Covid-19 Drug Design via Quantum Mechanical Principles Yielded a New Corona-SARS Anti-Viral Candidate 2-Phosphono-Benzoic-Acid: Synthetic Methods for Production

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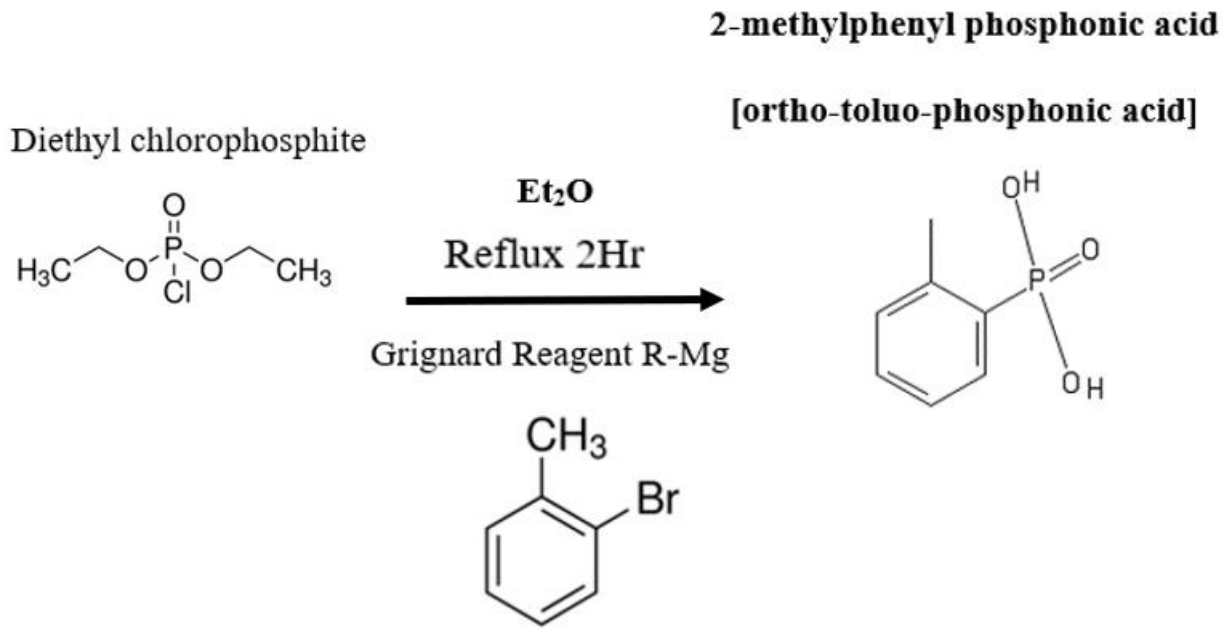
Background

The prior paper(s) on the new Corona-SARS Anti-Viral Candidate discussed principles and Proof Of Principle approaches in the Quantum Electrodynamics of non-conventional drug design with a turnaround time for the target disease measured in days, not years. This represents a breakthrough in Rational Drug Design by orders of magnitude efficiency in dealing with Pandemic and Epidemic scenarios. Rather than the hit and miss approach in Structural Assignment Relationships and consequent lengthy [measured in years] process of determining efficacy, toxicity, and so on, the direct principles of Quantum Electrodynamics, not meaning to include conventional Computational Chemistry approaches, a single compound of maximized parameters bubbles to the top. Many of the basic features of the descriptions of exacting mechanisms of action, complete with CG imaging of Polymerase Total Binding leading to complete inhibition, selectively, of DNA/RNA viruses was discussed in the prior paper.[Covid-19 Drug Design via Quantum Mechanical Principles Leads to a New Corona-SARS Anti- Viral Candidate 2-Phosphono-Benzoic-Acid April 2020 DOI: 10.13140/RG.2.2.17515.28962/1]. Since the turnaround time in novel drug design exceeded expectation against classic Structural Assignment Relationships which normally have a turnaround time of about 3 to 5-years as a shotgun approach with several concurrent analogues in the pipeline: The total development time for the 2-phosphonobenzoic acid was three days. Amidst numerous requests for a synthetic route to the otherwise very difficult ortho substitution on an aryl motif for a phosphonic acid, this paper will focus on the synthetic route to ortho-phosphonobenzoic acid in a straightforward, inexpensive, high yield, 2-step reaction. The total synthesis can be done in a day. Some summary of the two prior papers on the drug design will follow as an Appendix in this paper, with a few clarifications, as there were additional questions regarding some of the Quantum Electrodynamic approaches; wherein I will again avoid the transcendental maths and simplify the descriptions suitable for chemists and biochemists.

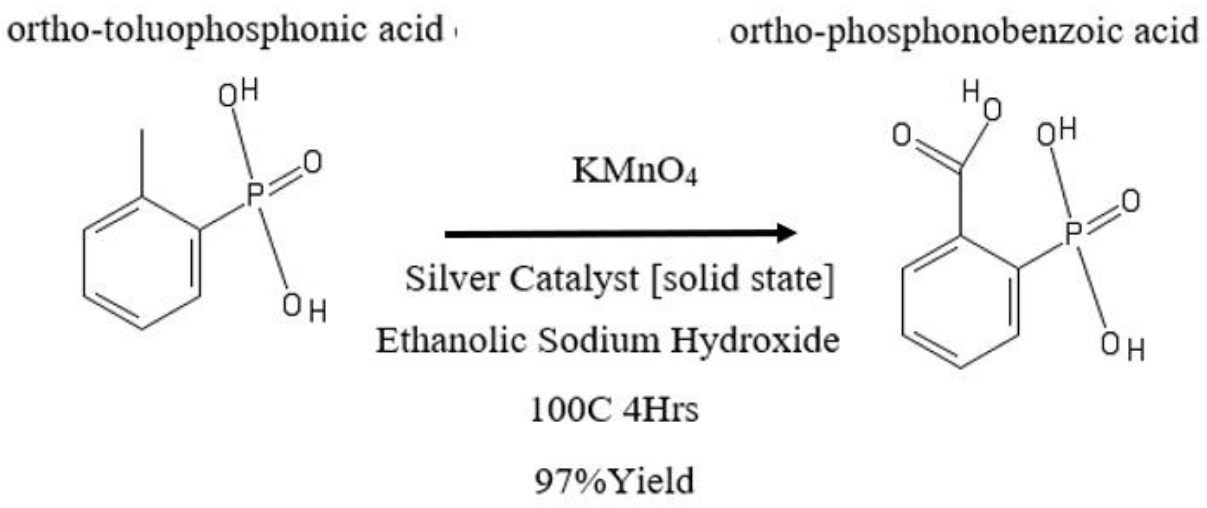
Synthetic Methods

2-Tolylphosphonic Acid (10, Ar1 = 2-Tol); Typical Procedure Using a Grignard Reagent:[65]

A solution of diethyl chlorophosphite (17.2 g, 0.10 mol) in Et2O (50 mL) was slowly added to a stirred solution of the Grignard reagent prepared from Mg (2.4 g, 0.1 mol) and 2-TolBr (18.8 g, 0.11 mol) in Et2O (75 mL) under an atmosphere of dry N2. Reflux began after approximately half of the phosphate had been added and continued for 15 min after addition had been completed. After refluxing for a further 2 h, the mixture was cooled and poured onto a mixture of crushed ice and diluted HCl. The aqueous layer was separated and washed with Et2O (2 ×), and the ethereal extracts were added to the organic layer and the whole dried (Na2SO4). Evaporation of the solvent afforded an oil, which was distilled to give the phosphonate ester; yield: 11.5 g (50%); bp 148–150°C/14 Torr. The oil was stirred in concentrated HCl (50 mL) and the mixture was then concentrated to give a syrup from which crystals of the acid precipitated; yield: 50%; mp 139–141°C.



A simple route from the ortho-tolyl-phosphonic acid to the ortho-phosphonobenzoic acid seems to have evaded common thinking, however, the reaction is a single step. A solution of saturated ethanolic sodium hydroxide is prepared via heating, stirring, sonication, essentially beating on the solution for several hours, then the ethanolic sodium hydroxide is decanted off. It is essential that the silver catalyst is a solid, surface, not a salt, and free of oxidation. A few links from a silver bracelet actually works quite well with high yields. Excess potassium permanganate and the 2-methylphenylphgosphonic acid are placed in a reaction vessel, together in the ethanolic sodium hydroxide and heated to 90C with stirring for 4 hours. Passing the *hot solution* through a paper filter clears it of permanganate and allowed to dry. Yield 95 to 97%.



If for some reason the reaction does not produce the desired yield it is likely the condition of the silver catalyst which is remedied by *sanding it free* of surface oxidation and the temperature raised significantly above 90C on the second try.

Appendix Discussion:

Background Quantum Electrodynamic Drug Design

Much of this section is a repeat of the original [April] paper with a few clarifications as requested. Covid-19 Anti-Viral activity is modeled for a new, small molecule, Ortho-phosphono-benzoic acid [2-phosphono-benzoic acid; 2-PBA], which is the aryl motif of the aliphatic phosphonic acids Foscarnet and Fosfonet. Tan et al demonstrated in 2004 that phosphonoformic acid [as Foscarnet] is potent and efficacious in inhibiting SARS Coronavirus [1]. Included in the appendix is a very long list of Viral and Cancer types that phosphonoformic and acetic acids have high efficacy in combating. It can be said that the phosphonic acids have the widest utility range on DNA/RNA Polymerase inhibition in Anti-Viral and Anti-Cancer regimens.

The aryl structure of 2-PBA has less [predicted] nephrotoxicity, which is the limiting factor in phosphonic acids for both amount and duration of dosing. In addition, the effective pKa *for the desired ionization species* is greater than two orders of magnitude in favor of 2-PBA over that of phosphonoformic acid, which has demonstrated global Anti-Viral activity in DNA/RNA viruses, including Corona-Covid [Cov] viruses.

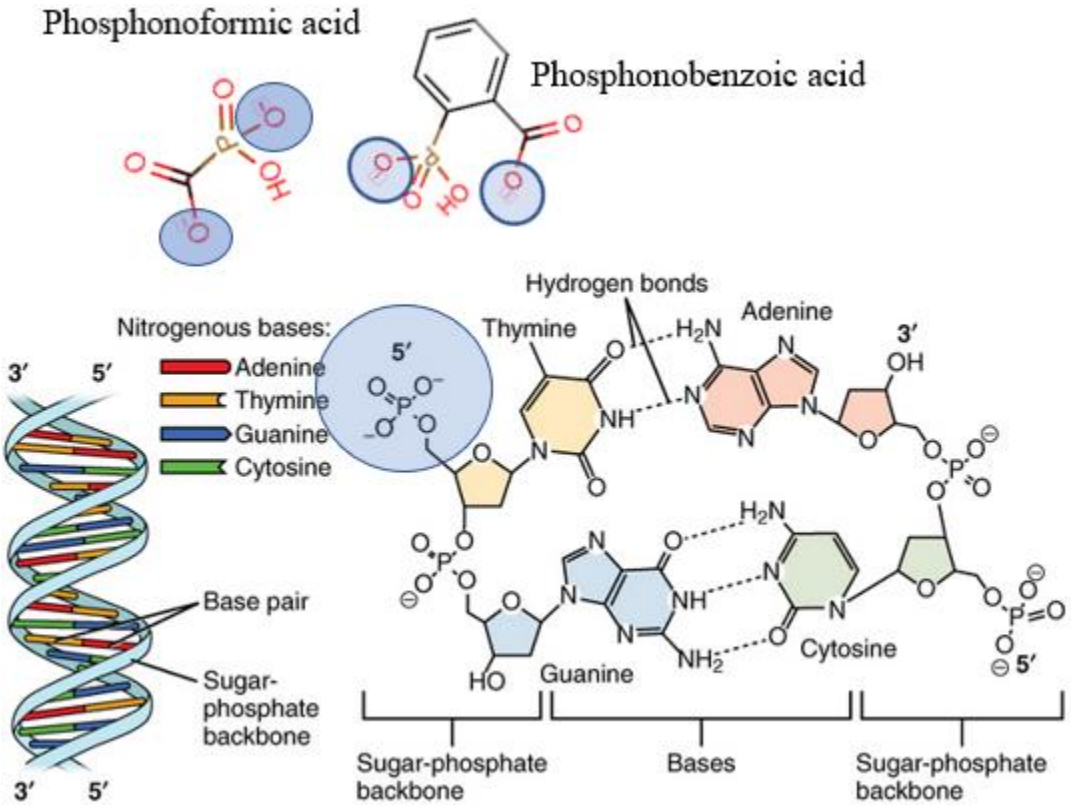
It is in particular the unique characterization of the C-P bond that yields the properties of the phosphonic acids as polymerase inhibitors. Where phosphonoformic acid [Foscarnet] shows high efficacy and selectivity for viral and cancer target cells, it is quite limited by the formic acid motif, in being an aliphatic has considerable nephrotoxicity. This is due to the metabolism to formic acids. In addition to enhancing the chemical [and thus Anti-Viral] properties, the aromatic structure of 2-Phosphono-Benzoic Acid drops off in nephrotoxicity, cytotoxicity, and so on by 2-orders of

magnitude. It is the P450 cleavage of the phosphonoformic [Foscarnet] and phosphonoacetic [Fosfonet] that yields formic acid, as the direct source of the toxicity issues for these aliphatic motifs of the phosphonic acids. By making the compound an aromatic with minimal substitutions the cleaved products are essentially non-toxic. The only metabolites are benzoic acid and phosphate ion.

It is this toxicity associated with the aliphatics [phosphono formic and acetic acids] that limits the utility and number of indications for Foscarnet and Fosfonet; essentially limited to HIV *salvage therapy* as a last line of Anti-Viral Defense; which is another indication of their unique Anti-Viral Potency. Given the aromatic structure for [2] Ortho-phosphonobenzoic acid, there is no such limitations, allowing the drug to be delivered in high dosages over indefinite periods of time.

There is also a structural, steric issue between phosphonoformic vs acetic acids in purely bond length from one end of the carboxylic acid group to the key C-P bond. pK may not be the exacting factor that determines the formic motif's greater affinity for the pyrophosphate leaving group. We look at some basic chemistry.

The effective pK's of phosphonoformic acid *at biological pH* is ~2, and phosphonobenzoic acid ~0.1 favoring the 2-PBA over that of PFA by one to two orders of magnitude in this induction driven reaction:



The activity of phosphonoformic acid over that of pyrophosphates is not related to the phosphate end structure, but rather obviously the key is the C-P bond. Zahn et al [11] determined that the

aliphatic motif phosphonoformic acid fits in directly bonding to the metal chelate(s) ions and the end charges on the Polymerase enzyme, the 3D imaging is shown further on. Thus, the phosphonic acids mode of inhibition is direct bonding to the enzyme site, as well as chelating the metal cations [and anions] that are necessary for transcription. It is a unique mode of action from any other Anti-Viral drug.

The Aromatic Effect of placing the phosphonic group in an ortho [2] position on the aromatic ring from the carboxylic acid group in fact increases the Electrodynamics and activity of the compound by two orders of magnitude over that of Foscarnet [phosphonoformic acid], which is proven effective against earlier strains of Corona-SARS in 2004. This, in addition to the lack of nephrotoxicity makes 2-Phosphonobenzoic acid a prime candidate as a drug in the treatment of Covid-SARS (19), which is the immediate goal.

The current mode is to treat the compound as an 'Open Access' compound for immediate investigation. Like aspirin, anyone can produce and distribute the drug, but not hold a patent on it; as there is significant prior art [here] that defines the drug rather completely. Nonetheless, if commerce can be gathered from it, so be it.

The compound is delivered as the tri-sodium salt in order to prevent hydrogen bonding. In a biological system, at pH 7.2 the ionization species of 2-PBA has no hydrogen bonding steric structure.

It is as a phosphonic acid a DNA/RNA Polymerase Inhibitor. The phosphonic acids have demonstrated great selectivity in Anti-Viral activity vs cellular DNA activity for a variety of viral classes and types. Tan et al demonstrated in 2004 that phosphonoformic acid [as Foscarnet] is potent and efficacious in inhibiting SARS Coronavirus [1]. The list of target diseases includes HSV, cytomegalovirus, HIV, and several other viral classes and also includes a wide variety of systemic cancers, carcinoma, sarcoma, melanoma, lymphoma, and leukemia. Because ortho-phosphono-benzoic acid is an aromatic rather than aliphatic the nephrotoxicity is negligible, where this is in fact the limiting factor in aliphatic phosphonic acids. This factor, taken together with the two-order magnitude [calculated] increase in efficacy regards ortho-phosphono-benzoic acid as an extremely worthwhile candidate for further investigation.

It should also be noted that ortho-phosphonobenzoic acid is highly insoluble in organic solvents such as DMSO, which is common to most high throughput in vitro assays for determining activity, as well as several other organic solvents. It is at the time of yield essentially only a water-soluble compound, making the necessitation for *in vivo assay* essential in determining activity for any penchant of viral or cancer motifs. Assays of the cell culture type, however, are invariably aqueous and therefore suited for investigating the drug's efficacy.

In addition, the water solubility of the ortho-phosphonobenzoic acid is a strong indication of a higher activity in the sense of P_k, Log_p, and strong Electrodynamic effects.

202.0 Da; **<u>At pH 7.2:</u>** LogD: -6 LogP: -0.006 LogS [solubility] 2.0 [high, saturated] pI -2.8

Bioavailability of *the oral dose* was determined to be about 17% [21]. The use of i.v. drips has been limited by the *quantities* of Foscarnet necessary, on the 1000mg dosing scale. This is a major

factor in the nephrotoxicity, 2000mg i.v. drip per day. This would certainly be limited to about 100-200mg per [calculated] 8-hours for the 2PBA motif. In addition, again, lacking the nephrotoxicity limitations, the duration of dosing a patient can tolerate has no such bound.

In general, one can regard the London forces along an aliphatic as a strong determining factor with respect to the PO_3H_2 substitute at the end of the carbon chain. Basic Structural Assignment Relationships [SAR] in Medicinal Chemistry are built upon this principle, as well as the aromatic effect of para to meta to ortho substitution around the aryl structure. Because the C-P bond is more electrodynamic by an order of magnitude over that of a C-O-P bond, it is this unique property of phosphonic acids that favors total blockade of the phosphate linkages in a DNA/RNA polymerase reaction. In addition, there is structural chelating of metal ions specific to the polymerase reaction. Because a C-P bond does not have an electrodynamic well afforded by the oxygen atom, the phosphonic acids can take on either/both electron donating-withdrawing depending on the environmental conditions. In a biological system at biological pH this is exceptionally more so, and in turn the 'environment' is afforded by the electrodynamics of the endpoints of the polymerase enzymes, along with the metal ions that catalyze the biological reactions. This is imaged in the later section of this paper.

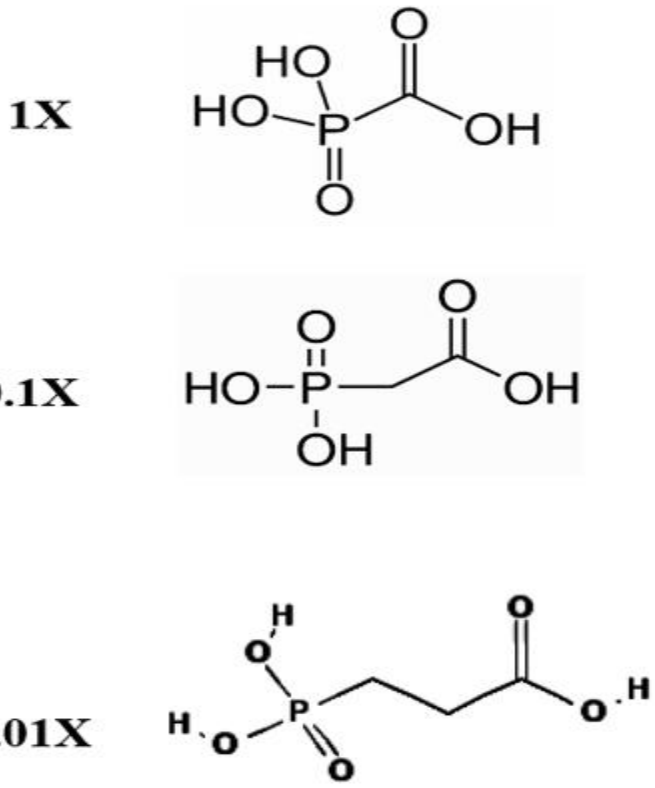
Phosphonoformic acid belongs to a class of antiviral drugs that inhibit the human cytomegalovirus DNA polymerase (UL54) by mimicking the pyrophosphate leaving group of the nucleotide transfer reaction. [11]. This occurs at the PP(i) mimic and two basic residues absolutely conserved in the fingers domain of family B polymerases. PFA also chelates metal ion B, the cation that contacts the triphosphate tail of the incoming nucleotide.

It is the ElectroDynamics of the C-P bond as opposed to the C-O-P, where we have the oxygen functioning as a stopgap, an electron well of ElectroDynamic Entropy. The affinity for the leaving group is based on this enhancement over the naturally occurring motif. In general, everything in nature is made of the least possible changes in enthalpies. C-P bonds are essentially impossible to make without metal catalysts. This *can happen* in some extremophiles [archaea], but not in a virus cell.

As we back off to two carbons from the C-P bond, phosphonoacetic acid has much less affinity for these active sites. Given the mechanism on the gene coding it is therefore rational to conclude that the active site is the C-P bond, and that the Electronegative state of that electron donating-withdrawing balance, as indicated by metal chelation, is the means to increase DNA/RNA Polymerase inhibition. In general, phosphonic acids resonate between electron donating and withdrawing based on the environmental conditions. They have extremely negative DLogP's, indicating that they are essentially a water bound substance. However, again, this water solubility characteristic makes them ideally bioavailable. They would therefore also be rapidly cleared from body tissues and fluids. Thus, iv dripping is typical for phosphonic acid Anti-Virals.

Classic organic chemistry indicates that placing the C-P bond ortho to the carboxylic acid group increases the pi activity, both electron donating and withdrawing effects, which are adjusted initially by pH. The inductive effect of the aromatic ring stabilizes the Lewis activities of the Electrodynamically active sites. Hydrogen bonding is not a problem in a trisodium salt system,

which inhibits steric effects. Thus, we see the Ortho-Phosphonobenzoic acid as ideally suited for the inhibition of the pyrophosphate leaving group of DNA/RNA Polymerases as well as stronger metal chelation by perhaps one or two pH points, again, two orders of magnitude. The lack of nephrotoxicity allows for much higher and prolonged dosing. This is because, again, the C-P bond has an order of magnitude greater Electrodynamic affinity for the polymerase endpoint ions over that of the C-O-P bond, which has the electrodynamic well of the oxygen mediating charge transfer, rendering it a one-way process; inductive withdrawing effects are classically oxygen directed. Phosphorous can form strong complexes with metals, however, oxygen attenuates this, which is not the case in a C-P bond [phosphonic acids].



Given the target diseases for Foscarnet [formic motif], Fosfonet, [acetic motif], and propionic motifs cover a broad range of hundreds of viral types as well as cancer types, a detailed listing is not practical here for the sake of length constraints. In addition, these aliphatic phosphonates are in general, soluble in organic solvents used in in vitro assays, where the arylPO_3H_2 type will not be soluble in solvents typical to in vitro assays. Aggregation is also an issue observed with ortho-phosphonobenzoic acid when even small amounts of organic solvents are present. This phenomenon is observed even during synthesis, the compound can be seen visually dropping out of solution to an aggregate as it forms in the heated ethanolic sodium hydroxide. However, filtered and transferred to water dissolves instantly without agitation.

Structural Assignment Relationships

Phosphonoformic acid, Foscarnet, is the formic aliphatic, phosphonoacetic acid goes under the name Fosfonet, is the 2-carbon [acetic] motif, the 3-carbon phosphonopropionic acid has no established trade name but is a sub-potent inhibitor of DNA/RNA polymerase. As one would expect from the basic electrodynamics, the activity of the phosphonic aliphatics diminishes with increasing carbon chain length:

This is a generalized scheme that is true across the activity of the aliphatic motifs of phosphonic acids and their inhibition of DNA/RNA polymerase in viral models and also holds true for the oncological [anti-cancer] applications that the aliphatic phosphonates have been applied against in treatment. The activity drops roughly tenfold, as one would expect, with each increasing carbon on the aliphatic chain.

Although Nagarajan et al [3] noted that ortho substitution on an aryl phosphonic system resulted in steric hindrance of electron donating character, as did Jaffe [4], neither investigated ortho-substitution of a carboxylic acid group to the phosphonic motif. The reason is that the synthetic route shown on the previous page was not available. The simple surface chemistry approach of using solid phase silver catalyst hadn't occurred to the authors at the time. More importantly Jaffe (1955) based his associations on a limited variety of chloro, bromo, and methoxy, which under such conditions are only weakly inductive. An important feature is the aryl motif of 2-carboxyphosphonic acid should result in significantly less nephrotoxicity.

In general, phosphonates resonate between electron donating and withdrawing depending on various factors. The problem to date with a compound such as ortho-phosphonobenzoic acid has been a chemical pathway. The successful and high yield chemistry of ortho-toluophosphonic acid with potassium permanganate in ethanolic sodium hydroxide in the presence of solid-state silver catalyst at 97% yield negates the problem of a chemical route. Ortho-toluophosphonic acid is rare but not difficult to synthesize, several pathways are available throughout the literature.

DNA/RNA Polymerase Inhibitor. Highly selective with respect to target vs cellular polymerase. Oberg [6] notes that the range, variety, and efficacy of phosphonoformic over that of phosphonoacetic are catalogued. In general, the acetic [2-carbon] motif [phosphonoacetic acid, Fosfonet] has less efficacy, and a much narrower range of viral targets than phosphonoformic acid [Foscarnet; 1-carbon]. The key is that phosphonopropionic acid, the 3-carbon motif, has little if any efficacy in viral handling. We see the general scheme in efficacy in the image a few pages back where th4e efficacy relationship in the Structural Assignment Relationship takes the form:

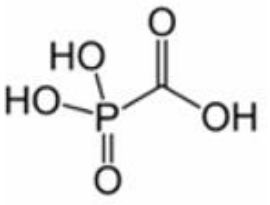
Structural Assignment Relationship **Efficacy** Rating:

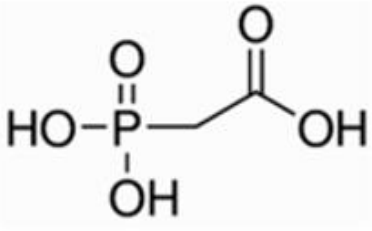
- 1-carbon C-P bond phosphonoformic acid baseline of 1X
- 2-carbon C-P bond phosphonoacetic acid 0.1X
- 3-carbon C-P bond phosphonopropionic acid 0.01X

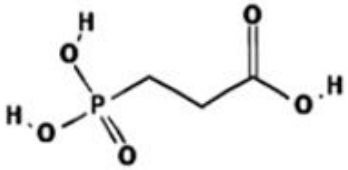
Structural Assignment Relationship **Toxicity** Rating:

- 1-carbon C-P bond phosphonoformic acid baseline of 1X
- 2-carbon C-P bond phosphonoacetic acid 0.1X
- 3-carbon C-P bond phosphonopropionic acid 0.01X

Note that the toxicity of the aliphatics are directly related to the formic, acetic, propionic motifs that are liberated as early as gut pH and rather deliberately by P450. This is a very rapid degradation, in turn requiring greater dosing and more frequently so. The efficacy is in fact limited by this rapid turnover to metabolites.



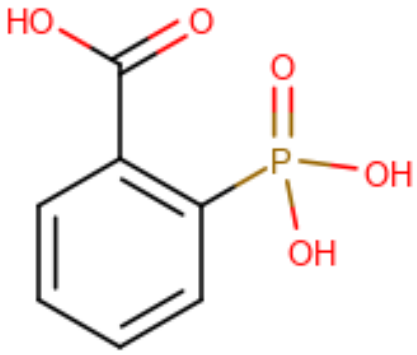




1X **0.1X** **0.01X**

Again, the primary limiting factor in phosphonic acids is the nephrotoxicity in an acute clinical setting limits the dosing and duration of dosing a patient can tolerate. This is primarily due to the aliphatic nature and structure of the phosphonic acids currently in use. The sections on Indications shows a vast spectrum of Anti-Viral, Anti-Bacterial, and Anti-Cancer applications that the aliphatic phosphonic acids have a high degree of efficacy for. However, this limitation of how much a patient can be titrated with a phosphonic acid in both dosing and duration of dosing is the key factor that limits their use. Phosphonoformic acid is typically regarded as a 'salvage therapy,' for example in refractive recurring cancer second round treatments, such as Refractory Ovarian Cancer and HIV in its full-blown AIDS stage of development.

The rather obvious next step is to place the phosphonic and acid groups ortho to one another on a benzene ring:



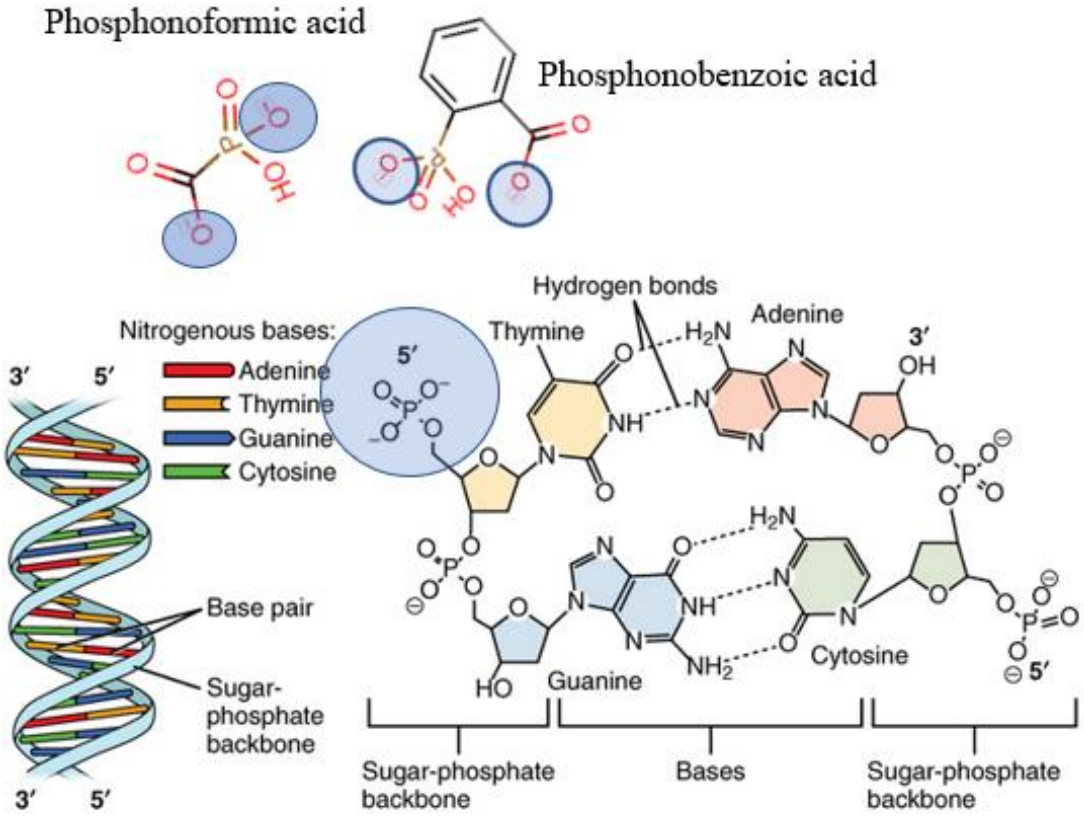
The aryl structure in Structural Assignment Relationships puts this down into the negligible domain in toxicity, as well as being more difficult to eliminate so quickly. Thus, much higher dosing for vastly prolonged and even indefinite periods of time can be titrated to the patient without concern. This opens the vast, wide spectrum of Anti-Viral, Anti-Bacterial, and Anti-Cancer applications for the phosphonic acids to be utilized in treatments of the host of applications where aliphatic phosphonic acids cannot.

Effective pKa

pKa [and pKb] is often the defining factor in a drug substance's efficacy. pKa is a function that defines ionization, the Electrodynamics, even solubility, as LogP, LogD, and so on. Effective pKa takes into account the biological pH, which is the only relevant pH [~7.2] and the ionization form, the ionization species at biological pH.

Only the ion form of interest is relevant, and only at biological pH. Characteristically, most pKa algorithms only report a max charge at max pH, which is misleading and incorrect information.

First, we reiterate: Phosphonoformic acid belongs to a class of antiviral drugs that inhibit the human cytomegalovirus DNA polymerase (UL54) *by mimicking the pyrophosphate leaving group of the nucleotide transfer reaction.* [11]. This occurs at the PP(i) mimic and two basic residues absolutely conserved in the fingers domain of family B polymerases. PFA also chelates metal ion B, the cation that contacts the triphosphate tail of the incoming nucleotide:



The ionization state and species of the phosphonic acids has to be suitable to fulfill the diagram above: Mimicking the phosphate leaving group of the nucleotide, as per the actual visualization by [11] via X-ray crystallography and TEM. In addition, there is a metal ion chelating characteristic that is estuary to the nucleotide's resequencing during replication, and that ionization state and species of the phosphonic acid *is different* from that species that mimics the phosphate leaving group.

This is basic Quantum Chemistry in the classic Quantum Mechanics sense that has not been addressed in the school of Rational Drug Design, but completely overlooked, in all likelihood because the math is sublime, even transcendent for a chemist. The approach of Structural Assignment Relationships in this way, which is the correct approach, simply do not occur in drug development, the 'hit and miss' approach of moving functional groups around rung structures is employed instead. This is why any-one drug company has millions of compounds in its compound libraries. Combinatorial Chemistry was the epitome of this failure.

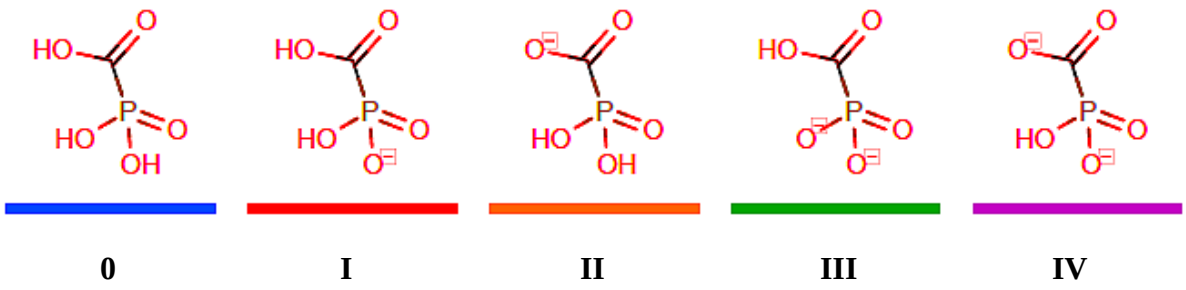
The calculated pKa for 2-phosphonobenzoic acid is 1.37. Phosphonoformic acid pKa -0.1 ***However,*** these are the generalized sum of ionization at max K; which is:

1. Not at biological pH and therefore irrelevant information.
2. Of the 4-ionization species, not the desired ionization state nor species.

Ortho-phosphono-benzoic acid [2-phosphono-benzoic acid] is a phosphonic acid [salt] that is derived and based on the Electrodynamics of efficacy based on activity vs carbon chain length of analogs of phosphonoformic acid [Foscarnet] to phosphonoacetic, phosphonopropionic acids as anti-viral drugs. The electron withdrawing effect of the carboxylic acid at some carbon number on an aliphatic formic, then acetic, and so on is interdependent with the C-P bond of the phosphonic group as the seat of activity for phosphonoformic acid [Foscarnet]. The addition of another carbon to phosphonoacetic [Fosfonet] drops off an order of magnitude, then so on to propionic, etc.

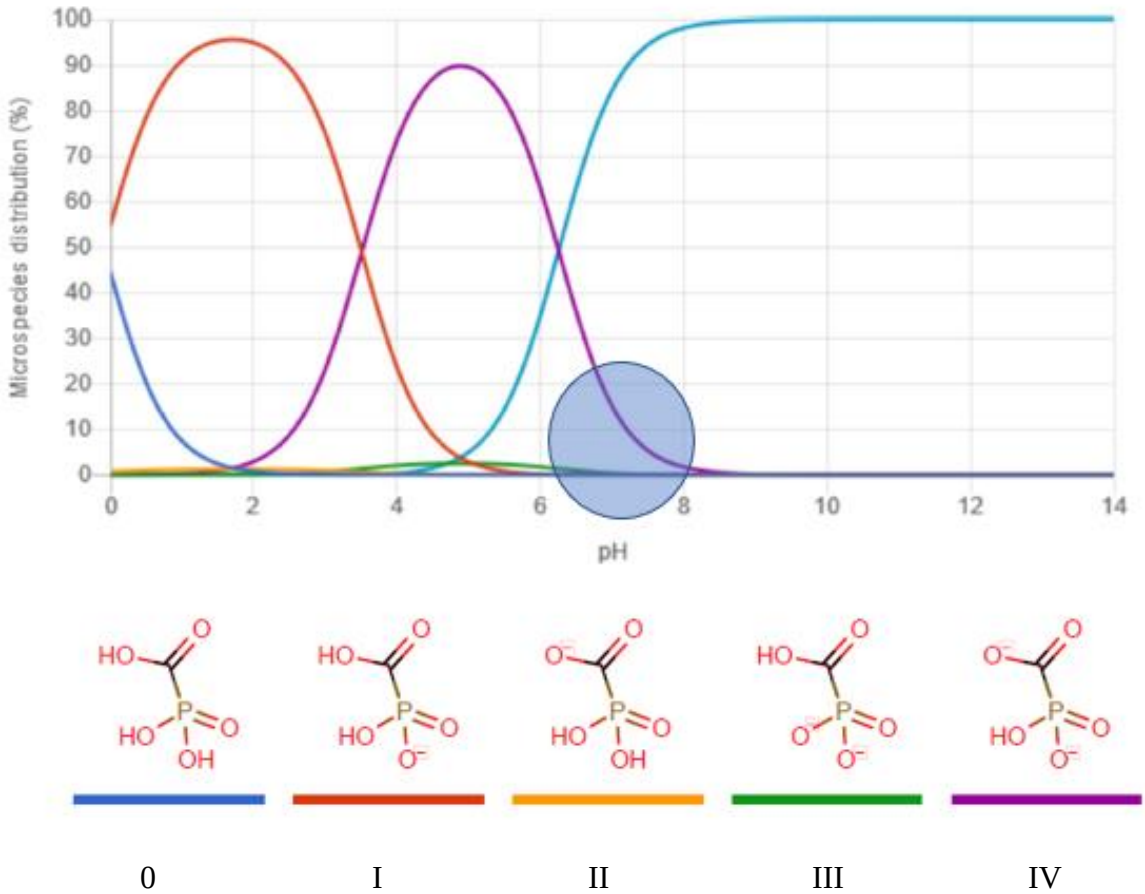
The reason the C-P bond does what it does is because unlike a C-O-P bond, where the oxygen functions as a [literal] *capacitance*, it is a well, a drop that favors a unidirectional flow of Electrodynamic energy. The C-P bond *easily resonates* equally in both directions according to the conditions, even in the subtlety of change that occurs in proximal relationship to another ion, in this case, the phosphate leaving group of the nucleotide during replication in an Electric environment described completely by biological pH. This is why phosphonoformic acid has such a vast range of indications. Phosphonoformic acid is however limited because of 1) size, steric issues, 2) nephrotoxicity limits both dosing and duration of use. Ortho-phosphonobenzoic acid is the correction to this.

As a general rule of thumb going from an aliphatic to an ortho-substituted [aryl] electron-withdrawing-donating groups is a 2-order of magnitude increase in pKa. However, 1) phosphonic acids have *4 PKa's* as a Permutation of ionized states:



2) the resonant forms from electron donating to withdrawing characteristic of phosphonic acids does not follow this rule for this reason 3) hydrogen bonding on an aryl structure may have a steric effect on pK. 4) the *Effective pK* is the p*K*, the degree of ionized species for the desired ionization state at biological pH; no other ionization state nor pH is of relevance.

The key is in understanding the most fundamental aspects of how compounds, particularly diprotic species, ionize. In the phosphate leaving group [Zahn et al 2011] we need to see form IV above in mimicking the triphosphate leaving group of the nucleotide. At biological pH, phosphonoformic acid must be in form IV so as to mimic a triphosphate leaving group of a nucleotide. However, in this modeling [Chemaxon, Chemicalize, similar to ACD] we can see that the microspecies, form IV, is nearly absent:



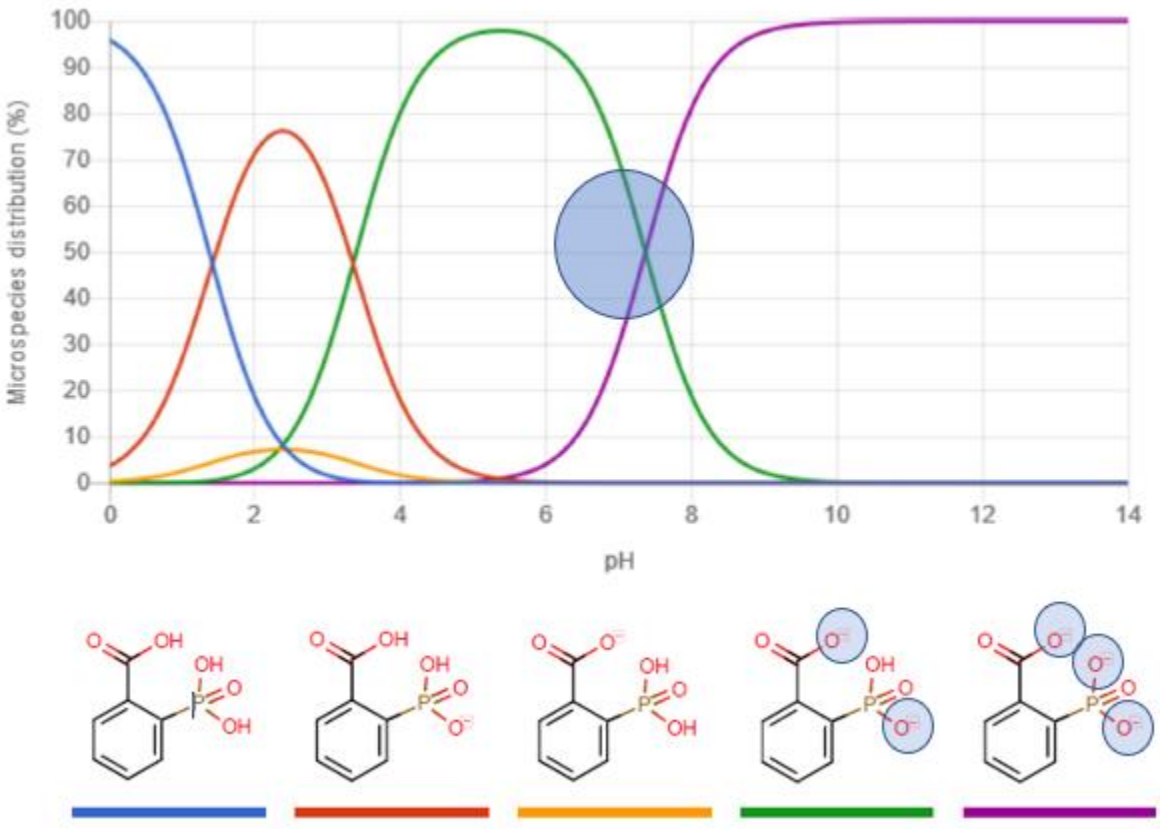
0 I II III IV

The diprotic species is only present in the tiniest fraction of a percent at biological pH. In fact, at biological pH, the best is 10% ionization for species IV, making its *effective pK* about (+)1.0. Salts do not address this because at such time they enter the biological system the metal ions are taken up; if that were not the case they would aggregate back out. Species IV will be visualized as that species which is the desired ionization state and form for the molecule. Just running a pK on a software platform will not typically reveal these features, Chemicalize and ACD both list the pKa as about -0.1, which is the sum of the species and in this case, at very high [non-biological] pH, which is not only irrelevant, but misguiding information.

As it turns out, trying to extrapolate from the graph, the *effective* pKa for species IV at biological pH 7 is somewhere between 1 and 2. This is not entirely surprising, as most drug substances are only weakly acidic or basic. Thus, it is not the pKa for the absolute species at near 100% ionization for the sum of the species, as only one species is effectively the drug substance [to interact as the phosphate leaving group of the nucleotide], with the caveat that *only pH 7*, biological pH is of any concern. That is, we can see the sum of species are effectively ionized at pH 10, however, that is non-sequitur, as there is no condition in a biological system; pH 10. Only the ionization of the desired species, and only at biological pH is of any importance.

I say these things clearly because I have seen far too many Medicinal Chemists merely look at the pKa's of a batch process, such as ACD Labs running a high throughput mode in Combinatorial Chemistry. They see the pKa's, which are not at all related to biological pH *for the desired ionization species,* not taking into consideration that the details of the ionization species and only at pH 7 is of value. Entire Combinatorial runs get tossed without having been properly evaluated.

Consequently, this is another reason phosphonoformic acid as Foscarnet is forced into a rather high dosage regimen. When we look at 2-phosphonobenzoic acid, the desired ionizable form is present at the 50% level: **2-PBA**



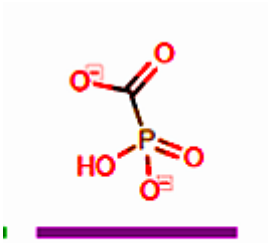
Note that species 3 and 4 are an order of magnitude greater ionized [in solution] at biological pH over that of PFA. This is a strong indication of ***maximized bioavailability.*** In this case the

bioavailability superiority is true for oral dosing as well as iv. ***Many drugs in the Anti-viral category do not have good bioavailability, none at 50%.***

The *diprotic species* is present, as form III and is ionized at about the 50% [59.39%] concentration level. At biological pH the species III *effective pK* is about 0.2 In addition, the triprotic species, missing in phosphonoformic acid, is abundant in 2-PBA. The triprotic species may take a significant role in chelating other metal ions, such as iron, cobalt, manganese, chromium, and so on.

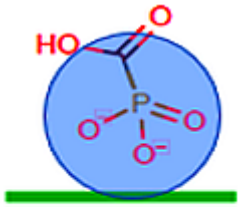
Noting that these are negative Log values, this is between one and two orders of magnitude in concentration difference over that of phosphonoformic acid. *When you sum together species III and IV*, the total ion abundance is one hundred-fold over that of species III for phosphonoformic acid. The *effective pK* for each and the sum of species III and IV is much more electronegative in this [biological pH] environment, which is the reason for its higher microspecies concentration. Meaning; *each ion* will have a greater affinity for the targets, which are the Transcription Enzyme and the metal ions that catalyze translocation. Again, form IV may play a significant role in chelating of higher oxidation transition metals in the Transcription process (e.g., +3).

Going back to metal chelation for phosphonoformic acid, it is abundantly obvious that this is species IV:



IV

NOT



III

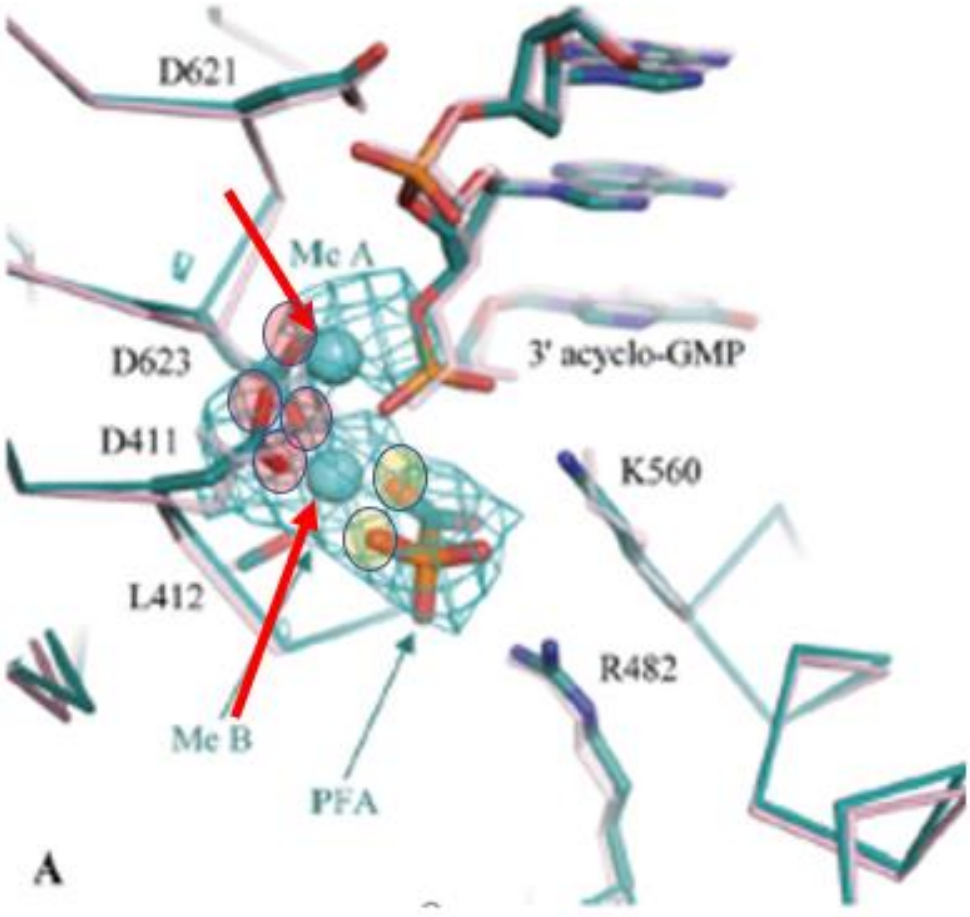
Species III is not going to chelate a metal ion. The steric placement of the two oxygens when maximized in 3D should be clear that this is the case. Species IV can chelate a metal ion. In a chelation the phosphorous has to be placed in the middle of that chelation bond, which is not going to happen in species III. So, the observation of metal chelation is species IV, and the effective mimicking of the phosphate leaving group of the nucleotide is species IV. Just a quick note that the mode of action for the acetic acid motif is likely purely a chelation via species III [phosphonoacetic acid]. For 2-Phosphonobenzoic acid, both species III [diprotic] and IV [triprotic] play a significant role in blocking DNA/RNA Polymerase Transcription by direct bonding and chelation.

Given that species IV for phosphonoformic acid is only ~10% ionized at biological pH, vs that of phosphonobenzoic acid, where both species III and IV are *each* abundant at the 50-percentile; this difference in *effective pK* predicts again, a hundred-fold increase in efficacy again, lacking the nephrotoxicity that limits phosphonoformic acid's utility as a drug substance. The nephrotoxicity

limitation limits both the amount and duration of dosing a patient can tolerate, which has kept the drug on the sidelines.

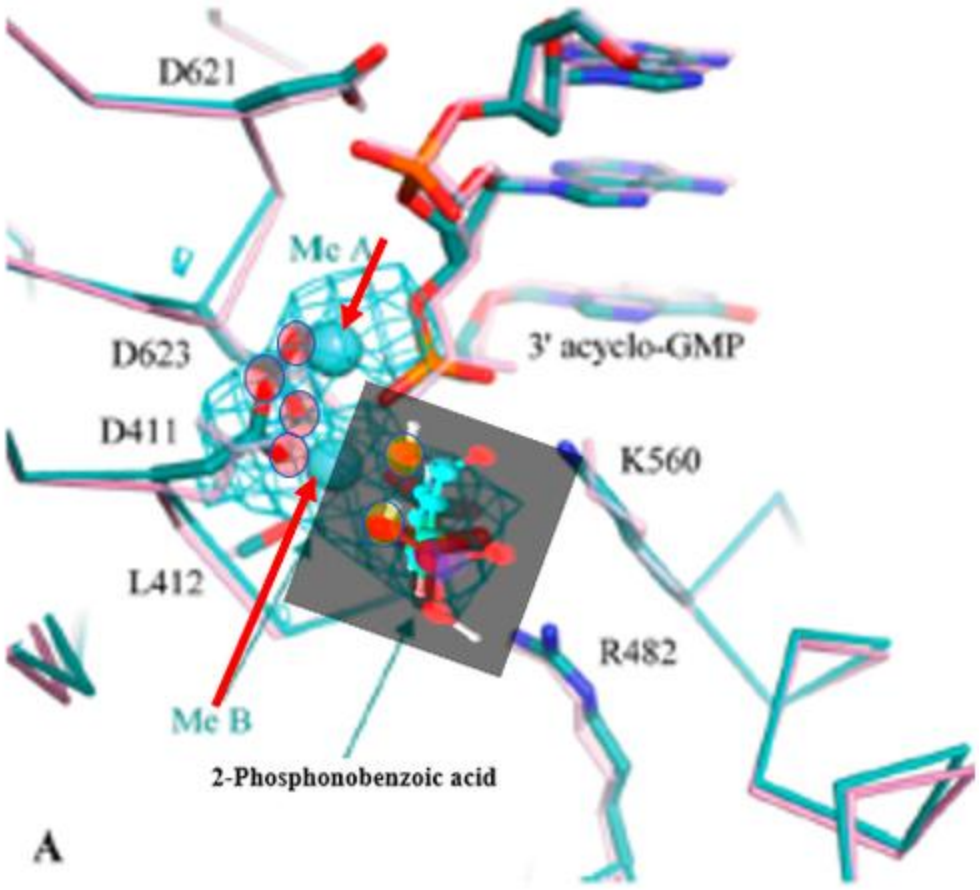
Binding Affinity and Imaging

Taken from the model of Zahn et al [11], we look at how they fit phosphonoformic acid into the binding matrix, then fit 2-Phosphonobenzoic acid into the binding site and find that they are exactly the same:



Above, phosphonoformic acid [labeled PFA] in an example of chimeric RB69, the phosphonic acid has a much higher affinity for the metal ions A and B [blue balls], as well as 'unbiased' superiority over that of the ASP [D]-411 on the enzyme, which changes conformation upon binding to the phosphonic acid, rendering it useless. Translocation cannot occur. Note that the two ion sites on PFA I have circled in yellow. The binding sites on the enzyme(s) are circled in red, the metal ions indicated by the red arrows. In every case, every binding site and metal catalyst involved in transcription has been bound. Transcription has been brought to a halt.

When we put Ortho-[2] Phosphonobenzoic acid in the mix:



Ortho-phosphonobenzoic acid fits precisely and taking into account its 2-orders of magnitude greater abundance as well as its much higher [electronegative] charge state, is a more efficacious drug substance. In addition, note that species III [diprotic] *and IV*, which is the triprotic form, is blocking any potential incoming group, in this example, R482 and K560.This may be the case with phosphonoformic acid, however given its abundance is nearly zero, plays no such role.

Properties

202.0 Da

At pH 7.2:

LogD: -6 LogP: -0.006 LogS [solubility] 2.0 [high, saturated]

pI -2.8

Indications

The following is a list of indications for phosphonoformic and acetic acids, where 2-phosphonobenzoic acid should have >100X the efficacy as well as being able to dose a patient

for prolonged periods of time due to the lack of nephrotoxicity, as there is no formic nor acetic motif present on the molecule.

The covalently closed circular (CCC) DNA of hepatitis B virus (HBV) functions as the only viral transcriptional template capable of producing all viral RNA species and is essential to initiate and sustain viral replication. Phosphonoformic acid inhibits Hepatitis B Reverse Transcriptase [14]. This is a key model in the use of phosphonic acids in global Anti-Viral mechanistics. The mechanism is irreversible DNA/RNA chain termination, which extends to the Anti-Viral domain of its efficacy in vivo [16]. Phosphonic acids possess activity as inhibitor of the FosA enzyme which in Gram-negative microorganisms. [17]

- Corona-SARS-covid species [1].
- Influenza, rhino [6].
- DNA Polymerase inhibition of influenza [7].
- Herpes virus DNA Polymerase [8].
- Phosphonoformic differs from phosphonoacetic in DNA Polymerase inhibition [9-10].
- Muscle Cell Calcification [VSMC] [12].
- Teratogenic carcinoma ATDC5 [13].
- Hepatitis B [14].
- Non-Small Cell Lung Cancer [15].
- HIV-1 Reverse Transcriptase [18].
- Epstein Barr Virus [19, 6]
- Epstein Bar Lymphoproliferative Disease [19, 6].
- Burkitt Lymphoma [19]
- Hodgkin's Lymphoma [19].
- Large B Cell Lymphoma [19].
- Human Cytomegalovirus [20].
- HSV-I [6]
- HSV-II [6]
- **Varicella**-zoster **virus [6]**

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