140. The Donor-Acceptor-Acceptor Purine Analog: Transformation of 5-Aza-7-deaza-1H-isoguanine (= 4-Aminoisimidazo[1,2-a]-1,3,5-triazin-2(1H)-one) to 2'-Deoxy-5-aza-7-deaza-isoguanosine Using Purine Nucleoside Phosphorylase

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A new synthesis is reported for 4-aminoisimidazo[1,2-a]-1,3,5-triazin-2(1H)-one (= 5-aza-7-deaza-isoguanosine; 8), a purine analog that, when incorporated into an oligonucleotide chain, presents a H-bond donor-acceptor-acceptor pattern to a complementary pyrimidine analog. A protected ribose derivative was coupled to 8 to yield 4-amino-8-(beta-D-ribofuranosyl)imidazo[1,2-a]-1,3,5-triazin-2(8H)-one (= 5-aza-7-deaza-isoguanosine; 11) after deprotection. Alternatively, direct synthesis of both the ribo derivative 11 and the corresponding deoxyribo derivative 17 as the beta-D-anomers was achieved using the enzyme purine nucleoside phosphorylase in a one-pot reaction. This adapts a known synthetic approach to yield a new strategy for obtaining diastereoisomerically pure deoxyribonucleoside analogs on 1-gram scales.

Introduction. – In the preparation of ribonucleosides and their analogs, anhimeric assistance from substituents at the 2'-position of the D-ribose ring readily yields products having a beta-D-configuration at the anomeric center [2-5]. In deoxyribonucleosides, similar assistance is not available, and reactions that couple a heterocycle and a 2'-deoxy-D-ribose derivative generally yield mixtures of alpha- and beta-D-isomers [6-13]. This is the key problem in the synthesis of many deoxyribosides, and much work has been devoted to solving it.

Enzyme-catalyzed coupling of deoxyribose derivatives and heterocycles offers one possible strategy for obtaining anomerically pure nucleoside analogs. E.g., both N-deoxyribosyltransferase and purine nucleoside phosphorylase (PNPase) transfer heterocycles on and off deoxy-D-ribose derivatives, with the beta-D-anomer being the exclusive product [14][15]. The use of enzymes as synthetic tools often encounters other difficulties, however, including inconvenient reactants and products, narrow substrate specificity, and idiosyncrasies and unreliability of reaction procedures.

Hennen and Wong [1] recently reported a particularly elegant procedure for addressing the first difficulty in ribose transferase reactions. In their procedure, 7-methylguanosine (12) serves as the glycosyl donor (see below, Scheme 3). Ribose 1-phosphate is formed in situ with the concomitant precipitation of essentially insoluble 7-methylguanine (14) as by-product. The ribose 1-phosphate can then accept a wide range of heterocycles (see below, Scheme 3).

The narrow substrate specificity of many glycotransferases remains problematic. For example, N-deoxyribosyltransferase does not accept substrates that differ only slightly in structure from the natural substrates (e.g., 7-deazapurine) [16]. Thus, this enzyme cannot
serve as a general tool for preparing nucleoside analogs. This problem is alleviated using PNPase, which can accept a wider range of heterocycles [17–21]. Further, 2'-deoxyribo-nucleosides are substrates for PNPase [17] [20] [22] [23]. However, the Hennen-Wong procedure with 2'-deoxy-7-methylguanosine (18) as glycosyl donor has, to our knowledge, never been explored with deoxyribose derivatives and PNPase.

As part of work in this laboratory aimed at expanding the genetic alphabet [24], we had need for both 9-(ribosyl) [25] and 9-(2'-deoxyribosyl)-5-aza-7-deaza-9H-isoguanine (11 and 17, resp., see below Scheme 3). The heterocycle borne by these nucleoside analogs presents a H-bond donor-donor-acceptor pattern to a pyrimidine analog on a complementary strand of DNA.

Rosemeyer and Seela prepared a small amount of a different (but related) β-D-nucleoside, 2'-deoxy-5-aza-7-deazaguanosine, by incubating 2'-deoxy-β-D-ribofuranose 1-phosphate, the heterocycle, and PNPase [11]. Therefore, we thought it should be relatively straightforward to use the procedure of Hennen and Wong to prepare 5-aza-7-deazaisoguanosine (11) and 2'-deoxy-5-aza-7-deazaisoguanosine (17). Unfortunately, our work encountered the third problem commonly observed in enzyme-assisted synthetic reactions: idiosyncrasies. When 7-methylguanosine (12) [26] was incubated with PNPase under the conditions of Hennen and Wong (0.25m phosphate buffer, pH 7.8 room temperature) [1] in the presence of 5-aza-7-deaza-1H-isoguanine, essentially none of the desired product was obtained.

Notably, the enzyme used by Hennen and Wong was obtained from Toyoba Chemical Co., ours was from Sigma. While we do not know whether this difference fully accounts for the failure of the reaction with 5-aza-7-deaza-1H-isoguanine and for the extremely low conversion rates with adenine in our hands using Hennen-Wong conditions (see below), the Sigma enzyme is more accessible to many workers. Therefore, an optimal set of reaction conditions was developed using the Sigma enzyme with adenine as a substrate. Then, these conditions were applied to the preparation of 5-aza-7-deazaisoguanosine (11).

**Results and Discussion.** – For comparison purposes, 5-aza-7-deazaisoguanosine (11) was first prepared by a classical approach starting from aminimidazolium sulfate 1. The free base 2 of 1 was obtained by neutralization of an aqueous solution of 1 with Na₂CO₃, followed by extraction with EtOH or MeCN [27]. In analogy to syntheses performed with 2-aminothiazole [28] [29], the condensed triazine ring system 5 was synthesized by reaction of 2-amino-1H-imidazole (2) with (phenylthio)carbonyl isothiocyanate (4), which was prepared in situ from S-phenyl chlorothioformate (3, Scheme 1). The polarity of the
product made it difficult to isolate. Therefore, crude 5 was treated directly with benzyl bromide, yielding dibenzylated product 6, which was easily separated from other alkylated products by chromatography. The benzyl-sulfide group was then replaced with ammonia in MeOH, yielding the benzylated nucleoside base 7. Conditions could not be found for removal of the benzyl group of 7 by catalytic hydrogenation using Pd catalysis [30–33]. But reaction of 7 with Na in liquid ammonia [34] yielded the desired 5-aza-7-deaza-1H-isoguanine (8; Scheme 2).

![Scheme 2]

The glycosylation of 5-aza-7-deaza-1H-isoguanine (8) failed, remarkably, under the conditions of the silyl modification of the Hilbert-Johnson reaction using trimethylsilyl triflate as catalyst developed by Vorbrüggen and coworkers [3–5]. Therefore, the classical method (silylated base, SnCl₂/dichloroethane) [35], already applied successfully to the synthesis of 5-aza-7-deazaguanosine by Kim et al. [36], was used. This procedure applied to 8 and 9 led to the protected ribonucleoside 10 in a yield of 60%. Cleavage of the benzoyl protecting groups with NH₃ in MeOH yielded the target 5-aza-7-deazaisoguanosine (11) as a crystalline white solid (Scheme 2). The analytical data agreed with those reported by Prisbe et al. [25], and this material served as a standard in the enzymatic work.

A systematic study was then made to find optimal conditions for enzymatic coupling of adenine to ribose, the latter being generated as the 1-phosphate derivative from 7-methylguanosine (12) according to Hennen and Wong [1] (Scheme 3). The high concentrations of phosphate (250 mm) recommended by Hennen and Wong reduced the yield of the expected adenosine (13) by ca. 80% with the Sigma enzyme employed by us. Of three buffer concentrations tested (250, 100, and 50 mm), best results were achieved with 50 mm. The yield was insensitive to the pH between 7.0 and 7.8. The enzyme tolerated temperatures of 60°, and the yield of product 13 was highest at this temperature (the by-product 7-methylguanine 14 precipitated).

The optimal reaction conditions (phosphate buffer 0.05M, pH 7.0, 60°) were then used to prepare 5-aza-7-deazaisoguanosine (11) from 12 and 5-aza-7-deaza-1H-isoguanine (8) according to Scheme 1. HPLC analysis showed more than 50% product formation after
10 days (Fig. 1), establishing that 11 can be prepared enzymatically. As the chemical synthesis appears preferable for the preparation of large amounts of 11 the workup was not optimized. The structure of the product was proven by comparison with material synthesized via the nonenzymatic route. A comparison between adenine and 8 showed that 8 reacted to 11 ca. 5 times slower than adenine to 13.

This procedure was then directed towards the synthesis of the corresponding deoxyribonucleoside. The 2′-deoxy-7-methylguanosinium iodide (15) was prepared by alkylation of 2′-deoxyguanosine [26] and used as the glycosyl donor (Scheme 3). Reaction conditions were optimized using 15 and adenine for the formation of 2′-deoxyadenosine

![Chemical structures and reaction scheme](image)

**Fig. 1. Enzymatic formation of 5-aza-7-deazaG2Guanosine (11) by Route A (see Scheme 2). Reaction conditions: 5-aza-7-deaza-1H-isoguanine (8), 8 μmol; 7-methylguanosine (12), 32 μmol; PNPase, 4 u; phosphate conc., 0.05M; volume, 1.66 ml; pH 7.0; temp. 60°. Yields by HPLC.**

**Fig. 2. Enzymatic formation of 2′-deoxyadenosine (16) by Route B (see Scheme 3). Reaction conditions: adenine, 8 μmol; 2′-deoxy-7-methylguanosinium iodide (15), 32 μmol; PNPase, 9 u; phosphate conc., 0.05M; volume, 1.66 ml; pH 7.4; time 16 h. Yields by HPLC.
(16) (see Fig. 2). The optimal temperature was found to be 45°; at higher temperature, product formation decreased rapidly. Moreover, rather high enzyme concentrations (23 units/8 μmol of heterocycle) were necessary to achieve high conversion rates (70% of 16) in a reasonable time (16h).

The optimal reaction conditions (45°, 23 units PNPase, 50 mM phosphate, 32 μmol of 15, 8 μmol free base, 1.66 ml) were then used for the synthesis of 2'-deoxy-5-aza-7-deazaadinosine (17) from 15 and 8 (Scheme 3). A comparison between 8 and adenine showed that 2'-deoxy-5-aza-7-deazaadinosine (17) was formed ca. three times slower than 2'-deoxyadenosine (16). With 2'-deoxy-7-methylguanosine iodide (15), yields of 17 (based on heterocycle) were never higher than 20%, even with very high enzyme concentrations. Variation of pH and temperature did not increase the yields. Interestingly, however, the glycosyl donor influenced greatly the yield of the reaction. Yields after 24 h using 15 were ca. 50% below those obtained under analogous conditions with the zwitterionic 2'-deoxy-7-methylguanosine (18; Scheme 3), prepared from 15 by neutralization with MeNH₂/H₂O [26]. Moreover, with iodide salt 15, the pH of the reaction mixture changed upon cleavage of 7-methylguanine, making high concentrations of this glycosyl donor incompatible with low concentrations of buffer.

By varying the concentration of 2'-deoxy-7-methylguanosine (18) and PNPase, conditions useful for the synthesis of 2'-deoxy-5-aza-7-deazaadinosine (17) could be found. Doubling the glycosyl-donor concentration led to an increase in the yield of 17 of 40%. Further increase in the concentration of 18 increased yields only slightly. The amount of 17 isolated was a linear function of the amount of enzyme added, and high enzyme concentrations were used for an efficient synthesis of 17. After equilibrium was attained, the enzyme was recovered by ultrafiltration and recycled. Typically, ca. 5% of enzyme activity was lost during each recycling step. The starting concentration of PNPase was chosen so that at least five cycles could be run. By this technique, 1120 units of PNPase and 780 μmol of 5-aza-7-deaza-1H-isoguanine (8) yielded 54% of isolated deoxyribonucleoside 17.

In the deoxy series, a NOE from H−C(1') to H−C(4') established the β-d-configuration of the principal product (a NOE from H−C(1') to H−C(3') expected in the α-d-isomer was missing) [37]. The position of glycosylation (N(8)) of the imidazotriazine base was assigned by comparison of the 1H- and 13C-NMR data of 17 with the corresponding ribonucleoside 11 and the N(1)-linked ribonucleoside, both known by independent chemical synthesis [25] [38].

This work showed PNPase to be a servicable tool for preparing β-d-configurated purine nucleoside analogs on a 1-gram scale. Further, by using deoxymethylguanosine 18 as a glycosyl donor, a glycosyl exchange reaction proved to be satisfactory, avoiding the need for a second enzyme to generate the intermediate ribose 1-phosphate.

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Experimental Part

General. Fluka solvents (puriss. p.a., puriss., or purum) were used for all syntheses. MeCN was distilled over CaH₂ and DMF dried over molecular sieves (4 Å, 2-3 mm). The 7-methylguanosine (12) was synthesized according to [26]. The following chemicals were purchased from Fluka: adenine (purum), 1-O-acetyl-2',3',5'-tri-O-benzoyl-β-D-ribofuranose (purum), 2'-deoxyguanosine monohydrate (> 98%), benzyl bromide (purum), 1,1,1,3,3,3-hexamethyldisilazane (HMDS; purum, freshly distilled before use), MeNH₂-purum, Na₂CO₃, and Et₃N. From Aldrich were purchased: 2-aminomimidazole sulfate and SnCl₄, KSCN was obtained from Mallinkrodt Chemical Works, (phenylthio)carbonyl chloride (= 5-phenyl chloroethioformate) from Alfa Products, and K₃PO₄ from Merck. From Sigma were purchased: guanosine (Sigma Grade) and bacterial purine nucleoside phosphorylase (= PNase; lyophilized powder (60% enzyme), 14 μg/mg; 1 unit is the amount of enzyme that phosphorylates 1.0 μmol of inosine to hypoxanthine and ribose 1-phosphate at 25°C and pH 7.4). PNase was recycled via ultrafiltration through a Centricon-10 filtration apparatus purchased from Amicon, Dialflo ultrafiltration membranes of the type PM 10 were used. Column chromatography: reversed-phase silica gel (CF8, 40 μm) from J.T. Baker. Flash chromatography (FC): standard flash silica gel 60. Ion-exchange chromatography: Dowex 1 × 8 resin, 200–400 mesh, OH form (Bio-Rad) 1.5 × 16 cm column; 10–15 ml fractions; elution with linear gradient 0–1.0 M (Et₄N)HCO₃, total volume 400 ml; detection by UV at 254 nm, single fractions by HPLC or HPTLC. HPLC: all enzymatic reactions with PNase were followed by measuring the UV absorbance at 254 and 277 nm in the HPLC; the integration of the UV absorbance at 254 nm formed the basis for calculating the yields of reactions that were not worked up for deoxyadenosine, and anal. C8 reversed-phase column (Brownlee Labs Aquapore RP-300, 22 cm × 4.6 mm, particle size 7 μm) was used, flow rate 1 ml/min, elution buffer 0.1 M (Et₄N)HNO₃, pH 7.5, without gradient, for all other reactions, a semi-prep. C18 reversed-phase column (Supelco LC-18-DB, 25 cm × 10 mm, particle size 5 μm) was used, flow rate 2 ml/min, elution buffer 0.1 M (Et₄N)HAc, pH 7.5, with gradient A (0–4% MeCN in 5 min, then increase to 8% MeCN in another 25 min) or gradient B (0–4% MeCN in 30 min). UV spectra: in nm. IR Spectra: in cm⁻¹. NMR Spectra: at 93.94 kg (400 MHz for H, 100 MHz for 13C) or 70.46 kg (300 MHz for H, 75 MHz for 13C), δ in ppm rel. to Me₄Si (= 0.00 ppm) as internal standard; OH and NH assignments were confirmed by D₂O exchange. MS: m/z (rel. intensity in %).

Nenzymatic Syntheses. 2-Amino-1H-imidazole (2). Following literature procedures [27], 1 (3.30 g, 25 mmol) was dissolved in H₂O (15 ml) together with Na₂CO₃ (2.65 g, 25 mmol). The H₂O was removed by evaporation and the residue extracted with EtOH (6 × 20 ml). The solvent was evaporated and the remaining brown oil dried overnight under high vacuum: 2 (1.65 g, 79%), which was used in the next reaction.

A similar reaction on larger scale of 1 (13.77 g, 104 mmol) gave 2 (6.51 g, 75%).

3,4-Dihydro-4-thioxoimidazol-1,2-a-1,3,5-triazin-2(1H)-one (5). KSCN (8.00 g, 75 mmol) was suspended in abs. MeCN (70 ml). 5-Phenyl chloroethioformate (3; 9.40 ml, 75 mmol) was added dropwise at r.t. The product 4, formed in situ after 5 min stirring, was immediately used in the subsequent cyclization. A larger-scale reaction was also carried out with a larger amount of 3 (21 ml, 168 mmol) and KSCN (18.65 g, 192 mmol).

To the suspension of 4 in MeCN was added Na₂CO₃ (2.65 g, 25 mmol) and then dropwise a soln. of 2 (1.65 g, 20 mmol) in MeCN (15 ml). The mixture was stirred for 1 h. The yellow precipitate was filtered off, washed with MeCN and Et₂O, and dried under high vacuum: crude 5 (10.03 g). Yellow brown solid, which was used directly for the next reaction.

A larger-scale reaction with more 2 (6.50 g, 78 mmol) gave 5 (22.05 g).

1-Benzyl-4-(benzylthio)imidazole [1,2-a-1,3,5-triazin-2(1H)-one (6). In a soln. of 5 (10.03 g) in DMF (70 ml), Na₂CO₃ (2.65 g, 25 mmol) was suspended and benzyl bromide (5.95 ml, 50 mmol) injected. The soln. was stirred 1.5 h at r.t., then diluted with CH₂Cl₂, and extracted with sat. aq. NH₄Cl soln., H₂O, and sat. aq. NaCl soln. The org. phase was dried (MgSO₄) and evaporated. Chromatography (silica gel (300 g), Et₂O/hexane 8:2) yielded 6 (1.06 g, 15% over 2 steps). Light yellow solid, which was used directly for the next reaction. Ring-benzylated products, not useful for subsequent synthesis, were the primary side products. An anal. sample of 6 was obtained via recrystallization from Et₂O/hexane 3:1. M.p. 134–135°C. UV (EtOH): 234 (11.300), 260 (10.700), 300 (2600). IR (CHCl₃): 3060, 3025, 1680, 1610, 1525, 1490, 1450, 1370, 1350, 1230, 1170, 1040, 960. 1H-NMR (CDCl₃): 4.59 (s, CH(Si)); 5.34 (s, CH₂N); 7.04 (d, J = 1.9, H−C(6)); 7.07 (d, J = 1.9, H−C(7)); 7.24–7.43 (m, 4 H; 4 H); 7.57–7.61 (m, 2 H). 13C-NMR (CDCl₃): 35.74 (t, CH₂S); 46.94 (t, CH₂N); 109.35 (d, C(6)); 128.10 (d, aro. CH); 128.29 (d, aro. CH); 128.53 (d, aro. CH); 129.97 (d, aro. CH); 129.34 (d, aro. CH); 129.42 (d, aro. CH); 131.07 (d, C(7)); 134.50 (s, C₆H₅); 135.60 (s, C₊); 144.47 (s, C(8a)); 151.01 (s, C(2)); 160.40 (s, C(4)). MS: 349 (6, [M+1]⁺), 348 (24, M⁺), 257 (8), 200 (12), 91 (100). Anal. calc. for C₂₇H₂₆N₄O₂S (348.43): C 65.50, H 4.63, N 16.08; found: C 65.26, H 4.70, N 16.07.

A scaled-up reaction with 5 (22.0 g) gave 6 (3.32 g, 12% over 2 steps).
4-Amino-1-benzylimidazo[1,2-a]1,3,5-triazin-2(1H)-one (7). To 6 (835 mg, 2.4 mmol) in a dry flask, MeOH sat. with NH₃ (40 ml) was injected at low temp. The flask was sealed and the mixture stirred for 1 h r.t. The NH₃ was evaporated and the solvent removed under vacuum to yield 7 (0.594 g). Slightly impure white solid, which was used without further purification for the next reaction. An anal. sample of 7 was obtained via recrystallization from MeCN. M.p. 238° (dec.). UV (EtOH): 232 (9100), 263 (3700). IR (KBr): 3340, 3150, 3125, 3025, 2740, 1680, 1635, 1549, 1430, 1370, 1230, 1080, 1650. 1H-NMR (D₂O): 5.13 (s, CH₃); 7.04 (d, J = 1.8, H-C(6)); 7.21-7.37 (m, 5 arom. H); 7.63 (d, J = 1.8, H-C(7)); 8.19-8.49 (br. s, NH₂). 13C-NMR (D₂O): 44.34 (s, CH₃); 108.53 (d, C(6)); 126.03 (d, C₅); 126.58 (d, C₆); 127.10 (d, C₇); 132.88 (d, C(7)); 135.06 (s, C₉); 144.25 (s, C(8a)); 148.52 (s, C(7)); 152.07 (s, C(4)). MS: 242 (S, [M + H]⁺), 241 (S, [M⁺]), 199 (16), 91 (100). Anal. calc. for C₁₇H₁₁N₂O₁ (241.25): C 59.74, H 4.60, N 29.03; found: C 59.72, H 4.85, N 29.14.

An analogical reaction with more 6 (3.30 g, 9.5 mmol) gave 7 (2.48 g).

4-Aminoimidazo[1,2-a]1,3,5-triazin-2(1H)-one (8). To a suspension of 7 (372 mg, 1.5 mmol) in liquid NH₃ (30 ml), Na was added in small pieces until the blue color persisted. Excess Na was destroyed with NH₃Cl and the NH₃ evaporated. The residual solid was dissolved in H₂O (7 ml) and the pH of the soln. adjusted to 5 with 2M HCl. The soln. was cooled and the resulting precipitate isolated by filtration: 8 (203 g, 85% over 2 steps). White solid. An anal. sample was obtained by recrystallization from MeOH/H₂O 8:1. M.p. > 300° (EtOH): 239 (6500), 255 (2500). IR (KBr): 3250, 3130, 2760, 1710, 1660, 1570, 1470, 1300, 1205, 1080. 1H-NMR (D₂O): 6.97 (d, J = 1.8, H-C(6)); 7.55 (d, J = 1.8, H-C(7)); 8.08 (br. s, NH₂). 13C-NMR (D₂O): 108.06 (d, C(6)); 128.88 (d, C(7)); 144.83 (s, C(8a)); 150.53 (s, C(2)); 152.44 (s, C(4)). FAB-MS (thioglycercine): 152 ([M + H]⁺). Anal. calc. for C₁₇H₁₁N₂O₁: 0.5 H₂O (160.14): C 37.49, H 3.78, N 43.74; found: C 37.58, H 3.54, N 43.60.

An analogical reaction of 7 (1.30 g, 5.4 mmol) gave 8 (668 mg, 87% over 2 steps).

4-Amino-8-[2',3',5'-tri-O-benzoyl-β-D-ribofuranosyl]imidazo[1,2-a]1,3,5-triazin-2(8H)-one (10). Dry 8 (23.7 mg, 0.157 mmol) was suspended in freshly distilled HMDS (0.5 ml). A small amount of NH₃Cl was added and the mixture refluxed for 24 h. The excess HMDS was removed by distillation under high vacuum. The resulting white solid was dried and then dissolved in dichloroethane (0.4 ml). A soln. of 9 (79.2 mg, 0.157 mmol) in dichloroethane (0.4 ml) was added, followed by SnCl₅ (25 µl). The mixture was stirred for 4 h at r.t., then diluted with CH₂Cl₂ and extracted with sat. aq. NaHCO₃ and NaCl solns. The organ. phase was dried (Na₂SO₄) and the solvent evaporated. The isolated oil was purified by chromatography (small silica-gel column, CH₂Cl₂/EtOH 9:1): 10 (56 mg, 60%) as light brown solid. UV (EtOH): 218 (42000), 220 (46000). IR (KBr): 3420, 3130, 3060, 1730, 1690, 1600, 1540, 1450, 1315, 1270, 1180, 1090, 1025. 1H-NMR (D₂O): 4.64-4.83 (m, H-C(4'), 2 H-C(5')); 6.03 (dd, J = 5.5, 5.9, H-C(3')); 6.19 (dd, J = 5.1, 5.9, H-C(2')); 6.30 (d, J = 5.1, H-C(1')); 7.42-7.70, 7.88-8.04 (2m, 15 arom. H, H-C(6), H-C(7)); 7.79 (br. s, NH₂). 13C-NMR (D₂O): 63.62 (t, C(5')); 70.56 (d, C(3')); 72.55 (d, C(2')); 78.99 (d, C(4')); 85.39 (d, C(1')); 107.42 (d, C(6)); 115.73 (d, C(7)); 128.19 (s, C(9)); 128.45 (s, C(9a)); 128.68 (d, C(9a)); 129.26 (d, C(9a)); 133.44 (d, C(9)); 133.79 (d, C(9)); 143.96 (s, C(8a)); 150.49 (s, C(2)); 161.53 (s, C(4)); 164.43 (s, C(6)); 164.51 (s, C(8a)); 145.32 (s, C(8a)); 150.49 (s, C(2)); 161.53 (s, C(4)); 164.43 (s, C(6)); 164.51 (s, C(8a)); 145.32 (s, C(8a)). FAB-MS (3-nitrobenzyl alcohol): 618 ([M + Na]+), 596 ([M + H]+), 445 (ribose part), 152 (free base).

An analogical reaction with 8 (50 mg, 0.33 mmol) gave 10 (82 mg, 44%) as light brown foun.
Enzymatic Synthesis. – Adenosine (13). To a soln. of adenine (1.1 mg, 8 μmol) and 7-methylguanosine (12: 11.1 mg, 32 μmol) inaq. phosphate buffer (1.50 ml; 0.05M K_H_2PO_4, pH 7.0) was added PNPase (4 units, 0.16 ml of a freshly prepared soln. containing 25 μl). Immediately a white precipitate formed. The mixture was allowed to stand at 60°C in the water bath with gentle shaking. HPLC monitoring (gradient A): adenine (t_R 22 min), 14 (t_R 23 min), 13 (t_R 29 min). The mixture was not worked up. HPLC analysis after 22 h gave a yield of 70% for 13.

4-Amino-8-([β-d-ribofuranosyl]imidazo[1,2-a]-1,3,5-triazin-2(8H)-one (5-Aza-7-deazaisoguanosine; 11). As described for 13, with 8 (50.0 mg, 0.3 mmol), 12 (416.0 mg, 1.3 mmol), phosphate buffer (50 ml; 0.05M K_H_2PO_4, pH 7.0), and PNPase (9.0 mg, 126 units). HPLC monitoring (gradient B): 8 (t_R 16 min), 11 (t_R 17 min), 14 (t_R 26 min). After 230 h, the mixture was worked up. The precipitate was recovered by centrifugation and the supernatant soln. evaporated until it turned slightly cloudy. The soln. was then loaded on an ion-exchange column (for exper. details, see General). 11 (0.35-0.45M (Et_3NH)HCO_3) followed by 8 (0.5-0.6M (Et_3NH)HCO_3). The ribonucleoside fractions were lyophilized. The white lyophilisate was dissolved in H_2O and 11 precipitated with MeCN. Drying under high vacuum gave 11 (19 mg, 22%) as white powder. Anal. data: in agreement with those given above.

2'-Deoxy-7-methylguanosinium Iodide (15). The synthetic procedures [26] were modified to achieve reproducible results in our hands. Deoxyguanosine hydrate (20.0 g, 70.1 mmol) was dissolved in DMSO (150 ml) at r.t. Mel (20 ml, 315 mmol) was added at r.t. and the mixture stirred at r.t. for 1 h. The mixture turned orange. Excess Mel was removed by purging with N_2. The soln. was diluted with CHCl_3 (1200 ml) at r.t. and then cooled to 0°C for 3 h. The resulting precipitate was isolated by filtration and washed with cold EtOH (60 ml) and cold Et_2O (80 ml). Drying gave 15 (22.0 g, 77%) as a white powder.

2'-Deoxy-7-methylguanosine (18). Following a slightly modified procedure of [26], 15 (22.0 g, 54 mmol) was suspended in EtOH (1100 ml) at 0°C and treated with 40% MeNH_2/H_2O (220 ml). The suspension was stirred at 0°C for 1 h. The white precipitate was immediately centrifuged and washed with EtOH (350 ml) and Et_2O (400 ml). Drying under high vacuum gave 18 (10.9 g, 66%) as white powder.

2'-Deoxyadenosine (16). To a soln. of adenine (1.1 mg, 8 μmol) and 15 (13 mg, 32 μmol) inaq. phosphate buffer (1.50 ml; 0.05M K_H_2PO_4, pH 7.4) was added PNPase (23 units, 0.16 ml of a freshly prepared soln. containing 145 μl). A white precipitate was immediately formed, clouding the initially clear soln. The mixture was allowed to stand at 45°C in the water bath under gentle shaking by HPLC monitoring: 14 (t_R 8 min), adenine (t_R 11 min), 16 (t_R 20 min). The mixture was not worked up. HPLC analysis after 16 h gave a yield of 70% for 16.

4-Amino-8-([β-d-2'-deoxyxribofuranosyl]imidazo[1,2-a]-1,3,5-triazin-2(8H)-one (2'-Deoxy-5-aza-7-deazaisoguanosine; 17). The reaction was performed in 5 cycles. After reaching the reaction equilibrium, the enzyme was recovered and used for the next cycle. Each cycle was performed as follows: To a soln. of 8 (25 mg, 156 μmol) and 18 (380 mg, 1.25 mmol) inaq. phosphate buffer (32 ml; 0.05M in K_H_2PO_4, pH 7.4) was added PNPase (80 mg, 1120 μl). Immediately a white precipitate formed making the soln. cloudy. The mixture was allowed to stand at 45°C in the water bath with gentle shaking. HPLC monitoring (gradient B): 8 (t_R 16 min), 17 (t_R 19 min), 14 (t_R 26 min). After reaching equilibrium (first cycle 24 h, later up to 36 h), the mixture was worked up. The precipitate was recovered by centrifugation and the enzyme recycled (for exper. details, see General) from the supernatant soln. After 5 cycles, the supernatant solns. were pooled and lyophilized. The lyophilisate was dissolved in little H_2O and loaded on an ion-exchange column (for exper. details, see General): 17 (0.25-0.4M (Et_3NH)HCO_3) followed by 8 (0.5-0.6M (Et_3NH)HCO_3). The deoxyribonucleoside fractions were lyophilized. The lyophilisate was desalted by reversed-phase chromatography (30 g of silica gel RP-C18, 3 ml fractions, H_2O (70 ml), then H_2O/MeOH 4:1 (10 ml). Again the deoxyribonucleoside fractions were lyophilized. Drying under high vacuum gave 17 (112 mg, 54%) as white powder. An anal. sample was obtained by recrystallization from EtOH. M.p. 210°C (dec.), UV (EtOH): 246 (12900). IR (KBr): 3380, 3330, 3140, 3100, 3010, 2940, 2880, 1675, 1635, 1610, 1530, 1460, 1380, 1280, 1245, 1120, 1070. ^1H NMR (D_2O): 2.46 (dd, J = 4.0, 6.6, 14.0, H_5-C(2')); 2.58 (dd, J = 6.6, 6.6, 14.0, H_5-C(2')); 3.71 (dd, J = 4.8, 12.6, H_5-C(5')); 3.79 (dd, J = 3.5, 12.6, H_5-C(5')); 4.04-4.07 (m, H-C(4')); 4.52-4.55 (m, H-C(3')); 6.28 (dd, J = 6.6, 6.6, H-C(1')); 7.39 (d, J = 3.0, H-C(6)); 7.42 (d, J = 3.0, H-C(7)). ^13C-NMR (D_2O): 41.08 (t, C(2')); 64.10 (t, C(5')); 73.49 (d, C(3)); 86.46 (d, C(4')); 89.72 (d, C(1')); 110.45 (d, C(6)); 119.23 (d, C(7)); 152.93 (s, C(2')); 154.08 (s, C(8a)); 168.60 (s, C(4)). FAB-MS (3-nitrobenzyl alcohol: 268 [M + H]^+), 152 (base free). Anal. calc. for C_12H_13N_2O_2·H_2O: C 42.10, H 5.30, N 24.55; found: C 42.18, H 5.16, N 24.26.

A scaled-up run was performed in 8 cycles using 8 (800 mg, 4.96 mmol) overall and yielded 17 (830 mg, 59%) as a white solid, contaminated with Et_3NH^+ salts. After resolution of the mixture with ion-exchange chromatography, the fractions containing 17 were lyophilized. The product was then recovered by precipitation with EtOH. This simplified procedure avoided the desalting by reversed-phase chromatography.
REFERENCES

[34] V. du Vigneaud, O. K. Behrens, J. Biol. Chem. 1937, 117, 27.