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Inhibitors of Human Immunodeficiency Virus Type 1 (HIV-1) Attachment 6. Preclinical and Human Pharmacokinetic Profiling of BMS-663749, a Phosphonooxymethyl Prodrug of the HIV-1 Attachment Inhibitor 2-(4-Benzoyl-1-piperazinyl)-1-(4,7-dimethoxy-1*H*-pyrrolo[2,3-*c*]pyridin-3-yl)-2-oxoethanone (BMS-488043)

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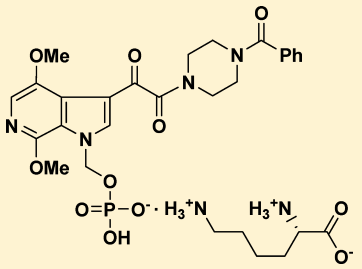
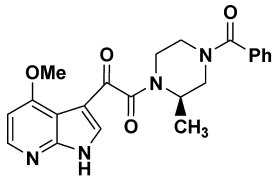
Supporting Information

ABSTRACT: BMS-663749, a phosphonooxymethyl prodrug **4** of the HIV-1 attachment inhibitor 2-(4-benzoyl-1-piperazinyl)-1-(4,7-dimethoxy-1*H*-pyrrolo[2,3-*c*]pyridin-3-yl)-2-oxoethanone (BMS-488043) (**2**) was prepared and profiled in a variety of preclinical in vitro and in vivo models designed to assess its ability to deliver parent drug following oral administration. The data showed that prodrug **4** had excellent potential to significantly reduce dissolution rate-limited absorption following oral dosing in humans. Clinical studies in normal healthy subjects confirmed the potential of **4**, revealing that the prodrug significantly increased both the AUC and C_{max} of **2** compared to a solid capsule formulation containing the parent drug upon dose escalation. These data provided guidance for further efforts to obtain an effective HIV-1 attachment inhibitor.

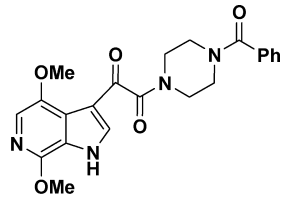


INTRODUCTION

A significant number of approved single antiviral agents and fixed-dose combinations that span six mechanistic classes are now available for use in the treatment of HIV-1 infection.[1] Despite the considerable advances made in effectively controlling viremia in HIV-1-infected patients using combination antiretroviral therapy, a cure for this disease is not imminent and there is an expanding population of HIV-1 patients relying on existing therapies whose medical needs are not completely satisfied.[2] The increasing occurrence of drug-resistant strains and the emergence of comorbidities associated with long-term highly active combination antiretroviral therapy (cART) strongly suggests that the need for mechanistically distinct, non-cross-resistant drugs will continue to be an imperative.[3] Members of a previously disclosed class of orally bioavailable, small molecule HIV-1 attachment inhibitors (AIs) have been shown to bind to and induce conformational changes within the HIV-1 viral envelope gp120 protein that interfere with its interaction with the cellular CD4 receptor, the initial point of host cell engagement by the virus.[4−9] Potent and selective inhibition has been observed in vitro against macrophage-, T-, and dual-tropic HIV-1 strains[7] by BMS-378806[10] (**1**) and BMS-488043[11] (**2**), AIs that have been advanced into clinical studies (Figure 1).[10−15]



1
BMS-378806



2
BMS-488043

Figure 1.

1 was the first AI advanced into clinical studies, but the plasma exposure in normal healthy human volunteers was inadequate to progress this compound into proof-of-concept trials in HIV-1 infected subjects.[11,12,14] AI **2** represented an advance from **1** that allowed evaluation of the antiviral activity, safety, and tolerability in a multiple ascending dose study conducted in HIV-1-infected adults. In a placebo-controlled trial, 800 and 1800 mg doses of **2** were administered orally twice daily for 8 days with daily viral load (VL) monitoring up to 14 days.[13,15] In subjects receiving an 1800 mg dose of **2**, 67% (8/12) experienced a VL decline of >1.0 $\log_{10}$, with 42% (5/

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12) having a VL decline >1.5 $\log_{10}$. This contrasted with the placebo-treated subjects, all of whom experienced a VL reduction of <0.4 $\log_{10}$. Inhibitor **2** was well tolerated, and there were no serious adverse events and no discontinuations from the study. This proof-of-concept study validated the AIs as a novel class of small molecules with clinically relevant antiviral activity in HIV-1-infected subjects and has stimulated further efforts to identify a drug candidate in this class.

In the clinical studies with **2** in HIV-1 infected patients, both a high dose (800 or 1800 mg BID) and concomitant administration of a high fat meal were required in order to achieve adequate exposure and antiviral activity. A single 200 mg dose administered as a solution had 2-fold greater exposure than that obtained from a single dose of a clinical capsule without a concomitant high fat meal (capsule containing wet milled crystalline active pharmaceutical ingredient (API) (95% <23 μm, SA = 0.8 m^2/g), data summarized in Figure 2. The

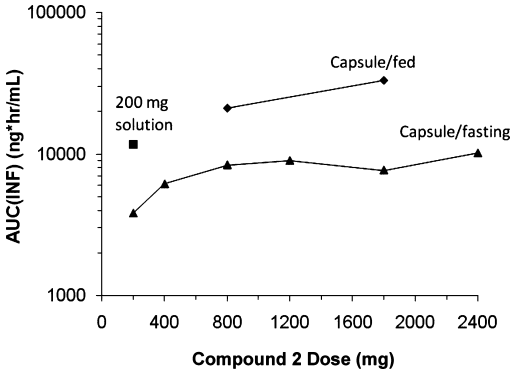

Figure 2. AUC (INF) vs dose after single oral administration of **2** as a solution or capsule formulation under fasting or fed (high fat meal) conditions.

evidence available suggested that the absorption of **2** was significantly limited by poor dissolution and/or poor solubility, with exposure in humans ostensibly reaching a plateau at a dose of 800 mg in the absence of a high fat meal. Both the high fat meal and the excessive pill burden necessitated by these dose regimens were considered to be liabilities that ultimately precluded further development of the parent compound.

The performance of **2** in vivo in which the area under the concentration−time curve (AUC) plateaus at a dose of 800 mg with fasting is consistent with its experimentally measured low solubility (Table 1) and high intrinsic membrane permeability,

Table 1. Physicochemical Properties of 4b vs 2

	4b phosphate prodrug	**2** parent compound
aqueous solubility at room temperature (mg/mL)	0.22 at pH 1.4	~0.04 at pH 4−8
	>12 at pH 5.4	0.9 at pH 1.5
	>12 at pH 8.9	0.25 at pH 10
pK_a	2.2	2.6
	6.1	9.3
lipophilicity	<−1.0 (log *D* at pH 6.5)	1.5 (log *P* at pH 6.5)

leading to its characterization as a BCS class 2 molecule.[16] On the basis of this appreciation, a number of alternate delivery strategies were explored that were designed to improve the exposure of **2**. Nanosizing and amorphous formulation approaches were examined with some success, providing approximately 5- and 9-fold enhancements in exposure in dogs, respectively, compared to the formulation used in the clinical capsule.[17] These data, along with the data from solution dosing, provided evidence that addressing dissolution-limited absorption had the potential to improve plasma exposure of **2** following oral administration.

Phosphate prodrugs, either directly attached to a molecule or incorporated via linkers, have been used to successfully enhance the solubility and exposure of a range of compounds administered by either an intravenous (IV) or oral route.[18−33] The physicochemical properties predictive of success with an oral phosphate prodrug approach have been studied, but due to the number of variables, the potential for utility must be assessed experimentally.[30,31] Previous experience with phosphate-based prodrugs of the antifungal agent ravuconazole and taxane derivatives suggested that a phosphonooxymethyl prodrug moiety might be readily attached to the indole nitrogen of **2**. It was anticipated that this moiety would provide parent drug via the intermediacy of a short-lived hydroxymethyl intermediate **3** after phosphatase cleavage of prodrug **4** (BMS-663749)[34] and would offer considerable potential to provide a functional solution to the formulation problem (Scheme 1).[28,33,35,36] The purpose of this work was to prepare prodrug **4** and assess its potential to remedy the high dose and solubility limited absorption that were two significant reasons parent **2** was not a viable HIV-1 attachment inhibitor for use in combination therapy to treat HIV-1 infection.

Synthesis. The synthesis and identification of a stable form of **4**, ideally a crystalline salt, which could be reproducibly prepared and used to perform definitive profiling, was achieved in the following manner The chemistry initially used to prepare prodrug **4** from parent **2** was an adaption of the process used to prepare a phosphonooxymethyl prodrug of ravuconazole and is summarized in Scheme 2.[28]

The route shown in Scheme 2 comprises of four steps, the last two of which can be accomplished without isolation of the penultimate free acid intermediate **4**. To ensure reproducible reactivity, di-*tert*-butyl chloromethyl phosphate (**6**) was freshly prepared from **5** and chloroiodomethane for each individual preparative experiment. An initial survey of conditions revealed that deprotonation of **2** was most effectively performed with 3 equiv of NaH and that the iodine-catalyzed alkylation proceeded most efficiently using 5 equiv of **6**. This reaction was typically performed on batches of 8−10 g of **2** based on the preparative reproducibility of the alkylating agent. Silica gel chromatography provided the diester **7** in purity sufficient to allow the subsequent deprotection, salt formation, and crystallization steps to produce material of consistently high purity. A crystallization of a final salt form was found to be the most expeditious method to produce material of sufficient purity for toxicology studies. TFA in dichloromethane was initially used to deprotect diester **7**, followed by isolation of crude material and then generation of the desired salt, typically in an acetone/water solvent system in the case of lysine. However, the yield and purity of both the crude material and final salt was not always reproducible as scales increased to tens of grams and additional manipulation was required to get the crude phosphate product ready for salt formation and

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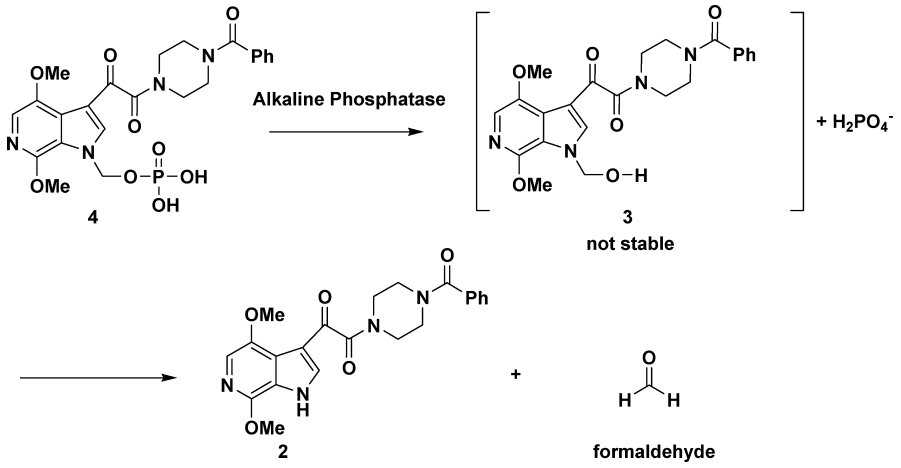
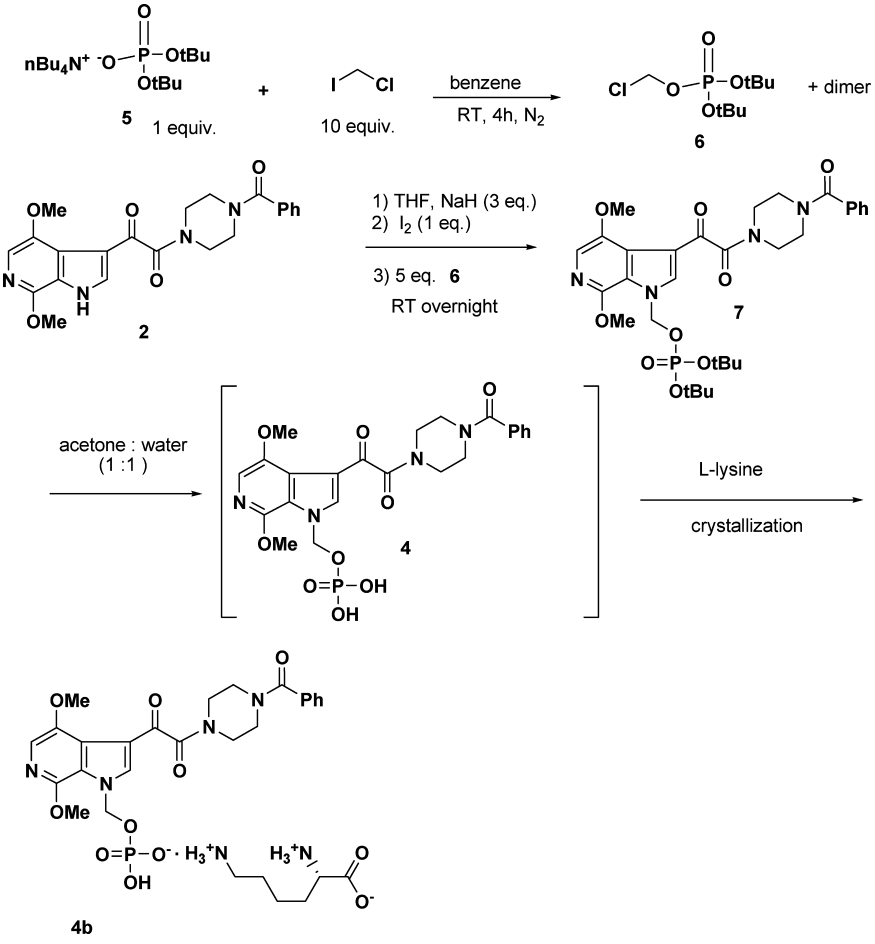
Scheme 1



Scheme 2. Synthesis of 4b



crystallization. A more preferable procedure was developed which avoided the use of highly acidic TFA and the need to isolate the crude phosphate free acid. Thus, deprotection of **7** was accomplished by heating at 40 °C in a mixture of acetone and water. The use of acetone/water as solvent allowed for efficient deprotection of the phosphate group, subsequent in situ salt formation, and crystallization. The discovery of these conditions provided an advance over alternative conditions to produce and isolate the prodrug in higher yields and purity on large scales. The phosphate prodrug **4** could be isolated as either the free acid or as a range salts. A survey of organic and inorganic salts identified the mono (*S*)-(+)-lysine salt mono hydrate as the most suitable candidate for advancing into key in vivo studies with a crystalline form that could be reproducibly prepared and crystallized to meet purity and stability requirements. The overall yield of the lysine salt mono hydrate starting from **2** was approximately 56%.

Preclinical Profiling of 4b. The physical and pharmaceutical characteristics of the lysine salt of **4** confirmed that this prodrug possessed significantly improved solubility and

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sufficient chemical stability in both the solid state and solution to support oral dosing studies (Table 1). Two pK_a values of 2.2 and 6.1 were measured by potentiometric titration of the diacid **4**. Lysine salt **4b** crystallized as an off-white powder containing a small amount of amorphous material, and the solubility at room temperature increased from 0.22 mg/mL at pH 1.4 to >12 mg/mL at pH 5.4 and pH 8.9. Concentrations of >100 mg/mL have been achieved in water for in vivo toxicology studies. In comparison, the aqueous solubility at room temperature of the parent compound **2** was determined to be approximately 0.04 mg/mL in the pH range of 4−8, 0.9 mg/mL at pH 1.5 and 0.25 mg/mL at pH 10.0, as crystalline material.

Prodrug **4** is hydrolyzed by alkaline phosphatase (ALP) to form **2**.[30,37,38] Because multiple ALP isoforms are widely distributed in various tissues, quantitative in vitro to in vivo correlations were not attempted.[39−43] Therefore, the studies were limited to a qualitative assessment of ALP-dependent hydrolysis in different tissues. As shown in Scheme 1, the hydroxymethyl indole adduct **3** arises from enzyme-mediated dephosphorylation of the phosphonooxymethyl prodrug **4**. This intermediate has not yet been observed in any in vitro or in vivo studies and apparently collapses spontaneously to **2**, simultaneously liberating one molecule of formaldehyde per molecule of **2** (Scheme 1). This is not surprising because the measured pK_a of the indole N−H of **2** is 9.3, and literature precedent suggests that the hydroxymethyl indole intermediate **3** would be unstable when the pK_a of the indole N−H is less than 11.[44] On the basis of the available toxicologic information on orally administered formaldehyde, other pharmaceuticals, and dietary sources, the risks associated with chronic low level oral exposure to formaldehyde are minimal when compared to the potential benefits of realizing a new efficacious agent for the treatment of HIV.[45] Clinical studies with salts of prodrug **4** or similar prodrugs of closely related molecules have to date not identified toxicities attributed to formaldehyde exposure.[46] The Caco-2 permeability coefficient (P_c) of the parent molecule **2** is high, >100 nm/s at pH 6.5, which is comparable to metoprolol, a highly membrane permeable marker that displays 100% absorption in humans.[11] Parent compound **2** was rapidly formed following IV administration of **4b** to rats, dogs and monkeys. The IV AUC conversion ratios were 1.5 in rats, 0.80 in dogs, and 0.70 in monkeys, suggesting good conversion from **4b** to **2** (The ratios were calculated from **2** AUC after prodrug **4** dosing/**2** AUC after **2** dosing for IV dosing). No or very low levels of **4** were detected in rat, dog, and monkey plasma after oral administration of **4b**. The absolute bioavailability of **2** following oral administration of **4b** was determined to be 62%, 93% and 67% in rats, dogs, and monkeys, respectively (Table 2). These data are consistent with reports describing the high levels of ALP expression in the gut that efficiently cleave the prodrug presystemically.[47,48]

In an oral dose escalation study conducted in rats administered an aqueous solution of the mono lysine salt **4b**, the increase in AUC of **2** was approximately dose-proportional over the doses of 16, 72, and 267 mg/kg (free acid). Comparisons of the C_{max} and AUC obtained in rats dosed with either **2** or **4b**, with doses plotted in parent drug equivalents, are shown in Figure 3. The dosages of the free acid of prodrug **4** studied correspond to molar equivalent dosages of 13, 57, and 211 mg/kg, respectively, of the parent **2** (Figure 3). In all experiments, the AUC of the prodrug in plasma was ≤0.03% that of the parent.

Table 2. Summary of the Pharmacokinetic Parameters of Prodrug 4 and Parent 2 Following IV and PO Administration of Prodrug Lysine Salt 4b in Various Species[a]

parameters	rat	dog	monkey
IV dosing	n = 3	n = 3	n = 3
dose (mg/kg)[b]	1.4 (1.1)	1.2 (0.95)	1.3 (1.1)
Prodrug 4			
Cl (mL/min/kg)	14 ± 4.2	70 ± 16	4.4 ± 0.18
Vss (L/kg)	0.12 ± 0.019	0.30 ± 0.094	0.048 ± 0.017
$T_{1/2}$ (h)	0.16 ± 0.052	0.15 ± 0.010	1.0 ± 0.78
MRT (h)	0.14 ± 0.020	0.070 ± 0.0057	0.18 ± 0.059
IV AUC conversion ratio[c]	1.5	0.80	0.70
PO dosing	n = 3	n = 3	n = 3
dose (mg/kg)[b]	7.9 (6.3)	6.6 (5.2)	7.1 (5.6)
Parent 2 Formed from Prodrug 4b			
bioavailability (%)[d]	62	93	67
C_{max} (nM)	3943 ± 983	14017 ± 1261	16354 ± 6381
T_{max} (h)	0.83 ± 0.29	0.25	0.83 ± 0.14
AUC_{tot} (nM·h)	13168 ± 1452	82844 ± 16499	34183 ± 2668
$T_{1/2}$ (h)	1.5 ± 0.24	4.1 ± 0.52	4.8 ± 1.3

[a]Data from IV and PO dosing of parent **2** in all three species has been published.[11] [b]Free acid of **4b** (equivalent dose of **2**). [c]The ratios were calculated from ((**2** AUC after prodrug **4** dosing)/(**2** AUC after **2** dosing)) for IV dosing. [d]The absolute oral bioavailability was calculated from the dose-normalized AUC of **2** after oral dosing of **4b** divided by the historical IV AUC^{tot} data (3348 nM·h at 1 mg/kg) of **2**.[1]

As can be seen from the data, **4b**, when dosed orally at ∼200 mg/kg equivalent of **2**, provided ∼3 fold higher C_{max} and ∼2-fold higher AUC of **2** in rats when compared to the AUC from **2** administered as a suspension at the similar dose.

The findings from in vitro and in vivo studies in animals as well as from in vitro human tissues predicted that **2** would be formed after oral administration of **4b** to human subjects. The data from preclinical studies supported further development of **4b**, and the compound was advanced sequentially into pre-IND characterization and phase I studies in normal healthy volunteers.[35,49]

Human Clinical Studies with 4b. The lysine salt **4b** was formulated as a solution from drug in bottle and evaluated in a single ascending dosing study in normal healthy human volunteers (N = 6 per dose cohort) at doses equivalent to 25, 50, 100, 200, 400, and 800 mg of parent **2**. The results from this study are compiled in Figures 4, 5, and 6 and Table 3 and were compared with those from the historical studies in which **2** was dosed in a clinical capsule formulation and as a 200 mg solution (data summarized in Figure 7).[15] Following dosing of **4**, parent drug **2** was observed in plasma with T_{max} achieved within 30 min, suggesting that the parent drug was both released from the prodrug and rapidly absorbed. As can be seen from Figures 4−6, and Table 3, overall exposure and both C_{max} and AUC increased at least proportionally with dose over the range studied, 25−800 mg. The 800 mg dose equivalent of prodrug **4** resulted in a significantly higher AUC of **2** but a somewhat lower C_{12} than that obtained from 800 mg of **2** administered as solid in a capsule concomitantly with a high fat meal (Table 3). Only relatively low levels of the prodrug **4** were detectable in human plasma at the 800 mg dose of **4** in 4 out of 5 subjects up to 1.5 h postdose. The $T_{1/2}$ of **2** measured from 2 to 12 h after an 800 mg dose of **4b** was 1.5 ± 0.2 h, significantly

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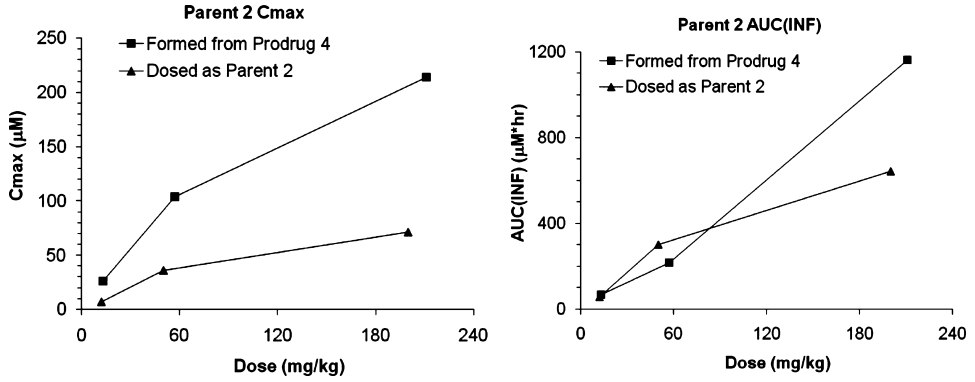

Figure 3. Comparison of C_{max} and AUC of **2** in male rats administered either **2** or **4b**. Data for **4b** are from a single-dose study and data for **2** are from day 1 of a 2 week exploratory oral dose toxicity study. Dosages of **4b** are presented as molar equivalents of **2**.

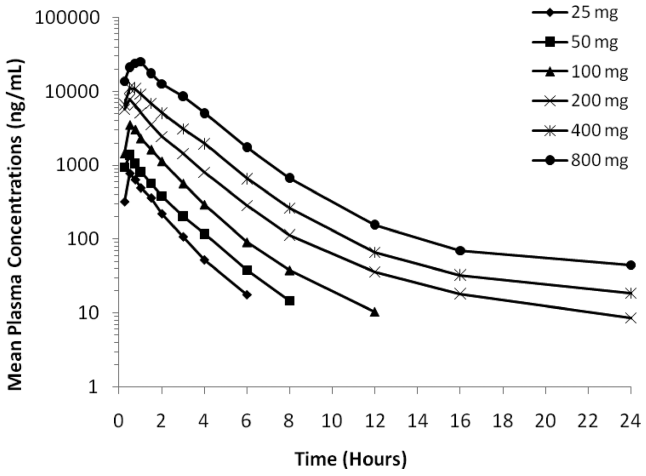

Figure 4. Comparative mean profiles of **2** in humans after single oral administration of 25−800 mg equivalents of **2** as prodrug (lysine salt) **4b**.

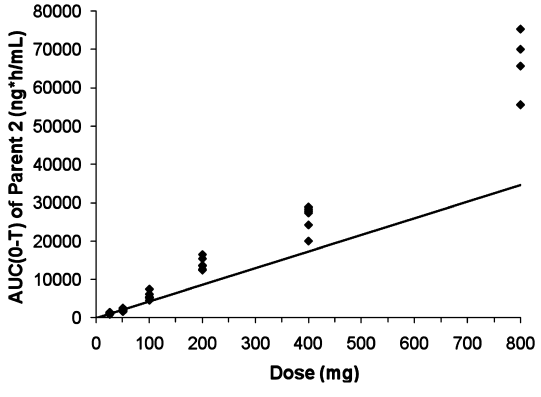

Figure 5. AUCt vs dose relationship in humans after single doses of 25−800 mg equivalents of **2** as prodrug **4b**. The diagonal line depicts a hypothetical linear relationship starting from the data at the lowest dose for comparison.

shorter than the ∼10 h $T_{1/2}$ observed for **2** following oral dosing of parent **2**, and approximately half of the 3 h measured after dosing parent **2** as a solution. These data are consistent with poor dissolution prolonging the absorption phase of the parent drug and some precipitation occurring from solution.

Qualitatively, when comparing the data from dosing **2**, either as a solid in the clinical capsule or as a solution, with that of the prodrug **4**, dosing of the prodrug more closely mimicked that of

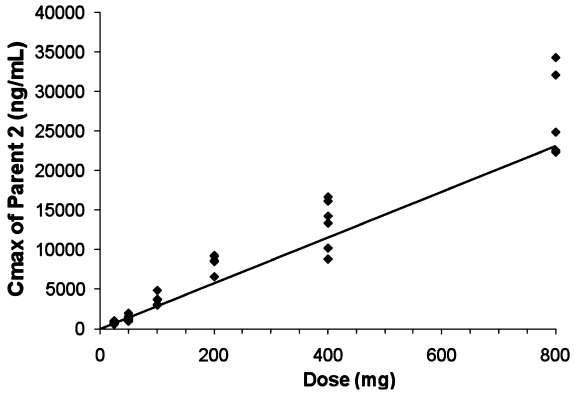

Figure 6. C_{max} versus dose relationship in humans after single doses of 25−800 mg equivalents of **2** as prodrug **4b**. The diagonal line depicts a hypothetical linear relationship starting from the data at the lowest dose for comparison.

parent when dosed as solution, exhibiting high bioavailability, a very high C_{max}, and a short $T_{1/2}$, as summarized in Figure 7. The oral solution data suggest complete conversion of **4b** to **2** in vivo after prodrug administration via the oral route.

In the human SAD study, a 400 mg dose of prodrug was administered concomitantly with a high fat meal to a single subject and the data compared to that obtained from the dosing of the same dose of prodrug alone. The high fat meal appeared to delay T_{max} by about 0.75 h and decreased both the C_{max} and AUC by 57% and 32%, respectively, in this limited sample set.

DISCUSSION

The phosphonooxymethyl prodrug methodology succeeded in overcoming the solubility- and dissolution-limited absorption encountered with the solid formulation of the parent AI **2** when dosed to human subjects in the initial clinical trials. Consistent with its improved ability to deliver parent to plasma following oral administration, the prodrug **4b** achieved a higher AUC of parent than the solid formulation at the highest dose studied (800 mg) without the need for a high fat meal to boost exposure. Indeed, the limited data obtained suggest that in humans, coadministration with food is actually somewhat detrimental to the exposure of the parent **2** from prodrug **4**. This suggests that the solubilization of the parent from the prodrug is already highly optimized and food may actually interfere with access of the prodrug to the gut wall. The dosing of prodrug **4b** at 200 mg equivalent of parent closely mimicked that from dosing a solution of the parent, affording a $T_{1/2}$ just

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Table 3. Human PK Parameters of Oral Dosing of 4b vs Oral Dosing of 800 mg 2 with a High Fat Meal[a]

PO treatment	C_{max} geometric mean (%CV) (ng/mL)	T_{max} median (range) h	AUC(INF) geometric mean (%CV) (ng/mL·h)	$T_{1/2}$ mean (SD) h	C_{12} geometric mean (%CV) (ng/mL)
800 mg **4b**	26762 (21)	1 (0.5−1)	64131 (13)	11 (5.4)	150(32)
400 mg **4b**	12906 (24)	0.63 (0.25−1)	26168 (13)	13 (15)	59 (43)
200 mg **4b**	8419 (11)	0.5 (0.25−0.75)	14157 (12)	6.7 (6.0)	33 (40)
100 mg **4b**	3653 (18)	0.5 (0.5−0.75)	5727 (18)	1.9 (0.42)	12 (17)
50 mg **4b**	1371 (25)	0.5 (0.25−0.5)	2151 (17)	1.4 (0.13)	<LLOQ[b]
25 mg **4b**	784 (22)	0.5 (0.5−0.75)	1162 (18)	1.0 (0.09)	<LLOQ[b]
800 mg **2** (capsule with high fat meal)	4100 (13)	4.0 (2.0−4.0)	21191 (20)	14 (6.3)	175 (88)

[a]Analyte for all data is parent compound **2**. [b]LLOQ: lower level of quantitation.

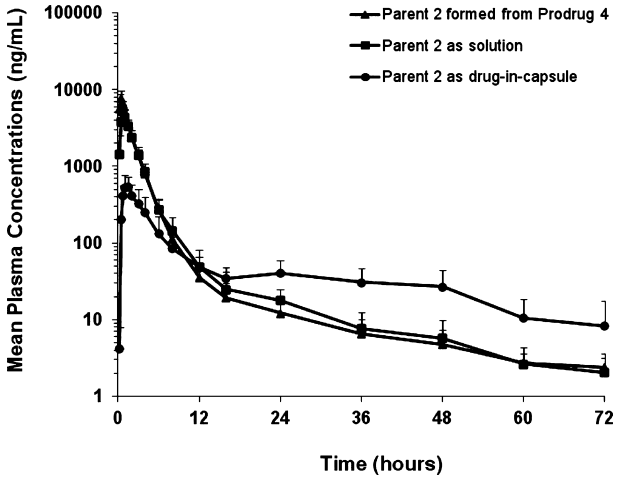

Figure 7. Human plasma profiles of parent **2** from dosing of solution, drug-in-capsule formulation, or prodrug **4b**. The dose was 200 mg of parent **2** or equivalent.

slightly longer than that from solution. The ∼10 h $T_{1/2}$ observed after dosing of the parent drug was apparently lengthened due to a major contribution from the effect of extended and prolonged absorption, a function of the limited solubility and slow dissolution in the gut. The solution dosing also probably demonstrates some component of prolonged absorption that was absent in the dosing of prodrug. A critical contributor to the success of an oral phosphate approach is that the rate of release of parent drug from the pro-moiety must be commensurate with the rate of absorption and the data for this prodrug suggest that this release is very rapid. In addition, the parent drug generated from unmasking of the prodrug presystemically must remain in solution long enough to be absorbed and this also seems to be occurring with **2** in a near optimal manner.

While the performance of the prodrug met expectations with respect to improving the plasma delivery of **2**, as demonstrated by increased C_{max} and AUC and reduced T_{max}, the intrinsic pharmacokinetic properties of **2** in humans are less than ideal, with a short $T_{1/2}$ in plasma. As a consequence, the C_{12} of the drug in humans falls below the targeted protein binding-adjusted EC_{50} measured in vitro that would give confidence that this compound could be developed as a twice daily dosing regimen. The C_{12} at the 800 mg dose of prodrug **4b** was 149.57 ± 32.32 ng/mL, which is less than the protein binding-adjusted EC_{50} for **2**, determined in vitro vs a panel of HIV subtype B clinical virus isolates to be 156 ng/mL (370 nM). Because the convenience of twice daily drug dosing was considered to be a critical element to its future utility, further development of the compound was not pursued in favor of candidates with improved pharmacokinetic and antiviral properties.[15,46,50]

CONCLUSION

A phosphonooxymethyl prodrug of an azaindole-based HIV-1 attachment inhibitor was found to provide improved biopharmaceutics properties, successfully overcoming the dissolution- and solubility-limited absorption associated with the parent drug and the requirement for concomitant dosing of a high fat meal in order to achieve targeted plasma levels of active drug. The parent drug was considered to be a good candidate for prodrug methodology based on its high intrinsic permeability, low solubility, and the projected need for a high dose. Although the plasma levels achieved with parent drug **2** dosed with a high fat meal and the prodrug **4** dosed under fasted conditions, were shown to be sufficient to demonstrate clinical antiviral activity and establish proof-of-concept for this drug class, the predicted dosing regimen of the drug fell short of objectives. Administration of the prodrug **4** more fully revealed inherent deficiencies in the pharmacokinetic properties of **2**, particularly the short $T_{1/2}$, which may have been masked by the slow absorption of parent drug, leading to a flip-flop kinetic profile. Nevertheless, the clinical data established the value of this prodrug methodology to overcome dissolution- and solubility-limited absorption of a BCS class 2 molecule and provided a platform for drug delivery via a prodrug that is currently being used for a compound of this mechanistic class, BMS-663068, that was formulated as an extended release tablet, and demonstrated improved pharmacokinetic and antiviral properties.[15,46,50]

EXPERIMENTAL SECTION

Procedures for the preparation of **4b** (mono-L-lysine salt): {3-[(4-benzoylpiperazin-1-yl)(oxo)acetyl]-4,7-dimethoxy-1*H*-pyrrolo[2,3-*c*]-pyridin-1-yl}methyl dihydrogen phosphate, L-lysine salt (1:1).

Preparation of **6**: di-*tert*-butyl chloromethyl phosphate.[28] The tetrabutylammonium salt of di-*tert* butyl phosphate, (45.1 g, 0.1 mol) and chloroiodomethane (200 g, 1.14 mol) were combined in 100 mL of benzene and the mixture stirred at room temperature for 4 h. The benzene was removed under vacuum, a portion of 500 mL of Et_2O added to the residue, and the insoluble solid filtered away. Concentration of the filtrate in vacuo and removal of the volatiles on a vacuum pump provided crude bis-*tert*-butyl chloromethyl phosphate **6** as a light-yellow or light-brown oil which was utilized in the next step without further purification.

Preparation of **7**: (3-(2-(4-benzoylpiperazin-1-yl)-2-oxoacetyl)-4,7-dimethoxy-1*H*-pyrrolo[2,3-*c*]pyridin-1-yl)methyl di-*tert*-butyl phosphate. NaH (2.4 g, 60 mmol, 60% in oil) was added slowly to a suspension of **2** (8.4 g, 20 mmol) in dry THF (120 mL) and the mixture stirred for an hour at room temperature. Iodine (5 g, 20 mmol) dissolved in dry THF (10 mL) was added slowly and

cautiously to the stirred solution at such a rate that foaming was kept under control. Following completion of the addition, the mixture was stirred at ambient temperature for an additional 15 min and **6** (bis-*tert*-butyl chloromethyl phosphate ~0.1 mol), obtained as described in step one, was added. After stirring for 16 h, the reaction mixture was poured into iced NH_4OAc solution (30%, 120 mL) and extracted with EtOAc (3 × 300 mL). The combined organic extracts were washed with H_2O (100 mL) and brine (100 mL), dried over Na_2SO_4, and concentrated in vacuo to afford a residue which was purified by silica gel chromatography. Elution with EtOAc/Et_3N (100/1) followed by EtOAc/MeOH (100/1) gave diester **7** (9.0–10.3 g, AP ~75%) as a light-yellow solid in yields of 70–80% over several runs.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09 (s, 1H), 7.48 (s, 1H), 7.40 (b, 5H), 6.15 (d, 2H, J = 11.5 Hz), 4.05 (s, 3H), 3.90 (s, 3H), 3.90–3.30 (b, 8H), 1.39 (s, 18H); ^{13}C NMR (125 MHz, $CDCl_3$) 185.5, 170.7, 166.5, 146.9, 146.2, 139.6, 135.3, 130.2, 128.7, 128.4, 127.2, 124.5, 122.0, 120.8, 115.8, 83.8, 73.2, 57.3, 53.5, 46.1, 41.7, 29.8; MS m/z: $(M + H)^+$ calcd for $C_{31}H_{42}N_4O_9P$, 645.27; found, 645.10.

Preparation of 4. A mixed solution of TFA (50 mL) and dichloromethane (450 mL) was added to a round-bottom flask containing 43.3 g of diester **7**. After stirring at room temperature for 16 h, the reaction mixture was concentrated under vacuum to offer a residue of crude **4** which was used in further steps without any purification.

Preparation of {3-[(4-benzoylpiperazin-1-yl)(oxo)acetyl]-4,7-dimethoxy-1*H*-pyrrolo[2,3-*c*]pyridin-1-yl}methyl dihydrogen phosphate, L-lysine salt (1:1) (**4b**). First, 55 g of crude product **4** from the preceding procedure was added to an aqueous solution of L-lysine (1.36M, 70 mL) at room temperature. The resulting suspension (pH = 1.83) was added to a lysine solution (1.36 M, ~40 mL) to reach pH 4.88. The resulting suspension was filtered through a pad of Celite. The clear light-yellow filtrate (~200 mL) was mixed with acetone (200 mL) and heated to 45 °C. Acetone (1400 mL) was added over 2 h at 45 °C. The clear solution was seeded and stirred at 45 °C for 2 h and slowly cooled to room temperature (5 h) and the suspension stirred overnight. The white solid was collected by filtration and dried under house vacuum at 50 °C over 24 h to afford 41.2 g of **4b** as an off-white solid. The above solid was dissolved in 1:1 water–acetone (560 mL) at 45 °C. Acetone (700 mL) was added over a period of 1 h at 45 °C. The clear solution was seeded and stirred at 45 °C for 2 h, slowly cooled to room temperature (5 h), and the suspension stirred at room temperature overnight. The white solid was collected by filtration and dried under house vacuum at 50 °C over 36 h to afford 33 g of **4b** as an off-white solid. The material was >99% by LC/MS (method below) and determined to be >98% pure based on the combustion analysis and water determination.

4b was a 1.70 molar hydrate (4.20% w/w water) and 1.14 molar lysine salt. Residual solvent analysis did not find any residual solvents. ^{1}H NMR (500 MHz, D_2O, 60 °C) δ 8.72 (s, 1H), 7.84 (m, 6H), 6.44 (d, 2H, J = 10 Hz), 4.41 (s, 3H), 4.27 (s, 3H), 4.3–3.7 (m, 8H), 4.10 (t, 1H, J = 5 Hz), 3.39 (t, 2H, J = 5 Hz), 2.30–1.80 (m, 6H). ^{13}C NMR (125 MHz, D_2O, 27 °C) δ 186.7, 174.9, 173.2, 167.9, 147.7, 145.7, 142.6, 134.3, 131.1, 129.2, 127.1, 124.3, 122.4, 120.1, 113.8, 73.5, 57.1, 54.9, 54.4, 47.7, 47.1, 46.3, 45.7, 42.6, 42.1, 42.0, 41.5, 39.5, 30.2, 26.8, 21.8. HRMS m/z: $(M - \text{lysine} + H)^+$ calcd for $C_{23}H_{26}N_4O_9P$, 533.1437; found, 533.1425. Anal. Calcd for $C_{23}H_{26}N_4O_9P$: C, 49.11; H 6.13; N, 12.05. Found: C, 48.93; H 6.26; N 12.07. Melting point 168–172 °C. HPLC/MS RT = 17.75 min. Method details: Waters 2695 HPLC with Finnigan UV 6000LP PDA detection and LCQ Classic MS (electrospray probe); column, Waters Atlantis dC18; 150 mm × 2.1 mm ID; 5 μm (at 30C); mobile phase A, 0.05% TFA/water; mobile phase B, 0.05% TFA/acetonitrile; flow, 0.30 mL/min 5–98%; B gradient, hold 5%B 0–2 min, 5–35%B 2–23 min, 35–98%B 23–35 min, hold 98%B 35–37 min, 98–5%B 37–37.3 min, hold 5%B 37.3–45 min; UV detection, UV at 260 nm sampling: 2 Hz, bandwidth 7 nm, step 5 nm.

Optimized procedure for preparation of {3-[(4-benzoylpiperazin-1-yl)(oxo)acetyl]-4,7-dimethoxy-1*H*-pyrrolo[2,3-*c*]pyridin-1-yl}methyl dihydrogen phosphate, L-lysine salt (1:1) (**4b**) using hydrolytic acetone/water deprotection/insitu salt formation. Diester **7** (500 mg, 0.77 mmol) was dissolved in a solution of H_2O (3 mL) and acetone (3 mL). The mixture (pH: not determined) was stirred at 40 °C for 16 h to allow the solvolysis to reach completion. To this mixture (pH 2.18; prodrug 4–69 AP) was added 4 M aqueous lysine solution to adjust the pH to 4.83 and the solution heated to 55 °C. Acetone (35 mL) was added slowly into the reaction mixture over 30 min at 45–50 °C. At 45 °C, and after adding about 15 mL of the acetone, the clear solution was seeded with crystalline **4b** and stirring at this temperature was continued for 45 min. After complete addition of acetone, the solution was cooled to room temperature over ~4 h and stirred overnight to complete the crystallization of **4b**. The solid was collected by filtration and dried via suction under N_2 for 2 h. The white crystalline solid was dried under house vacuum at 50–55 °C for 24 h to afford 343 mg (93%) of **4b** with purity and characterization data similar to that obtained in the TFA procedure above.

In Vivo Studies. The studies described used the monolysine salt of **4b**, unless stated otherwise. All in vivo PK studies in rats, dogs, and monkeys were performed using aqueous solutions of prodrug for PO and IV dosing.

Human Clinical Studies with 4b. The Lysine salt of **4b** was formulated as a solution from drug in bottle and evaluated in a single ascending dosing study in healthy volunteers with cohorts of six patients each at doses 25, 50, 100, 200, 400, and 800 mg of two parent equivalents. The results from this study were compared to those from the historical dosing of **2** in a clinical capsule formulation and from a 200 mg solution dose.[15]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Detailed experimental procedures for assays, preclinical in vitro and in vivo studies, and human clinical studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

API, active pharmaceutical ingredient; AUC, area under the concentration–time curve; BCS, The Biopharmaceutics Classification System; cArt, combination antiretroviral therapy; C_{max}, maximum concentration; INF, from time zero to infinity; IV, intravenous; PO, oral

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