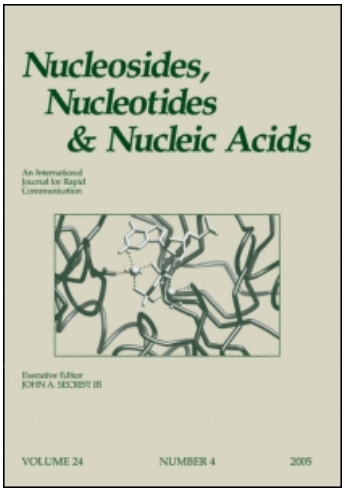

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Synthesis, Transformation Chemistry, and Biological Activity of Guanine Nucleosides and Analoges

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SYNTHESIS, TRANSFORMATION CHEMISTRY, AND BIOLOGICAL ACTIVITY OF GUANINE NUCLEOSIDES AND ANALOGUES[1]

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Abstract: Regiospecific (9-) and regioselective (7-) coupling procedures have been developed with easily prepared guanine derivatives. Acyclovir and 9-(D-pentofuranosyl)guanine nucleosides have been prepared without observed formation of 7-isomers. Guanosine has been functionalized and transformed into a variety of base and/or sugar modified analogues. Potent activity against HIV and a hepadnavirus has been found.

The chemistry of guanine nucleosides and analogues presents significant experimental challenges relative to that involved with the other four major nucleic acid bases. Guanine 7- and 9-substituted compounds are amphoteric, essentially insoluble in most solvents (unless protected with lipophilic groups), and often form gels in solution when impure. There are few generally applicable purification techniques for unprotected guanine nucleosides and analogues. These considerations and the prior absence of clinical success with guanine derivatives had led to some neglect of such compounds until quite recently.

The discovery[2] of 9-[(2-hydroxyethoxy)methyl]guanine (acyclovir) as the first "acyclonucleoside" analogue with potent herpes antiviral activity and low human toxicity stimulated a world-wide renaissance of research activity in the nucleoside/analogue field in general and in the guanine analogue area in particular. Other examples of potent antiviral activity of guanine-type nucleoside analogues also exist[3].

Several methods for the synthesis of guanine nucleosides have been used. Fischer and Helferich reported the first preparation of a guanine nucleoside in 1914[4]. Coupling of "acetobromoglucose" with the silver salt of 2,8-dichloroadenine followed by deprotection and selective dechlorination at C8 gave the "2-chloroadenine glucoside". Hydrolytic deamination at C6 followed by aminolysis at C2 gave 9-(β-D-glucopyranosyl)guanine. Todd and co-workers used this sequence with 2,3,5-tri-*O*-acetyl-D-ribofuranosyl chloride[5] to provide the first synthesis of naturally occurring guanosine. Davoll and Lowy acetylated 2,6-diaminopurine, and coupled[6] the 2,6-diacetamidopurine chloro-mercury salt with the latter sugar derivative[5]. Methanolic ammonia at 0°C effected selective ammonolysis of the 6-*N*-acetyl function and concomitant removal of the sugar *O*-acetyl groups. Hydrolytic deamination and removal of the 2-*N*-acetyl group gave guanosine[6].

Russian workers prepared the chloromercury salt of 2-*N*-acetylguanine. Coupling of this organometallic derivative with the protected glucosyl bromide resulted in formation of a mixture of 7- and 9-substituted isomers[7]. Walton and co-workers[8] utilized this procedure with 2,5-di-*O*-benzoyl-3-deoxy-D-*erythro*-pentofuranosyl bromide and obtained 3'-deoxyguanosine and its 7-regioisomer after deprotection. All subsequently reported coupling procedures that employed a "directly-protected derivative" of guanine have produced mixtures of 7- and 9-isomers. Japanese workers have employed a variety of "direct fusion" approaches[9]. One that involved iodine-catalyzed fusion of 1-*O*-acetyl-2,3,5-tri-*O*-benzoylribofuranose and diacetylguanine gave a complex anomeric mixture of 7- and 9-regioisomers of guanosine[10].

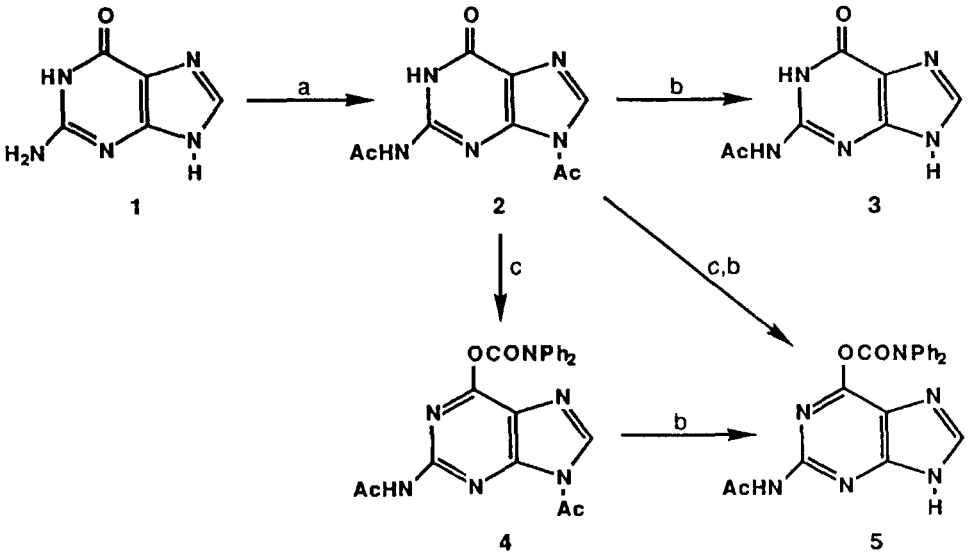
Reist and Goodman utilized a fusion procedure for coupling of 2,6-dichloropurine with tetra-*O*-acetylxylofuranose. Treatment of the product with methanolic ammonia effected selective ammonolysis at C6 and concomitant deprotection of the sugar to give 2-chloro-9-(β-D-xylofuranosyl)adenine[11]. A series of sugar transformations followed by the Fischer-Helferich[4] hydrolytic deamination and ammonolysis sequence gave 9-(β-D-arabinofuranosyl)guanine. Goodman and coworkers[12a] reported improved methods for obtaining 2-amino-6-substitutedpurine 9-nucleosides

in 1972. Acetylation of 2-amino-6-chloropurine followed by formation of an ill-defined chloromercury compound had been used[12b] for coupling with protected glycosyl halides to give 2-acetamido-6-chloropurine nucleosides. Trimethylsilylation of 2-amino-6-chloropurine or 2-acetamido-6-chloropurine followed by coupling of these solubilized bases with protected glycosyl halides in the presence of mercury(II) cyanide gave good yields of the 9-glycosyl products[12a]. Conversion of the 6-chloro function to 6-oxo and deprotection gave guanine-9-nucleosides.

Difficulties with purification of nucleosides from trace quantities of toxic mercuric ions that have led to spurious biological testing conclusions[13] made Vorbruggen's tin(IV) chloride and trimethylsilyl trifluoromethanesulfonate (TMS triflate) catalyzed couplings of trimethylsilylated bases and 1-*O*-acetyl-2,3,5-tri-*O*-benzoylribofuranose a welcome and convenient improvement in nucleosidation methodology[14]. It was reported in 1981 that such coupling of trimethylsilylated 2-*N*-acetylguanine followed by deprotection provided guanosine in 66% yield[15]. However, no characterization of that "pure guanosine" product other than TLC comparison with the natural product was noted. Ogilvie and co-workers observed formation of equivalent amounts of 7- and 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine derivatives[16] upon treatment of trimethylsilylated 2-*N*-acetylguanine with a protected chloromethyl ether in the presence of tetrabutylammonium iodide. Dudycz and Wright's studies[17] on glycosylations of trimethylsilylated 2-*N*-substituted guanine derivatives demonstrated that the 7-isomer was formed as the kinetic product, and a mixture rich in the more thermodynamically stable 9-isomer could be obtained by heating. Very recently, Garner and Ramakanth have extended these conclusions[18].

We now report studies on the highly regioselective kinetic formation of 7-(D-pentofuranosyl)guanines by ambient temperature coupling of tetra-*O*-acetyl-D-pentofuranoses and trimethylsilylated 2-*N*-acetylguanine with tin(IV) chloride as catalyst; and successful applications of trimethylsilylated 2-*N*-acetyl-6-*O*-diphenylcarbamoylguanine for the high yield preparation of 9-(D-pentofuranosyl)guanines without contamination by 7-isomers[19].

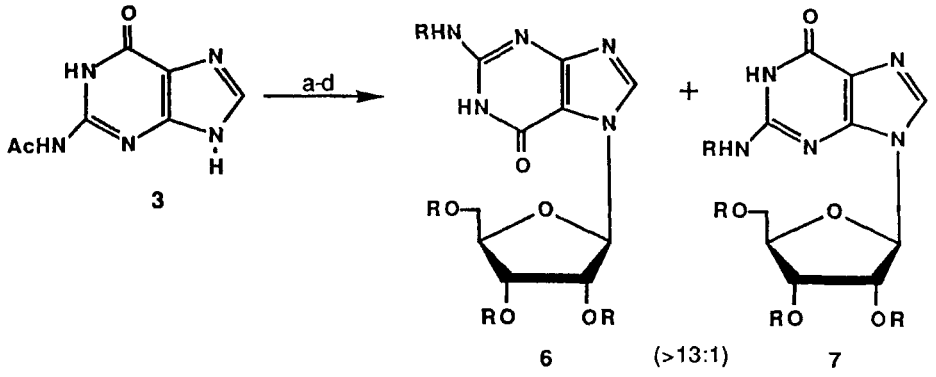
Acetylation of guanine (**1**) with acetic anhydride in hot DMAC (*N*,*N*-dimethylacetamide) gave 2-acetamido-9-acetylhypoxanthine (**2**),[20] which readily undergoes solvolysis of the 9-acetyl group[21] in aqueous ethanol to give 2-*N*-acetylguanine (2-acetamidohypoxanthine, **3**) in high yield. Treatment of a pyridine solution of **2** with diphenylcarbamoyl chloride in the presence of ethyldiisopropylamine at ambient temperature according to the general procedure of Hata and co-workers[22] gave 2-acetamido-9-acetyl-6-diphenylcarbamoyloxypurine (**4**). Selective solvolysis of the 9-acetyl group also occurred with **4** to give 2-acetamido-6-diphenycarbamoyloxypurine (**5**). Execution of this two-stage procedure without isolation of intermediate **4** gave pure **5** as a stable crystalline product in 91% overall yield.



(a) Ac_2O/DMAC/Δ. (b) EtOH/H_2O/Δ. (c) Ph_2NCOCl/EtN(iPr)$_2$/C_5H_5N.

Trimethylsilylation of **3** (hexamethyldisilazane/ammonium sulfate) and coupling of the resulting tris(trimethylsilyl) derivative with tetra-*O*-acetyl-D-arabinofuranose under the general Vorbruggen conditions (TMS triflate catalyst in 1,2-dichloroethane [DCE] at 80°C overnight[15]) gave a mixture of 9-α and 7-α isomers in a ratio of ~2:1. Repetition of this experiment gave ratios of 2-2.5:1 (400 MHz ^{1}H NMR spectroscopy). This led us to repeat the

reported conditions[15] with both tetra-*O*-acetylribofuranose and 1-*O*-acetyl-2,3,5-tri-*O*-benzoylribofuranose. As noted by Garner and Ramakanth[18], the benzoylated sugar gave higher relative ratios of the 9-isomer. Our ^{1}H NMR results[19] indicated that ~2-5:1 ratios of 9/7-isomers of guanosine were formed in couplings with these two sugar derivatives. Similarly, tetra-*O*-acetylxylofuranose gave 9/7-isomer ratios of ~2:1.



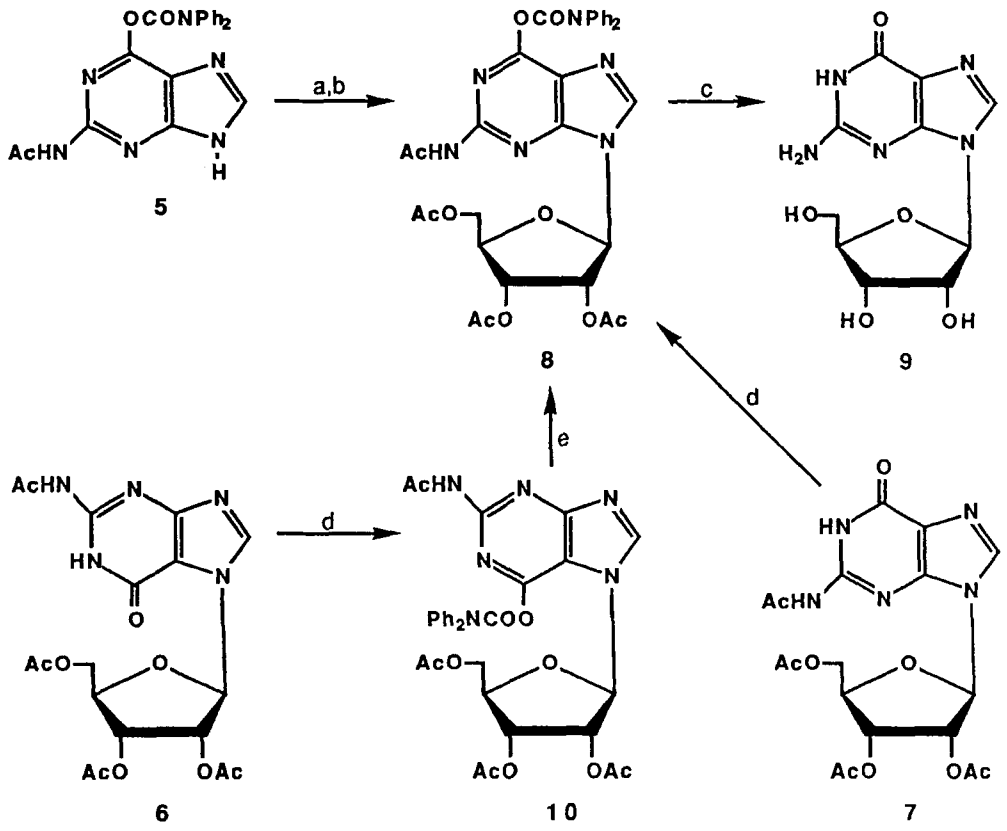
(a) BSA/DCE/Δ. (b) $SnCl_4$. (c) 1,2,3,5-Tetra-*O*-acetyl-D-pentofuranose. (d) NH_3/MeOH/H_2O/Δ.

We next examined kinetic formation of the 7-isomers. Treatment of 2-*N*-acetylguanine (**3**) with bis(trimethylsilyl)acetamide (BSA) in DCE at 80°C gave a clear solution of the tris(trimethylsilyl) derivative in 2.5-3 hours. Addition of 2.14 molar equivalents of tin(IV) chloride to the cooled solution resulted in formation of an active organometallic complex. Treatment of a solution of the respective tetra-*O*-acetyl-D-pentofuranose in DCE with this complex at ambient temperature overnight resulted in formation of high yields of a mixture of 7/9-isomers. Flash chromatography gave the pure 7-isomers in yields of 70% (**6**, R = Ac, β-ribo), 72% (α-arabino), and 76% (β-xylo). NMR analysis of the crude coupling mixtures indicated 7/9-isomer ratios of >13:1 (**6**:**7**, R = Ac, ribo), ~18:1 (arabino), and ~15:1 (xylo). The xylo 9-isomer also was isolated in 3% yield by column chromatography. Deprotection and crystallization of the purified coupling products gave analytically pure hemihydrates of the 7-(D-pentofuranosyl)guanines in yields of 78% (**6**, R = H, β-ribo), 85% (α-arabino), and 86% (β-xylo).

In parallel with observations of Garner and Ramakanth[18], solvent, catalyst, and reaction condition modifications failed to significantly alter our ratios of 9/7-isomer compositions with reactions conducted under higher temperature thermodynamic conditions[19]. We then focused our attention on development of a guanine derivative with different intrinsic thermodynamic attributes. As noted above, Goodman and co-workers had observed that 2-amino-6-chloropurine underwent couplings to give 9-glycosyl isomers[12]. Similarly, couplings with 2,6-dichloropurine[11] and 2,6-diacetamidopurine[6] proceeded without noted 7-isomer formation. It appears that constriction of the guanine system into a "6-enolate derivative" results in enhancement of the relative 9/7-isomer thermodynamic stability ratio. We reasoned that a bulky, electron withdrawing group attached to O6 of 2-*N*-acetylguanine might give a derivative that would undergo kinetic coupling at N9; and also that the resulting 9-isomer would be the thermodynamically more stable product.

Trimethylsilylation of **5** with BSA/DCE at 80°C was complete in ~15 minutes. The clear solution of the bis(trimethylsilyl) derivative was evaporated and the syrupy residue was dissolved in **dry** toluene. This was added to a solution of tetra-*O*-acetylribofuranose in dry toluene and stirred with catalytic TMS triflate at 80°C for 1 hour. Examination of the crude coupling mixture by NMR indicated formation of 2-acetamido-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-6-diphenylcarbamoyloxypurine (**8**) with none of the 7-isomer detected. Column chromatographic purification gave **8** in 91% yield. Application of this procedure with the corresponding arabinose and xylose derivatives provided 9-α (82%) and 9-β (86%) protected products, respectively. Quantitative deprotection was effected smoothly with aqueous methanolic ammonia at 60°C to give analytically pure crystalline hemihydrates of guanosine (**9**) (75%), 9-(α-D-arabinofuranosyl)guanine (84%), and 9-(β-D-xylofuranosyl)guanine (67%). These yields represent recrystallization recoveries.

Trace quantities of bis(sugar)-substituted by-products were separated from the protected intermediates by column chromatography. Their 2:1 sugar/base compositions were indicated by NMR and FAB mass spectra. The



(a) BSA/DCE/Δ. (b) 1,2,3,5-Tetra-*O*-acetyl-D-pentofuranose/TMSOTf/$C_6H_5CH_3$/80°C. (c) NH_3/MeOH/H_2O/Δ. (d) Ph_2NCOCl/EtN(iPr)$_2$/C_5H_5N. (e) TMSOTf/$C_6H_5CH_3$/80°C.

^{1}H-coupled ^{13}C NMR spectrum of the bis(xylosyl) by-product had 3-bond coupling to C2 of the guanine base in harmony with attachment of the second sugar residue at N2. Hydrolysis of that product produced 9-(β-D-xylofuranosyl)guanine. No NMR signals corresponding to 7-isomers were detected in spectra of the deprotected nucleosides or in the crude protected coupling mixtures.

Diphenylcarbamoylation of the tetraacetyl 7-isomer (**6**) of guanosine occurred in the usual manner to give 2-acetamido-7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-6-diphenylcarbamoyloxypurine (**10**). Subjection of **10** to our standard coupling conditions with TMS triflate in dry toluene at 80°C

resulted in its rearrangement to the 9-glycosyl isomer (**8**). Nearly 2 hours was required for completion of the 7 (**10**) to 9 (**8**) isomerization process, in contrast with the 1 hour employed for 9-glycosylation with the tetra-*O*-acetyl sugars. A solution of bistrimethylsilylated-**5**, tetra-*O*-acetylribofuranose, and TMS triflate in dry toluene was stirred at ambient temperature for 4 days. Progressive formation of the 9-glycosyl isomer occurred to give a good yield of **8**, without observed formation of the 7-isomer (**10**).

These two experiments indicate that our 9-isomer (**8**) is the kinetic *and* thermodynamic product. The absence of detected 7-isomer in the ambient temperature coupling suggests kinetic formation of **8**. The longer time required for rearrangement of **10** to **8** relative to the usual glycosylation reaction period at 80°C also argues against formation of 7-isomer and its subsequent conversion to the 9-glycosyl product *in situ*. However, the isomerization of **10** to **8** under the condensation reaction conditions is convincing proof of the enhanced thermodynamic stability of the 9/7-isomers of this 6-*O*-DPC pair relative to that of **7/6** (the 6-oxo containing pair).

Hata and co-workers had logically assigned the 6-*O*-DPC structures to their guanine nucleoside diphenylcarbamoylation products[22]. However, no direct proof of this assignment was given. Treatment of 2-*N*-2',3',5'-tri-*O*-tetraacetylguanosine (**7**) with diphenylcarbamoyl chloride gave a compound that was identical to **8** (TLC migration and ^{1}H and ^{13}C NMR and UV spectral comparison). Analogous coupling of (2-acetoxyethoxy)methyl bromide with bistrimethylsilylated-**5** in dry toluene (in the absence of TMS triflate)[23] gave the protected "acyclovir" product, 2-acetamido-9-[(2-acetoxyethoxy)methyl]-6-diphenylcarbamoyloxypurine, plus a small amount of the N2,N9-bis by-product. X-ray crystallographic analysis of the major acyclovir derivative verified the position of the 6-*O*-DPC linkage[23].

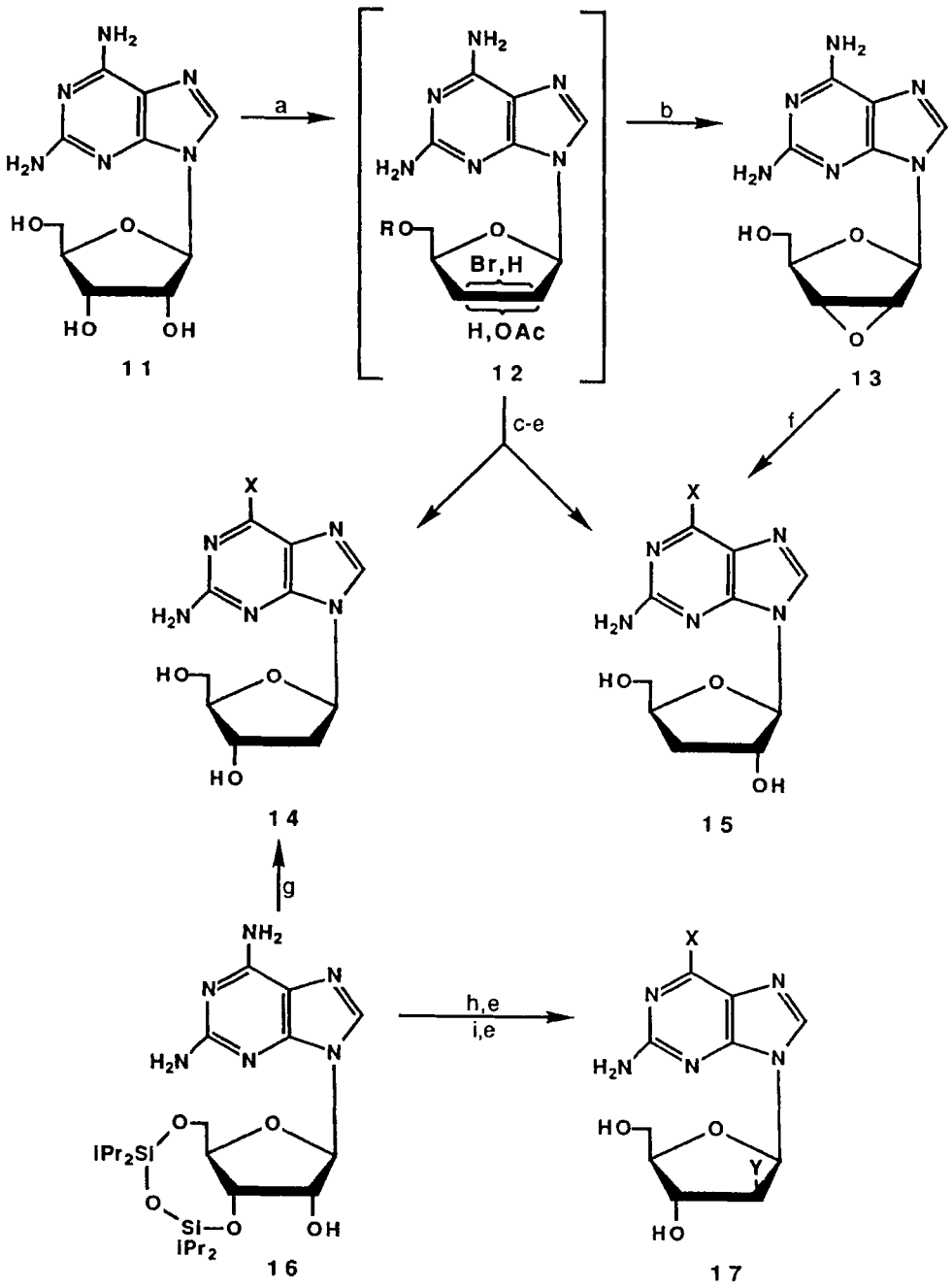
Our procedure is very sensitive to moisture and has been found to proceed well only in **anhydrous** toluene. This is a serious caveat on its generality, and additionally, attempted alkylations of trimethylsilylated-**5** with "normal" alkyl halides (in contrast with α-halo ethers and glycosyl derivatives) were not successful. However, we have achieved our original goal, within these

limitations, of developing a readily accessible derivative of guanine that undergoes regiospecific coupling with α-halo ethers and sugar derivatives to give the desired 9-isomers without observed contamination by 7-isomers under kinetically or thermodynamically controlled conditions.

We also have examined transformation chemistry of the base and sugar moieties of guanosine. Efficient procedures for acetylation and chlorination of guanosine to give 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-2-amino-6-chloropurine have been reported[24]. Evaluation of the formation and disappearance of a transient 6-dichlorophosphoryloxy intermediate by ^{31}P NMR gave the mechanistic rationale for addition of a source of solubilized chloride anion to enhance formation of the 2-amino-6-chloro product. Our earlier use of the hygroscopic tetraethylammonium chloride[24] has now been supplanted by one of the more stable and conveniently handled phase transfer catalysts such as benzyltriethylammonium chloride.

Antimony(III) halide catalyzed non-aqueous diazotization/dediazoniation of the 2-amino-6-chloropurine derivative with *tert*-butyl nitrite in a compatibly-halogenated solvent (to avoid contamination by minor amounts of crossed-halide product from radical processes) gave good yields of 2-halo-6-chloropurine nucleosides[25]. The 2-halo-6-fluoropurine nucleosides were prepared by initial phase transfer catalyzed replacement of chloride by fluoride[24]. An extremely rapid non-aqueous diazotization/dediazoniation of the 2-amino function with *tert*-butyl nitrite in hydrogen fluoride/pyridine gave 2-fluoro-6-halopurine derivatives[25]. These 2- and 6-halopurine nucleosides provide a versatile array of substrates for nucleophilic replacement reactions to give guanine-type nucleosides and analogues.

Adenosine deaminase (adenosine aminohydrolase E.C.3.3.5.4) readily accepts 2,6-diamino-9-(β-D-ribofuranosyl)purine (2-aminoadenosine, 2,6-diaminopurine riboside, DAPR, **11**) as an alternative substrate[26]. Owing to the enhanced experimental problems with guanosine relative to adenosine, we decided to utilize DAPR as a "masked guanosine" precursor for sugar transformations that we had developed in the adenosine series. Our initial studies[27] demonstrated that virtually quantitative monomethylation of the cis-



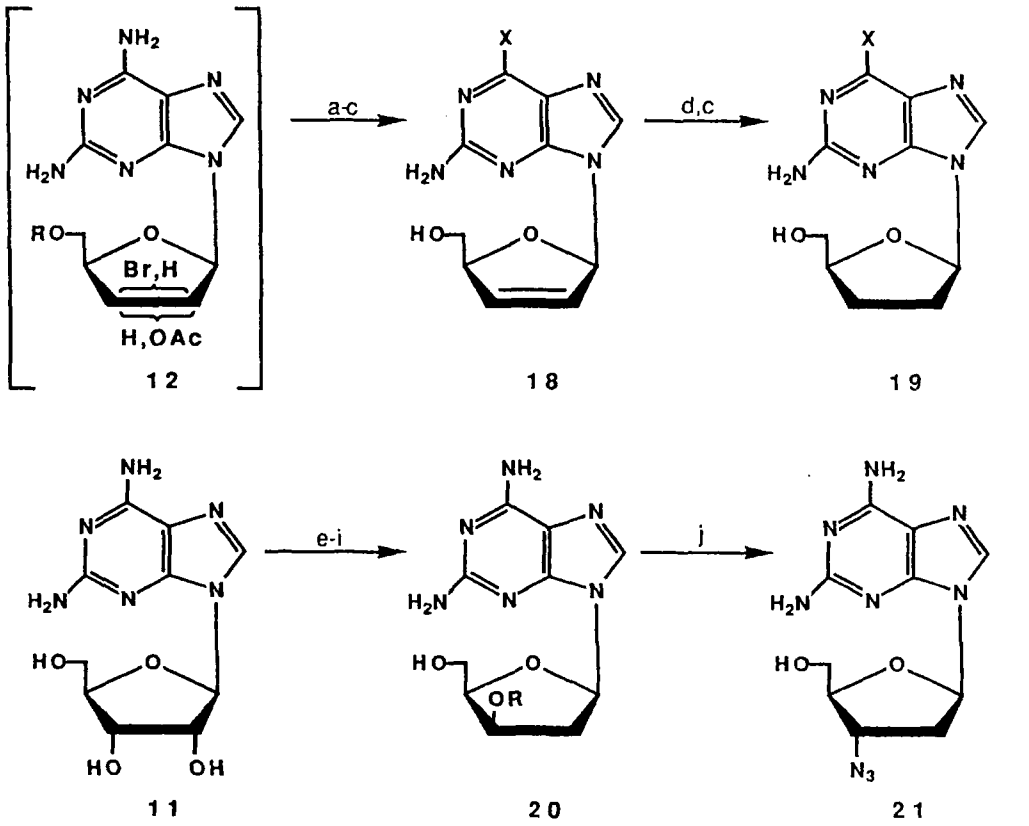
(a) $(CH_3)_2C(OAc)COBr/CH_3CN/H_2O$. (b) Dowex 1X2 ($OH^-$)/MeOH. (c) $Bu_3SnH/AIBN/C_6H_5CH_3/\Delta$. (d) NH_3/MeOH. (e) Adenosine deaminase. (f) $LiEt_3BH$/THF/DMSO. (g) (i) C_6H_5OCSCl/DMAP/CH_3CN. (ii) [(c)]. (iii) Bu_4NF/THF. (h) (i) CF_3SO_2Cl/DMAP/CH_2Cl_2. (ii) LiN_3/DMF/Δ. (iii) [(g) (iii)]. (iv) N_2H_4/Raney nickel/H_2O/EtOH. (i) (i) Ac_2O/DMSO. (ii) $NaBH_4/H_3BO_3$/EtOH. (iii) [(g) (iii)].

glycol system of DAPR could be effected with diazomethane under tin(II) chloride catalysis[28]. Enzymatic deamination gave 2'-*O*-methyl and 3'-*O*-methylguanosine in >90% combined overall yield[27]. Facile separation of the methylated regioisomers at the 2,6-diaminopurine nucleoside level was effected by Dowex 1x2(OH^-) column chromatography[29].

Treatment of DAPR (**11**) with α-acetoxyisobutyryl bromide in "moist" acetonitrile[30,31] resulted in formation of a mixture of 3'(2')-bromo-2'(3')-*O*-acetyl nucleoside products (**12**) analogously to the adenosine series. Treatment of this mixture (**12**) with Dowex 1X2(OH^-) resin in methanol gave the ribo-epoxide (2',3'-anhydro-DAPR, **13**) in 81% overall yield. Tributylstannane-mediated hydrogenolysis of the mixture (**12**) followed by deprotection with methanolic ammonia and separation by ion exchange chromatography[29] gave 2'-deoxy-DAPR (**14**, X = NH_2) and 3'-deoxy-DAPR (**15**, X = NH_2) in 16% and 59% yields, respectively, from **11**. Both deoxynucleosides are substrates of adenosine deaminase, and provide access to 2'-deoxyguanosine (**14**, X = OH) and 3'-deoxyguanosine (**15**, X = OH). Specific routes to these compounds were also developed. Application of our four-stage procedure[32] [selective protection[33] of **11** to give 3',5'-*O*-(1,1,3,3-tetraisopropyldisilox-1,3-diyl)-DAPR (**16**), functionalization of O2' with phenoxythiocarbonyl chloride, tributylstannane-mediated hydrogenolysis, and deprotection with tetrabutylammonium fluoride] gave **14** (X = NH_2). Our regiospecific reduction[31] of **13** with lithium triethylborohydride in tetrahydrofuran/dimethylsulfoxide gave **15** (X = NH_2).

Triflation of **16** at O2' followed by nucleophilic displacement of triflate by azide and deprotection with tetrabutylammonium fluoride gave the 2'-azido-2'-deoxy-arabino analogue (**17**, X = NH_2, Y = N_3). Hydrogenolysis of the azido function gave **17** (X = Y = NH_2) which underwent deamination to provide 9-(2-amino-2-deoxy-β-D-arabinofuranosyl)guanine (**17**, X = OH, Y = NH_2). Oxidation[34] of **16** followed by sodium borohydride reduction of the 2'-keto intermediate and deprotection gave araDAP (**17**, X = NH_2, Y = OH). This product was deaminated to give 9-(β-D-arabinofuranosyl)guanine (**17**, X = Y = OH).

Treatment of the bromo acetate mixture (**12**) with zinc-copper couple in dimethylformamide[30] followed by deprotection gave 2,6-diamino-9-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)purine (**18**, X = NH_2). This unsaturated nucleoside analogue is a very poor alternative substrate of adenosine deaminase[19,35], but extensive exposure at ambient temperature resulted in formation of the somewhat unstable 9-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)guanine (**18**, X = OH). Hydrogenation of **18** (X = NH_2) gave 2,6-diamino-9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)purine (ddDAPR, **19**, X = NH_2). Enzymatic deamination of ddDAPR to give 2',3'-dideoxyguanosine (**19**, X = OH) proceeded more readily[19,35].



(a) Zn/Cu/DMF. (b) NH_3/MeOH. (c) Adenosine deaminase. (d) H_2/Pd/C. (e) (i) Bu_2SnO/MeOH/Δ. (ii) TsCl. (f) $LiEt_3BH$/THF/DMSO. (g) $TrCl/C_5H_5N$. (h) $MsCl/C_5H_5N$. (i) $HOAc/H_2O$/Δ. (j) LiN_3/DMF/Δ.

Application of the Imazawa and Eckstein transglycosylation procedure[36] with 3'-azido-2',3'-dideoxythymidine as donor and 2,6-diacetamidopurine as acceptor gave the α and β anomers of 3'-azido-2',3'-dideoxy-DAPR in low yield with an anomeric ratio of ~1:1. Tosylation of DAPR (**11**) by the two-stage Moffatt procedure[37] gave 2'-*O*-tosyl-DAPR. Subjection of this product to our hydride-shift rearrangement[38] with lithium triethylborohydride in tetrahydrofuran/dimethylsulfoxide gave 2,6-diamino-9-(2-deoxy-β-D-*threo*-pentofuranosyl)purine (**20**, R = H) in high yield. Selective protection, mesylation, and deprotection gave the 3'-*O*-mesyl product (**20**, R = Ms). Treatment of this compound with lithium azide in hot dimethylformamide gave a stereodefined synthesis of the desired 2,6-diamino-9-(3-azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)purine (**21**, AzddDAPR)[39].

Potent inhibition of the human immunodeficiency virus (HIV) was found with both ddDAPR (**19**, X = NH_2) and AzddDAPR (**21**). In the Balzarini and De Clercq test system, the 50% inhibitory concentrations for HIV were determined to be: ~3.8 μ*M* (ddDAPR)[40] and ~0.3 μ*M* (AzddDAPR)[39]. As reported for 3'-azido-2',3'-dideoxythymidine[41], a pronounced increase in the 1-octanol/water partition coefficient was found[39] with the 3'-azido compound (**21**). This might present advantages for intracellular accessibility of these agents since they apparently do not utilize the usual nucleoside transport system[41]. It appears most likely that ddDAPR (**19**, X = NH_2) functions as a prodrug form of 2',3'-dideoxyguanosine[39,40] (by *in vivo* application of our adenosine deaminase-mediated synthetic sequence of 2,6-diaminopurine to guanine nucleosides[19]). However, the 3'-azido derivative (AzddDAPR, **21**) appears to have independent inhibitory activity[39a] as well as serving as a prodrug of 3'-azido-2',3'-dideoxyguanosine.

Dramatic inhibitory activity against duck hepatitis B virus (DHBV) has been found with ddDAPR (**19**, X = NH_2)[42]. The potent IC_{50} = ~0.07 μg/mL (0.3 μM) value evaluated in an *in vitro* duck hepatocyte system has been found to extrapolate to therapeutic success *in vivo*. Treatment of persistently infected Pekin ducks at a level of 10 mg/kg twice daily effected complete

clearance of DHBV DNA from their sera[42]. Extended treatment appears to offer excellent antiviral potential with no apparent overt toxicity.

In summary, we have developed coupling methods that allow a highly regioselective synthesis of 7-glycosylguanine derivatives with catalysis by tin(IV) chloride at ambient temperature; and a route to guanine 9-nucleosides and analogues employing 2-acetamido-6-diphenylcarbamoyloxypurine that proceeds without observed contamination by 7-isomers. Halogenation of the purine ring of acetylated nucleosides at C2 and C6 has been effected in high yields under mild reaction conditions to give protected derivatives for elaboration to guanine and 2-substituted adenine analogues. Guanosine can be converted into 2,6-diamino-9-(β-D-ribofuranosyl)purine in high yield. The sugar moiety of this nucleoside has been manipulated to give a variety of modified 2-aminoadenosine analogues. Deamination of these modified products by adenosine aminohydrolase has provided a mild and usually quantitative route to the corresponding guanine nucleoside analogues. The 2,6-diamino-9-(β-D-ribofuranosyl)purine 2',3'-dideoxy (ddDAPR) and 3'-azido-2',3'-dideoxy (AzddDAPR) derivatives have potent inhibitory activity against the human immunodeficiency virus and duck hepatitis B virus. Further development of ddDAPR as a promising chemotherapeutic agent for human hepatitis B infection is in progress.

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REFERENCES

1. This publication constitutes Nucleic Acid Related Compounds. 55. For the previous paper in this series see: Robins, M. J.; Wnuk, S. F. *Tetrahedron Lett.* (in press).

2. Elion, G. B.; Furman, P. A.; Fyfe, J. A.; DE Miranda, P.; Beauchamp, L.; Schaeffer, H. J. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 5716.
3. Borthwick, A. D.; Butt, S.; Biggadike, K.; Exall, A. M.; Roberts, S. M.; Youds, P. M.; Kirk, B. E.; Booth, B. R.; Cameron, J. M.; Cox, S. W.; Marr, C. L. P.; Shill, M. D. *J. Chem. Soc., Chem. Commun.* **1988**, 656.
4. Fischer, E.; Helferich, B. *Chem. Ber.* **1914**, *47*, 210.
5. Davoll, J.; Lythgoe, B.; Todd, A. R. *J. Chem. Soc.* **1948**, 1685.
6. Davoll, J.; Lowy, B. A. *J. Am. Chem. Soc.* **1951**, *73*, 1650.
7. Shabarova, Z. A.; Polyakova, Z. P.; Prokof'ev, M. A. *J. Gen. Chem.. USSR* **1959**, *29*, 218.
8. Jenkins, S. R.; Holly, F. W.; Walton, E. *J. Org. Chem.* **1965**, *30*, 2851.
9. Sato, T.; Shimadate, T.; Ishido, Y. *Nippon Kagaku Zasshi* **1960**, *81*, 1440; 1442.
10. Imai, K.; Nohara, A.; Honjo, M. *Chem. Pharm. Bull.* **1966**, *14*, 1377.
11. Reist, E. J.; Goodman, L. *Biochemistry* **1964**, *3*, 15.
12. (a) Lee, W. W.; Martinez, A. P.; Goodman, L.; Henry, D. W. *J. Org. Chem.* **1972**, *37*, 2923. (b) Tong, G. L.; Ryan, K. J.; Lee, W. W.; Acton, E. M.; Goodman, L. *J. Org. Chem.* **1967**, *32*, 859.
13. Skoda, J.; Bartosek, I.; Sorm, F. *Collect. Czech. Chem. Commun.* **1962**, *27*, 906.
14. Vorbruggen, H. *in* "Nucleoside Analogues: Chemistry, Biology, and Medical Applications" **1979**, Walker, R. T.; De Clercq, E.; Eckstein, F.; Eds. NATO Advanced Study Institute Series, Vol. 26A, Plenum Press, New York, pp. 35-69.
15. Vorbruggen, H.; Krolikiewicz, K.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1234.
16. Ogilvie, K. K.; Cheriyan, U. O.; Radatus, B. K.; Smith, K. O.; Galloway, K. S.; Kennell, W. L. *Can. J. Chem.* **1982**, *60*, 3005.
17. (a) Wright, G. E.; Dudycz, L. W. *J. Med. Chem.* **1984**, *27*, 175. (b) Dudycz, L. W.; Wright, G. E. *Nucleos. Nucleot.* **1984**, *3*, 33.
18. Garner, P.; Ramakanth, S. *J. Org. Chem.* **1988**, *53*, 1294.
19. Zou, R. *Ph.D. Dissertation* **1986**, The University of Alberta.

20. Ishido, Y.; Hosono, A.; Isome, S.; Maruyama, A.; Sato, T. *Bull. Chem. Soc. Jpn.* **1964**, *37*, 1389.
21. Novak, J. J. K.; Sorm, F. *Collect. Czech. Chem. Commun.* **1973**, *38*, 1173.
22. (a) Kamimura, T.; Tsuchiya, M.; Koura, K.; Sekine, M.; Hata, T. *Tetrahedron Lett.* **1983**, *24*, 2775. (b) Kamimura, T.; Tsuchiya, M.; Urakami, K.; Koura, K.; Sekine, M.; Shinozaki, K.; Miura, K.; Hata, T. *J. Am. Chem Soc.* **1984**, *106*, 4552.
23. Zou, R.; Robins, M. J. *Can. J. Chem.* **1987**, *65*, 1436.
24. Robins, M. J.; Uznanski, B. *Can. J. Chem.* **1981**, *59*, 2601.
25. Robins, M. J.; Uznanski, B. *Can. J. Chem.* **1981**, *59*, 2608.
26. (a) Chilson, O. P.; Fisher, J. R. *Arch. Biochem. Biophys.***1963**, *102*, 77. (b) Baer, H.-P.; Drummond, G. I.; Duncan, E. L. *Mol. Pharmacol.* **1966**, *2*, 67.
27. Robins, M. J.; Hansske, F.; Bernier, S. E. *Can. J. Chem.* **1981**, *59*, 3360.
28. Robins, M. J.; Naik, S. R.; Lee, A. S. K. *J. Org. Chem.* **1974**, *39*, 1891.
29. Dekker, C. A. *J. Am. Chem. Soc.* **1965**, *87*, 4027.
30. Robins, M. J.; Hansske, F.; Low, N. H.; Park, J. I. *Tetrahedron Lett.* **1984**, *25*, 367.
31. Hansske, F.; Robins, M. J. *Tetrahedron Lett.* **1985**, *26*, 4295.
32. Robins, M. J.; Wilson, J. S.; Hansske, F. *J. Am. Chem. Soc.* **1983**, *105*, 4059.
33. Markiewicz, W. T. *J. Chem. Res. (S)* **1979**, 24.
34. Hansske, F.; Madej, D.; Robins, M. J. *Tetrahedron* **1984**, *40*, 125.
35. Balzarini, J.; Robins, M. J.; Zou, R.; Herdewijn, P.; De Clercq, E.; *Biochem. Biophys. Res. Commun.* **1987**, *145*, 277.
36. Imazawa, M.; Eckstein, F. *J. Org. Chem.* **1978**, *43*, 3044.
37. Wagner, D.; Verheyden, J. P. H.; Moffatt, J. G. *J. Org. Chem.* **1974**, *39*, 24.
38. Hansske, F.; Robins, M. J. *J. Am. Chem. Soc.* **1983**, *105*, 6736.

39. (a) Balzarini, J.; Baba, M.; Pauwels, R.; Herdewijn, P.; Wood, S. G.; Robins, M. J.; De Clercq, E. *Mol. Pharmacol.* **1988**, *33*, 243. (b) Robins, M. J.; Wood, S. G.; Dalley, N. K.; Herdewijn, P.; Balzarini, J.; De Clercq, E. (manuscript submitted).
40. Pauwels, R.; Baba, M.; Balzarini, J.; Herdewijn, P.; Desmyter, J.; Robins, M. J.; Zou, R.; Madej, D.; De Clercq, E. *Biochem. Pharmacol.* **1988**, *37*, 1317.
41. (a) Zimmerman, T. P.; Mahony, W. B.; Prus, K. L. *J. Biol. Chem.* **1987**, *262*, 5748. (b) Domin, B. A.; Mahony, W. B.; Zimmerman, T. P. *Biochem. Biophys. Res. Commun.* **1988**, *154*, 825.
42. (a) Suzuki, S.; Lee, B.; Luo, W.; Tovell, D.; Robins, M. J.; Tyrrell, D. L. J. *Biochem. Biophys. Res. Commun.* **1988**, *156*, 1144. (b) Lee, B.; Luo, W.; Suzuki, S.; Robins, M. J.; Tyrrell, D. L. J. *Antimicrob. Agents Chemother.* (in press).