).* The bis(benzyloxycarbonylamino) derivative (**17**) was prepared as described during the preparation of **18** [63.6 mg (0.25 mmol) of **16**, and 123 mg (0.49 mmol) of *N*-(benzyloxycarbonyloxy)succinimide]. To 50 ml of 50% aqueous ethanol eluate from the Dowex column, trioctylamine (0.14 ml, 0.32 mmol) was added and the mixture was evaporated with dry pyridine (32 ml, 8 ml×4) and finally dissolved in dry pyridine (10 ml). To the solution, adenosine 5′-phosphoromorpholidate *N,N′*-dicyclohexyl-4-morpholinecarboxamidine salt (Sigma, 193 mg, 0.27 mmol, dried prior to use) was added and the mixture was stirred at room temperature for 11 d. After evaporation of the mixture, the residue was dissolved in 7 ml of 50% aqueous ethanol and the solution was hydrogenated at room temperature for 68 h in the presence of 10% palladium on charcoal (30 mg) under 390 kPa initial hydrogen atmosphere. Cellulose TLC (*n*-BuOH : AcOH : H_2O=5 : 2 : 3) revealed five compounds (R_f 0.66, 0.51, 0.32, 0.25 and 0.18, all UV and/or ninhydrin-positive with the latter compound predominant). The reaction mixture was applied to a Dowex 1×8 column (formate form, 85 ml wet volume, 22 mm×250 mm), which was washed with water (80 ml), then gradient eluted with aqueous formic acid (0.0 M to 0.1 M, 4.5-ml fractions). Fractions showing R_f 0.18 (fractions 33 to 36) were combined and evaporated with water (12 ml, 6 ml×2) to afford 47.9 mg (33%) of TLC-homogeneous **20** as a colorless glass: $[\alpha]_{\mathrm{D}}^{23}$ +26.9° (*c* 1.21, H_2O); ^1H NMR (D_2O, 100 MHz, TMS) δ 2.93—4.50 (m, 11H, H-2′, H-3′, H-4′, H-5′ of D-ribose, and H-2″, H-3″, H-4″, H-5″, H-6″ of neosamine C), 5.82 (dd, 1H, $J_{1,2}$=5.2 Hz, H-1′), 8.05 (s, 1H, H-2 of adenine), 8.34 (s, 1H, H-8); ^{13}C NMR (D_2O, 25 MHz, TMS) δ 40.3 (s, C-6″), 53.7 (d, $J_{C,P}$=8.8 Hz, C-2″), 65.4 (d, $J_{C,P}$=6.7 Hz, C-5′), 69.1, 69.3, 70.1, 71.0 (all s's, C-2′ C-3″, C-4″, C-5″), 74.3 (s, C-3′), 83.3 (d, $J_{C,P}$=9.4 Hz, C-4′), 87.1 (s, C-1′), 92.2 (d, $J_{C,P}$=5.4 Hz, C-1″), 117.9 (s, C-5), 139.6 (s, C-8), 148.2 (s, C-4), 151.4 (s, C-2), 154.1 (s, C-6); FABMS *m/z* 588 (M+H).

References

1) K. L. Rinehart, Jr., "Biosynthesis and Mutasynthesis of Aminocyclitol Antibiotics," in "Aminocyclitol Antibiotics," ACS Symposium Series 125, ed by K. L. Rinehart, Jr., and T. Suami, American Chemical Society, Washington, D. C. (1980), p. 335 and references cited therein.

2) N. K. Kochetkov and V. N. Shibaev, *Adv. Carbohydr. Chem. Biochem.* **28**, 307 (1973).

3) H. Grisebach, *Adv. Carbohydr. Chem. Biochem.*, **35**, 81 (1978).

4) J. L. Strominger, "Nucleotide Derivatives of Amino Sugars," in "The Amino Sugars," ed by R. W. Jeanloz, Academic Press, New York (1969), p. 375.

5) T. Yamazaki, C. D. Warren, A. Herscovics, and R. W. Jeanloz, *Carbohydr. Res.*, **79**, C9 (1980).

6) F. Maley, G. F. Maley, and H. A. Lardy, *J. Am. Chem. Soc.*, **78**, 5303 (1956).

7) K. L. Rinehart, Jr., K. Tadano, T. Suami, and T. Tsuchiya, *J. Am. Chem. Soc.*, to be submitted.

8) C. J. Morel, *Helv. Chim. Acta*, **41**, 1501 (1958).

9) S. Ogawa, H. Fujimori, and T. Suami, *Bull. Chem. Soc. Jpn.*, **49**, 2585 (1976).

10) L. M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," 2nd ed, Pergamon Press, London (1969), p. 351.

11) J. G. Moffatt, *Methods Enzymol.*, **8**, 136 (1966) and references cited therein.

12) S. Roseman, J. J. Distler, J. G. Moffatt, and H. G. Khorana, *J. Am. Chem. Soc.*, **83**, 659 (1961).

13) D. E. Dorman and J. D. Roberts, *Proc. Natl. Acad. Sci. U. S. A.*, **65**, 19 (1970).