Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 19, pp. 2259-2262, 1996

Published by Elsevier Science Ltd
Printed in Great Britain
PII: S0960-894X(96)00391-5
0960-894X/96 $15.00 + 0.00

SYNTHESIS OF WATER SOLUBLE PRODRUG OF DMP323

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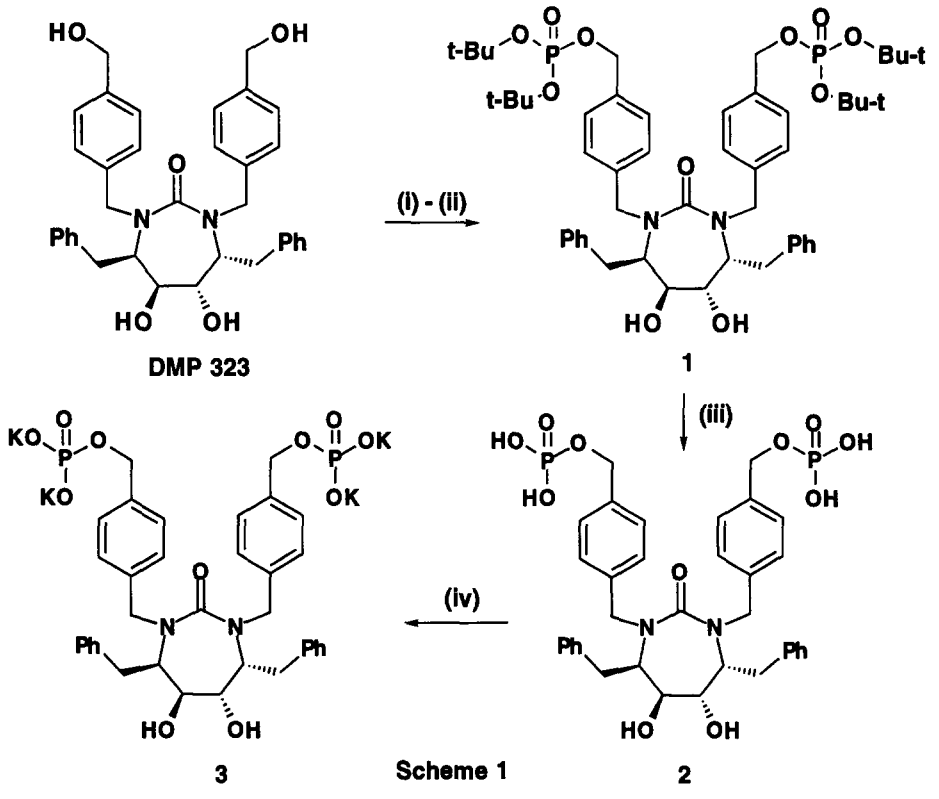
Abstract: A water soluble bisphosphate prodrug of DMP323 was synthesized from DMP323. Pharmacokinetic studies of the prodrug indicate that it is orally absorbed as the parent drug, after hydrolysis of the phosphate ester in the gastrointestinal tract.

Human immunodeficiency virus type-1 (HIV-1), the causative agent of acquired immunodeficiency syndrome (AIDS) encodes a proteinase (HIV-1 protease) that proteolytically processes the *gag* and *gag-pol* polyproteins. The action of this enzyme is essential for the proper assembly and maturation of fully infectious virions.[1-5] DMP323, the first clinical candidate from the cyclic urea class of HIV-1 protease inhibitors,[6,7] has reasonably good oral bioavailability in rats (15-27%) and dogs (37-38%) when dosed with glycol-based solutions at 3-10 mg/kg doses,[7] but at higher doses (35 mg/kg) oral bioavailability was greatly reduced. There was a negligible oral absorption in animals dosed with aqueous suspensions. DMP323 was withdrawn from phase I clinical studies due to variable bioavailability in humans. This was presumably due to precipitation of the drug from the vehicle upon dilution with the aqueous contents of the gastrointestinal tract. DMP323 has poor solubility in water (~10 μg/mL). The solubility and oral absorption of DMP323 could be improved by transforming it into a water soluble prodrug.[8b] Phosphate esters are very attractive prodrugs since they can be hydrolyzed to parent drugs by phosphatase enzymes in the gastrointestinal tract.[8]

The phosphate esters of the diol moiety are sterically hindered and may not serve effectively as prodrugs. Consequently, hydroxymethyl groups of DMP323 were chosen for attaching the phosphate ester moiety. Thus, DMP323[9] on treatment with di-*tert*-butyl *N,N*-diethylphosphoramidite[10] in the presence of tetrazole provided a phosphite ester intermediate which was oxidized in situ to the phosphate ester (**1**) in an overall yield of 78% after purification by flash chromatography. The *tert*-butyl ester groups of the phosphate ester (**1**) were slowly hydrolyzed on silica gel. Consequently, it is necessary to minimize the residence time of the product on the column during flash chromatography. Deprotection of the *tert*-butyl esters of (**1**) in refluxing methanol in the presence of Amberlyst 15 ion exchange resin provided bis dihydrogen phosphate (**2**). The phosphate intermediate (**2**) was then converted into bis dipotassium phosphate salt (**3**) by treatment with potassium bicarbonate. The phosphate prodrug of DMP323 (**3**) dissolves rapidly in water with a solubility of >100 mg/mL.



(i) $(Et)_2NP(OBu\text{-}t)_2$/tetrazole/THF/0 °C (5 min); 23 °C (15 min) (ii) m-chloroperbenzoic acid/-40 °C (5 min); 23 °C (15 min) (iii) Amberlyst 15/CH_3OH/reflux/5 h (iv) 4 equiv $KHCO_3$/THF/23 °C

One criterion that a successful prodrug must satisfy is that of its conversion to the pharmacologically active species in vivo. To test this, in vitro metabolism studies were performed with the prodrug (**3**). Fresh dog plasma and a 10% (w/v) homogenate of rat small intestine in pH 7.4 phosphate buffer were used as the media. Prodrug of DMP323 was added to these media at 37 °C, and at various times aliquots were removed and assayed for DMP323 using HPLC. Assuming that the formation of DMP323 correlates with disappearance of prodrug (**3**), we estimated that prodrug (**3**) was converted to DMP323 with half-lives of 7.8 h in dog plasma and 2.7 h in the 10% rat intestinal homogenate. Because a dilute intestine preparation was used, these results suggested that prodrug (**3**) could be fairly rapidly converted to DMP323 upon contacting the intestinal wall or during the course of its intestinal absorption. It was previously shown that a phosphate prodrug of phenytoin was stable in rat, dog, and human plasma, but was rapidly converted to phenytoin in homogenates of rat intestine or liver or dog liver.[8]

Prodrug (**3**) was administered to beagle dogs at 100 mg and 350 mg doses using hard gelatin capsules filled with only the solid drug substance. Dogs were fasted overnight before dosing. Separate dogs were dosed with DMP323 intravenously (5 mg/kg using a polyethylene glycol solution vehicle), so that oral bioavailability could be estimated. Blood samples were collected, and plasma assayed for DMP323 concentrations. This was

done by organic phase extraction and HPLC. There were 3 dogs in each group. A summary of some DMP323 pharmacokinetic properties after dosing with the prodrug is given in Table 1. DMP323 phosphate prodrug was well absorbed when administered as the neat powder. The area under the plasma concentration vs. time curve (AUC) and the maximum observed plasma concentration (C_{max}) increased in proportion to the increase of dose. The time of the maximum observed plasma concentration (t_{max}) was short, reflecting rapid absorption and conversion of the prodrug to DMP323. The extent of oral bioavailability (F %) was similar to that previously reported for low DMP323 doses administered using glycol-based solution vehicles.[7] Moreover, oral bioavailability was maintained at the higher dose. In contrast, high doses of DMP323 administered to dogs using glycol-based vehicles resulted in bioavailability <5%. Another group of dogs was similarly dosed orally with the free acid form of DMP323 phosphate (**2**). Oral bioavailability was 28.1 ± 5.1%, not different from that after dosing with the potassium salt. The phosphate prodrug thus provides considerable advantages when compared with DMP323.

Table 1. DMP323 oral bioavailability in dogs after dosing with (**3**).†

Dose (mg/kg DMP323 equiv)	7.7	27.5
DMP323 C_{max} (μg/mL)	1.0 ± 0.1	2.6 ± 0.5
DMP323 t_{max} (h)	0.5 ± 0	1.4 ± 0.7
DMP323 AUC	1.5 ± 0.2	5.2 ± 1.2
DMP323 F (%)	28.7 ± 1.9	27.2 ± 5.9

†(**3**) was dosed as solid with capsules containing (**3**). Values represent the mean ± SE of 3 dogs.

EXPERIMENTAL

[4R-(4α,5α,6β,7β)]-[Tetrahydro-5,6-dihydroxy-2-oxo-4,7-bis(phenylmethyl)-1H-1,3-diazapine-1,3(2H)-diyl]bis(methylene-4,1-phenylenemethylene)bis[bis(1,1-dimethylethyl) phosphate] (1): DMP323[9] 11.340 g (20 mmol) was dissolved in 60 mL tetrahydrofuran and cooled to 0 °C. Intermediate A 13.00 g (52 mmol) in 40 mL tetrahydrofuran was slowly added followed by addition of 7.28 g (104 mmol) of tetrazole. The contents were stirred for 5 min in the 0 °C ice bath and then for 15 min in a water bath at 23 °C. The contents were cooled in a -40 °C bath and 21.5 g (62.4 mmol) of 50% m-chloroperoxybenzoic acid in 100 mL dichloromethane added over a 10 min period and stirred for 5 min in the same bath and then 15 min in a water bath at 23 °C. The reaction mixture was cooled at 0 °C and quenched with 250 mL of 10% aqueous sodium bisulfite. The aqueous layer was extracted with ether (100 mL). The organic extracts were combined and washed with sat. sodium bicarbonate (100 mL), 10% aqueous sodium bisulfite (250 mL) and then sat. sodium bicarbonate (100 mL). The organic layer was separated and dried over magnesium sulfate and the filtrate taken to dryness. The residue was purified on silica gel using chloroform followed by 1% CH_3OH in $CHCl_3$, followed by 1.5% CH_3OH in $CHCl_3$ to provide 14.410 g (75.8% yield) of the desired phosphate ester (**1**) as a white foam. (Caution! The flash chromatography column should be done as fast as possible to avoid any decomposition of (**1**) on silica gel). ^1H NMR spectra were determined on a Varian spectrometer (300 MHz, $CDCl_3$) δ 1.44 (36H, d, J = 4 Hz), 2.97 (6H, m), 3.35 (2H, s), 3.49 (4H, m), 4.86 (2H, d, J = 8 Hz), 4.92 (4H, d, J = 14 Hz), 7.11 (10H, t, J = 8 Hz), 7.29 (8H, m); ^{31}P NMR spectra were determined on a Varian spectrometer (121 MHz, $CDCl_3$) δ -9.8 (s, decoupled).

[4R-(4α,5α,6β,7β)]-[Tetrahydro-5,6-dihydroxy-2-oxo-4,7-bis(phenylmethyl)-1H-1,3-diazepine-1,3(2H)-diyl]bis(methylene-4,1-phenylenemethylene)bis(dihydrogen phosphate) (2): The intermediate (**1**) 36 g (37.85 mmol) was dissolved in 200 mL methanol and to this solution was added 3.6 g of Amberlyst 15 ion exchange resin. The contents were stirred at reflux for 5 h. The mixture was filtered through a celite pad and concentrated to afford 27 g (98.2%) of the phosphate prodrug (**2**) as a white solid. ^{1}H NMR (DMSO-d_6) δ 2.84 (4H, m), 2.95 (2H, m), 3.34 (2H, s), 3.42 (2H, d, J = 11 Hz), 4.66 (2H, d, J = 4.2 Hz), 4.82 (4H, d, J = 6.9 Hz), 7.02 (4H, d, J = 6.9 Hz), 7.08 (4H, d, J = 7.7 Hz), 7.27 (10H, m); ^{31}P NMR (DMSO-d_6) δ -0.48 (s, decoupled).

[4R-(4α,5α,6β,7β)]-[Tetrahydro-5,6-dihydroxy-2-oxo-4,7-bis(phenylmethyl)-1H-1,3-diazepine-1,3(2H)-diyl]bis(methylene-4,1-phenylenemethylene)bis(dipotassium phosphate) (3): 2.178 g (3 mmol) of white foam (**2**) was slowly added to a stirred solution of 1.024 N potassium bicarbonate (11.71 mL, 12 mmol). After the addition was complete the solution was filtered through a sintered glass filter funnel and lyophilized to afford (**3**) as white powder (2.433 g; 92.3% yield).

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(*Received in USA* 22 *July* 1996; *accepted* 20 *August* 1996)