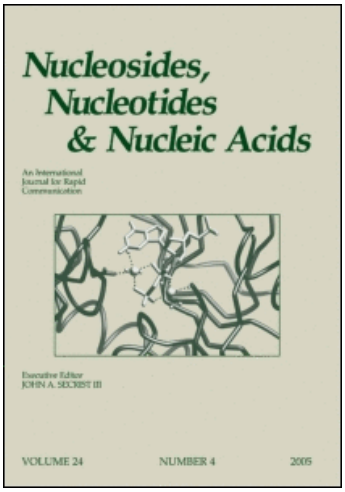

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SYNTHESIS AND PHARMACOKINETIC AND PHARMACODYNAMIC EVALUATION OF THE FORODESINE HCl ANALOG BCX-3040

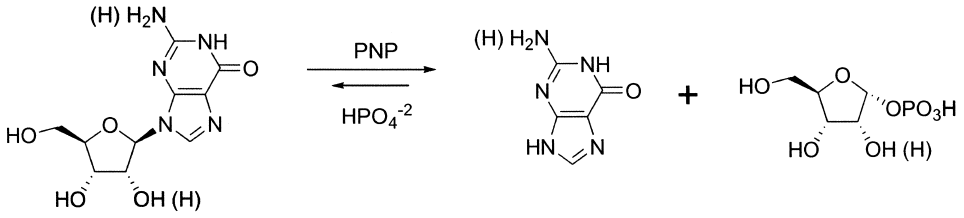
Hollis S. Kezar III, J. Michael Kilpatrick, Deborah Phillips, Debbie Kellogg, Jianwen Zhang, and Philip E. Morris, Jr. □ *BioCryst Pharmaceuticals, Inc., Birmingham, Alabama, USA*

□ *Forodesine HCl is a potent inhibitor of the enzyme purine nucleoside phosphorylase (PNP) and is currently in clinical trials for the treatment of leukemia and lymphoma. Animal models indicated that forodesine HCl would have low oral bioavailability in humans and it was initially developed as an intravenous formulation. We were interested in identifying analogs of forodesine HCl with improved oral bioavailability. The 2′-deoxy analog (BCX-3040) was synthesized and its pharmacokinetic and pharmacodynamic properties compared with forodesine HCl.*

Keywords Forodesine HCl; Purine nucleoside phosphorylase; PNP; T-cell

INTRODUCTION

Forodesine HCl (BCX-1777) is a novel inhibitor of the enzyme purine nucleoside phosphorylase (PNP) and is in human clinical trials for the treatment of leukemia and lymphomas.[1–5] The enzyme purine nucleoside phosphorylase (PNP, EC 2.4.2.1) catalyzes the reversible cleavage of purine nucleosides to the corresponding purine base and sugar phosphate in the purine salvage pathway as shown below.[6]



Dedicated to the memory of John A. Montgomery.
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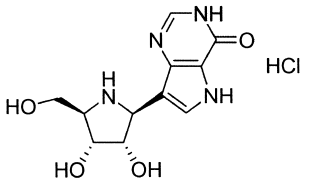
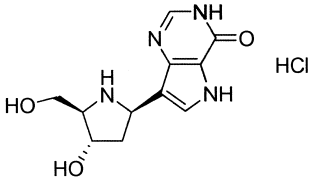
In the absence of PNP, nucleoside substrates such as 2′-deoxyguanosine (dGuo) accumulate. dGuo accumulation has been observed in children with inherited PNP deficiency and as a consequence, these children exhibit severe T-cell immunodeficiency but retain normal or exaggerated B-cell function.[7] T-cell cytotoxicity is due to phosphorylation of dGuo (via 2′-deoxycytidine kinase, dCK, (EC 2.7.1.74)) to 2′-deoxyguanosine triphosphate (dGTP). dGTP allosterically inhibits the enzyme ribonucleotide diphosphate reductase (EC 1.17.4.1), preventing DNA synthesis and hence T-cell proliferation.[8] The relatively unique sensitivity of T-cells is attributed to their relatively high level of dCK compared to other cells. This observation has led to the development of PNP inhibitors for the treatment of T-cell cancers and T-cell autoimmune indications. The biochemical basis for the use of PNP inhibitors as well as the various classes of inhibitors developed has been reviewed.[9,10] The use of forodesine HCl as an immunosuppresent has also been demonstrated.[11] More recently, clinical trial experience in psoriasis with the PNP inhibitor BCX-34 has been described.[12]

When bound to the PNP enzyme, forodesine mimics the charged ribosyl oxocarbenium ion complex formed during the transition state of the enzyme-catalyzed C-N bond cleavage of nucleosides. Forodesine is a slow-onset, tight-binding inhibitor with a K_i* of 23 pM and is one of the most potent inhibitors known for the enzyme.[13] Initial pharmacokinetic experiments conducted in mice indicated an oral bioavailability of 63%. However, in rats the oral bioavailability dropped to 13% and in primates the measured oral bioavailability was <11%.[14] Based on these results, forodesine HCl was initially developed as an intravenous formulation. As part of our development program, we were interested in identifying analogs of forodesine HCl with improved oral pharmacokinetic characteristics.

That dGuo binds tightly to PNP suggests that the 2′-OH group contributes little to binding and is not required for activity. Based on this observation, we proposed that the 2′-deoxy analog (**1**) of forodesine HCl would retain the potency of forodesine HCl and would have improved oral bioavailability. In this manuscript the synthesis of **1** is presented with a pharmacokinetic and pharmacodynamic comparison to forodesine HCl.



Forodesine HCl



1 (BCX-3040)

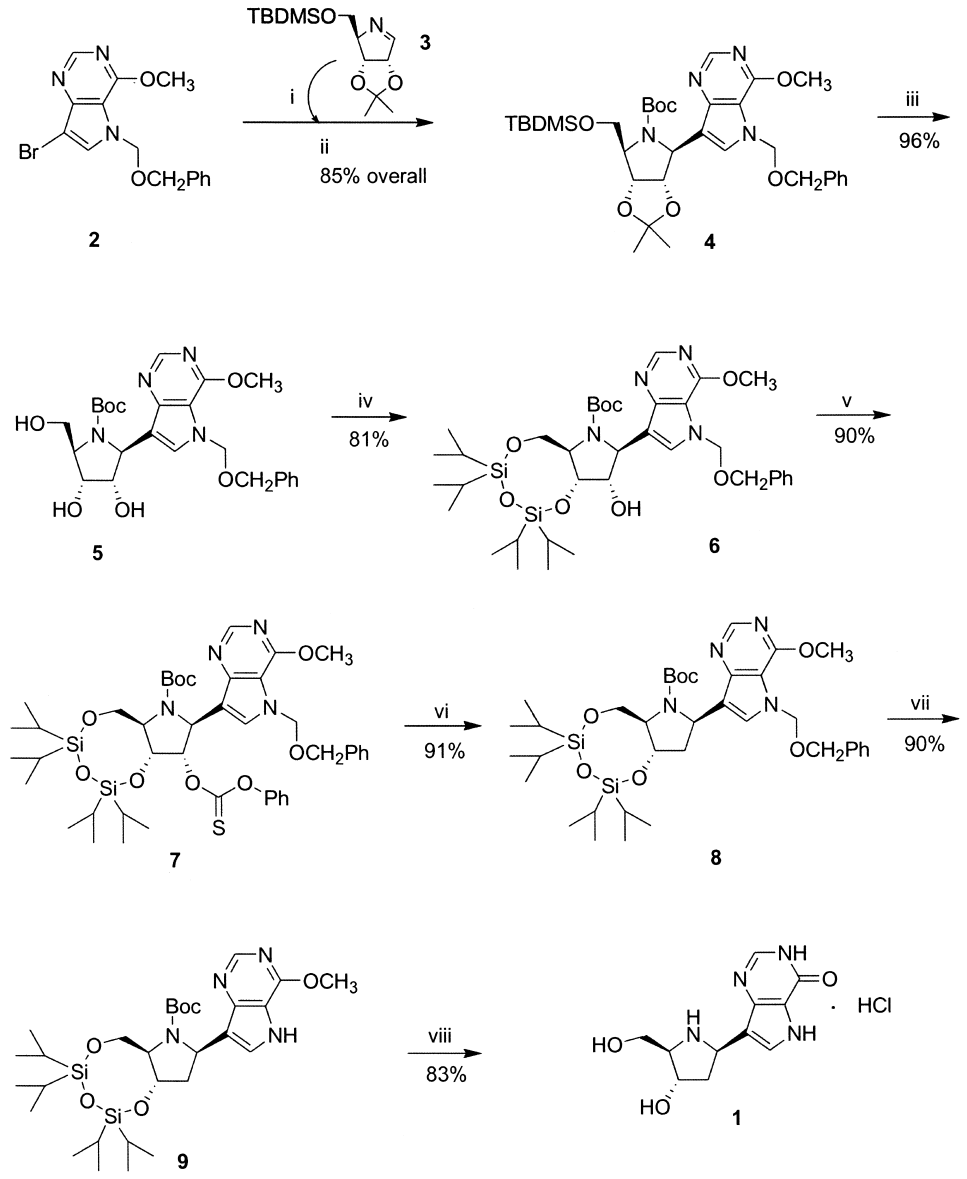
RESULTS AND DISCUSSION

Chemistry

BCX-3040 (**1**) was synthesized according to Scheme 1. We have previously described the synthesis of **4** via addition of the lithiated 9-deazapurine derivative **2** to the carbohydrate derived cyclic imine **3**.[15] With **4** in hand, we envisioned the title compound **1** could be obtained through a sequence of selective deprotection, protection and followed by deoxygenation of the unprotected 2′-OH group. Previous unpublished work from our laboratory had shown that under acidic conditions the relative stabilities of the acid labile protecting groups of **4** was $PhCH_2OCH_2 > OCH_3 >$ Boc > isopropylidene ≅ TBDMS. Partial deprotection of **4** was accomplished with 1*N* HCl in methanol, which selectively cleaved only the more labile TBDMS and isopropylidene groups affording the triol **5** in excellent yield. The 3′ and 5′-OH groups were protected as the 1,1,3,3-tetraisopropyldisiloxyl ether, which afforded **6**. The 2′-OH group was converted to the thiocarbonate **7** in excellent yield. Deoxygenation was accomplished by heating **7** with 1,1′-azobis(cyclohexane-1-carbonitrile) in toluene at reflux to give **8**. While it is possible to directly convert **8** to the title compound **1** under acidic conditions we chose a two-step procedure in which the $PhCH_2OCH_2$ (BOM) protecting group is cleaved first with catalytic hydrogenation followed by acid hydrolysis of the remaining protecting groups. The advantage of this route is it obviates the need for the strongly acidic conditions required to cleave the BOM group, which often results in formation of tarry material, which can be difficult to remove from the final product. Thus **8** was hydrogenated with 20% $Pd(OH)_2$ in ethanol containing a small amount of NH_4OH. The presence of NH_4OH is required since catalytic removal of BOM groups under neutral conditions is sometimes complicated by formation of the intermediate NCH_2OH, which easily converted in situ in the presence of base to the desired NH compound **9**. Acid hydrolysis of **9** afforded **1**, which was isolated as the hydrochloride in an overall yield of 40% from **4** over seven steps.

Biology: A Comparison of the Pharmacokinetic and Pharmacodynamic Properties of BCX-3040 and Forodesine HCl

After a single 5 mg/kg IV dose of BCX-3040 there is a rapid decrease in plasma BCX-3040 (Figure 1) with a terminal half life ($\lambda t½$) of 0.97 h (Table 1). The BCX-3040 clearance, 5.8 mL/min (348 mL/h) (Table 1), is greater than the rat glomerular filtration rate (1.3 mL/min).[16] The dGuo response follows a similar pattern (Figure 2) with a slightly delayed C_{max} (Table 1) and a terminal half life of 1.2 h. The normal plasma concentration of dGuo is below the limit of detection for both methods used.



SCHEME 1 Reagents. i. nBuLi/anisole/ether/−70°C; ii. Boc-anhydride/CH_2Cl_2; iii. 1N HCl/MeOH/30°C; iv. 1,3-dichloro-1,1-3,3-tetraisopropyldisoloxane/pyridine; v. phenyl chlorothionoformate/MeCN; vi. 1,1′-azobis(cyclohexane-1-carbonitrile)/toluene reflux; vii. 20% Pd $(OH)_2$, H_2, ethanol NH_4OH (trace); viii. HCl/heat.

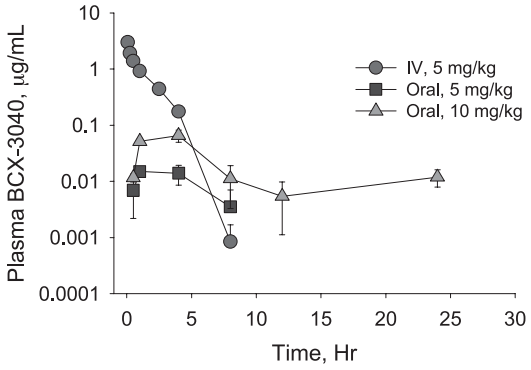


FIGURE 1 Concentration time curves of BCX-3040 after 5 mg/kg IV dosing, and 5 and 10 mg/kg oral dosing of BCX-3040. Shown are the mean ± standard error bars.

TABLE 1 Pharmacokinetics of BCX-3040 and dGuo in Rats after Single Doses of 5 and 10 mg/kg BCX-3040

Pharmacokinetic parameters BCX-3040				
Parameter	Units			
Route		IV	Oral	Oral
Dose	mg/kg	5	5	10
C_{max}	µg/mL	3.0 ± 0.31	0.018 ± 0.0029	0.078 ± 0.0169
T_{max}	h	0.083 ± 0.00	1.6 ± 0.80	3.2 ± 0.75
AUC_{all}	µg·h/mL	3.7 ± 0.028	0.093 ± 0.0318	0.50 ± 0.114
λt½	h	0.97 ± 0.086	ND	ND
CL_{obs}	mL/h	348.5 ± 33.55	—	—
V_z	mL	537.2 ± 32.66	ND	ND
F	%	—	2.5 ± 0.87	6.8 ± 1.52
n		3	4	4
Pharmacokinetic parameters of plasma dGuo				
Parameter	Units			
Route		IV	Oral	Oral
Dose	mg/kg	5	5	10
C_{max}	µg/mL	0.40 ± 0.045	0.005 ± 0.00189	0.041 ± 0.0033
T_{max}	h	0.83 ± 0.167	6.0 ± 1.15	4.0 ± 0.00
AUC_{all}	µg·h/mL	1.2 ± 0.64	0.02 ± 0.006	0.23 ± 0.030
λt½	h	1.2 ± 0.008	ND	1.3 ± 0.39
n		3	4	4

ND, Not determined; there were not 3 points available for analysis only 2 for λt½ had 3 points available for analysis, but only one of these had a correlation coefficient greater than 0.9. Therefore, an accurate value for this parameter could not be determined from these data.

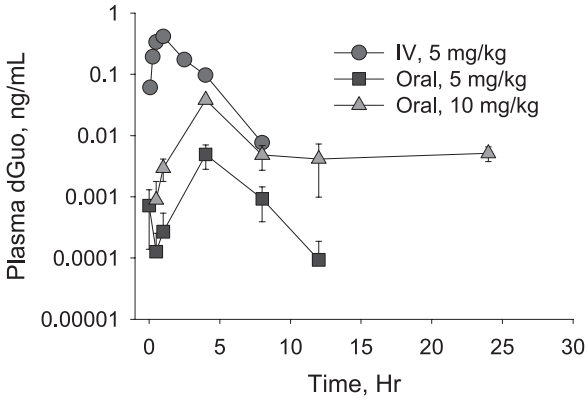


FIGURE 2 Concentration time curves of plasma dGuo after 5 mg/kg IV dosing, and 5 and 10 mg/kg oral dosing of BCX-3040. Shown are the mean ± standard error bars.

TABLE 2 Pharmacokinetics of Forodesine and dGuo in Rats after Single Doses of 1, 5, and 10 mg/kg Forodesine HCl

Pharmacokinetic parameters forodesine HCl				
Parameter	Units			
Route		IV	Oral	Oral
Dose	mg/kg	1	5	10
C_{max}	μg/mL	2.0 ± 0.20	0.24 ± 0.078	0.20 ± 0.157
T_{max}	h	0.083 ± 0.00	3.0 ± 1.29	2.0 ± 1.00
AUC_{all}	μg·h/mL	2.6 ± 0.54	1.4 ± 0.26	1.5 ± 0.27
λt½	h	1.1 ± 0.11	7.0 ± 3.25	6.8 ± 2.58
Cl_{obs}	mL/h	101.5 ± 17.89	—	—
V_z	mL	151.4 ± 22.53	ND	ND
F	%	—	10.6 ± 1.82	5.6 ± 1.03
N	—	5	7	7
Pharmacokinetic parameters of plasma dGuo				
Parameter	Units			
Route		IV	Oral	Oral
Dose	mg/kg	1	5	10
C_{max}	μg/mL	0.75 ± 0.197	0.33 ± 0.050	0.35 ± 0.0393
T_{max}	h	1.6 ± 0.60	8.6 ± 2.82	4.7 ± 0.94
AUC_{all}	μg·h/mL	5.0 ± 1.26	2.9 ± 0.33	3.0 ± 0.48
λt½	h	3.7 ± 0.71	4.9 ± 2.00	3.8 ± 0.59
N		5	7	7

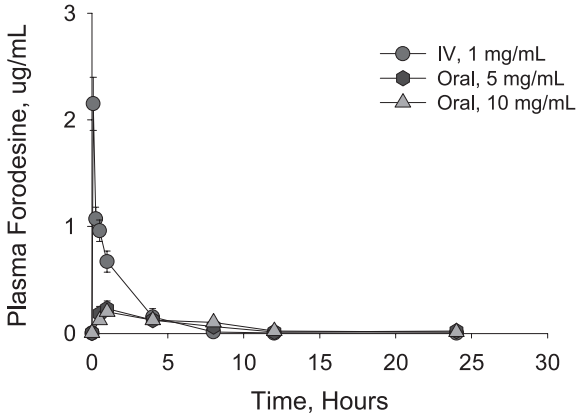


FIGURE 3 Concentration time curves of Forodesine after 1 mg/kg IV dosing, and 5 and 10 mg/kg oral dosing of Forodesine. Shown are the mean ± standard error bars.

IV dosing of rats with 1 mg/kg forodesine HCl produced a slightly lower C_{max} than did dosing with 5 mg/kg BCX-3040 (Tables 1 and 2, Figures 1 and 3). Both C_{max}s were observed 5 min after the bolus doses and were the first samples collected. The relatively low BCX-3040 C_{max} is probably due to the more rapid clearance of BCX-3040.

Oral dosing produced mean oral BCX-3040 bioavailabilities of 2.5 and 6.8% for the 5 and 10 mg/kg doses, respectively. The terminal half-life for BCX-3040 and dGuo after the 5 mg/kg dose could not be determined due to low plasma concentration of both analytes. For 10 mg/kg oral the mean terminal half life was 1.3 h. Compared to the dGuo response produced by forodesine, the dGuo response produced by BCX-3040 was low (Tables 1 and 2, Figures 2 and 4). Using the more sensitive LC/MS/MS method, the

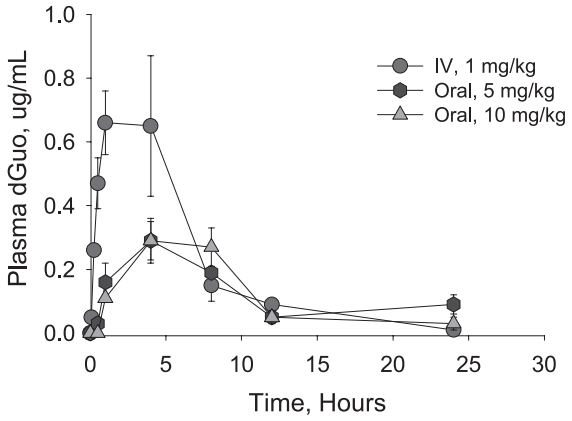


FIGURE 4 Concentration time curves of plasma dGuo after 1 mg/kg IV dosing, and 5 and 10 mg/kg oral dosing of Forodesine. Shown are the mean ± standard error bars.

dGuo response was barely quantitatable after the 5 mg/kg oral dose of BCX-3040 and was 8 times less than the corresponding forodesine-dGuo response after dosing orally with 10 mg/kg BCX-3040.

Both BCX-3040 and forodesine are potent inhibitors of PNP in erythrocyte extracts with IC_{50}s of 3.1 ± 0.50 nM and 1.2 ± 0.21 nM[12] (mean $\pm$ SE), respectively. These IC_{50}s are higher than would be found using purified enzyme but are sufficient for comparison.

BCX-3040 bioavailability and BCX-3040-dependent dGuo response after oral administration of BCX-3040 are significantly lower compared to forodesine HCl (Table 2). Oral 5 mg/kg forodesine HCl resulted in a 12.6-fold higher dGuo response than did the 10 mg/kg BCX-3040 oral treatment. Additionally, plasma terminal terminal half life of forodesine after oral administration of 10 mg/kg was 5 times longer than the corresponding oral dose of BCX-3040. The dGuo response after a single IV dose of 1 mg/kg forodesine HCl was not quite twofold greater than the 5 mg/kg IV BCX-3040 dGuo response. This corresponds to their respective plasma C_{max}s and clearances for the IV dosed groups. The combination of rapid elimination and poor bioavailability make BCX-3040 a poor in vivo inhibitor of PNP as related to forodesine HCl. Another factor that could play a role would be a slow extended absorption of forodesine leading to a longer apparent half-life.[14] The C_{max} and AUC for the oral doses of both BCX-3040 and forodesine did not increase in proportion to the dose. It is hypothesized that this may be due to a saturable transporter.[14]

BIOLOGY EXPERIMENTAL

Animal Housing

The rats (male, Sprague Dawley, Charles River) fed and watered ad libitum. They were housed in a humidity-, light-, and temperature-controlled environment.

Pharmacokinetic Study Design

BCX-3040 was dosed at 5 mg/kg IV, and 5 and 10 mg/kg orally in groups of 3, 4, and 4 rats, respectively. Forodesine HCl was dosed IV in 5 rats/group as a bolus at 1 mg/kg IV and given orally in 7 rats/group at 5 and 10 mg/kg. Previous studies indicated that forodesine dosed IV at 1 mg/mL produced the maximum plasma dGuo response, and preliminary experiments indicated that 1 mg/mL BCX-3040 was inadequate. Additionally, dosing 5 mg/kg IV and oral with BCX-3040 was used to get a more accurate indication of bioavailability. For the IV studies blood samples were collected pre, 5 min, 15 min, 30 min, 1 h, 4 h, 8 h, 12 h, and 24 h of the IV dose; and for the single dose oral studies blood was collected pre, 30 min,

1 h, 4 h, 8 h, 12 h, and 24 h following the oral doses. The animals were weighed immediately before dosing to determine the dose volume.

Blood Collection and Plasma Preparation

For all the studies blood was collected in BCX-34 (to achieve a final concentration of 50 uM) spiked tubes. This acted as a preservative for dGuo. Plasma samples were collected after centrifugation of the blood stored at around −80°C.

BIOANALYTICAL ANALYSIS

Plasma BCX-3040 and dGuo Analysis

For plasma BCX-3040 and dGuo analysis the determinations were made within the same run. The plasma concentration of BCX-3040 and dGuo was determined by LC-MS-MS after ultrafiltration of the plasma using Amicon Centrifree filters. The filtrates were then analyzed using C-3 (Zorbax SB-C3 2.1 X 40 mm, 5 μm) reverse phase HPLC and selected reaction monitoring (SRM, multiple reaction monitoring, MRM) method of mass spectrometric detection (Q1/Q3 Masses: 251.30/215.10 amu for BCX-3040 and 268/152 for dGuo). The concentrations of BCX-3040 and dGuo were determined using standard curves of each prepared in rat plasma. Quality control samples were distributed throughout the run. A PE Sciex API 2000 MS/MS with a Hewlett-Packard 1100 HPLC were used for the analysis.

Plasma Forodesine and dGuo Analyses

For forodesine and dGuo analysis the determinations were made in separate methods due to the different chromatographic properties of the two. Forodesine was determined by LC/MS analysis after solid-phase extraction. A Zorbax SB C-3, 3.0X150 mm column was used for the chromatography. An internal standard method was used with quadratic regression analysis, using inosine-U-$^{15}N_4$ as the internal standard.

Mass Spectrometry, positive electrospray in single ion monitoring mode was used for BCX-1777 set at 208 m/z with the fragmentor set at 130 volts. A Hewlett-Packard 1100-MSD was used for the analysis.

dGuo concentrations were determined by HPLC, Hewlett-Packard 1100, high-performance liquid chromatography system equipped with a binary pump, solvent degassing module, autosampler, and a UV detector. Plasma samples were ultrafiltered using Centrifree YM-30 filters as per the manufacturers instructions. The analytes were eluted from a Hewlett-Packard Purospher RP C-18 12 × 150 mm column with 5% DMSO in 10 mM ammo-

nium formate, pH 5.0. The flow rate was 0.5 mL/min and the run time was 20 min.

For both assays quality control samples were included throughout the batch, and standard curves were run in pooled rat plasma that was spiked with the appropriate analyte prior to extraction or filtration.

PNP Analysis

Inhibition enzymatic activity of PNP in erythrocyte extracts was determined by monitoring the PNP dependent hydrolysis of inosine spectophotometrically. A Cary 3 Spectrophotometer, Vavian Model 1001206 equipped with Cary WinUV software was used for these studies. The IC50 was determined by determining the concentration of compound, which reduced the rate of inosine hydrolysis by 50% compared to the buffer control. Specifically, the PNP is prepared from rat erythrocytes after lysis of the erythrocytes with 0.2% Triton X-100 and adjusted to the required specific activity. Ten μL of inhibitor and water are added to 40 μL of PNP containing $\sim$1 mUnit of PNP. This is then added to 1450 μL of 50 mM phosphate buffer containing 0.5 mM inosine and excess, 80 mU/mL, xanthine oxidase. One unit of PNP causes the phosphorolysis of 1 μmol of inosine to hypoxanthine and ribose 1-phosphate per minute at pH 7.2 at 25°C. In the presence of excess xanthine oxidase, the hypoxanthine is hydrolyzed to uric acid. Generation of uric acid is monitored at 293 nm. An inhibition curve was generated using 0.625, 1.25, 5, 10, and 50 nM. The concentration of inhibitor that resulted in 50% inhibition of the rate of generation uric acid is reported as the IC_{50}.

Pharmacokinetic Analysis

Data were reported as mean $\pm$ S.E. Pharmacokinetic analysis was performed using WINNONLIN Standard 3.1, EXCEL, and Sigma Plot. Noncompartmental analysis was used to calculate the following parameters:

C_{max}: maximum observed plasma concentration of BCX-1777 or 2′-deoxyguanosine obtained after the each dose.

t_{max}: time of maximum observed plasma concentration of BCX-1777 or 2′-deoxyguanosine obtained after the each dose.

AUC_{all}: area under the curve for a single dose over a 24-h period.

Lambda z t ½: (λt½) terminal half-life of the terminal elimination phase of drug disposition.

Clearance (Cl_{obs}): millimeters of drug cleared of drug per unit time.

Volume of distribution (V_z): base on the terminal elimination phase; $V_z = \text{dose}/\lambda 2 \cdot AUC_{inf}$.

Oral bioavailability (F): percent drug absorbed after oral administration base on the IV and oral AUC_{all}.

CHEMISTRY EXPERIMENTAL

General

Melting points were determined on a Meltemp II melting point apparatus and are uncorrected. The ^{1}H-NMR spectra were recorded on a Bruker Avance 300 at 300.13 MHz. Chemical shifts (ppm) are referenced to internal tetramethylsilane and spectra were recorded at ambient temperature. IR spectra were obtained on a Bio-Rad FTS-7 FT-IR. Mass spectra were recorded on a Micromass ZMD in the positive electrospray mode with a scan range of 0–1000 m/z and cone voltage setting of 20 V. A solution of the sample (≅100 μg/mL) in methanol (100%) was introduced into the source via a Waters 2690 autosampler. Flash chromatographic separations were performed on Whatman silica gel, 60 Å or using a Biotage Flash 40i or 75i with prepacked silica gel (60 Å) cartridges. Thin-layer chromatography (TLC) was performed using aluminum backed silica gel 60 plates from E. Merck. Inosine was purchased from Sigma Chemical Company, St. Louis, Missouri; dGuo was obtained from Reliable Biohemicals, St. Louis, Missouri; and BCX-1777 (forodesine HCl) and BCX-34 were synthesized at BioCryst. Inosine-U-^{15}Na was obtained from Cambridge Isotope Laboratories, Sorbax C3 and Purospher C18 columns from Agilent, and phenyl boronic acid solid-phase cartridges from Varian. Centrifree YM-30 1 mL Micropartition Devices were obtained from Millipore.

(1S)-*N*-*tert*-Butoxycarbonyl-1′-*C*-{4-methoxypyrrolo[3,2-*d*]pyrimidin-9-*N*-benzyloxo-methyl)}-1′,4′-dideoxy-1′,4′-imino-D-ribitol (5). A sample of the silyl ether **4** (42.5 g, 64.9 mmol) was dissolved in methanol (1.5 L) under an Argon atmosphere. To this 1*N* HCl (∼650 mL) was added. After 1 h an additional 150 mL of 1*N* HCl was added and solution placed in a water bath at ∼30°C. After 1.5 h the solution was cooled in an ice/water bath and made slightly basic (∼pH 8) with concentrated aqueous NH_4OH (∼60 mL). After concentrating the resulting syrup was partitioned between chloroform and a small amount water. The organic layer was dried (Na_2SO_4) and concentrated to give a crude foam. The crude product was purified by chromatography (Flash 75, 800 g SiO_2, EtOAc) and relevant fractions combined to give 31.2 g (62.3 mmol, 96%) of **5** as a white foam. An analytical sample was obtained from a center fraction giving **5** as a white foam. IR (KBr) 2935, 1695, 1614, 1539, and 1365 cm^{-1}; MS (*m/z*, ES+) 501.1 (100%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (d, J = 17.5 Hz, 1H), 7.47–7.25 (m, 6H), 5.71 (s, 1H), 5.67 (dd, J = 10.5, 59.6 Hz, 1H), 4.78–4.48 (m, 4H), 4.31–3.96 (m, 3H), 4.07 (s, 3H), 3.68 (d, J = 11.5 Hz, 1H), 3.19 (br s, 1H, D_2O exchangeable), 1.31 and 1.05 (pair s, 9H total). Analysis calculated for $C_{25}H_{32}N_4O_7$: C, 59.99; H, 6.44; N, 11.19. Found: C, 59.91; H, 6.50; N, 10.89.

(1S)-*N*-*tert*-Butoxycarbonyl-1′-*C*-{4-methoxypyrrolo[3,2-*d*]pyrimidin-9-*N*-benzyloxo-methyl)}-1′,4′-dideoxy-1′,4′-imino-3′,5′-*O*-(1,1,3,3-tetraisopropyl-disiloxa-1,3-diyl)-D-ribitol (6). A sample of the triol **5** (28.5 g, 57.0 mmol) was dissolved in anhydrous pyridine (250 mL) under an Argon atmosphere. To this 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (20.0 mL, 62.6 mmol, 1.1 eq.) was added. After 15 h the solution was concentrated azeotroping with toluene (2 × 200 mL). The resulting residue was partitioned between chloroform and water. After separating layers the aqueous layer was extracted with chloroform (2×). The organic layer was dried (Na_2SO_4) and concentrated. The crude product was purified by chromatography (Flash 75, 800 g SiO_2, gradient 10 → 15 → 25 → 50% EtOAc-hexanes) and relevant fractions combined to give 34.35 g (46.2 mmol, 81%) of **6** as a white foam. An analytical sample was obtained from a center fraction giving **6** as a white foam. IR (KBr) 2946, 2868, 1699, 1612, 1538, and 1367 cm^{-1}; MS (*m/z*, ES+) 743.2 (100%); ^1H NMR (300 MHz, $CDCl_3$) δ 8.47 (s, 1H), 7.45 (br s, 1H), 7.35–7.23 (m, 5H), 5.76 (br d, $J = 9.9$ Hz, 1H), 5.58 (d, $J = 10.6$ Hz, 1H), 5.22 (br s, 2H), 4.50–4.41 (m, 4H), 4.19–4.16 (m, 1H), 4.10 (s, 3H), 3.83 (br s, 1H), 3.25 (br s, 1H, D_2O exchangeable), 1.41 (br s, 59H), 1.11–0.96 (m, 28H). Analysis calculated for $C_{37}H_{58}N_4O_8Si_2$: C, 59.81; H, 7.87; N, 7.54. Found: C, 59.89; H, 7.77; N, 7.52.

(1S)-*N*-*tert*-Butoxycarbonyl-1′-*C*-{4-methoxypyrrolo[3,2-*d*]pyrimidin-9-*N*-(benzyloxo-methyl)}-1′,4′-dideoxy-1′,4′-imino-2′-*O*-[phenoxy(thiocarbonyl)]-3′,5′-*O*-(1,1,3,3-tetra-isopropyldisiloxa-1,3-diyl)-D-ribitol (7). A sample of the alcohol **6** (10.0 g, 13.4 mmol) and DMAP (4.60 g, 37.7 mmol, 2.8 eq.) was dissolved in anhydrous acetonitrile (200 mL) under an Argon atmosphere. To this mixture was added phenyl chlorothionoformate (2.0 mL, 14.8 mmol, 1.1 eq.) in one portion. After 12 h the solution was concentrated and the resulting residue partitioned between dichloromethane and water. The organic layer was washed with water (2×), dried (Na_2SO_4), and concentrated. The crude product was purified by chromatography (Flash 75, 200 g SiO_2, 20% EtOAc-hexanes) and relevant fractions combined to give 10.63 g (12.1 mmol, 90%) of **7** as a white foam. An analytical sample was obtained from a center fraction giving **7** as a white foam. IR (KBr) 2946, 2868, 1701, 1611, 1539, and 1368 cm^{-1}; MS (*m/z*, ES+) 879.2 (100%); ^1H NMR (300 MHz, DMSO-d_6) δ 8.38 (s, 1H), 7.77 (s, 1H), 7.51–7.46 (m, 2H), 7.36–7.13 (m, 8H), 6.37 (br s, 1H), 5.79–5.72 (m, 2H), 5.49 (br s, 1H), 5.28 (s, 1H), 4.48 (s, 2H), 4.18–4.13 (m, 2H), 4.05 (s, 3H), 3.63–3.57 (m, 1H), 1.38 (br s, 9H), 1.05–0.88 (m, 28H). Analysis calculated for $C_{44}H_{62}N_4O_9S_1Si_2$: C, 60.11; H, 7.11; N, 6.37. Found: C, 60.42; H, 7.21; N, 6.34.

(1S)-*N*-*tert*-Butoxycarbonyl-1′-*C*-{4-methoxypyrrolo[3,2-*d*]pyrimidin-9-*N*-(benzyloxo-methyl)}-1′,4′-dideoxy-1′,4′-imino-2′-deoxy-3′,5′-O-(1,1,3,3-tetra-isopropyldisiloxa-1,3-diyl)-D-ribitol (8). A sample of the thiocarbonate **7** (31.2 g, 35.5 mmol) and azobis-(cyclohexane-1-carbonitrile (ACCN, 2.86 g,

11.7 mmol, 0.33 eq.) was dissolved in anhydrous toluene (600 mL) under an Argon atmosphere. The solution was sparged with argon for 30 min followed by addition of trimethylsilane (23 mL, 74.5 mmol, 2.1 eq.). The resulting solution was placed in a pre-heated 100°C oil bath. After 1.5 hrs the solution was concentrated. The crude product was purified by chromatography (Flash 75, 800 g SiO_2, gradient 15 → 20% EtOAc-hexanes) and relevant fractions combined to give 23.52 g (32.3 mmol, 91%) of **8** as a clear glass. IR (KBr) 3054, 2947, 2868, 1687, 1610, 1535, and 1264 cm^{-1}; MS (*m/z*, ES+) 727.3 (100%); ^1H NMR (300 MHz, $CDCl_3$) δ 8.50 (s, 1H), 7.40 (s, 1H), 7.34–7.23 (m, 5H), 5.75 (br d, $J = 9.0$ Hz, 1H), 5.57 (d, $J = 10.5$ Hz, 1H), 5.36 (d, $J = 8.9$ Hz, 1H), 4.96 (br s, 2H), 4.49–4.38 (m, 2H), 4.16–4.11 (m, 1H), 4.10 (s, 3H), 3.68–3.62 (m, 1H), 2.58 (br s, 1H), 2.42–2.31 (m, 1H), 1.40 (br s, 9H), 1.08–0.88 (m, 28H). Analysis calculated for $C_{37}H_{58}N_4O_7Si_2$: C, 61.12; H, 8.04; N, 7.71. Found: C, 61.46; H, 7.97; N, 7.72.

(1S)-*N*-*tert*-Butoxycarbonyl-1′-*C*-{4-methoxypyrrolo[3,2-*d*]pyrimidin-7-yl}-1′,4′-dideoxy-1′,4′-imino-2′-deoxy-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxa-1,3-diyl)-D-ribitol (9). A mixture of **8** (24.54 g, 33.8 mmol) and 20% $Pd(OH)_2$ on C (25 g) in EtOH (∼150 mL) containing concentrated aqueous NH_4OH (∼1 mL) was shaken under an H_2 atmosphere (Parr shaker, 40 psig) for 1.5 h. The mixture was filtered through Celite and the pad washed with EtOH (75 mL). After the filtrate was concentrated the residue was partitioned between dichloromethane and water. The organic extract was washed with water, dried (Na_2SO_4), and concentrated to give 18.55 g (30.6 mmol, 90%) of **9** as a white foam. IR (KBr) 2947, 2868, 1697, 1675, 1627, 1537, and 1392 cm^{-1}; MS (*m/z*, ES+) 607.2 (100%); ^1H NMR (300 MHz, $CDCl_3$) δ 8.86 (br s, 1H, D_2O exchangeable), 8.46 (br s, 1H), 7.26 (br s, 1H), 5.37 (d, $J = 8.8$ Hz, 1H), 4.89 (br s, 2H), 4.34–4.10 (m, 2H), 4.10 (s, 3H), 3.68–3.62 (m, 1H), 2.63 (br s, 1H), 2.40–2.30 (m, 1H), 1.44 (br s, 9H), 1.12–0.88 (m, 28H). Analysis calculated for $C_{25}H_{50}N_4O_6Si_2$: C, 57.39; H, 8.30; N, 9.23. Found: C, 57.39; H, 8.32; N, 9.22.

(1S)-2′-Deoxy-1′-*C*-{4-hydroxypyrrolo[3,2-*d*]pyrimidin-7-yl}-1′,4′-imino-D-ribitol Hydrochloride (1). A sample of **9** (18.05 g, 29.7 mmol) was dissolved in methanol (100 mL) under an Argon atmosphere. To this concentrated aqueous HCl (100 mL, ∼1200 mmol, ∼40 eq.) was added and the mixture refluxed for 3 h. The solution was concentrated and the residue taken up in a small amount of water (40 mL). Absolute EtOH was added and the resulting mixture concentrated and the treatment with EtOH repeated three times. The crude product was recrystallized from 1*N* HCl/EtOH to give 7.05 g (24.6 mmol, 83%) of **1**, m.p. > 280°C (dec). IR (KBr) 3472, 3422, 3118, 2845, 1683, 1660, and 1597 cm^{-1}; MS (*m/z*, ES+) 251.0 (100%); ^1H NMR (300 MHz, DMSO-d_6 and $CDCl_3$) δ 12.37 (s, 1H, D_2O exchangeable), 12.17 (br s, 1H, D_2O exchangeable), 10.6–8.9 (br s, 1H, D_2O exchangeable),

7.90 (s, 1H), 7.69 (s, 1H), 5.64 (d, $J = 3.6$ Hz, 1H, D_2O exchangeable), 5.42 (br s, 1H, D_2O exchangeable), 5.02 (dd, $J = 6.2$, 11.9 Hz, 1H), 4.35 (d, $J =$ 2.0 Hz, 1H), 3.78–3.68 (m, 1H), 3.52–3.47 (m, 1H), 2.62–2.52 (m, 1H), 2.21 (dd, $J = 6.6$, 13.2 Hz, 1H). Analysis calculated for $C_{11}H_{14}N_4O_3 \cdot HCl$: C, 46.08; H, 5.27; N, 19.54; Cl, 12.37. Found: C, 46.34; H, 5.30; N, 19.70; Cl, 12.36.

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