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Submicromolar Phosphinic Inhibitors of *E. coli* Aspartate Transcarbamoylase

Laëtitia Coudray[a], **Evan R. Kantrowitz**[b], and **Jean-Luc Montchamp**[a,*]

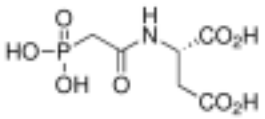
[a] Department of Chemistry, Box 298860, Texas Christian University, Fort Worth, Texas 76129, USA

[b] Department of Chemistry, Boston College, Merkert Chemistry Center, Chestnut Hill, MA 02467-3807, USA

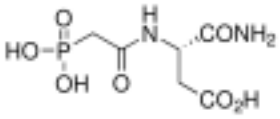
Abstract

The design, syntheses, and enzymatic activity of two submicromolar competitive inhibitors of aspartate transcarbamoylase (ATCase) are described. The phosphinate inhibitors are analogs of N-phosphonacetyl-L-aspartate (PALA) but have a reduced charge at the phosphorus moiety. The mechanistic implications are discussed in terms of a possible cyclic transition-state during enzymatic catalysis.

Since the discovery of N-phosphonacetyl-L-aspartate **1** (PALA) in 1971, and its subsequent evaluation for use in cancer chemotherapy, the inhibition of aspartate transcarbamoylase (ATCase) has received considerable attention.[1] To date, no inhibitor has been reported to be more potent than PALA.[2] Recently, based on crystallographic studies, Kantrowitz designed inhibitor **2**, which is nearly as potent as PALA, but is less highly charged due to the presence of an amide group in the aspartyl moiety.[3] To the best of our knowledge, inhibitors of ATCase, which are restricted to a monoanionic species where PALA's phosphonate group is located, have not been studied. Herein, we report the first phosphinate inhibitors **3** and **4** of ATCase. Inhibition with these phosphinates has mechanistic implications and may provide a new direction for the design of compounds with a reduced charge and improved pharmacological profiles.



1 PALA



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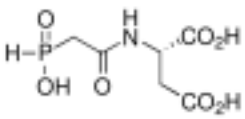

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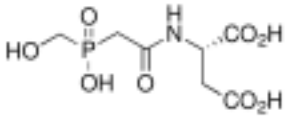

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Analyzing the role of the charge at the phosphonate moiety of **1** is interesting based on the proposal of an ordered cyclic transition-state for the proton transfer from nitrogen to oxygen (Scheme 1).[4] Additionally, *H*-phosphinate **3** could constitute a prodrug of PALA if oxidation takes place *in vivo*.

H-Phosphinate **3** was synthesized as shown in Scheme 2. Cinnamyl alcohol was converted into cinnamyl-*H*-phosphinic acid **5** using a palladium-catalyzed allylation reaction.[5] Reaction of

*Corresponding author. Tel.: +1 817 257 6201; e-mail: j.montchamp@tcu.edu.

5 with triethyl orthoacetate provided the protected intermediate **6** in excellent yield.[6] Ozonolysis of **6** to the aldehyde, followed by oxidation[7] gave **7**, which was reacted without purification with L-aspartic acid dibenzyl ester to form amide **8**. Deprotection through catalytic hydrogenation, followed by acid hydrolysis provided **3** cleanly, and in good yield after purification by ion-exchange chromatography. The possible presence of PALA was ruled out based on NMR analysis of the final product, as well as the ion-exchange purification.

Hydroxymethyl phosphinate **4** was synthesized as shown in Scheme 3. Hydroxymethyl-*H*-phosphinic acid[8] was silylated and reacted[9] with the bromoacetyl derivative of aspartic acid in a well-known Arbuzov-like reaction. Esterification with diphenyldiazomethane[10] provided **10**, which was purified by column chromatography over silica gel. Catalytic debenzylation provided the desired phosphinate **4** cleanly and in quantitative yield. No ion-exchange purification was necessary in this case.

Compounds **3** and **4**, as well as PALA, were evaluated against the catalytic subunit of ATCase which was purified from the strain/plasmid combination pEK17/EK1104 as previously described.[12] The results are shown in Table 1. Both phosphinates are weaker inhibitors than PALA by one to two orders of magnitude (12 – 26 fold). This is not unexpected if ATCase binds the phosphate dianion as suggested in Scheme 1. However, the loss in inhibition observed with the monoanionic phosphorus moieties of **3** and **4**, is perhaps not as pronounced as what such a profound modification would entail. For example, in the case of 3-dehydroquinate synthase (DHQ synthase), which is an example of an enzyme exploiting its substrate charged state for catalytic gain, a similar modification results in a three to four orders of magnitude loss in inhibition.[13] Carboxylate **11** was synthesized (Scheme 4) and evaluated in order to establish if simply providing a single negative charge is responsible for the inhibition observed with **3** and **4**. Compound **11** was completely inactive against ATCase, thus confirming the importance of the phosphorus geometry for inhibition with the phosphinate inhibitors.

Interestingly, *H*-phosphinate **3** might also be oxidized *in vivo* to PALA thus providing a potential prodrug of the anticancer compound.[6a, 14] In fact, there is some precedent for the oxidation of *H*-phosphinate compounds into the corresponding phosphonates: for example, 3-aminopropyl-*H*-phosphinate (CGP 27492), a potent $GABA_B$ receptor agonist, is oxidized to a significant extent when administered to rats.[6a] In terms of inhibitor design, the possibility of synthesizing cyclic phosphinate inhibitors to mimic the transition-state shown in Scheme 1 should be investigated. Even with an initial loss in binding affinity, the resulting transition-state analogs might lead to potent time-dependent inhibition.

In conclusion, we have synthesized the first phosphinate inhibitors of ATCase. Although the observed inhibition constants are consistent with the enzyme initially binding to the phosphate dianion of carbamyl phosphate, inhibitors **3** and **4** show significant potency. In fact, these appear to be the most potent non-phosphonate inhibitors of ATCase. Additionally, *H*-phosphinate **3** could function as an oxidatively activated PALA prodrug. Implementing the charge reduction simultaneously in both the phosphorus moiety as in **3** and **4**, and in the aspartate moiety as in **2** might result in useful inhibition while decreasing the overall charge of PALA. This, and cyclic inhibitors to mimic the postulated transition-state, will be the object of future studies.

Acknowledgments

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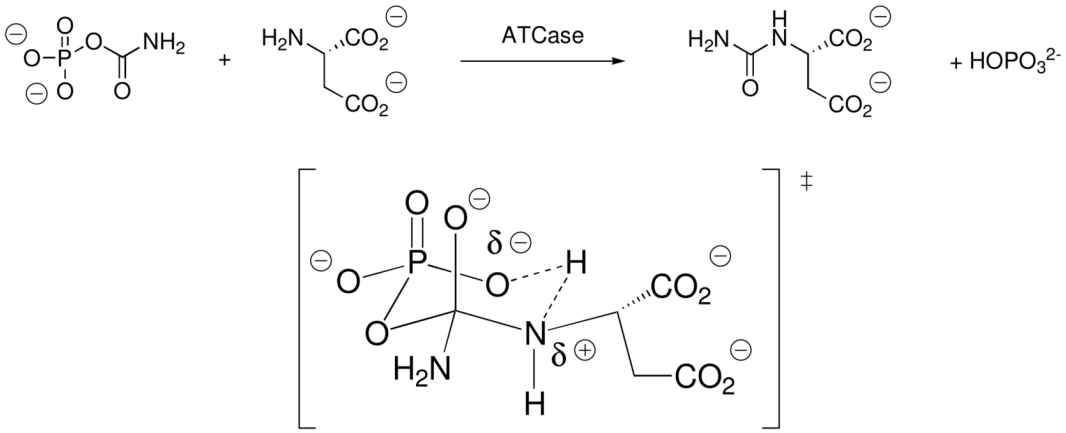
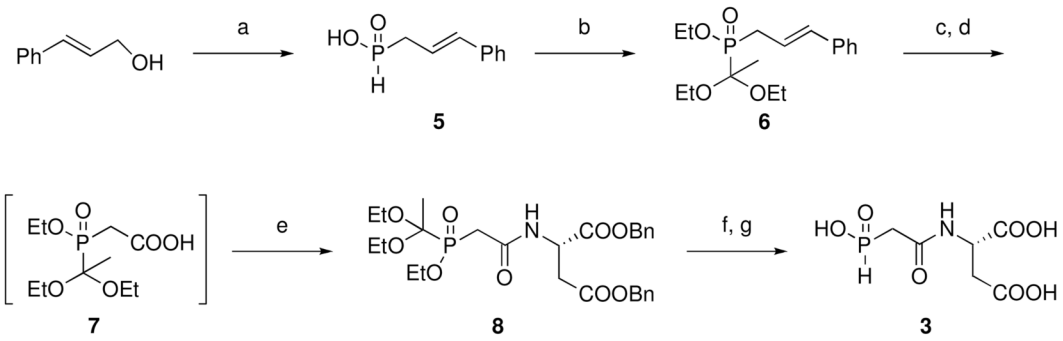
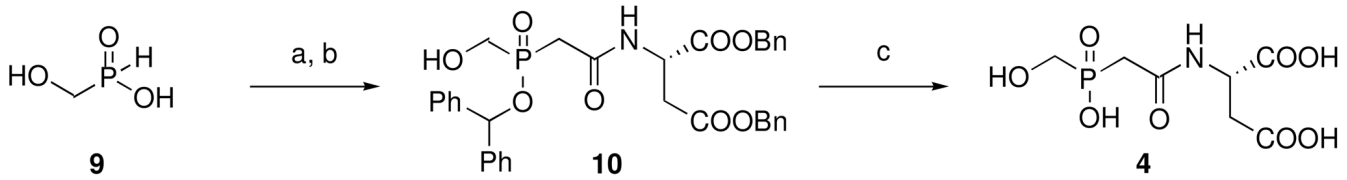


Scheme 1.
ATCase-catalyzed transformation and postulated transition-state



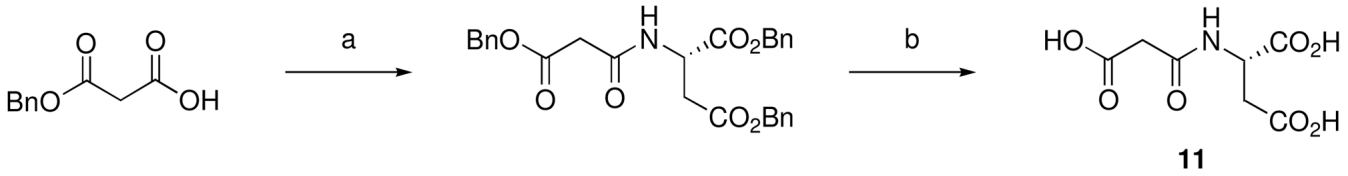
Scheme 2.

Reagents and conditions: (a) H_3PO_2 (2.0 equiv.), $Pd(OAc)_2$ (0.2 mol %), xantphos (0.22 mol %), DMF, 85 °C, N_2, 7 h, 95 %; (b) $CH_3C(OEt)_3$ (6.0 equiv.), BF_3•OEt_2 (0.16 equiv.), rt, N_2, 24 h, 80 %; (c) O_3, CH_2Cl_2, −78 °C then Me_2S (6.8 equiv.), −78 °C to rt, N_2, 14 h; (d) $NaClO_2$ (1.5 equiv.), $NaH_2PO_4.H_2O$ (1.5 equiv.), 2-methyl-2-butene (2.0 equiv.), *t*BuOH/H_2O, 0 °C then rt, 1 h; (e) L-aspartic acid dibenzyl ester *p*-toluenesulfonate (1.8 equiv.), DMAP (2.5 equiv.), EDC•HCl (3.5 equiv.), Et_3N (2.0 equiv.), THF, rt, N_2, 16 h, 70 % from **6**; (f) H_2, Pd/C, THF/H_2O, 17 h; (g) Amberlite IR 120 plus, THF/H_2O, 80 °C, 15 h, 57 % from **8** after ion exchange chromatography.



Scheme 3.

Reagents and conditions: (a) *N*-(bromoacetyl)-L-aspartic acid dibenzyl ester (1.0 equiv.), HMDS (2.5 equiv.), TMSCl (2.5 equiv.), toluene, reflux, 14 h; (b) Ph_2CN_2, toluene, rt, 10 min, 50 % from **9**; (c) H_2, Pd/C, THF/H_2O, 24 h, 100 %.



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Scheme 4.

Reagents and conditions: (a) L-aspartic acid dibenzyl ester *p*-toluenesulfonate (1.0 equiv.), DMAP (2.5 equiv.), EDC•HCl (2.5 equiv.), Et_3N (1.1 equiv.), THF, rt, N_2, 5 h, 78 % from **6**; (b) H_2, Pd/C, THF/H_2O, 24 h, 83%.

Table 1

[a] Inhibition constants for PALA and phosphinates **3** and **4**

Inhibitor	Inhibition Constant (K_i)[b]	Inhibition Type
PALA **1**	16 ± 2 nM	competitive
H-phosphinate **3**	417 ± 85 nM	competitive
Hydroxymethylphosphinate **4**	193 ± 32 nM	competitive

[a] Colorimetric assay detecting the formation of *N*-carbamoyl-L-aspartate.[11] The *E. coli* catalytic subunit of ATCase was used.

[b] Inhibition relative to carbamyl phosphate K_m = 28 ± 7 μM.