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Synthesis of 2-Phenylaminoadenosine from Imidazole Nucleosides

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Three methods for the synthesis of 2-phenylaminoadenosine (**1**, CV-1808), a potent coronary vasodilator with prolonged action, were exploited.

1) The reaction of 5-amino-4-cyano-1-(β-D-ribofuranosyl)imidazole (**7**) with phenyl isothiocyanate gave 7-imino-5-phenylamino-3-(β-D-ribofuranosyl)imidazo[4,5-*d*][1,3]-thiazine (**11**), which, on alkaline treatment, rearranged to 6-mercapto-2-phenylamino-9-(β-D-ribofuranosyl)purine (**12**). On methylation, **12** gave the 6-methylmercapto derivative (**14**), which was converted to **1** by treatment with ammonia.

2) 5-Amino-4-cyano-1-(β-D-ribofuranosyl)imidazole (**7**) reacted with phenyl cyanamide in methanolic ammonia, giving **1** and 2-aminoadenosine as a by-product.

3) Ethyl 5-amino-1-(β-D-ribofuranosyl)-4-carboximidate (**21b**) was directly obtained by treatment of 5-amino-1-(2,3,5-tri-O-propionyl-β-D-ribofuranosyl)imidazole-4-carboxamide (**4**) with Meerwein's reagent followed by deacylation, and this was led to **1** by the reaction with phenyl cyanamide.

Keywords——CV-1808; 2-phenylaminoadenosine; phenyl cyanamide; imidazole nucleoside; coronary vasodilator; imidazo[4,5-*d*][1,3]thiazine

Adenosine is well known to possess coronary vasodilatory action and has been proposed to be a physiological mediator for regulating coronary vascular tone.[1] Because of its rapid uptake into erythrocytes and myocardial tissue, and its conversion to nucleotides by adenosine kinase or to inosine by adenosine deaminase,[2] its therapeutic application has been limited to the intraarterial route.

In order to obtain compounds having long-lasting action even on oral administration, many N^6- and 2-substituted adenosines[3] and adenosine derivatives with a modified ribose moiety[4] have been synthesized, and some of them were shown to have stronger and longer-lasting activities than adenosine.

We have reported the synthesis and coronary vasodilatory action of 2-(substituted amino)-adenosines,[5] among which 2-phenylaminoadenosine (**1**, CV-1808) showed most desirable activity on oral administration.[6] Compound **1** has been synthesized from 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (**2**) which is available in quantity[5] as follows: **2**→2-mercaptoinosine→2-bromoinosine→2-phenylaminoinosine→2′,3′,5′-tri-O-acetyl-2-phenylaminoinosine→2′,3′,5′-tri-O-acetyl-6-chloro-2-phenylaminonebularine→**1**. However, this method consists of six steps and the overall yield is low. The present study deals with new, facile synthetic methods for **1**.

In the first step[7] of the above synthesis, 5-(N′-phenylthioureido)-1-(β-D-ribofuranosyl)-imidazole-4-carboxamide (**3**) was assumed to be an intermediate, but it could not be isolated. In order to detect this intermediate, 5-amino-1-(2,3,5-tri-O-propionyl-β-D-ribofuranosyl)-imidazole-4-carboxamide (**4**) was allowed to react with phenyl isothiocyanate in dimethylformamide (DMF) to give the expected 5-(N′-phenylthiocarbamoyl)amino-1-(2,3,5-tri-O-propionyl-β-D-ribofuranosyl)imidazole-4-carboxamide (**5**), which, under heating, rapidly liberated aniline to give 2′,3′,5′-tri-O-propionyl-2-mercaptoinosine (**6**) (Chart 1). In view of this finding it was expected that **1** could be synthesized by the reaction of 5-amino-4-cyano-1-(β-D-ribofuranosyl)imidazole[8] (**7**) with phenyl isothiocyanate followed by methylation and ammonolysis (**8**→**9**→**10**) (Chart 1).

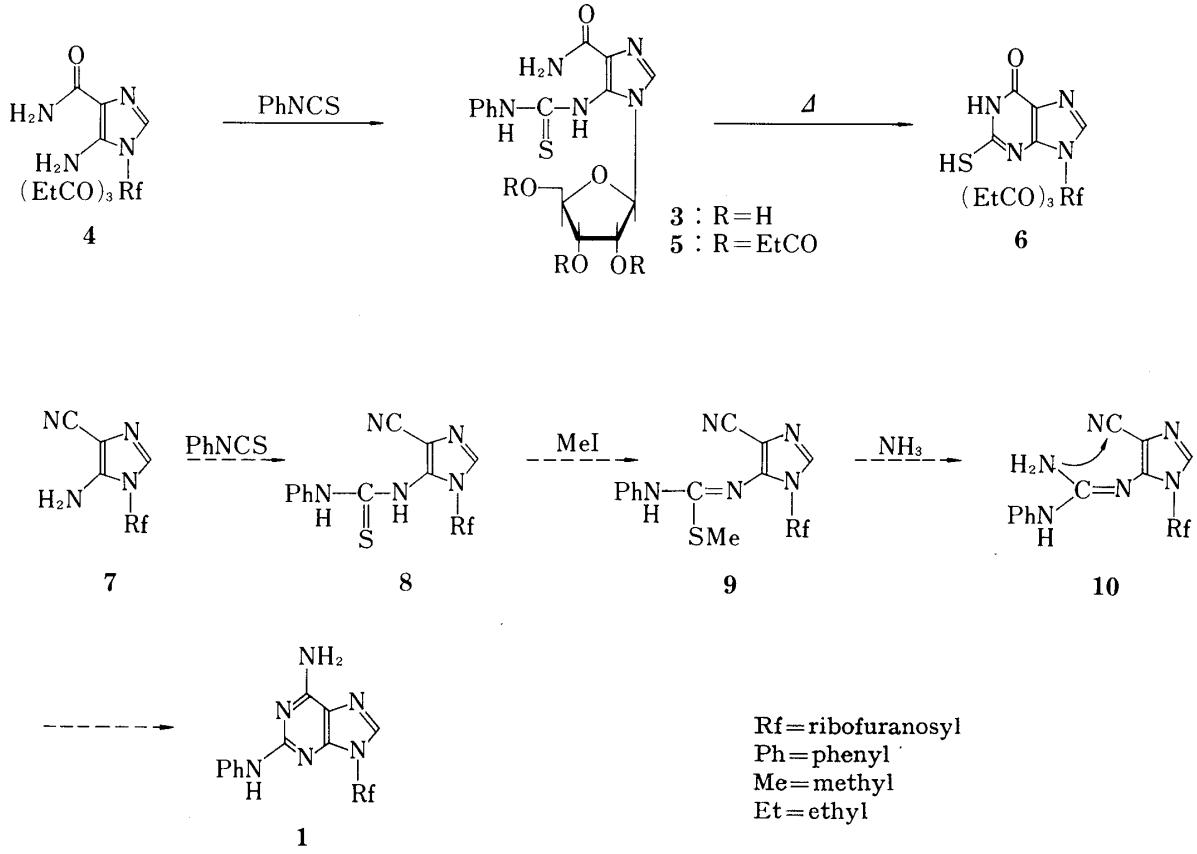

Chart 1

The reaction of **7** with phenyl isothiocyanate in DMF gave yellow crystals, which were assumed to be 5-(N′-phenylthioureido)-4-cyano-1-(β-D-ribofuranosyl)imidazole (**8**) on the basis of their elemental analysis. However, inspection of the IR spectrum revealed no peak near 2200 cm^{-1} due to the cyano group. This nucleoside was identified as 7-imino-5-phenylamino-3-(β-D-ribofuranosyl)imidazo[4,5-*d*][1,3]thiazine (**11**) by conversion of it into 6-mercapto-2-phenylamino-9-(β-D-ribofuranosyl)purine (**12**). The structure of **12** was confirmed by comparison with an authentic sample synthesized by an unambiguous route, *i.e.*, thiolation of 2-phenylaminoinosine-triacetate (**13**) followed by deacetylation. We have previously reported a similar reaction of **7** with carbon disulfide in pyridine.[5] Similarly, certain cyclic *o*-aminonitriles are known to give analogous ring closure compounds.[9] Compound **12** was methylated with methyl iodide to give 6-methylthio-2-phenylamino-9-(β-D-ribofuranosyl)purine (**14**),

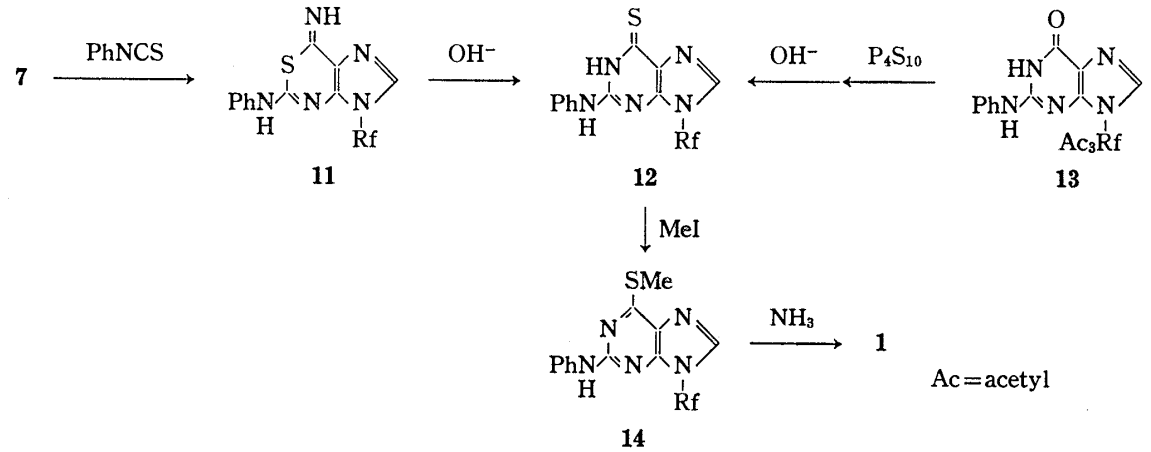

Chart 2

which was heated in methanolic ammonia at 180°C for 20 h to give **1** (Chart 2). However, this method is not satisfactory, because the yield of the last step was low.

It is known that tautomerism of phenyl cyanamide between a cyanamide structure (A) and a carbodiimide structure (B) is possible.[10] The structure (B) seems to be a nitrogen isostere of phenyl isothiocyanate. Furthermore, addition reactions of phenyl cyanamide with nucleophiles are well known.[11] Therefore, if **7** adds to phenyl cyanamide and the adduct (**10**) cyclizes to **1**, a one-step synthesis of **1** would be possible. In fact, in methanolic ammonia at 150°C, **1** was formed in about 40% yield [determined by thin-layer chromatography (TLC)] accompanied by an almost equal amount of 2-aminoadenosine[12] (**15**). Crystallization of the crude reaction product gave pure **1** in 21% yield. The yield of **1** was scarcely affected by the content of ammonia in the solvent. The same yield was obtained in methanolic propylamine, but in methanolic triethylamine complicated by-products were formed. Though detailed studies on the reaction mechanism are still lacking, **7** may either add to phenyl cyanamide or be displaced by phenylguanidine,[13] which may be formed from phenyl cyanamide and ammonia, to give 5-phenyldiaminomethyleneamino-4-cyano-1-(β-D-ribofuranosyl)imidazole (**10**), whose amino group attacks the cyano group to provide **1**. In the case of methanolic ammonia, displacement of the phenylamino group of **10** by ammonia may give 5-diamino-methyleneamino-4-cyano-1-(β-D-ribofuranosyl)imidazole (**16**), which cyclizes into **15** (Chart 3).

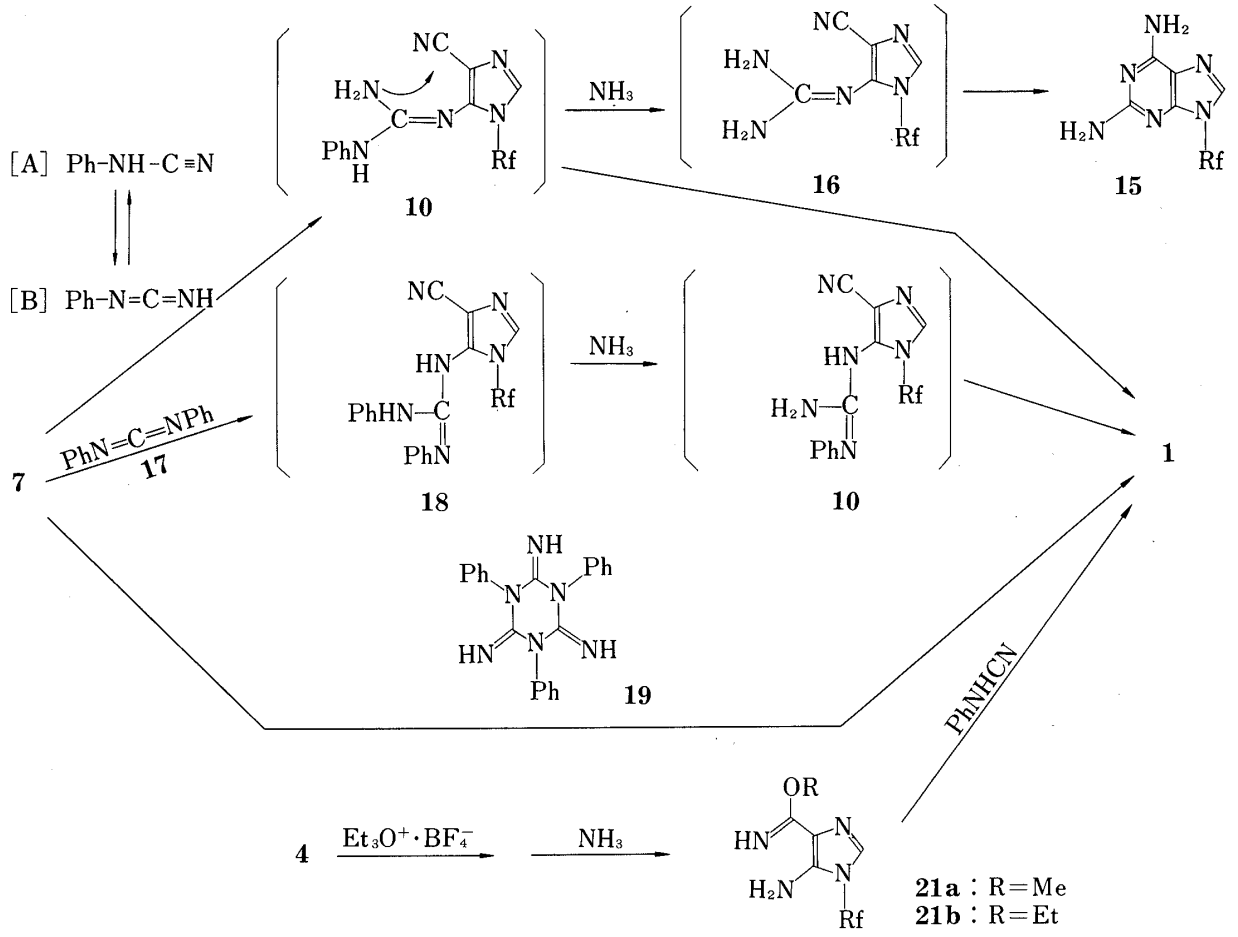

Chart 3

Similar reactions using N,N′-diphenyl carbodiimide (**17**) in place of phenyl cyanamide gave **1**, the possible intermediate being a guanidine derivative (**18**) (Chart 3). It is well known that phenyl cyanamide changes slowly into triphenylmelamine (**19**) and trianilino-s-triazine (**20**) under mild conditions.[14] Thus, the reactions of these compounds (**19**, **20**) with **7** were

examined and it was found that **1** was derived from **19**, and not from **20** (Chart 3). The reaction mechanism is not clear.

Although facile syntheses of **1** from **7** were achieved as described above, we sought yet another synthetic method for **1**. It seemed to us that methyl 5-amino-1-(β-D-ribofuranosyl)-imidazole-4-carboximidate (**21a**) might react with phenyl cyanamide to give **1**.

Compound **21a** was first prepared by reacting **7** with methanolic hydrogen chloride.[15] We found that the reaction of **4** with Meerwein's reagent followed by deacylation with methanolic ammonia gave **21b** in good yield. Compound **21b** reacted with phenyl cyanamide in methanolic ammonia to give **1** in good yield (Chart 3).

In conclusion, we have found three new methods for the synthesis of **1**. The second method was most favorable, and about eighty analogs of **1** were synthesized by this method. Structure-activity relations of the analogs will be reported elsewhere.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. NMR spectra were recorded on a Varian EM-390 spectrometer (90 MHz) using tetramethylsilane as an external standard.

The following silica gels (E. Merck) were used: "DC Alufolien Kieselgel F_{254}" for thin-layer chromatography (TLC) and "Kieselgel 0.05—0.2 mm" for column chromatography.

5-Amino-1-(2,3,5-tri-O-propionyl-β-D-ribofuranosyl)imidazole-4-carboxamide (4)——A solution of **2** (258 g) in pyridine (350 ml) and propionic anhydride (400 ml) was stirred at room temperature for 16 h. The mixture was evaporated to dryness *in vacuo*, and the residue was triturated in H_2O (2.5 l) to give crystals, mp 115—116°C (355 g, 83%). Recrystallization of an aliquot from EtOH–Et_2O afforded colorless needles, mp 117—118°C. *Anal.* Calcd for $C_{18}H_{26}N_4O_8$: C, 50.70; H, 6.15; N, 13.14. Found: C, 50.60; H, 6.10; N, 13.21.

5-(N′-Phenylthioureido)-1-(2,3,5-tri-O-propionyl-β-D-ribofuranosyl)imidazole-4-carboxamide (5)——PhNCS (10 g) was added to a solution of **4** (5 g) in DMF (30 ml), and the solution was heated at 50°C for 5 h. The reaction mixture was diluted with H_2O (200 ml) then extracted with AcOEt (100 ml×3), and the extract was washed with H_2O (100 ml×3). The extract was evaporated to dryness and the residue was chromatographed on silica gel (100 g) using $CHCl_3$–MeOH (20: 1) as an eluent. Fractions containing **5** were evaporated to dryness to give a colorless resin (2.17 g, 33%). NMR ($CDCl_3$) δ: 0.95—1.3 (9H, m, CH_3CH_2×3), 2.15—2.5 (6H, m, CH_3CH_2×3), 4.35 (3H, broad, $H_{4'}$, $2H_{5'}$), 5.25 (1H, t, $H_{3'}$), 5.6—5.75 (1H, m, $H_{2'}$), 6.2 (1H, d, $H_{1'}$), 7.0—7.6 (5H, m, Ph), 7.7 (1H, s, H_2), 9.0 (1H, broad, NH), 9.6 (1H, broad, NH). *Anal.* Calcd for $C_{25}H_{31}N_5O_8S$: C, 53.47; H, 5.56; N, 12.47; S, 5.71. Found: C, 52.83; H, 5.47; N, 12.11; S, 5.68. The mass spectrum (MS) of this compound revealed a fragment peak (*m/e* 486, M^+-PhNH_2) instead of the desired molecular ion peak (*m/e* 561). This indicates ready thermal conversion of **5** into **6**.

7-Imino-5-phenylamino-3-(β-D-ribofuranosyl)imidazo[4,5-*d*][1,3]thiazine (11)——PhNCS (25 g) was added to a solution of **7** (5 g) in DMF (50 ml) and the solution was set aside at room temperature for 4 days. After heating at 50°C for 8 h, the solution was cooled and diluted with H_2O (400 ml) and Et_2O (100 ml), whereupon a yellow crystalline solid separated. The solid was filtered off and thoroughly washed with H_2O and Et_2O. This product was dissolved in a minimum amount of DMF and the solution, on addition of Et_2O (200 ml), formed two layers. MeOH was added until a homogeneous solution was obtained. On standing at room temperature, crystals separated. They were collected and washed with EtOH and Et_2O to give yellow crystals (6.55 g, 88%), mp 150—170°C (dec.). NMR (DMSO-d_6) δ: 5.86 (1H, d, $H_{1'}$), 6.9—8.0 (5H, m, Ph), 8.20 (1H, s, H_8), 10.52 (1H, s, PhNH), 10.8—11.4 (1H, NH). *Anal.* Calcd for $C_{16}H_{17}N_5O_4S$: C, 51.20; H, 4.57; N, 18.66. Found: C, 51.50; H, 4.36; N, 18.06. MS *m/e*: 375 (M^+).

6-Mercapto-2-phenylamino-9-(β-D-ribofuranosyl)purine (12)——A mixture of **11** (4 g) and 1 N NaOH (40 ml) was heated at 100°C for 30 min and after cooling, the solution was slightly acidified with 10% aq. HCl to deposit a pale yellow powder (3.13 g, 78%), which was recrystallized from 70% MeOH to give pale yellow crystals, mp 235—240°C (dec.). NMR (DMSO-d_6) δ: 5.75 (1H, d, $H_{1'}$), 6.9—7.7 (5H, m, Ph), 8.20 (1H, s, H_8), 9.0 (1H, s, PhNH), 11.9 (1H, s, NH or SH). *Anal.* Calcd for $C_{16}H_{17}N_5O_4S$: C, 51.20; H, 4.57; N, 18.66. Found: C, 51.17; H, 4.59; N, 18.67. MS *m/e*: 375 (M^+).

6-Methylthio-2-phenylamino-9-(β-D-ribofuranosyl)purine (14)——MeI (160 mg) was added to a solution of **12** (375 mg) in 1 N NaOH (1.2 ml). The solution soon began to precipitate colorless crystals, which were filtered off and washed with H_2O sufficiently to give **14** (0.23 g, 83%), mp 245—248°C. *Anal.* Calcd for $C_{17}H_{19}N_5O_4S$: C, 52.44; H, 4.92; N, 17.99. Found: C, 52.33; H, 5.03; N, 17.50. NMR (DMSO-d_6) δ: 2.67 (3H, s, Me), 5.88 (1H, d, $H_{1'}$), 6.7—7.9 (5H, m, Ph), 8.28 (1H, s, H_8), 9.42 (1H, s, PhNH). Compound **14** (150 mg) in 20% NH_3–MeOH (10 ml) was heated at 180°C for 3 h in an autoclave. The yield of **1** was determined by TLC (solvent, $CHCl_3$–MeOH (2: 1)) to be 30%.

Synthesis of 12 from 13——A mixture of **13** (1.5 g), P_4S_{10} (2.5 g), pyridine (35 ml), and H_2O (0.2 ml) was heated under reflux for 4 h. The solution was evaporated to dryness and hot H_2O (60 ml) added. The mixture was boiled for 15 min, and the crystals that separated on cooling were filtered off and chromatographed on silica gel (80 g) using $CHCl_3$–MeOH (20: 1) as an eluent. Fractions containing the triacetate of **12** were concentrated and the residue was treated with Et_2O to give the triacetate of **12** as an amorphous powder (640 mg, 41%), mp 128—132°C. This product (0.4 g) was dissolved in 20% NH_3–MeOH (10 ml) and the solution was kept at room temperature overnight and then evaporated to dryness. The residue was recrystallized from 70% EtOH to give **12** (0.2 g, 67%), mp 236—240°C (dec.), undepressed on admixture with a specimen of **12** derived from **11**.

Reaction of 7 with PhNHCN in NH_3–MeOH——A solution of **7** (100 g) and PhNHCN[16] (110 g) in 20% NH_3–MeOH (1.5 l) was heated at 150°C for 5 h in an autoclave. The brown reaction mixture was evaporated to dryness and the residue was triturated with EtOH (500 ml) to give a crystalline solid. Recrystallization from hot H_2O (10 l) gave crude **1** as brown needles (34 g). Recrystallization of these from 20% EtOH and then from hot H_2O afforded pure **1** as colorless needles (31 g, 21%), mp 247—248°C. *Anal.* Calcd for $C_{16}H_{18}N_6O_4$: C, 53.62; H, 5.06; N, 23.45. Found: C, 53.45; H, 4.99; N, 23.24.

The mother liquor, after isolation of crude **1**, was concentrated to a small volume and kept at 0°C for several days. Compound **15** separated slowly as stout rods (21 g), mp 239—240°C (dec.) lit.[12] mp 245—247°C from 50% EtOH. UV: $\lambda_{max}^{pH\ 1}$ 251, 290 nm, $\lambda_{max}^{pH\ 13}$ 255, 278 nm.

Reaction of 7 with PhNHCN in Methanolic Propylamine——In the manner described for the above experiment, **7** (1 g), PhNHCN (1.2 g), and 33% *n*-$PrNH_2$–MeOH (30 ml) were heated and the colored solution was evaporated to dryness to give a brown resin. It was triturated with $CHCl_3$ and the deposited solid was filtered off. Recrystallization from hot H_2O afforded **1** (0.3 g) as pale yellow needles, mp 245—248°C.

Reaction of 7 with N,N′-Diphenylcarbodiimide (17)——A mixture of **7** (1 g), **17** (2 ml), and 20% methanolic ammonia (20 ml) was heated at 180°C for 5 h in an autoclave. The reaction mixture was evaporated to dryness and the residue was crystallized from EtOH to give **1** (150 mg), mp 243—245°C.

Reaction of 7 with Triphenylmelamine (19)——Compound **19** was obtained from PhNHCN according to Arndt's method.[14] In a manner similar to that described for the experiment using PhNHCN, a mixture of **7** (1 g), **19** (mp 224—225°, 1.2 g), and 20% NH_3–MeOH (20 ml) was treated to give **1** (0.25 g), mp 243—245°C.

Ethyl 5-Amino-1-(β-D-ribofuranosyl)imidazole-4-carboximidate (21b)——Epichlorohydrin (4.95 g) was added dropwise to a stirred and ice-cooled solution of $BF_3 \cdot OEt_2$ (47%, 15 g) in anhydrous Et_2O (40 ml). The solution was left at room temperature for 30 min, whereupon a viscous resin ($Et_3O^+ \cdot BF_4^-$) separated. The supernatant liquid was decanted, and the resin was washed with Et_2O, and dissolved in CH_2Cl_2 (40 ml). A solution of **4** (10 g) in CH_2Cl_2 (60 ml) was added dropwise to this solution under ice-cooling and stirring. After cooling overnight, the reaction mixture was diluted with $CHCl_3$ (300 ml) and washed with cold aq. $NaHCO_3$ twice. The organic layer was concentrated to give the tri-O-propionate of **21b** as a viscous oil (10.6 g, 100%).

The oil (10 g) was dissolved in 20% NH_3–MeOH (150 ml) and kept at 0°C overnight. The solution was concentrated to 20 ml and cooled. It deposited crystals of **21b** (3.2 g, 67%), which were recrystallized from MeOH–Et_2O to yield colorless crystals, mp 179—181°C. *Anal.* Calcd for $C_{11}H_{18}N_4O_5$: C, 46.15; H, 6.34; N, 19.59. Found: C, 45.68; H, 6.43; N, 19.01. MS *m/e*: 286 (M^+). NMR (DMSO-d_6) δ: 1.29 (3H, t, CH_3CH_2), 4.25 (2H, q, CH_3CH_2), 5.44 (1H, d, $H_{1'}$), 7.38 (1H, s, H_2).

Reaction of 21b with PhNHCN——A mixture of **21b** (1 g), PhNHCN (1.2 g), and 20% NH_3–MeOH (30 ml) was heated at 150°C for 5 h. The reaction mixture was evaporated to dryness and the residue was chromatographed on silica gel (30 g) using $CHCl_3$–MeOH (10: 1) as an eluent. The fractions containing **1** were collected and the solvent was evaporated off to give crystals of **1** (0.27 g), mp 244—246°C.

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