Nucleosides of 2-Azapurines and Certain Ring Analogs†

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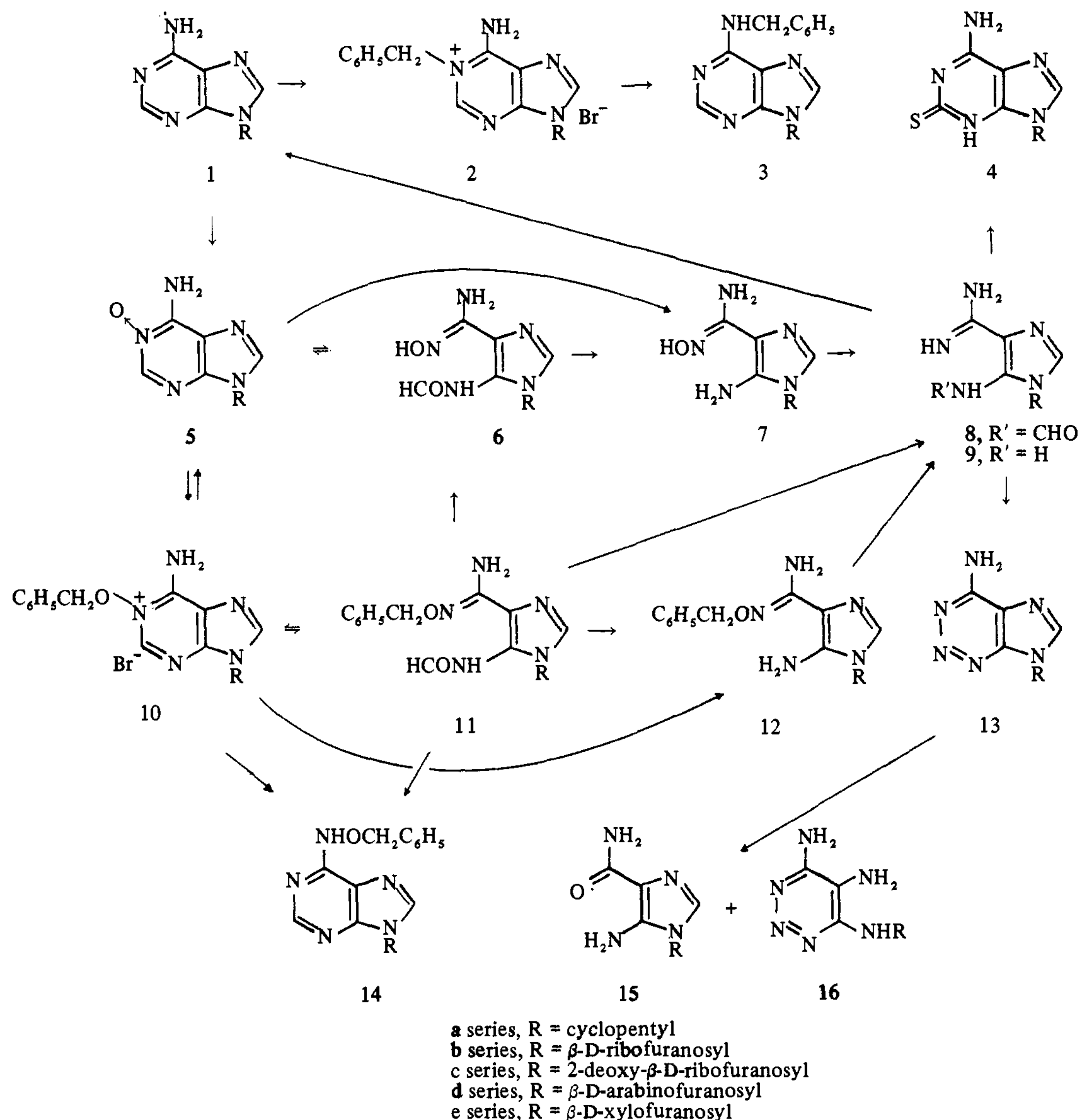
A convenient synthesis of 2-azapurine nucleosides (7-glycosylimidazo[4,5-*d*]-*v*-triazines) in 5 steps from the corresponding purine nucleosides is described. 2-Azaadenosine, 2′-deoxy-2-azaadenosine, and 4-amino-1-β-D-ribofuranosylpyrazolo-4-carboxamidine are all cytotoxic at low concns, and 2-azaadenosine has shown consistent activity against L1210 leukemia on both chronic and single-dose schedules.

Azapurines have shown anticancer activity and other interesting biological properties.[1] The ribonucleosides of certain 8-azapurines (*v*-triazolo[4,5-*d*]pyrimidines) have been prepared[2-5] and have shown biological activity as the intact nucleosides,[1,6,7] but the synthesis of a 2-azapurine (imidazo[4,5-*d*]-*v*-triazine) nucleoside has not previously been described.‡ Stevens, *et al.*, described the preparation of 2-azaadenosine 1-oxide but were unable to reduce this material to 2-azaadenosine (4-amino-7-β-D-ribofuranosylimidazo[4,5-*d*]-*v*-triazine, **13b**). It occurred to us that an adaptation of the procedure developed by us for the synthesis of 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide (**15b**)[9] might provide an intermediate suitable for the preparation of nucleosides of 2-azaadenine. However, such an approach to the preparation of imidazolecarboxamidines from adenine nucleosides was complicated by the ease of reclosure of the proposed intermediates of the Dimroth rearrangement of 1-substituted adenines. Thus Taylor,[10] and later Leonard,[11] observed that 1-substituted adenines rearrange quantitatively in boiling water to N-substituted adenines; no imidazole intermediates were isolated.

Our initial studies were carried out on a model compound, 9-cyclopentyladenine (**1a**), which was benzylated in the usual manner[12] to give 1-benzyl-9-cyclopentyladenine hydrobromide (**2a**) and converted to its 1-oxide (**5a**) by the procedure of Stevens, *et al.*[13] Treatment of **2a** with reflux-



a series, R = cyclopentyl
b series, R = β-D-ribofuranosyl
c series, R = 2-deoxy-β-D-ribofuranosyl
d series, R = β-D-arabinofuranosyl
e series, R = β-D-xylofuranosyl

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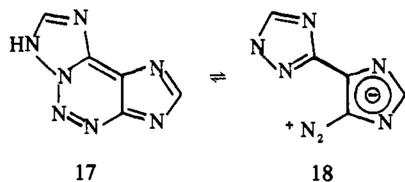
‡A preliminary communication describing part of this work has appeared.[8]

ing aq ethanolic NaOH (excess) caused the Dimroth rearrangement to occur in agreement with previous observations[10,11] giving *N*-benzyl-9-cyclopentyladenine (3a), although a better yield was obtained if the reaction was carried out in aq EtOH containing only 1 equiv of NaOH. In either case, no intermediate was detected. Base hydrolysis of the 1-oxide **5a** gave 1-cyclopentyl-5-formamidoimidazole-4-carboxamidoxime (**6a**), which was deformylated in acid to 5-amino-1-cyclopentylimidazole-4-carboxamidoxime (7a). 7a could also be produced directly from **6a** by acid treatment.[14] Catalytic reduction of the carboxamidoxime **7a** with Ra Ni gave 5-amino-1-cyclopentylimidazole-4-carboxamidine (9a), which was isolated as the HCl salt and converted to 9-cyclopentyl-2-mercaptoadenine (4) by treatment with CS_2 in DMF and to 9-cyclopentyl-2-azaadenine (4-amino-7-cyclopentylimidazo[4,5-*d*]-*v*-triazine, 13a) by treatment with aq $NaNO_2$. Hot, dil aq NaOH caused cleavage of both rings of **13a** to give approximately equal amounts of 5-amino-1-cyclopentylimidazole-4-carboxamide (15a)[9] and 4,5-diamino-6-cyclopentylamino-*v*-triazine (**16a**). Since aq base is known to convert adenosine to 4,5,6-triaminopyrimidine (**16**, R = H) exclusively, these results indicate that the *v*-triazine ring is more labile to base than the pyrimidine ring. Since previous work from this laboratory showed that imidazo[4,5-*e*]-*s*-triazolo[1,5]-*v*-triazine (**17**) exists in equilibrium with 3-[5(4)-diazoimidazol-4(5)-yl]-*s*-triazole (**18**) and that only **18** was detected in CF_3CO_2H solution of **17**,[15] we examined the ir spectrum of **13a** in CF_3CO_2H for the presence of a diazo band; its absence indicated that if there is an equilibrium between **13a** and the corresponding diazo compound, the equilibrium favors **13a** almost exclusively.



Although the route described above provided the desired model compounds for our nucleoside work, it is not amenable to nucleosides since the acidic cleavage of the pyrimidine ring would also result in cleavage of the glycosyl linkage. Furthermore, in contrast to the results of Stevens, *et al.*,[16] we found that aq base treatment of **5a** opened the pyrimidine ring but did not cause deformylation of the resultant formamido compound **6a**. Also, as mentioned above, Stevens, *et al.*, were unable to reduce 2-azaadenosine 1-oxide to the desired 2-azaadenosine (**13b**).[16] Benzylation of the 1-oxide **5a** gave, in agreement with the results of Fujii,[17] 1-benzyloxy-9-cyclopentyladenine bromide (**10a**), an unstable compound that could be purified by careful recrystallization from EtOH. Boiling in EtOH or H_2O, however, caused debenzylation back to the 1-oxide. In boiling H_2O the free base from **10a** was converted to *N*-benzyloxy-9-cyclopentyladenine (14a). If, however, the crude bromide was carefully neutralized, and the neutral EtOH-H_2O solution was allowed to stand 2 days at room temp, ring opening occurred to give *N*-benzyloxy-1-cyclopentyl-5-formamidoimidazole-4-carboxamidine (**11a**), a result also in general agreement with the findings of Fujii.[17] An attempt to deformylate **11a** with methanolic HCl gave only 1-benzyloxy-9-cyclopentyladenine (**10a**), in contrast to the Dimroth rearrangement obtained in boiling H_2O (**10a** or **11a** → 14a). Hydrogenolysis of **11a** with Ra Ni gave primarily 9-cyclopentyladenine (**1a**), whereas hydrogenolysis with Pd/C gave primarily 9-cyclopentyladenine 1-oxide (**5a**), resulting from hydrogenolysis of the N–O bond with Ra Ni and of the C–O bond with Pd/C with concomitant ring closure in both cases. It was not possible to prevent cyclization of **11a** or its reduction products (**6a** and **8a**). It seemed possible that deformylation of **11a** might be accomplished by a transamidation reaction that could compete effectively with the intramolecular ring-closure reaction. Treatment of **11a** with methanolic NH_3 (satd at 0°) at 80° for 2 days gave the desired 5-amino-*N*-benzyloxy-1-cyclopentylimidazole-4-carboxamidine (**12a**) in good yield. Hydrogenolysis of the benzyloxy group of **12a** with Ra Ni then gave 5-amino-1-cyclopentylimidazole-4-carboxamidine (**9a**). It seemed probable that, despite the precedent of the Dimroth rearrangement, 1-benzyloxy-9-cyclopentyladenine hydrobromide (**10a**) could be converted directly to **12a** by the methanolic NH_3 treatment, and this turned out to be the case. The conditions employed in this route to **9a** are completely compatible with nucleosides.

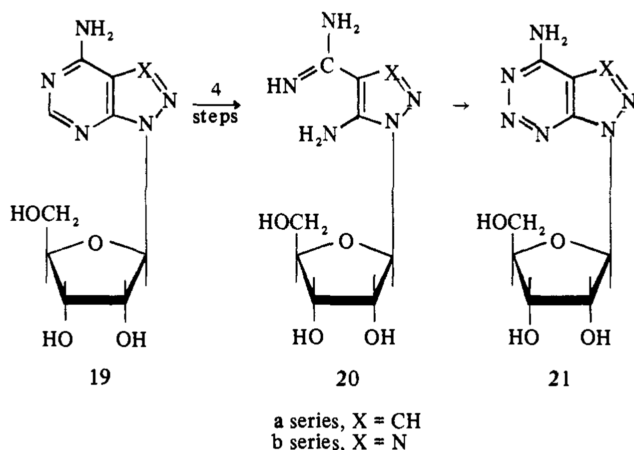
Table I. Cytotoxicity of 2-Azaadenine Nucleosides

Compound	ED_{50}, μmole/l.[a]
2-Azahypoxanthine	1.5
2-Azaadenine	4.0
9-Cyclopentyl-2-azaadenine	49
2-Azaadenosine	0.22
2'-Deoxy-2-azaadenosine	<2.0
9-β-D-Arabinofuranosyl-2-azaadenine	>100
9-β-D-Xylofuranosyl-2-azaadenine	>100
4-Amino-7-β-D-ribofuranosylpyrazolo-[3,4-*d*]-*v*-triazine	20

[a]The concn required to inhibit the growth of treated cells to 50% of that of untreated controls as measured by colony counts.[7]

1-Benzyloxyadenosine hydrobromide (**10b**), prepared by the method described above, behaved exactly as the cyclopentyl compound; refluxing a neutralized EtOH-H_2O solution of **10b** gave *N*-benzyloxyadenosine (**14b**), while standing 3 days at room temp gave the imidazole **11b**, which could be deformylated with methanolic NH_3 to **12b**. It was also possible to convert **10b** directly to **12b**. In order to establish whether the particular conditions of this ring-opening procedure or the benzyloxy group permitted ring-opening followed by deformylation rather than reclosure (Dimroth rearrangement), we prepared 1-benzyladenosine and subjected it to the methanolic NH_3 treatment, which gave *N*-benzyladenosine exclusively.§ Thus, the decreased basicity of the *N*-benzyloxyamidine (**11b**) compared to the *N*-benzylamidine is essential for successful deformylation, but the use of methanolic NH_3 is also necessary (see above). Ra Ni-catalyzed hydrogenolysis of the benzyloxy group of **12b** then gave 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamidine (**9b**). Thus, the 5-amino-1-glycosylimidazole-4-carboxamidines requisite for conversion to 2-azaadenine nucleosides (**13**) can now be obtained from the corresponding adenine nucleosides (**1**) by a relatively simple 4-step procedure. Treatment of the aminoamidine (**9b**) with $NaNO_2$ in aq AcOH gave 2-azaadenosine (**13b**). This procedure was also used to prepare 2′-deoxy-2-azaadenosine (4-amino-7-(2-deoxy-β-D-*erythro*-pentofuranosylimidazo[4,5-*d*]-*v*-triazine, **13c**), 9-β-D-arabinofuranosyl-2-azaadenine (4-amino-7-β-D-arabinofuranosylimidazo[4,5-*d*]-*v*-triazine, **13d**), 9-β-D-xylofuranosyl-2-azaadenine (4-amino-7-β-D-xylofuranosylimidazo[4,5-*d*]-*v*-triazine, **13e**), and 4-amino-

§This result was anticipated, since treatment of 1-benzyl-9-β-D-ribofuranosylpurine-6(1*H*)-thione in a similar manner gave *N*-benzyladenosine.[18]



7-β-D-ribofuranosyl-7*H*-pyrazolo[3,4-*d*]-*v*-triazine (**21a**) from 4-amino-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (**19a**)[19] and 2,8-diazaadenosine (7-amino-3-β-D-ribofuranosyl-*v*-triazolo[4,5-*d*]-*v*-triazine, **21b**) from 8-azaadenosine (**19b**)[20] *via* the corresponding pyrazole (**20a**) and triazole (**20b**).

Biologic Evaluations. The cytotoxicities of the 2-azapurines to human epidermoid carcinoma cells No. 2 in culture[7] are given in Table I. 2-Azaadenosine (**13b**) is 5 times as toxic as 8-azaadenosine,[7] 20 times as toxic as 2-azaadenine, and about 7 times as toxic as 2-azahypoxanthine, indicating that neither enzymatic sugar cleavage nor deamination followed by sugar cleavage appear to be important to the activity of this nucleoside. Of the other nucleosides of 2-azaadenine, only 2'-deoxy-2-azaadenosine (**13c**) appears to be cytotoxic, but its ED_{50} value has not been accurately determined. 4-Amino-7-β-D-ribofuranosylpyrazolo[3,4-*d*]-*v*-triazine (**21a**), the 2-aza analog of the ribonucleoside of 4-aminopyrazolo[3,4-*d*]pyrimidine (4-APP) is only 0.005 as toxic, but the synthetic intermediate to **21a**, 4-amino-1-β-D-ribofuranosylpyrazolo-4-carboxamidine (**20a**), is quite cytotoxic, having an ED_{50} value of 1.9 μmoles/l.

2-Azaadenosine (**13b**) given at levels of 75–400 mg/kg on day 1 only increased the life-span of BDF_1 mice injected ip with 10^5 leukemia L1210 cells by 30–35%. It was about equally active at levels of 8–23 mg/kg per dose when given chronically qd 1–9. None of the other 2-azapurines or the intermediates leading to them that have been tested has shown any significant activity against leukemia L1210.

Experimental Section

Melting points were detd with a Mel-Temp apparatus and are not corrected. Uv spectra were detd in aq soln with a Cary Model 14

Table II. 9-Pentofuranosyl-2-azapurines

R	Crude yield, %	Recrystn solvent	Mp, °C	Formula[a]
A. 9-Pentofuranosyladenine 1-oxides (5b–e)				
β-D-Ribofuranosyl[b]	30	H_2O	233–235	$C_{10}H_{13}N_5O_5$
2-Deoxy-β-D-ribofuranosyl[c]	64	H_2O	218–220	$C_{10}H_{13}N_5O_4 \cdot 0.25H_2O$
β-D-Arabinofuranosyl[d]	67	95%EtOH	220–222	$C_{10}H_{13}N_5O_5$
β-D-Xylofuranosyl[d]	74	H_2O	173–175	$C_{10}H_{13}N_5O_5 \cdot 0.4H_2O$
B. 5-Amino-*N*-benzyloxy-1-pentofuranosylimidazole-4-carboxamidines (12b–e)				
β-D-Ribofuranosyl[e,f]	97	H_2O[g]	146–150[h]	$C_{16}H_{21}N_5O_5$
2-Deoxy-β-D-ribofuranosyl[i]	31	MeOH[j]		$C_{16}H_{21}N_5O_4$
β-D-Arabinofuranosyl[e]	100	H_2O[g]	175–177[h]	$C_{16}H_{21}N_5O_5$
β-D-Xylofuranosyl[e]	54	H_2O[g]	166–168[h]	$C_{16}H_{21}N_5O_5$
C. 5-Amino-1-pentofuranosylimidazole-4-carboxamidines (9b–e)				
β-D-Ribofuranosyl[e]	48	H_2O[g]	192–194[h]	$C_9H_{15}N_5O_4$
2-Deoxy-β-D-ribofuranosyl[e]	25	H_2O[g]	150[h]	$C_9H_{15}N_5O_3$
β-D-Arabinofuranosyl[i]	57	MeOH[j]		$C_9H_{15}N_5O_4$
β-D-Xylofuranosyl[i]	33	H_2O[j]		$C_9H_{15}N_5O_4$
D. 9-Pentofuranosyl-2-azaadenines (13b–e)				
β-D-Ribofuranosyl	64	H_2O	240–241	$C_9H_{12}N_6O_4$
2-Deoxy-β-D-ribofuranosyl	31	H_2O[k]	203–204	$C_9H_{12}N_6O_3$
β-D-Arabinofuranosyl	43	H_2O[k]	236–238	$C_9H_{12}N_6O_4$
β-D-Xylofuranosyl	28	H_2O[k]	234–235	$C_9H_{12}N_6O_4$

[a]Analyzed for C, H, N. [b]See Ref 13. [c]H. Klenow and S. Frederiksen, *Biochim. Biophys. Acta*, 52, 384 (1961). [d]E. J. Reist, D. F. Calkins, and L. Goodman, *J. Med. Chem.*, 10, 130 (1967). [e]Analytical sample of picrate obtd. [f]Also prepd by deformylation in MeOH–NH_3 at 78° of *N*-benzyloxy-5-formamido-1-β-D-ribofuranosylimidazole-4-carboxamidine. [g]Recrystn of picrate. [h]Mp of picrate. [i]Analytical sample was not obtd. [j]Did not cryst. Sample was obtd by evapn of a soln of the compd in this solvent. [k]Isolated by chromatog on a thick silica gel plate before recrystn.

Table III. 4-Amino-7-β-D-ribofuranosyl-7*H*-pyrazolo[3,4-*d*]-*v*-triazine (21a)

Compound	Crude yield, %	Recrystn solvent	Mp, °C	Formula[a]
4-Amino-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine 5-oxide	74	H_2O	233–234	$C_{10}H_{13}N_5O_5$
5-Amino-*N*-benzyloxy-1-β-D-ribofuranosylpyrazole-4-carboxamidine	69	MeOH-H_2O	190–191	$C_{16}H_{21}N_5O_5 \cdot 0.25H_2O$
5-Amino-1-β-D-ribofuranosylpyrazole-4-carboxamidine[b]	60	H_2O[c]	175–176[d]	$C_9H_{15}N_5O_4$
4-Amino-7-β-D-ribofuranosylpyrazolo[3,4-*d*]-*v*-triazine	32	H_2O[e]	207–208	$C_9H_{12}N_6O_4$

[a]Analyzed for C, H, N. [b]Analyzed as the picrate. [c]Recrystn of picrate. [d]Mp of picrate. [e]Isolate as the picrate. Free base obtd by treatment of the picrate with Dowex 1-X8 (carbonate) ion-exchange resin.

Table IV. 2,8-Diazaadenosine (21b)

Compound	Crude yield, %	Recrystn solvent	Mp, °C	Formula[a]
9-β-D-Ribofuranosyl-8-aza-adenine 1-oxide	78	MeOH	208–210	$C_9H_{12}N_6O_5$
5-Amino-N-benzyloxy-1-β-D-ribofuranosyl-1,2,3-triazole-4-carboxamidine	54	MeOH	138–140	$C_{16}H_{20}N_6O_5 \cdot 0.5H_2O$
5-Amino-1-β-D-ribofuranosyl-1,2,3-triazole-4-carboxamidine[b]	40	H_2O[c]	181–182[d]	$C_8H_{14}N_6O_4$
2,8-Diazaadenosine	7.5	H_2O[e]	193–195	$C_8H_{11}N_7O_4$

[a]Analyzed for C, H, N. [b]Analyzed at the picrate. [c]Recrystn of picrate. [d]Mp of picrate. [e]Isolated by chromatog on a thick silica gel plate before recrystn.

spectrophotometer. Ir spectra were detd in pressed KBr discs with Perkin-Elmer Models 221-G, 521, and 621 spectrophotometers, pmr spectra in DMSO-d_6 (TMS) with a Varian A-60A spectrometer; chemical shifts quoted in the case of multiplets are measured from the approximate center. Chromatog analyses were carried out on tlc plates of silica gel H (Brinkmann). The spots were detected by uv light after spraying the plates with Ultraphor (WT, highly concentrated). Most of the chromatog purifications were carried out on Mallinckrodt SilicAR-7 with the solvents indicated. The analytical samples were dried over P_2O_5 (0.07 mm) for 16–20 hr at the temps given.

9-Cyclopentyladenine (1a). A. A soln of 5-amino-1-cyclopentylimidazole-4-carboxamidine (9) · 1.4HCl (64 mg, 0.26 mmole) in 98% formic acid (2 ml) was refluxed for 4 hr and then evapd to dryness. The pH of a soln of the residue in H_2O (15 ml) was raised to 10 with concd NH_4OH before it was extd with $CHCl_3$ (2 × 20 ml). The $CHCl_3$ ext was dried ($MgSO_4$) and evapd to dryness *in vacuo*. The residue crystd from C_6H_6 as a white solid: yield, 30 mg (57%). This material was identical in all respects (mp, ir and uv spectra) with an authentic sample of 9-cyclopentyladenine.[21]

B. A soln of 1-cyclopentyl-5-formamido-N-benzyloxyimidazole-4-carboxamidine (11, 327 mg, 1.0 mmole) in abs EtOH contg sponge Ni catalyst (50 mg) was hydrogenated at atm pressure for 24 hr. The catalyst was removed, fresh catalyst (50 mg) was added, and hydrogenation was resumed for another 24 hr. The process was repeated. The catalyst was then removed, and the soln was evapd to dryness. The residue crystd from MeOH as a white solid: yield, 68 mg (31%). The material was identical with an authentic sample of 9-cyclopentyladenine.[21]

1-Benzyl-9-cyclopentyladenine Hydrobromide (2a). A soln of 9-cyclopentyladenine (203 mg, 1.0 mmole) and benzyl bromide (342 mg, 2.0 mmoles) in DMA (20 ml) was heated for 20 hr at 110° and evapd to dryness *in vacuo*. After trituration with ether, the residue crystd from EtOH: yield, 241 mg (66%).

The analytical sample was obtd by recrystn from EtOH, and dried at 78°: mp 256–257°; λ_{max} nm ($\epsilon \times 10^{-3}$) 0.1 N HCl, 263 (13.1); pH 7, 262 (13.2); 0.1 N NaOH, 261 (13.5), 268 (sh) (12.1), 298 (sh) (3.52); $\bar{\nu}_{max}$ (cm^{-1}) 3300–2600 (br) (NH^+, CH); 1685 (C=N); 1630, 1575, 1510 (purine ring stretch); 1495, 730, 690 (C_6H_5). *Anal.* ($C_{17}H_{19}N_5 \cdot HBr$) C, H, N.

***N*-Benzyl-9-cyclopentyladenine (3a).** A. A soln of 2a (150 mg, 0.4 mmole) in 95% EtOH (20 ml) and 1 N NaOH (2 ml) was refluxed for 3 hr, neutralized with 1 N HCl, and evapd to dryness *in vacuo*. The combined $CHCl_3$ ext of the residue was dried ($MgSO_4$) and evapd to dryness *in vacuo*. The residue crystd from EtOH as a white solid: yield, 60 mg (53%); mp 102–103°; λ_{max} nm ($\epsilon \times 10^{-3}$) 0.1 N HCl, 268 (19.9); pH 7, 271 (20.1); 0.1 N NaOH (20.1); $\bar{\nu}_{max}$ (cm^{-1}) 3265 (NH); 3125, 3075, 3050, 3020 (arom CH); 2950, 2865 (aliph CH); 1625, 1580, 1530, 1490, 1485 (purine and C_6H_5 ring stretch); 730, 695 (C_6H_5). *Anal.* ($C_{17}H_{19}N_5$) C, H, N.

B. A soln of 2a (374 mg, 1.0 mmole) in EtOH (75 ml) and H_2O (100 ml) was neutralized with 1 ml of 1 N NaOH, refluxed for 24 hr, and evapd to dryness *in vacuo*. The residue crystd from aq EtOH (80%): yield, 246 mg (87%). This material was identical in all respects with that obtd from reaction A.

9-Cyclopentyl-2-mercaptoadenine (4a). A soln of 9 · 1.4HCl (230 mg, 0.94 mmole) in DMF (25 ml) contg a suspension of anhyd K_2CO_3 (276 mg, 2.0 mmoles) was stirred for 10 min before the addn of CS_2 (5 ml), after which it was stirred for 20 hr, and then poured into H_2O (150 ml). Evapn of the aq mixt gave a residue that crystd from 20% aq MeOH as a yellow solid: yield, 100 mg (45%). The analytical sample from DMF was dried at 100°: mp 321–323°; λ_{max} nm ($\epsilon \times 10^{-3}$) EtOH, 234 (19.3), 273 (10.8); $\bar{\nu}_{max}$ (cm^{-1}) 3310, 3160 (NH_2); 2950, 2865 (aliph CH); 1640, 1580 (purine ring stretch); 1450 (CH). *Anal.* ($C_{10}H_{13}N_5S$) C, H, N.

9-Cyclopentyladenine 1-Oxide (5a). A. A soln of 9-cyclopentyladenine (2.03 g, 10.0 mmoles) in glacial AcOH (50 ml) and 30% aq H_2O_2 (5 ml) was kept for 10 days with exclusion of light and then evapd to dryness *in vacuo* below 40°. The residue crystd from EtOH (125 ml) as a white solid: yield, 1.70 g (78%). The analytical sample, obtd from a previous run by recrystn from EtOH, was dried at 78°; mp 292° dec; λ_{max} nm ($\epsilon \times 10^{-3}$): 0.1 N HCl, 216 (28.6), 260 (12.0); pH 7, 233 (43.2), 263 (7.94), 295 (2.11); 0.1 N NaOH, 232 (27.2), 268 (8.45), 305 (3.99): $\bar{\nu}_{max}$ (cm^{-1}): 3365 3260, 3190, 3080 (NH); 2950, 2870 (aliph CH); 1660, 1565, 1490 (purine ring stretch); 1225 (N→O). *Anal.* ($C_{10}H_{13}N_5O$), C, H, N.

B. A soln of 1-cyclopentyl-N-benzyloxy-5-formamidoimidazole-4-carboxamidine (327 mg, 1.0 mmole) in EtOH (150 ml) contg 5% Pd/C (100 mg) was hydrogenated at room temp and atm pressure for 20 hr during which the theoretical uptake of H_2 was observed. The soln was filtered, and the filtrate was evapd to dryness *in vacuo*. The residue crystd from EtOH: yield, 144 mg (65%). The uv, ir, and pmr spectra of this material indicate that it is impure **5a**.

5-Amino-1-cyclopentyl-4-imidazolecarboxamidoxime (7a). A. A soln of 5a (16.05 g, 73.6 mmoles) in 3 N HCl (322 ml) was refluxed for 10 min and evapd to dryness *in vacuo*. When a soln of the residue in H_2O (200 ml) was neutralized with 1 N NaOH, the product pptd as a white solid: yield, 10.37 g (67.5%). The analytical sample was obtd in a previous run by recrystn from EtOH, and dried at 78°: mp 192–194°; λ_{max} nm ($\epsilon \times 10^{-3}$) 0.1 N HCl, 278 (9.63); pH 7, 257 (9.60); 0.1 N NaOH, 260 (9.50); $\bar{\nu}_{max}$ (cm^{-1}) 3435, 3140, 3295 (NH); 3140 (arom CH); 2960, 2870 (aliph CH); 3300–2200 (br) (OH); 1630 (C=N); 1565, 1495 (imidazole ring stretch); 1450 (CH). *Anal.* ($C_9H_{14}N_5O$) C, H, N.

B. A soln of 5a (1.00 g, 4.58 mmoles) in 1 N NaOH (10 ml) was refluxed for 30 min, neutralized with concd HCl, and extd with $CHCl_3$ (100 ml). After drying ($MgSO_4$), the ext was evapd to dryness. The residue crystd from EtOH as a white solid: yield, 188 mg (17%). Spectral data indicated that this material is 1-cyclopentyl-5-formamidoimidazole-4-carboxamidoxime (**6a**). From the filtrate, 391 mg (39%) of starting compd was obtained. The product, without purification, was refluxed for 10 min in 3 N HCl (4 ml). The soln was neutralized with dil NaOH and extd with $CHCl_3$ (4 × 25 ml). The $CHCl_3$ ext was dried ($MgSO_4$) and evapd to dryness. The residue crystd from EtOH as a white solid: yield, 22 mg. Further $CHCl_3$ extn of the residue from the aq soln gave another 20 mg: total yield, 37%. This material was identical in ir and uv spectra with an authentic sample of 5-amino-1-cyclopentyl-4-imidazolecarboxamidoxime (7).

5-Amino-1-cyclopentylimidazole-4-carboxamidine (9a). A. A soln of 7a (2.09 g, 10.0 mmoles) in MeOH (350 ml) contg Ra Ni catalyst (2 g) was hydrogenated for 24 hr at room temp and atm pressure. The catalyst was removed by filtration and washed with MeOH. The combined filtrate and washes were evapd to dryness *in vacuo*. A soln of the residue in MeOH was acidified with concd HCl and evapd to dryness. The residue crystd from EtOH as an HCl salt: yield, 2.18 g (90%). The analytical sample was obtd in a previous run by recrystn from EtOH and dried at 100° (0.07 mm) over P_2O_5: mp 265–266°; λ_{max} nm ($\epsilon \times 10^{-3}$) 0.1 N HCl, 283 (11.4); pH 7, 284 (12.1); 0.1 N NaOH, 267 (9.60); $\bar{\nu}_{max}$ (cm^{-1}) 3295, 3170 (NH); 2960, 2870 (aliph CH); 1560, 1645, 1575 (C=NH_2^+ and imidazole ring stretch). *Anal.* ($C_9H_{15}N_5 \cdot HCl \cdot 0.67H_2O$) C, H, Cl, N. A second crop was obtd from the soln of the analytical sample and dried at 100°: λ_{max} nm ($\epsilon \times 10^{-3}$) 0.1 N HCl, 283 (11.0); pH 7, 284 (11.8); 0.1 N NaOH, 266 (9.45). *Anal.* ($C_9H_{15}N_5 \cdot HCl \cdot 0.44H_2O$) C, H, N.

The identity of the fractional hydrates was further confirmed by conversion to 9-cyclopentyladenine (see above).

B. A soln of **7a** · HCl (970 mg, 2.90 mmoles) in H_2O (60 ml)

was made basic (pH 9) with 1 *N* NaOH. Repeated $CHCl_3$ extn of the resulting cloudy soln gave a clear aq layer. The combined $CHCl_3$ ext was dried ($MgSO_4$) and evapd to dryness *in vacuo*. The residue, a white cryst solid, was dissolved in EtOH (50 ml) and hydrogenated at room temp and atm pressure for 20 days in the presence of Ra Ni catalyst. The soln was filtered and evapd to dryness. An EtOH soln of the residue was acidified with concd HCl and evapd to dryness. The residue crystd in 2 crops from EtOH as a white solid (1.4 HCl): yield, 163 mg (23%); the second crop was a 1.2HCl: yield, 143 mg (21%). Both ir and uv data of these two crops are in agreement with those given in A above.

1-Benzyloxy-9-cyclopentyladenine Hydrobromide (10a). A soln of 9-cyclopentyladenine 1-oxide (218 mg, 1.0 mmole) in DMA (20 ml) contg $PhCH_2Br$ (684 mg, 4.0 mmoles) was kept for 4 days at 25° and evapd to dryness *in vacuo* without heating. The residue, after 2 triturations with Et_2O, crystd from EtOH as a light yellow solid: yield, 303 mg (78%). The analytical sample was obtd by careful recrystn from EtOH. It was dried at 78°: mp 177-182°; λ_{max} nm ($\epsilon \times 10^{-3}$) 0.1 *N* HCl, 262 (12.3); pH 7, 262 (12.1); 0.1 *N* NaOH, 258 (11.9), 266 (sh) (10.9); $\bar{\nu}_{max}$ (cm^{-1}) 3400 (OH); 3400-2400 (br) (NH_2^+); 1680 (C=NH_2^+); 1610, 1495 (C_6H_5); 1565 (purine ring stretch); 1450 (CH); 740, 690 (C_6H_5). *Anal.* ($C_{17}H_{20}BrN_5O \cdot 0.5C_2H_5OH$) C, H, N.

1-Cyclopentyl-5-formamido-*N*-benzyloxyimidazole-4-carboxamidine (11a). A soln of 10a (413 mg, 1.0 mmole) in H_2O (40 ml) and EtOH (15 ml) was neutralized with 1 *N* NaOH, kept for 2 days at 25°, and evapd *in vacuo* without heating to remove the alc. The ppt that formed in the remaining aq soln was collected as a white solid: yield, 268 mg (82%). The analytical sample was obtd by recrystn from EtOH and dried at 78°: mp 132-133°; λ_{max} nm ($\epsilon \times 10^{-3}$): 0.1 *N* HCl, 255 (8.84); pH 7, 255 (sh) (6.92); 0.1 *N* NaOH, 256 (12.6); $\bar{\nu}_{max}$ (cm^{-1}) 3490, 3400, 3345, 3300 (NH); 3160, 3100 (arom CH); 2940, 2920, 2865 (aliph CH); 1695 (C=O); 1690, 1680 (amide I); 1630 (C=N); 1600, 1490 (C_6H_5); 1530 (amide II); 735, 695 (C_6H_5). *Anal.* ($C_{17}H_{21}N_5O_2$) C, H, N.

5-Amino-*N*-(benzyloxy)-1-cyclopentylimidazole-4-carboxamidine (12a). A. A soln of 11a (6.54 g, 20.0 mmoles) in methanolic NH_3 (satd at 0°) (800 ml) was heated in a stainless steel reaction vessel at 80° for 2 days and then evapd to dryness. A soln of the residue in EtOH was acidified with concd HCl and evapd to dryness. The residue crystd from EtOH. The analytical sample, obtd by recrystn from EtOH, was a white solid: yield, 4.02 g, (60%); mp 179-181° dec; λ_{max} nm ($\epsilon \times 10^{-3}$): 0.1 *N* HCl, 279 (10.0); pH 7, 264 (10.9); 0.1 *N* NaOH, 264 (10.7); $\bar{\nu}_{max}$ (cm^{-1}) 3450, 3320 (NH); 3190, 3140, 3080, 3020, 3000 (arom CH); 2945, 2865 (aliph CH); 3200-2200 (br) (NH_2^+); 1650 (CH=NH_2^+); 1590, 1500 (C_6H_5); 1525, 1460 (imidazole ring stretch); 735, 685 (C_6H_5). *Anal.* ($C_{16}H_{21}N_5O \cdot HCl$) C, H, N. An 18% recovery of starting material was obtained from the filtrate.

B. A soln of 10a (827 mg, 2.0 mmoles) in methanolic NH_3 (satd at 0°) (200 ml) was heated in a stainless steel reaction vessel at 80° for 2 days and evapd to dryness *in vacuo*. A soln of the residue in EtOH was acidified with concd HCl and evapd to dryness. Two recrystn from EtOH gave the product as a white solid: yield, 528 mg (79%). This material was identical in all respects with that from the previous run.

2-Aza-9-cyclopentyladenine (13a). To a cold, stirred soln of 5-amino-1-cyclopentylimidazole-4-carboxamidine · 1.4HCl (1.15 g, 4.7 mmoles) in H_2O (40 ml) was added slowly a soln of $NaNO_2$ (690 mg, 10.0 mmoles) in H_2O (15 ml). The ppt that formed was collected after standing 2 hr in the cold: yield, 703 mg (73%). The analytical sample was obtd in a previous run by recrystn from H_2O and dried at 78°: mp 212-215°; λ_{max} nm ($\epsilon \times 10^{-3}$) 0.1 *N* HCl, 254 (7.88), 293 (4.27); pH 7, 257 (7.37), 297 (6.20); 0.1 *N* NaOH, 257 (7.37), 297 (6.17); $\bar{\nu}_{max}$ (cm^{-1}) 3400-3000 (br) (NH); 2945, 2870 (aliph CH); 1700, 1645, 1615, 1510 (azaadenine ring stretch). *Anal.* ($C_9H_{12}N_6$) C, H, N.

N-Benzyloxy-9-cyclopentyladenine (14a). A. A soln of 11a (327 mg, 1.0 mmole) in H_2O (60 ml) and EtOH (10 ml) was refluxed for 27 hr. The white solid that pptd on cooling was collected by filtration: yield, 204 mg (66%); mp 169-171°. The analytical sample was obtd by recrystn from EtOH and dried at 78°: λ_{max} nm ($\epsilon \times 10^{-3}$) 0.1 *N* HCl, 274 (14.3); pH 7, 270 (17.1); 0.1 *N* NaOH, 287 (13.2): $\bar{\nu}_{max}$ (cm^{-1}) 3100 (br), 3025 (NH); 2950, 2910, 2860 (aliph CH); 1645, 1525 (purine ring stretch); 1590, 1500, 730, 690 (C_6H_5). *Anal.* ($C_{17}H_{19}N_5O$) C, H, N.

B. A soln of 10a (390 mg, 0.95 mmole) in H_2O (60 ml) was neutralized with 1 *N* NaOH (0.95 ml), dild with EtOH (30 ml) to give a clear soln, and then refluxed for 8.5 hr. Upon cooling, a cryst solid pptd: yield, 107 mg (37%). This product was identical in all respects with that from the previous run. Evapn of the filtrate to 10 ml caused the pptn of another cryst solid that was identified by its uv and ir spectra as 1-cyclopentyl-5-formamido-*N*-(benzyloxy)imidazole-4-carboxamidine: yield. 111 mg (36%).

N-Benzyloxyadenosine (14b). A soln of 1-benzyloxyadenosine hydrobromide (908 mg, 2.0 mmoles) in H_2O (150 ml) to which 2 ml of 1 *N* NaOH was added was refluxed 9 hr and then evapd to dryness. The product was isolated by chromatog on a silica gel plate as a white glass that crystd from H_2O: yield, 118 mg (16%); mp 129-130°; λ_{max} nm ($\epsilon \times 10^{-3}$) 0.1 *N* HCl, 269 (17.0); pH 7, 269 (16.9); 0.1 *N* NaOH, 284 (13.4); $\bar{\nu}_{max}$ (cm^{-1}) 3380 (OH); 2920, 2860 (CH); 1650, 1590, 1530, 1505 (C=N, C=C); 1205 (COC); 1075, 1040 (CO); 830, 790 (C_6H_5). *Anal.* ($C_{17}H_{19}N_5O_5$) C, H, N.

Effect of Base on 2-Aza-9-cyclopentyladenine. 5-Amino-1-cyclopentylimidazole-4-carboxamide (15) and 4,5-Diamino-6-cyclopentylamino-*v*-triazine (16). A soln of 2-aza-9-cyclopentyladenine (375 mg, 1.84 mmoles) in 0.1 *N* NaOH (30 ml) was refluxed with stirring for 20 hr, then cooled, and the white cryst solid that pptd was collected by filtration (271 mg). Purification of this material was effected by chromatog on a thick plate using $CHCl_3$-MeOH (9:1) as developing solvent; 3 bands were obtd. Each was extd with hot MeOH. The slower moving band gave a white solid; yield, 125 mg (36%). The analytical sample of this material, identified as 4,5-diamino-6-cyclopentylamino-*v*-triazine (16), was obtd by recrystn from EtOH and dried at 78°: mp 275° dec; λ_{max} nm ($\epsilon \times 10^{-3}$) 0.1 *N* HCl, 204 (13.8), 252 (18.8), 289 (9.30), 374 (5.72); pH 7, 235 (25.4), 286 (7.06), 318 (4.85); 0.1 *N* NaOH, 236 (25.7), 286 (7.12), 318 (4.90); $\bar{\nu}_{max}$ (cm^{-1}) 3430, 3380, 3340, 3190 (NH); 2950, 2860 (aliphatic CH); 1680, 1610, 1575 (ring stretch); δ in ppm, 1.7 (m, CH_2); 4.4 (m, CH); 5.0, 5.7 (m, NH and NH_2). *Anal.* ($C_8H_{14}N_6$) C, H, N.

The middle band also gave a white solid: yield, 145 mg (41%). It was identified by spectral and analytical data as 5-amino-1-cyclopentylimidazole-4-carboxamide (15).[9]

The fast moving band gave a solid (6 mg, 1%) that was identified as 9-cyclopentyl-2-azaadenine (13).

Nucleosides. The 9-pentofuranosyl-2-azapurines (13b-e), 4-amino-7-β-D-ribofuranosylpyrazolo[3,4-*d*]-*v*-triazine (21a), and 2,8-diazaadenosine (21b) were all prepd in essentially the same manner as 9-cyclopentyl-2-azaadenine (1a → 5a → 10a → 12a → 9a → 13a). The details for each compd are given in Tables II-IV.

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N^6-Substituted Adenosines: Synthesis, Biological Activity, and Some Structure–Activity Relationships

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Nucleosides of N^6-substituted adenines, which possess cytokinin activity, inhibit the growth of tumor cells, while the corresponding adenines are relatively inactive. Addnl N^6-substituted adenosines have been prepd and tested to secure information regarding structure–activity relationships, if any. The new compds include N^6-butyl-, N^6-*n*-2-propoxyethyl, N^6-*n*-2-butoxyethyl-, N^6-cyclohexyl-, N^6-cyclopropylmethyl-, N^6-tetrahydrofurfuryl-, N^6-geranyl-, N^6-farnesyl-, and N^6-α-pyridoxyladenosine. They were prepd from 6-chloropurine riboside by nucleophilic substitution with the appropriate amine. The known cytokinin compds, 2-methylthio-N^6-isopentenyladenine, *cis*-6-(β-chloro-2-butenylamino)purine, and *trans*-6-(β-chloro-2-butenylamine)purine and their ribosides, and *trans*-zeatin riboside were examd for other biological activity. The alkylated adenosines show optimal cytokinin activity when the N^6-substituent contains a double bond. In *Escherichia coli* the compds were active at 10^{-6}–10^{-4} *M* with the trans isomers showing greater activity than the cis compds. As inhibitors of mouse adenocarcinoma cells (TA-3) in culture, some of the compds were active at 10^{-5}–10^{-6} *M* but in sarcoma S-180 cells in culture they were all less active. Redn of the double bond in the side chain lowered activity of these compds in the tumor cell cultures. The trans isomers are more active against tumor cells *in vitro* than the cis analogs, paralleling their activity as cytokinins. The presence of an OH group in the side chain diminished antitumor activity. A moderate increase in survival time of mice bearing leukemia L-1210 was produced by the compds bearing an ether linkage in the side chain.

We have previously reported[1,2] the synthesis of a series of N^6-substituted adenine ribosides which are potent cytokinins. Many of these compds inhibited the growth of neoplastic cells *in vitro* at concns of about 10^{-6} *M*. Most of these adenosine analogs had different effects on various leukemic cells and no effects on lymphocytes *in vitro*. It was also noted that at lower concns (e.g, 10^{-8}–10^{-7} *M*) some stimulation of human leukemic (line 6410) cell growth took place in contrast to the inhibitory effects that occurred at higher concns. The corresponding free adenines were relatively inactive against tumor cells. The more active tumor inhibitory nucleosides were found to be N^6-benzyl-, N^6-furfuryl-, N^6-ethoxyethyl-, N^6-phenyl-, and N^6-thenyladenosines. Like N^6-(3-methyl-2-butenyl)adenosine[12] (IPA), these analogs are also active as cytokinins.

The present communication† is an extension of our previous work. In order to secure further information on the structure–activity relationships in this series of adenosine derivatives, addnl analogs were prepd and examd for their biol properties. These include N^6-*n*-Bu- (I), N^6-*n*-2-propoxyethyl- (II), N^6-*n*-2-butoxyethyl- (III), N^6-cyclohexyl- (IV), N^6-cyclopropylmethyl- (V), N^6-tetrahydrofurfuryl- (VI), N^6-geranyl- (VII), N^6-farnesyl- (VIII), and N^6-α^4-pyridoxyladenosines (IX). The compds were tested for cytokinin activity in the tobacco callus bioassay, and in microbial and tumor systems. This series of compds was augmented by *trans*-N^6-4-hydroxymethyl-2-butenyladenosine (zeatin riboside), a potent cytokinin that was prepd in this work by the method of Shaw, *et al.*[4] The compds were chosen for synthesis and examn of properties for the following reasons.

(I) The *n*-Bu fragment represents the shortest C chain among a series of N^6-substituted adenines resulting in really good cytokinin activity.[5] (II, III) The propoxyethyl and butoxyethyl compds are homologs of N^6-2-ethoxyethyladenosine, a compd with antitumor activity *in vivo.*[2] (IV, VI) The cyclohexyl and tetrahydrofurfuryl compds are satd derivatives of unsatd analogs which showed biol and antitumor activity. (V) The cyclopropylmethyl derivative was prepd to examine the biol effect of the smallest satd ring structure. (VII, VIII) The geranyl and farnesyl side chains were chosen to det the effects of multiple isopentenyl fragments on a single side chain. (IX) The α^4-pyridoxyl side chain was included to evaluate the effect of a strong hydrophilic substituent which contains a biol active moiety.

Zeatin riboside (*trans*-N^6-(4-hydroxymethyl-2-butenyl)-adenosine was included because it is about the most active known cytokinin of the N^6-adenosine series.[6,7] The compds were prepd by condensing 6-chloropurine riboside (6-chloro-9-β-D-ribofuranosyl-9*H*-purine) with the corresponding amines by nucleophilic substitution in boiling EtOH, using $CaCO_3$ or Et_3N as ancillary acid acceptors.[1] The compds were isolated and purified by crystn. The purity of the products was confirmed by chromatog, elemental analyses, and by uv spectra. The physical data are given in Tables I and II.

The new compds were examd for cytokinin activity in a tobacco pith assay system by the method of Murashige and Skoog.[8] As shown in Table III, the compds vary in activity. The N^6-butyl and propoxyethyladenosines have good activity, although somewhat less than that of zeatin riboside. The other compds displayed marginal or no cytokinin activity noted since high concns had to be used for initial response.

†A portion of this material was presented by the author at the 160th National Meeting of the American Chemical Society.[3]