NUCLEIC ACID RELATED COMPOUNDS. 136. SYNTHESIS OF 2-AMINO- AND 2,6-DIAMINOPURINE DERIVATIVES VIA INVERSE-ELECTRON-DEMAND DIELS–ALDER REACTIONS[+]

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Dedicated to Professor Antonín Holý on his 70th birthday in appreciation of outstanding achievements in the area of nucleic acid chemistry and a long and pleasant friendship.

Thermal inverse-electron-demand Diels–Alder reactions of 5-aminoimidazoles and 2,4,6-tris(ethoxycarbonyl)-1,3,5-triazine (**2**) with spontaneous retro-Diels–Alder loss of ethyl cyanoformate and elimination of ammonia give 2,6-bis(ethoxycarbonyl)purines. A report that selective alkaline hydrolysis followed by acid-catalyzed decarboxylation gave 6-(ethoxycarbonyl)purine products was not in harmony with known reactions in purine chemistry. Our reinvestigation has shown that the 6-(ethoxycarbonyl) group undergoes preferential base-promoted hydrolysis, as expected, but regioselectivity for attack of hydroxide at the carbonyl group at C6 is not high (relative to hydrolysis of both C2 and C6 esters). The structure of 9-benzyl-2-(ethoxycarbonyl)purine was determined by X-ray crystallography and confirmed by Curtius rearrangement of the azidocarbonyl analogue to give 2-amino-6-benzylpurine. Acid-catalyzed decarboxylation of the 2,6-dicarboxylate formed during hydrolysis gave 9-benzylpurine, and Curtius rearrangement of 2,6-bis(azidocarbonyl)-9-benzylpurine gave 2,6-diamino-9-benzylpurine. Attempted applications of inverse-electron-demand Diels–Alder reactions of **2** with nucleoside derivatives were problematic.

Keywords: Purines; Curtius rearrangement; Diels–Alder inverse-electron-demand reactions; 5-Aminoimidazoles; Triazines; Nucleosides.

Holý and coworkers[2] have reported studies on organometallic cross-coupling reactions of halopurine derivatives and nucleosides. Hocek and coworkers[3] have made major contributions in this area, and reviews[4] are available. We recently have developed other cross-coupling methods[5] in-

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[+] Part 135 see ref.[1]

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cluding reactions with non-conventional leaving groups[5b,5c] to produce compounds with carbon-linked substituents on the purine ring of nucleoside derivatives.

Several years ago we were intrigued by a communication[6] that described the preparation of 2- and 6-(ethoxycarbonyl)-substituted purine bases and nucleosides by inverse-electron-demand Diels–Alder (DA) reactions[7]. That approach[6] employed cycloaddition of 2,4,6-tris(ethoxycarbonyl)-1,3,5-triazine (**2**) (Fig. 1) with electron-rich aminoimidazole derivatives such as 5-amino-1-benzylimidazole (**4**) (generated in situ by decarboxylation of 5-amino-1-benzylimidazole-4-carboxylic acid). The initially formed bicyclic adducts undergo spontaneous retro-DA loss of ethyl cyanoacetate and aromatization by loss of ammonia[7a,7b] to give 2,6-bis(ethoxycarbonyl)purines. The same process with 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide was reported[6] to give the corresponding purine nucleoside, and we envisioned use of this methodology for the synthesis of sugar-modified analogues that could be conjugated with amino acids and peptides at C2 and/or C6.

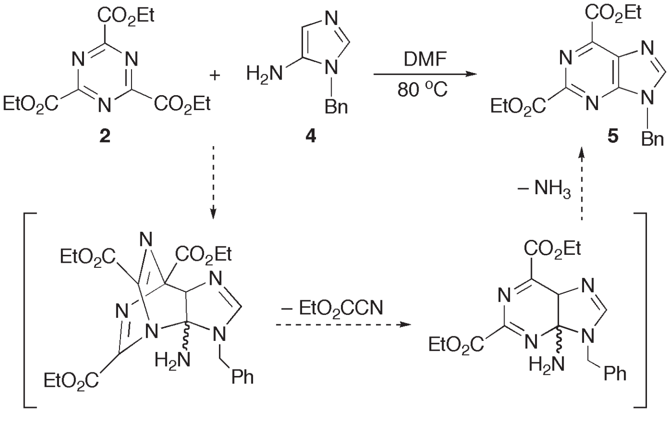


FIG. 1
The (inverse-electron-demand Diels–Alder cycloaddition, retro-Diels–Alder fragmentation, loss of ammonia) reaction cascade

It was stated: “Selective functionalization of the 2- or 6-ethoxycarbonyl group can be readily achieved because of the large difference in reactivity between the ester groups. For example, treatment of **5** with one equivalent of sodium hydroxide followed by decarboxylation gave 9-benzyl-6-ethoxycarbonylpurine (65%) with no detectable 9-benzyl-2-ethoxycarbonylpurine or 9-benzylpurine”[6]. That structure assignment was based on analogy with selective reduction of the 2-(ethoxycarbonyl) group on the model 6-amino-2,4-bis(ethoxycarbonyl)-5-(methylthio)pyrimidine (**A**) (Fig. 2) with sodium borohydride[7a]. Steric bias in opposition to reduction of the 6-(ethoxycarbonyl) group adjacent to the 5-substituent was invoked to rationalize selective reduction of the 2-(ethoxycarbonyl) group. However, steric effects of an endocyclic nitrogen (N7) in the purine ring (a fused imidazo[4,5-*d*]pyrimidine) of **5** are not equivalent to those of a 5-methylsulfanyl group on the monocyclic pyrimidine ring of **A**. Also the methylsulfanyl substituent might exert significantly different electronic effects, and reduction with $NaBH_4$ is substantially different than hydrolysis with NaOH.

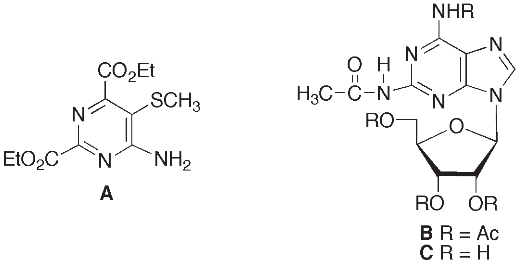


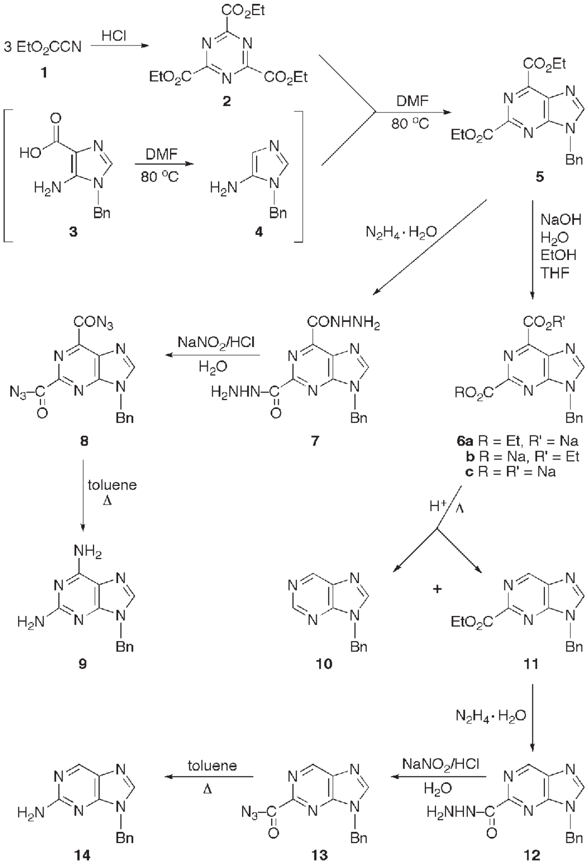
FIG. 2
6-Amino-2,4-bis(ethoxycarbonyl)-5-methylsulfanylpyrimidine (**A**);
2,6-Bis(acetamido)-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (**B**);
2-(Acetamido)-6-amino-9-(β-D-ribofuranosyl)purine (**C**)

Schaeffer and Thomas[8] had shown that treatment of a 2,6-dichloropurine nucleoside derivative with methanolic ammonia gave 2-chloroadenosine by displacement of chloride from C6, and that treatment of a 2-chloro-6-methoxypurine analogue with ammonia in methanol at elevated temperature resulted in displacement of methoxide from C6 rather than chloride from C2. Numerous examples of regiospecific displacement of the 6-substituent from 2,6-disubstituted purine derivatives[9] (especially with two identical groups) are known, and treatment of 2,6-bis(acetamido)-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (**B**) (Fig. 2) with methanolic ammonia at 0 °C resulted in selective ammonolysis of the acetamido group at C6 to give 2-acetamido-6-amino-9-(β-D-ribofuranosyl)purine[10] (**C**). Thus, C6 is more reactive than C2 with nucleophiles, and this effect also is observed at the carbonyl group of acetamido substituents. We considered it much more likely that treatment of 9-benzyl-2,6-bis(ethoxycarbonyl)purine (**5**) with one equivalent of hydroxide under controlled conditions would produce 9-benzyl-2-(ethoxycarbonyl)purine-6-carboxylate rather than the

9-benzyl-6-(ethoxycarbonyl)purine-2-carboxylate[6] regioisomer. Therefore, the model reactions were reinvestigated prior to the pursuit of analogous chemistry with nucleosides.

RESULTS AND DISCUSSION

Trimerization of ethyl cyanoformate (**1**) (Scheme 1) to give 2,4,6-tris-(ethoxycarbonyl)-1,3,5-triazine[11] (**2**) has been reported several times. We found the general method of Bozhkova and Heimgartner[11b] (with a roughly measured addition of dry HCl gas) to be most reliable. The unstable 5-amino-1-benzylimidazole-5-carboxylic acid (**3**) was prepared by hydrolysis[12] of its ethyl ester (prepared by a two-step in situ ring closure of ethyl



SCHEME 1
Synthesis of substituted purine derivatives

2-amino-2-cyanoacetate[13] with triethyl orthoformate and benzylamine[14]) followed by acidification. In situ decarboxylation of **3** in DMF at 80 °C produced the unstable 5-amino-1-benzylimidazole (**4**), an electronrich dieneophile for inverse-electron-demand Diels–Alder cycloaddition[7] with electron-deficient diene **2**. The 9-benzyl-2,6-bis(ethoxycarbonyl)purine (**5**) product was obtained in 76% yield when the reported[6] 2:1 ratio of **3**/**2** was used. The yield was diminished (67%) with a 1:1 ratio of these reactants, but **2** was more readily accessible than **3**.

Careful treatment of **5** with one equivalent of sodium hydroxide under the reported[6] conditions (0 °C for 1 h and 25 °C for 2 h) gave a mixture of recovered starting material **5** (22%) and three hydrolysis products. Sodium 9-benzyl-2-(ethoxycarbonyl)purine-6-carboxylate (**6a**) was the major product and sodium 9-benzyl-6-(ethoxycarbonyl)purine-2-carboxylate (**6b**) was the decidedly minor component. Disodium 9-benzylpurine-2,6-dicarboxylate (**6c**) was produced in significant amounts (typical ^{1}H NMR ratios were 67:8:25 for **6a**/**6b**/**6c**, respectively). Treatment of **5** under more vigorous conditions (2 equivalents of NaOH, 25 °C for 3 h) gave **6a**/**6b**/**6c** ratios of 20:4:76 (^{1}H NMR). Under a variety of conditions, hydrolysis of **5** was neither regiospecific nor was **6b** ever the major product. Monoester **6a** was the predominant isomer produced, as expected[8–10] from other reactions of identically 2,6-disubstituted purine compounds with nucleophilic reagents, but the <3:1 selectivity for base-promoted hydrolysis of the more susceptible C6 ester relative to hydrolysis of both C2 and C6 esters was low.

Also in contrast with the earlier report[6], thermal decarboxylation of the mixture from the more selective hydrolysis gave two isolated materials. The minor material was 9-benzylpurine (**10**) and the major (68% based on recovered starting material) was a mixture of two compounds (^{1}H NMR ratio of ~10:1). The predominant isomer was 9-benzyl-2-(ethoxycarbonyl)purine (**11**), which was confirmed by X-ray crystallography (Fig. 3) and conversion into 2-amino-9-benzylpurine[15] (**14**). Decarboxylation of the disodium salt **6c** gave 9-benzylpurine[16] (**10**).

Treatment of purified **11** with hydrazine monohydrate gave 9-benzyl-2-(hydrazinocarbonyl)purine (**12**), which underwent diazotization to give 9-benzyl-2-(azidocarbonyl)purine (**13**). A toluene solution of **13** was heated at 110 °C to effect Curtius rearrangement, and 2-amino-9-benzylpurine[15] (**14**) was obtained in 74% overall yield (two-stage conversion from **12**). We then probed the potential for selective hydrazinolysis of the esters as a possible route to purine derivatives with amino and carboxyl groups at C2 and/or C6. However, solutions of **5** in DMF treated with various molar ratios of hydrazine monohydrate at 0 °C showed slow and complex changes, whereas heating such solutions resulted in disappearance of starting **5** without formation of defined products. We found that treatment of **5** with $N_2H_4 \cdot H_2O$/EtOH (3:10) at 22 °C for 20 min gave a single product (TLC). Because concentration of such solutions resulted in formation of additional UV-active materials, H_2O was added and partially evaporated several times. $NaNO_2$ and HCl/H_2O were then added to the solution of intermediate **7**, and a toluene solution of the resulting diazotization product **8** was heated. The Curtius rearrangement product, 2,6-diamino-9-benzylpurine[17] (**9**), was isolated in 74% yield (overall for three steps from **5**).

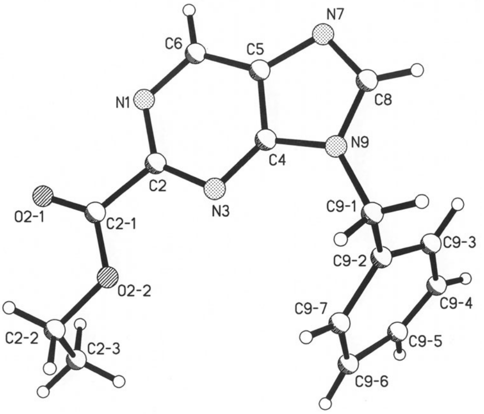
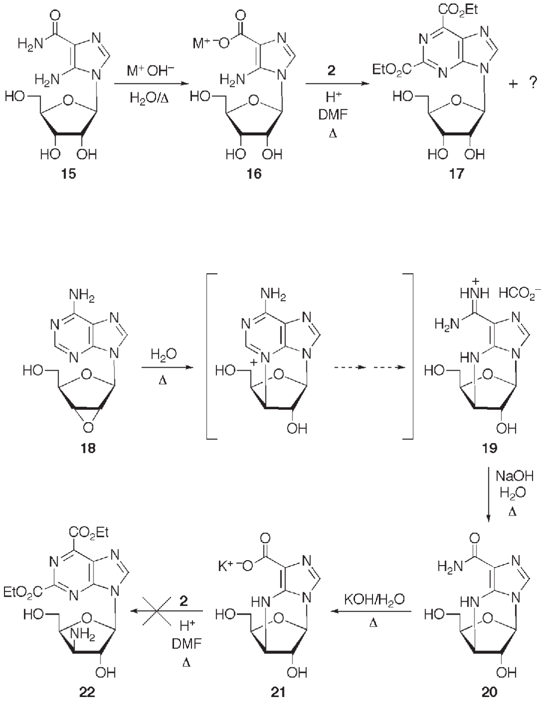


FIG. 3
X-ray crystal structure of 9-benzyl-2-(ethoxycarbonyl)purine (**11**) (CCDC 608646)

Attempts to duplicate the results and yields reported[6] for inverse-electron-demand DA reactions of **2** with 5-aminoimidazole riboside (derived from base-promoted hydrolysis of 5-aminoimidazole-4-carboxamide riboside[12] (**15**) (Scheme 2) followed by thermal decarboxylation under acidic conditions) were problematic. Both the sodium and potassium salts of 5-aminoimidazole-4-carboxylate riboside (**16**) were prepared as described by Robins and coworkers[12]. Subjection of either salt to variations of the reported general conditions[6] gave ≤30% yields of purified product **17** (contaminated with minor amounts of an unknown UV-active impurity after chromatography).

We had prepared 5-amino-3′,5-anhydro-1-(β-D-xylofuranosyl)imidazole-4-carboxamide[18] (**20**) by heating aqueous solutions of 2′,3′-anhydroadenosine (**18**) (formation of the charged cyclonucleoside and ring opening) followed by base-promoted hydrolysis of the resulting 5-carbox-



Scheme 2

Attempted applications of the reaction cascade with **2** and 5-aminoimidazole-4-carboxylic acid nucleoside derivatives

amidine **19**. Vigorous heating of **20** with KOH/H_2O[12] gave the potassium carboxylate **21**. Heating solutions of catalytically acidified **21** and triazine **2** under various conditions failed to produce 9-(3-amino-3-deoxy-β-D-xylofuranosyl)-2,6-bis(ethoxycarbonyl)purine (**22**), which might have been expected to result from an in situ reaction cascade (decarboxylation-inverse-electron-demand DA–retro-DA-elimination of amino sugar) analogous to the process with the model aminoimidazole **4**. It appears that in situ reaction cascades with **2** and 5-aminoimidazole nucleoside derivatives are problematic.

In conclusion, the Boger reaction cascade[7a,7b] with 2,4,6-tris(ethoxycarbonyl)-1,3,5-triazine (**2**) and 5-amino-1-benzylimidazole (**4**) proceeds as described[6]. However, the controlled, base-promoted hydrolysis of the resulting 9-benzyl-2,6-bis(ethoxycarbonyl)purine (**5**) is <3:1 selective for hydrolysis of one versus both ester groups, and the selectivity for mono-hydrolysis of the ester functions at C6:C2 is ≥5:1. The major product of controlled hydrolysis followed by decarboxylation is 9-benzyl-2-(ethoxycarbonyl)purine (**11**), and not the reported[6] 6-(ethoxycarbonyl) regioisomer (the derived carboxamide[6] must also be the C2 isomer). On the basis of our investigations, a more promising route is required for the preparation of novel nucleoside conjugates derived from richly functionalized platforms such as structure **22**. Oxidation of 2- and/or 6-(hydroxymethyl) substituents on purine and nucleoside derivatives (readily accessible by the methodology of Hocek and coworkers[3b]) to the carboxylate level might be a plausible new approach.

EXPERIMENTAL

General

Uncorrected melting points were determined with a capillary tube apparatus. UV spectra (λ, nm; ε) were determined with solutions in MeOH unless otherwise indicated. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra (δ, ppm; *J*, Hz) were determined with solutions in DMSO-d_6 (with internal Me_4Si) unless otherwise indicated. “Observed” ^{1}H NMR multiplicities are within quotation marks if more complex splitting patterns are expected. High-resolution mass spectra (MS) were determined under FAB conditions (with a glycerol or thioglycerol matrix) unless otherwise indicated; methane (CH_4) was used for chemical ionization (CI) mass spectral determinations. Reagent grade chemicals were used, and solvents were distilled before use. CH_2Cl_2, CH_3CN, and toluene were dried over and distilled from CaH_2. Volatile materials were flash evaporated at <35 °C under house vacuum (~10 mm Hg) or at mechanical oil-pump vacuum (<0.1 mm Hg). “Diffusion crystallization” was performed as described[19] with the solvent combinations indicated.

2,4,6-Tris(ethoxycarbonyl)-1,3,5-triazine (**2**)

Dry hydrogen chloride gas was bubbled (15–30 bubbles/s) for 20 min into ethyl cyanoformate (0.5 ml, 0.50 g, 5 mmol) at 0 °C, and the solution was stored at ambient temperature for 6 days. The resulting crystals were filtered, washed with Et_2O, and recrystallized from EtOH to give **2** (438.5 mg, 88%) as a white solid; m.p. 170–172 °C (lit.[11a] m.p. 170–171 °C). ^{1}H NMR (300 MHz, $CDCl_3$): 4.56 (q, $J = 7.2$, 2 H, CH_2); 1.46 (t, $J = 7.2$, 3 H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 167.1 (C=O), 161.4 (aromatic), 64.2 (CH_2), 14.2 (CH_3). MS (CI): *m/z* 298.1044 (MH^+ [$C_{12}H_{16}N_3O_6$] = 298.1039).

5-Amino-1-benzylimidazole-4-carboxylic Acid (**3**)

Saturated $NaHCO_3/H_2O$ (30 ml) was added with vigorous stirring to a suspension of ethyl cyanoglyoxylate oxime (10 g, 0.07 mol) in water (60 ml) under a nitrogen atmosphere. The resulting clear yellow solution was treated with 2-g portions of solid sodium dithionite (34 g, 0.21 mol) at a rate that gave controlled effervescence, and stirring was continued at ambient temperature for an additional 30 min. The solution was then extracted ($CHCl_3$, 3 × 50 ml) and dried (Na_2SO_4). Volatiles were evaporated to give ethyl 2-amino-2-cyanoacetate[13] (6.13 g, 68%) as an oil, which must be used immediately in the next step.

Triethyl orthoformate (1 ml, 0.89 g, 6.0 mmol) was added dropwise to a stirred solution of ethyl 2-amino-2-cyanoacetate (426 mg, 3.33 mmol) in CH_3CN (4 ml, freshly distilled), and the stirred solution was heated at reflux for 50 min and then cooled to ambient temperature. Benzylamine (0.50 ml, 0.49 g, 4.58 mmol) was added, and the red solution was stored at ambient temperature for 16 h. Volatiles were evaporated, and the residue was chromatographed (0.5 to 0.8% $MeOH/CH_2Cl_2$) to give ethyl 5-amino-1-benzylimidazole-4-carboxylate[14] (364 mg, 45%) as a yellow solid. Recrystallization of this material (EtOH) gave long needles; m.p. 167–170 °C (lit.[14] m.p. 156 °C). UV: λ_{max} 269 (13 800), λ_{min} 225 (6140). ^1H NMR (300 MHz, $CDCl_3$): 7.40–7.28 (m, 3 H, Ph): 7.23–7.13 (m, 3 H, Ph and H2); 5.05 (br s, 4 H, CH_2Ph and NH_2); 4.31 (q, J = 7.2, 2 H, CH_2); 1.35 (t, J = 7.2, 3 H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 164.4 (CO), 145.2 (C4), 134.3, 129.5, 128.8, 127.2 (Ph), 131.7 (C2), 111.8 (C5), 60.1 (OCH_2), 45.0 (CH_2Ph), 14.8 (CH_3). MS: *m/z* 246.1251 (MH^+ $[C_{13}H_{16}N_3O_2]$ = 246.1243).

A suspension of ethyl 5-amino-1-benzylimidazole-4-carboxylate (478 mg, 1.95 mmol) in EtOH (20 ml) and 0.5 M $NaOH/H_2O$ (20 ml) was refluxed for 5 h. Volatiles were evaporated, H_2O (5 ml) was added, and 5% HCl/H_2O was added to pH ~5. The off-white solid that precipitated was filtered and washed with one portion of H_2O. This material was dried under vacuum at ambient temperature for 30 min to give **3** (quantitative), which was used immediately in the next step.

9-Benzyl-2,6-bis(ethoxycarbonyl)purine (**5**)

A mixture of **2** (438 mg, 1.47 mmol) and **3** (664 mg, 3.05 mmol) in DMF (7.9 ml) was heated at 80 °C for 2 h under a nitrogen atmosphere, and then cooled to ambient temperature. Volatiles were evaporated, CH_2Cl_2 (10 ml) was added, and the suspension was filtered. The filtrate was concentrated and subjected to flash chromatography (EtOAc/hexanes, 1:1) to give **5** (398 mg, 76%) as an off-white solid. Diffusion crystallization[19] (EtOAc/hexanes) gave material with m.p. 145–146.5 °C (lit.[6] 143–144 °C). UV: λ_{max} 287 (8660), λ_{min} 253 (4060). ^1H NMR (300 MHz, $CDCl_3$): 8.43 (s, 1 H, H8); 7.42–7.30 (m, 5 H, Ph); 5.60 (s, 2 H, CH_2Ph); 4.66–4.52 (m, 4 H, 2 × CH_2); 1.53–1.46 (m, 6 H, 2 × CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 163.5 (6-CO), 163.2 (2-CO), 154.5 (C6), 150.8 (C4), 149.6 (C2), 146.0 (C8), 134.2 (Ph), 132.7 (C5), 129.5, 129.2, 128.5 (Ph), 63.2 (OCH_2), 48.2 (CH_2Ph), 14.4 (CH_3). MS: *m/z* 377.1235 (MNa^+ $[C_{18}H_{18}N_4O_4Na]$ = 377.1225).

9-Benzyl-2-(ethoxycarbonyl)purine (**11**)

A solution of **5** (198 mg, 0.559 mmol) in $THF/EtOH/H_2O$ (3:2:1, 7.6 ml) was cooled to 0 °C, treated with 1 M $NaOH/H_2O$ (0.56 ml, 0.56 mmol) under a nitrogen atmosphere, and stirred at 0 °C for 1 h and then at 25 °C for 2 h (as described[6]). Volatiles were evaporated, and the residue was partitioned ($EtOAc/H_2O$). The aqueous layer was extracted with EtOAc until no further UV active material was removed. Evaporation of the combined organic phase gave recovered **5** (43.3 mg, 22%). Evaporation of volatiles from the aqueous layer gave a mixture of sodium 9-benzyl-2-(ethoxycarbonyl)purine-6-carboxylate (**6a**), sodium 9-benzyl-6-(ethoxycarbonyl)purine-2-carboxylate (**6b**), and disodium 9-benzylpurine-2,6-dicarboxylate (**6c**). This mixture was treated with Ac_2O (2.3 ml, 2.5 g, 24 mmol) and AcOH (2.3 ml, 2.4 g, 40 mmol) and heated at 130 °C for 20 h. Volatiles were evaporated, the residue was partitioned between saturated $NaHCO_3/H_2O$ (25 ml) and CH_2Cl_2 (30 ml), and the aqueous layer was extracted with CH_2Cl_2 (3 × 25 ml). The combined organic phase was dried (Na_2SO_4), and volatiles were evaporated. The residue was chromatographed (1% EtOH/CH_2Cl_2) to give contaminated **11** (84 mg, 68% based on recovered starting material) and 9-benzylpurine (**10**; 13 mg, 14% based on recovered material) as white solids. A sample of **11** was purified by diffusion crystallization[19] (EtOAc/hexanes) to give crystals; m.p. 89–91 °C (lit.[6] m.p. 89–90 °C). UV: λ_{max} 269 (9850), λ_{min} 241 (3820). ^1H NMR (300 MHz, $CDCl_3$): 9.36 (s, 1 H, H6); 8.60 (s, 1 H, H8); 7.39 (s, 5 H, Ph); 5.62 (s, 2 H, CH_2Ph); 4.59 (q, *J* = 7.2, 2 H, CH_2); 1.51 (t, *J* = 7.2, 3 H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 163.5 (CO), 151.7, 151.1, 148.4, 147.6, 134.2, 133.1, 129.6, 129.3, 128.7, 63.2, 48.2. MS: *m/z* 283.1185 (MH^+ [$C_{15}H_{14}N_4O_2$] = 283.1195). The NMR spectra of **10** were identical to those of the sample prepared in the following experiment.

9-Benzylpurine (**10**)

A solution of **5** (202 mg, 0.570 mmol) in THF/EtOH/H_2O (3:2:1, 5 ml) was treated with 0.1 M NaOH/H_2O (12 ml, 1.2 mmol) under an argon atmosphere at 26 °C for 3 h. Volatiles were evaporated, and the residue was treated with Ac_2O (7.5 ml, 8.12 g, 80 mmol) and AcOH (7.5 ml, 7.87 g, 0.131 mmol) at 130 °C for 4 days under an argon atmosphere. The reaction mixture was then cooled, volatiles were evaporated, and the residue was partitioned between CH_2Cl_2 and saturated $NaHCO_3$/H_2O. The aqueous phase was extracted (CH_2Cl_2, 3×), and the combined organic phase was dried (Na_2SO_4). Volatiles were evaporated, and the residue was chromatographed (1% MeOH/CH_2Cl_2) to give **10** (81 mg, 68%) as an off-white solid. Recrystallization (Et_2O/hexanes) gave **10**; m.p. 97–99 °C (lit.[14] m.p. 100–101 °C). UV (MeOH): λ_{max} 264 (9900). ^1H NMR (300 MHz, $CDCl_3$): 9.19 (s, 1 H, H6); 9.07 (s, 1 H, H2); 8.16 (s, 1 H, H8); 7.42–7.20 (m, 5 H, Ph); 5.49 (s, 2 H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): 152.0 (C4), 147.5 (C2), 146.1 (C8), 134.8 (C6), 134.0 (C5), 129.5, 129.0, 128.2 (Ph), 47.6 (CH_2). MS: *m/z* 211.0989 (MH^+ [$C_{12}H_{11}N_{14}$] = 211.0983).

9-Benzyl-2-(hydrazinocarbonyl)purine (**12**)

A yellow solution of **11** (13.7 mg, 0.05 mmol) in hydrazine monohydrate (0.30 ml, 0.41 g, 8.2 mmol) was stirred at 30 °C for 20 min. Volatiles were evaporated, and the residue was adsorbed on a small amount (~1 ml) of silica gel that was added to a small column of silica gel. Chromatography (2 to 4% MeOH/CH_2Cl_2) gave **12** (11.4 mg, 88%) as an off-white solid; m.p. 143–146 °C. UV: λ_{max} 273 (10 700), λ_{min} 242 (6080). ^1H NMR: 10.11 (br s, 1 H, NHCO); 9.24 (s, 1 H, H6); 8.87 (s, 1 H, H8); 7.42–7.22 (m, 5 H, Ph); 5.59 (s, 2 H, CH_2); 4.64 (br s, 2 H, NH_2). ^{13}C NMR: 161.7 (CO), 152.0 (C2), 151.4 (C4), 148.7 (C6), 147.6 (C8), 136.4 (C5), 133.9, 128.8, 128.0, 127.7 (Ph), 46.2 (CH_2). MS (EI): *m/z* 268.1056 (MH^+ [$C_{13}H_{12}N_6O$] = 268.1073).

2-Azidocarbonyl-9-benzylpurine (**13**)

Et_2O (6 ml) was layered over a solution of **12** (56.9 mg, 0.21 mmol) in 5 M HCl/H_2O (5.7 ml), and the biphasic mixture was stirred and cooled at 0–2 °C. A solution of $NaNO_2$ (136 mg, 1.97 mmol) in H_2O (0.6 ml) was added dropwise to the cold mixture under a nitrogen atmosphere. The ether layer was removed after 20 min, and Et_2O/CH_2Cl_2 (1:1, 6 ml) was added. The organic layer was removed every 20 min and fresh Et_2O/CH_2Cl_2 was added to extract the product from the acidic aqueous solution. After 2 h, another portion of $NaNO_2$/H_2O (50 mg in 0.3 ml) was added to the mixture, and stirring was continued until no additional UV-active product was extracted into the organic layer. The combined organic phase was concentrated, washed once with H_2O, dried ($MgSO_4$) for 20 h, and filtered. The filtrate was concentrated, toluene was added and evaporated (2 × 10 ml) to ~10 ml, and this solution was used immediately for the next step.

2-Amino-9-benzylpurine (**14**)

Trifluoroacetic acid (0.30 ml, 0.44 g, 3.9 mmol) was added to a stirred solution of **13** (~59 mg) in toluene (~10 ml), and stirring was continued at 110 °C (oil bath temperature) for 48 h under a nitrogen atmosphere. Volatiles were evaporated, and the residue was chromatographed (3% MeOH/CH_2Cl_2) to give **14** (35.5 mg, 74%, based on starting **12**) as an off-white solid. This material was heated gently in EtOAc/MeOH (10:1) and filtered to give **14**; m.p. 179–180 °C (lit.[15] 180–182 °C). UV: λ_{max} 246, 310 (5100, 7400), λ_{min} 238, 266 (4700, 840). ^1H NMR: 8.60 (s, 1 H, H6); 8.16 (s, 1 H, H8); 7.40–7.20 (m, 5 H, Ph); 6.54 (br s, 2 H, NH_2); 5.29 (s, 2 H, CH_2). ^{13}C NMR: 160.7 (C2), 153.0 (C4), 149.2 (C6), 142.7 (C8), 137.0, 128.7, 127.7, 127.1 (Ph), 126.7 (C5), 45.4 (CH_2). MS: *m/z* 226.1082 (MH^+ [$C_{12}H_{12}N_5$] = 226.1092).

9-Benzyl-2,6-bis(hydrazinocarbonyl)purine (**7**)

Hydrazine monohydrate (0.40 ml, 0.412 g, 8.0 mmol) was added dropwise to a stirred suspension of **5** (86.2 mg, 0.24 mmol) in EtOH (4.5 ml) at ambient temperature. A clear yellow solution formed, and stirring was continued for an additional 20 min (TLC showed complete reaction of **5**). H_2O was added and the solution was concentrated (2 × 10 ml) to ~20 ml. This solution was used immediately for the next step.

2,6-Bis(azidocarbonyl)-9-benzylpurine (**8**)

Et_2O (20 ml) and HCl/H_2O (37%, 10 ml) were added dropwise to a stirred aqueous solution of **7** (~79 mg, 0.24 mmol) in H_2O (20 ml) at 0 °C. A solution of $NaNO_2$ (563 mg, 8.10 mmol) in H_2O (2 ml) was carefully added to the cooled biphasic mixture at a rate that kept effervescence under control, and stirring at 0 °C was continued. The Et_2O layer was removed after 20 min, and Et_2O/CH_2Cl_2 (1:1, 20 ml) was added. Continuous extraction of the product formed in the aqueous phase was effected by removal of the organic layer and addition of fresh solvents every 20 min. Solutions of $NaNO_2$ (500 mg) in H_2O (2 ml) were added after two and four hours, and stirring was continued until no further UV-active product was detected (TLC) in the aqueous layer. The combined organic phase was concentrated, washed once with H_2O/saturated aqueous $NaHCO_3$ (10:1), dried ($MgSO_4$), and filtered. Toluene (10 ml) was added to the filtrate, and the solution was concentrated. Toluene (10 ml) was again added, and the solution of **8** was concentrated and used immediately in the next step.

2,6-Diamino-9-benzylpurine (**9**)

Trifluoroacetic acid (0.20 ml, 0.30 g, 2.6 mmol) was added to a solution of **8** (~85 mg, 0.24 mmol) in toluene, and the stirred solution was heated at 100–110 °C (oil bath temperature) for 24 h. Volatiles were evaporated, and the residue was adsorbed on a small amount of silica gel and added to a small column of silica gel. Chromatography (2 to 3% MeOH/CH_2Cl_2)

gave **9** (43.1 mg, 74%) as an off-white solid that was recrystallized (H_2O) to give colorless needles; m.p. 178–180 °C (lit.[17] m.p. 180–181 °C). UV: λ_{max} 256, 281 (8300, 10 400), λ_{min} 237, 265 (5090, 6940). ^{1}H NMR: 7.81 (s, 1 H, H8); 7.38–7.18 (m, 5 H, Ph); 6.84 (br s, 2 H, 6-NH_2); 5.91 (br s, 2 H, 2-NH_2); 5.19 (s, 2 H, CH_2). ^{13}C NMR: 157.8 (C2), 154.2 (C6), 151.7 (C4), 138.8 (C8), 137.3, 128.7, 127.6, 127.1 (Ph), 112.4 (C5), 45.7 (CH_2). MS (EI): *m/z* 240.1130 (MH^+ [$C_{12}H_{12}N_6$] = 240.1124).

X-ray Crystal Data for **11**

Colorless crystals; formula: $C_{15}H_{14}N_4O_2$ (FW: 282.30); monoclinic; space group: *P*2(1)/*n*; unit cell dimensions: a = 9.7068(6) Å, b = 9.8131(6) Å, c = 15.7115(9) Å, α = 90°, β = 106.9370(10)°, γ = 90°; Z = 4; R = 0.0735; GOF = 1.040.

CCDC 608646 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge, CB2 1EZ, UK; fax: +44 1223 336033; or deposit@ccdc.cam.ac.uk).

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REFERENCES

1. Zhong M., Nowak I., Cannon J. F., Robins M. J.: *J. Org. Chem.* **2006**, *71*, 4216.
2. a) Hocek M., Holý A., Votruba I., Dvořáková H.: *J. Med. Chem.* **2000**, *43*, 1817; b) Hocek M., Holý A., Votruba I., Dvořáková H.: *Collect. Czech. Chem. Commun.* **2001**, *66*, 483.
3. See: a) Hocek M., Nauš P., Pohl R., Votruba I., Furman P. A., Tharnish P. M., Otto M. J.: *J. Med. Chem.* **2005**, *48*, 5869; b) Šilhár P., Pohl R., Votruba I., Hocek M.: *Collect. Czech. Chem. Commun.* **2005**, *70*, 1669; and references therein.
4. a) Hocek M.: *Eur. J. Org. Chem.* **2003**, 245; b) Agrofoglio L. A., Gillaizeau I., Saito Y.: *Chem. Rev.* **2003**, *103*, 1875.
5. a) Liu J., Janeba Z., Robins M. J.: *Org. Lett.* **2004**, *6*, 2917; b) Liu J., Robins M. J.: *Org. Lett.* **2004**, *6*, 3421; c) Liu J., Robins M. J.: *Org. Lett.* **2005**, *7*, 1149.
6. Dang Q., Liu Y., Erion M. D.: *J. Am. Chem. Soc.* **1999**, *121*, 5833.
7. a) Boger D. L., Honda T., Menezes R. F., Colletti S. L., Dang Q., Yang W.: *J. Am. Chem. Soc.* **1994**, *116*, 82; b) Boger D. L.: *Chem. Rev.* **1986**, *86*, 781; c) Fleming I: *Frontier Orbitals and Organic Chemical Reactions*. Wiley, London 1976.
8. Schaeffer H. J., Thomas H. J.: *J. Am. Chem. Soc.* **1958**, *80*, 3738.
9. a) Robins M. J., Robins R. K.: *J. Am. Chem. Soc.* **1965**, *87*, 4934; b) Robins M. J., Uznański B.: *Can. J. Chem.* **1981**, *59*, 2601; c) Robins M. J., Uznański B.: *Can. J. Chem.* **1981**, *59*, 2608; d) Janeba Z., Francom P., Robins M. J.: *J. Org. Chem.* **2003**, *68*, 989; and references therein.
10. Davoll J., Lowy B. A.: *J. Am. Chem. Soc.* **1951**, *73*, 1650.
11. a) Sugiyama Y., Sasaki T., Nagato N.: *J. Org. Chem.* **1978**, *43*, 4485; b) Bozhkova N., Heimgartner H.: *Helv. Chim. Acta* **1989**, *72*, 825.
12. Srivastava P. C., Mancuso R. W., Rousseau R. J., Robins R. K.: *J. Med. Chem.* **1974**, *17*, 1207.
13. Logemann F. I., Shaw G.: *Chem. Ind.* **1980**, 541.

14. Mackenzie G., Wilson H. A., Shaw G., Ewing D.: *J. Chem. Soc., Perkin Trans. 1* **1988**, 2541.
15. Liu F., Dalhus B., Gundersen L.-L., Rise F.: *Acta Chem. Scand.* **1999**, *53*, 269.
16. Montgomery J. A., Temple C., Jr.: *J. Am. Chem. Soc.* **1961**, *83*, 630.
17. Montgomery J. A., Hewson K.: *J. Am. Chem. Soc.* **1960**, *82*, 463.
18. Robins M. J., Fouron Y., Mengel R.: *J. Org. Chem.* **1974**, *39*, 1564.
19. Robins M. J., Mengel R., Jones R. A., Fouron Y.: *J. Am. Chem. Soc.* **1976**, *98*, 8204.